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Substitution Value of Cup Plant (*Silphium perfoliatum* L.) Silage in Dairy Cow Diet

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Abstract

Cup plant (*Silphium perfoliatum* L.) may serve as an alternative perennial forage crop in lowlands of the north central United States. Three feeding trials were conducted with Holstein cows (*Bos taurus*) at the early, mid, and late lactation stages to evaluate the impact of different cup plant silage substitution levels for alfalfa (*Medicago sativa* L.)-corn (*Zea mays* L.) silage mixture on milking cow performance. The concentrations of lignin and CP (crude protein) in the first cut and regrowth cup plant silage were lower than alfalfa silage. Substituting one-half of the silage reduced voluntary dry matter (DM) intake of early lactation stage cows by 11%. Although milk composition was not changed by the substitution, the 4% FCM (fat corrected milk) production was reduced by 7.5%. Another feeding trial tested substitution of one-third and two-thirds of the silage for mid-lactation cows. Increasing cup plant silage up to two-thirds of the forage portion in the diet reduced DM intakes and 4% FCM production by 21.8 and 8.7%, respectively. Milk composition did not change. Cow bodyweight was reduced as the substitution rate increased. Finally, a feeding trial with late-lactation cows indicated substitution of one-fourth of the silage performed equivalent in DM intake, milk composition, and milk production to those of cows fed a low forage diet (50% alfalfa-corn silage in diet), or a high forage diet (66% alfalfa-corn silage in diet). Based on the results of the three feeding trials, it is concluded that cup plant silage can substitute mixture of alfalfa-corn silage at up to 30% of the forage portion in diets without substantial negative impacts on the performance of dairy cows, especially during late lactation.

Keywords: *Silphium perfoliatum*, alfalfa, corn, silage, lactation

1. Introduction

Alfalfa (*Medicago sativa* L.) and corn (*Zea mays* L.) silages are the two primary forages in diets for lactating dairy cows in the north-central United States due to high crude protein (CP), or high energy concentrations. However, cropping systems based on alfalfa and corn silages are challenged on sites prone to intermittent flooding. Cup plant, a native perennial of wet to wet-mesic prairies of North America, is highly productive for biomass in soils too wet to maintain dependable production of alfalfa or corn (Albrecht & Goldstein, 1997). Cup plant has demonstrated superior biomass production and has been singled out for strategic use on wet “foot-slopes” in eastern South Dakota (Zilverberg et al., 2016).

Cup plant biomass production reaches more than 20 Mg DM/ha with a single harvest, and it has been promoted as an alternative to whole-plant corn for methane biogas production in Germany (Gansberger et al., 2015; Gerstberger et al., 2016). While research evaluating cup plant for forage utilization is limited, some research has documented potential feeding value as a silage crop with up to 240 g/kg DM of crude protein (CP) and 177 g/kg DM of water-soluble carbohydrates (Douglas et al., 1987; Han et al., 2000b; Pichard, 2012; Kowalska et al., 2020). In Russia, CP concentration of cup plant declined from 210 to 140 g/kg DM depending on the growth stage (Sokolov & Gritsak, 1972). In Wisconsin USA, Albrecht and Bures (1999) studied the nutritive value changes of cup plant between bud and flowering growth stages. Cup plant harvested at the bud stage contained 130 g CP/kg DM which was reduced to 96 g CP/kg DM at the late flowering stage. However, the neutral detergent fiber (NDF) concentration did not substantially change from 478 g/kg DM at the bud stage through late flowering.

The digestibility of fresh cup plant was 661 g/kg DM, while that of cup plant silage was 521 g/kg DM (Douglas et al., 1987). The *in vitro* true digestibility was around 700 g/kg DM and remained at that value even at the late-flowering stage (Albrecht & Bures, 1999). Results from an *in vitro* study demonstrated that the DM digestion rate and the extent of the NDF digestion of cup plant were similar to those of alfalfa when compared at similar NDF concentrations, however, cup plant digestion occurred at a slower rate than alfalfa (Han et al., 2000a). Therefore, DM intake potential of cup plant can be lower than that of alfalfa. A study conducted with cup plant in southern Chile reported *in vitro* DM digestibility ranging from 610 to 780 g/kg DM between the vegetative and seed-set stages (Pichard, 2012). Although the levels of CP, NDF, and digestibility need further evaluation to validate these results, the CP concentration and digestibility suggest that cup plant contains sufficient forage quality to be considered as a feed source for ruminant livestock.

In addition to the favorable CP and digestibility of cup plant, high biomass yield potential in a single harvest makes cup plant an attractive silage crop; however, thick stems and the moisture containing pith of cup plant require crushing the stem to speed the wilting process, which may cause some loss of nutritious leaves through mechanical damage. Limited research has investigated the feed value of cup plant in rations of ruminant livestock. Although the milk production cannot be compared to the current production in North America, Sokolov and Gritsak (1972) reported that cows fed cup plant silage produced 12% more milk than cows fed corn silage. However, a feeding trial with yearling beef cattle in Wisconsin USA suggested a limit of 60% cup plant silage substitution for corn silage in mixed diets (Lehmkuhler et al., 2007).

Based on the limited feeding trial reports, cup plant may serve as an alternative perennial forage crop where conventional forage crops cannot be productive. There is little information on optimum proportion of cup plant silage in dairy cattle diets. Therefore, three feeding trials were conducted to determine the impact on lactation performance when portions of an alfalfa-corn silage mixture were replaced with cup plant silage in the test diets of lactating dairy cows.

2. Methods

2.1 Forage Crop Production and Ensiling

Cup plant derived from a synthetic population of seed collected in Wisconsin, Minnesota, Illinois, and Iowa was grown on Plano silt loam soil at the University of Wisconsin Arlington Agricultural Research Station near Arlington, Wisconsin. Approximately 0.5 ha of cup plant was established in 90 cm row space at a density of approximately 40,000 plants per ha 2 years before forage was harvested for the feeding trials. During this period, biomass was cut and removed from the field in late summer. Nitrogen fertilizer was applied at 150 kg/ha each spring, and soil P and K were maintained at levels recommended for corn silage on that soil type. Cup plant foliage was cut with a New Idea model 5212 disk mower (AGCO, Georgia, USA) at the early bloom stage of maturity in mid-July, compressed through rubber rollers, and wilted for 30 h in the field. The wilted cup plant was chopped to a theoretical length of cut (TLC) of 1.5 to 2.0 cm and ensiled in plastic silo bags (Ag-Bag System, St. Nazianz, WI) for trials 1 and 2. In trial 3, cup plant second regrowth was harvested at the pre-bloom stage in October and ensiled as in trials 1 and 2.

The alfalfa used in all three trials was harvested at the bud stage and wilted to reach 350 g DM/kg before ensiling. Phosphorus and potassium were applied at 60 kg P_2O_5 and 180 kg K_2O /ha. Whole-plant corn was harvested between the 1/2 and 3/4 starch milk line of the kernel (Wiersma et al., 1993) in mid-September, chopped to a TLC of 2.0 cm, and ensiled. The DM concentrations of major ingredients in the diets were determined at the beginning of each trial to maintain the intended forage to concentrate ratios.

2.2 Feeding Trials

Three feeding trials were conducted with multiparous cows, weighing from 569 to 580 kg housed in a tie-stall barn at the Dairy Cattle Center located on the main campus of the University of Wisconsin, Madison. Cows were fed using individual pens twice a day (0900 and 1800 h), and the amount of feed offered and orts were weighed daily for each cow. Daily milkings were made twice a day at 0700 and 1600 h. All three trials were approved by the University of Wisconsin animal care and use committee.

Table 1. Feed composition and feed value (g/kg DM) of diet (total mixed ration) treatments in the three feeding trials

Item	Diet treatment[1]							
	Trial 1		Trial 2			Trial 3		
	CONT	$^{1/2}$CUPS	CONT	$^{1/3}$CUPS	$^{2/3}$CUPS	LF	HF	$^{1/4}$CUPS
Alfalfa silage	232	116	224	149	75	243	324	220
Corn silage	232	116	224	149	75	243	324	220
Cup plant silage	-	232	-	149	298	-	-	132
Corn gluten meal	126	126	83	83	83	-	-	-
Corn grain	212	212	283	284	283	-	-	-
Animal fat	19	19	9	9	9	-	-	-
Soybean meal (SBM)	145	145	111	111	111	51	36	77
Blood meal	10	10	9	9	9	-	-	-
Distiller grains	-	-	-	-	-	40	41	44
High moisture corn	-	-	-	-	-	308	162	176
Roasted SBM	-	-	-	-	-	101	101	110
Corn grain meal	-	-	36	36	36	-	-	-
Limestone	14	14	13	13	13	7	3	11
Dicalcium phosphate	4	4	4	4	4	4	5	4
Salt	5	5	3	3	3	3	3	4
Vitamin. A, D, E[2]	1	1	1	1	1	1	1	1
Feed value of diet								
DM	703	658	667	655	642	511	558	501
CP	202	191	168	162	165	153	153	157
aNDF	252	264	260	275	290	277	332	290
ADF	146	164	139	155	172	162	201	160

Note. [1] CONT: control diet containing alfalfa-corn silage in the forage portion at one half of total DM; $^{1/2}$CUPS: diet with one-half of the forage portion replaced with cup plant silage; $^{1/3}$CUPS: diet with one-third of the forage portion replaced with cup plant silage; $^{2/3}$CUPS: diet with two-thirds of the forage portion replaced with cup plant silage; LF: diet with low forage proportion; HF: diet with high forage proportion; $^{1/4}$CUPS: diet with one-fourth of the forage portion replaced with cup plant silage.

[2]Contains vitamin A > 3,300,000 IU/kg, vitamin D > 1,100,000 IU/kg; vitamin E > 11,000 IU/kg.

A diet containing 500 g/kg DM as the forage portion comprising an equal amount of alfalfa silage and corn silage was replaced with selected proportions of cup plant silage in the feeding trials and balanced according to NRC (1989) with concentrate feed ingredients, minerals, and vitamins (Table 1). Therefore, nutrient compositions in the diets slightly differed in the diet treatments.

The CP concentrations in the diets were balanced to 190 g/kg DM for early lactation cows (trial 1), 160 g/kg DM for-mid lactation cows (trial 2), and 150 g/kg DM for late lactation cows (trial 3) with corn gluten meal, soybean meal, and blood meal. The diet treatments were fed in the morning and afternoon. Milk yield, milk fat, and milk protein composition were measured for the last week of each experimental period. The amount of feed refusal, DM intake, and milk production were recorded daily for each cow. Milk and feed samples were collected twice a day for three consecutive days during the last week of each period.

2.2.1 Trial 1

The control diet (CONT) had a ratio of forage to concentrate of 46 to 54, and the forage portion was formulated with a 50:50 mixture of alfalfa and corn silage. A diet replacing one-half of the forage portion with cup plant silage ($^{1/2}$CUPS) was compared to the control diet. Six multiparous Holstein cows in early lactation (between 1 and 80 DIM, days in milk) were randomly assigned to one of the two diet treatments in a 2 × 2 Latin square design with three replications. Feeding periods were 14 d consisting of 9 d of diet adaptation and 5 d of sample and data collection.

2.2.2 Trial 2

The control diet (CONT) had a 45% forage portion formulated with a 50:50 mixture of alfalfa and corn silage. Test diets replacing one-third ($^{1/3}$CUPS), or two-thirds ($^{2/3}$CUPS) of the forage portion with cup plant silage were fed along with the CONT treatment. Twelve multiparous Holstein cows in mid-lactation (between 81 and 150 DIM) were randomly assigned to one of the three diet treatments in a 3 × 3 Latin square design with four replications. Feeding periods of 21-d, consisted of 11 d of diet adaptation followed by 10 d for sample and data collection.

2.2.3 Trial 3

Three diet treatments containing low, intermediate, and high levels of forage proportions were prepared in the trial. The $^{1/4}$CUPS was a diet replacing one-fourth of the intermediate level of standard forage (50:50 alfalfa-corn silage mixture) portion with cup plant silage. Therefore, the trial compared impact of the increasing forage level in diet, and especially when replaced limited portion of the intermediate level of forage with cup plant silage. The forage to concentrate ratios in the diets were 50:50, 65:35, and 58:42 for low forage (LF), high forage (HF), and cup plant silage ($^{1/4}$CUPS) treatments, respectively. Nine multiparous Holstein cows in late-lactation (> 150 DIM) were randomly assigned to the diet treatments in a 3 × 3 Latin square design with three replications. Feeding periods were 21 d, consisting of 11 d of diet adaptation followed by 10 d for sample and data collection.

2.3 Analysis of Feed Samples

Daily feed samples were prepared by treatment during each period. Subsamples of the diets were dried at 60 °C in a forced-air drying oven, and the dried feed samples were ground to pass a 1-mm screen with a Wiley Model 4 laboratory mill (Thomas Scientific, Swedesboro, NJ). A 1-g subsample was used to determine absolute DM by crucible drying in a 105 ºC oven for three hours. The aNDF and ADF concentrations of these samples were determined by procedures of Robertson and Van Soest (1981) using alpha-amylase and sodium sulfite as described by Hintz et al. (1996). Cup plant silage samples were analyzed by the same procedure as other feed samples after pre-extraction with acetone to minimize filtration problems incurred. Lignin (sa) in feed samples was determined with ADF as an intermediate step through non-sequential analysis (Goering & Van Soest, 1970). Total nitrogen (N) analysis was determined by the semi-micro-Kjeldhal procedure (Bremner & Breitenbeck, 1983), and CP was calculated as N × 6.25. Ash concentration was determined by modified AOAC 942.05 of Official Mehtods of Analysis (2005), igniting samples at 550 °C for 6 hours.

2.4 Statistical Analysis

Data obtained from feeding trials were analyzed using a mixed model analysis procedure of SAS (Littell et al., 1996) for replicated Latin square designs as the following model.

$$Y_{Ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_j + \alpha\gamma_{ik} + \varepsilon_{ijkl} \quad (1)$$

Where, Y_{Ijkl} = lactation performance, μ = overall mean, α_i = fixed effect of the diet treatment, β_j = effect of period, γ_k = effect of square, δ_j = effect of cow, $\alpha\gamma_{ik}$ = interaction between diet treatment and period, ε_{ijkl} = residual error. The least-square means of diet treatment were compared using PDIFF as a test option. The significance of the mean difference was determined at $P < 0.05$. The model considered treatment as a fixed effect; feeding period and cow were considered as random effects.

3. Results

3.1 Nutrient Value of Forages

Dry matter concentration of the first cut cup plant silage ranged from 275 to 295 g/kg DM in trials 1 and 2, indicating relatively high moisture concentration in cup plant even after the 30 hours of wilting (Table 2). Cup plant silage made with regrowth was wilted slightly more to attain a desirable DM range. Concentrations of aNDF in the first cut cup plant silage were greater than those in alfalfa silage or corn silage. However, the regrowth cup plant silage in trial 3 contained a lower fiber concentration than alfalfa silage.

Table 2. Chemical composition of the forage in the lactating cow diets (g/kg DM unless stated otherwise)

Item	Forage		
	Cup plant	Alfalfa	Corn
Trial 1[1]			
DM, g/kg	275±14[2]	580±12.1	358±10.0
aNDF	454±1.1	412±3.2	382±8.8
ADF	363±3.9	343±28.9	227±4.8
Lignin (sa)	36±3.9	69±1.2	22±0.9
CP	98±9.0	216±2.4	74±1.5
Ash	133±3.7	107±3.7	33±3.7
Trial 2[1]			
DM, g/kg	295±7.5	444±32.1	317±26.0
aNDF	493±8.9	396±9.3	385±2.6
ADF	349±7.5	268±8.0	203±15.0
Lignin (sa)	40±4.2	51±6.7	14±3.6
CP	118±1.0	250±2.4	79±1.5
Ash	150±3.0	121±3.0	37±3.0
Trial 3[1]			
DM g/kg	305±1.5	510±2.4	385±7.5
aNDF	392±4.4	415±14.5	291±3.2
ADF	220±1.6	311±8.6	217±1.2
Lignin (sa)	41±0.6	76±2.7	28±3.2
CP	115±1.4	241±3.7	73±0.7
Ash	165±3.0	104±3.0	29±3.0

Note. [1] In trials 1 and 2, the first growth cup plant was ensiled. In trial 3 second growth was ensiled.

[2] Values are mean±SE.

The ADF concentrations in cup plant were slightly higher or similar to those in alfalfa or corn silage. The lignin (sa) in cup plant silage ranged from 36 to 41 g/kg DM, which is lower than alfalfa silage by 35 g/kg DM, but greater than corn silage (Table 2). The CP concentrations in cup plant silage in the three trials were around 100 g/kg DM, which was lower than in the alfalfa silage but higher than in the corn silage. Ash concentrations of cup plant silage ranged from 133 to 165 g/kg DM (Table 2), which were greater values than the other two silages. The ash concentrations in cup plant silage were consistently higher than the other silages in the trials.

3.2 Diet Intake and Lactation Performance

3.2.1 Trial 1

Dry matter intake of cows fed $^{1/2}$CUPS was 2.7 kg/d lower than intake of cows fed CONT (Table 3). Acid detergent fiber and aNDF intakes were also lower for cows fed $^{1/2}$CUPS ($P < 0.01$). Milk yield, milk fat percentage, and milk protein percentage were not different between CONT and $^{1/2}$CUPS. Therefore, milk fat and milk protein production did not differ. However, the 4% FCM differed between cows fed CONT and those fed $^{1/2}$CUPS, and cows fed $^{1/2}$CUPS lost more bodyweight.

Table 3. Cow performance on control diet containing alfalfa-corn silage in the forage portion (CONT), and test diet with one-half of the forage portion replaced with cup plant silage ($^{1/2}$CUPS) in Trial 1.

Item	Diet treatment		SE
	CONT	$^{1/2}$CUPS	
Intake, kg/d			
DM	24.1[a1]	21.4[b]	0.73
CP	4.9[a]	4.1[b]	0.15
ADF	3.4[a]	2.7[b]	0.10
aNDF	5.9[a]	5.1[b]	0.18
Lactation performance			
Milk yield, kg/d	43.1	41.1	2.40
Milk fat, %	3.5	3.4	0.13
Milk protein, %	2.9	2.8	0.10
Milk fat yield, kg/d	1.5	1.4	0.11
Milk protein yield, kg/d	1.2	1.2	0.07
4% FCM[2], kg/d	40.1a	37.1b	2.49
Bodyweight change[3], kg	-34.3a	-41.7b	1.90

Note. [1] Means within a row with different superscripts differ ($P < 0.05$).

[2] Fat corrected milk production.

[3] Bodyweight change during last 21 days.

3.2.2 Trial 2

Dry matter, CP, aNDF, and ADF intakes decreased linearly ($P < 0.05$) as the cup plant silage in the diets increased (Table 4). Milk fat and milk protein composition were not affected by the proportion of cup plant silage.

Table 4. Cow performance on control diet containing alfalfa-corn silage in the forage portion (CONT), a test diet with one-third of the forage portion replaced with cup plant silage ($^{1/3}$CUPS), and a test diet with two-thirds of the forage portion replaced with cup plant silage ($^{2/3}$CUPS) in Trial 2

Item	Diet treatment			SE
	CONT	$^{1/3}$CUPS	$^{2/3}$CUPS	
Intake, kg/d				
DM	32.1[a1]	28.7[b]	25.1[c]	0.17
CP	6.7[a]	5.7[b]	4.7[c]	0.23
ADF	5.3[a]	4.2[b]	3.7[c]	0.21
aNDF	8.3[a]	7.4[b]	6.3[c]	0.30
Lactation performance				
Milk yield, kg/d	46.4[a]	44.2[b]	41.3[c]	1.17
Milk fat, %	3.6	3.5	3.4	0.14
Milk protein, %	3.1	2.9	2.8	0.82
Milk fat yield, kg/d	1.6	1.5	1.5	0.10
Milk protein yield, kg/d	1.0[a]	0.8[b]	0.7[c]	0.04
4% FCM[2], kg/d	42.5[a]	40.7[ab]	38.8[b]	1.65
Bodyweight change[3], kg	24.4[a]	6.6[b]	-14.2[c]	4.01

Note. [1] Means within a row with different superscripts differ ($P < 0.05$).

[2] Fat corrected milk production.

[3] Bodyweight change during last 21 days.

However, milk yield decreased as cup plant silage proportion increased to the $^{2/3}$CUPS treatment. The 4% FCM of $^{2/3}$CUPS was also lower than that of CONT. The cows on CONT and $^{1/3}$CUPS gained weight, while those on $^{2/3}$CUPS lost weight (Table 4).

3.2.3 Trial 3

Dry matter intakes of $^{1/4}$CUPS did not differ from LF or HF (Table 5). The CP intake also did not differ among the diet treatments. Cows on HF had greater aNDF and ADF intakes than LF and $^{1/4}$CUPS due to the high fiber concentration in the diet treatment, which was followed by $^{1/4}$CUPS and LF. The production parameters milk yield, 4% FCM, milk compositions, milk fat, and milk protein production were not different among the diet treatments. In all three diets, cows gained weight, and $^{1/4}$CUPS gained the least.

Table 5. Cow performance fed on diets with low forage (LF), high forage (HF), and one-fourth of the forage portion substituted with cup plant silage ($^{1/4}$CUPS) in Trial 3

Item	Diet treatment			SE
	LF	HF	$^{1/4}$CUPS	
Intake, kg/d				
DM	20.8	21.8	20.7	1.59
CP	3.6	3.6	3.6	0.07
ADF	3.0^{c1}	4.0^{a}	3.3^{b}	0.07
aNDF	5.4^{c}	6.7^{a}	5.6^{b}	0.12
Lactation performance				
Milk yield, kg/d	27.0	26.4	26.5	0.74
Milk fat, %	3.7	3.8	3.7	1.80
Milk protein, %	3.4	3.3	3.3	0.16
Milk fat yield, kg/d	1.0	1.0	1.0	0.06
Milk protein yield, kg/d	0.9	0.9	0.9	0.03
4% FCM[2], kg/d	25.9	25.6	25.3	0.98
Bodyweight change[3], kg	18.3^{b}	31.9^{a}	3.3^{c}	1.10

Note. [1] Means within a row with different superscripts differ ($P < 0.05$).

[2] Fat corrected milk production.

[3] Bodyweight change during last 21 days.

4. Discussion

As noted in the literature review, cup plant can serve as a buffer crop between fertile cropland and wetlands for years, capturing nutrients in runoff before they enter wetlands. Additionally, cup plant can serve as a wildlife crop because of its attractiveness to native pollinators and birds (Tuell et al., 2008). For these reasons, it is an ecologically attractive crop to incorporate into forage system in North America. As a native forb, cup plant has value as an alternative forage crop due to high biomass production potential and greater persistence than conventional forage crops on marginal land. With those merits, cup plant may serve as a forage crop if feeding value is proven for a range of ruminants.

The first growth of cup plant used in trials 1 and 2 was chopped and ensiled at marginally low levels of DM because of impending rain. The pH measurement of cup plant silage averaged near 6.0 in the study. The butyric acid and lactic acid concentrations in the cup plant silage averaged 2.24 and 2.45 g/kg DM, respectively. Although the unconsumed cup plant silage was not quantified in the trials, a substantial proportion of orts were identified as cup plant silage. The high concentration of acetic acid (data not presented) could be the reason for the low DM intake of silage with cup plant substituted for alfalfa and corn silage in diets in trials 1 and 2. Since soluble sugar concentration in biomass ranged from 120 to 210 g/kg DM (Daniel & Rompf, 1994), fermentable sugar does not seem to be a limiting factor to lactic acid fermentation. The DM concentration in fresh cup plant biomass can be as low as 15% (Douglas et al., 1987; Albrecht & Goldstein, 1997; Daniel & Rompf, 1994), wilting appears to be an essential step for this high yielding and high water containing biomass crop (Han et al., 2000b). However, in the Wisconsin climate, moisture reduction of biomass is always challenging. It can take several days to reach 300 g DM/kg even under full sun (Albrecht, unpublished data). Cup plant retains most of its moisture in thick stems, and mechanical conditioning is required at cutting to hasten the wilting process.

Compared with alfalfa or corn silage, the high fiber in cup plant silage is distinct. However, the higher fiber did not necessarily reason for low digestibility due to the different characteristics of the cell wall component (aNDF) in forage crops. Han et al. (2000a) reported a relatively slower increase of the lignin (sa) proportion in cup plant compared with the increase of aNDF, which was also confirmed through the lower lignin than alfalfa in the current study (Table 2). Since aNDF is a combined analytic value of cellulose, hemicellulose, lignin, mineral, and some detergent-insoluble nutrients, the actual digestibility of the forage should depend more on the indigestible portion rather than aNDF concentration. Maintaining relatively high *in vitro* true digestibility even at high aNDF reflects a moderate impact of increasing fiber on the digestibility of cup plant (Han et al., 2000a). Although lignin is a recalcitrant compound and demonstrates a particular relationship with indigestible aNDF in various forage crops (Van Soest et al., 2005; Raffrenato et al., 2018), the impact of lignin on digestibility differs across species, genetic background, and growth environment. The lignin proportions in aNDF of cup plant silage ranged from 8 to 10%, while that of alfalfa ranged from 12 to 18% (Table 2). Relatively lower lignin (sa) concentration in cup plant silage compared to that in alfalfa silage may explain the greater digestibility potential of cup plant when compared with that of alfalfa at similar aNDF levels (Han et al., 2000a). Therefore, cup plant may provide a wider harvest window than conventional forage while maintaining digestibility. The range of CP concentration of cup plant reported in the literatures varies (Pichard, 2012; Daniel & Rompf, 1994, Albrecht & Goldstein, 1997). It was greater than that in corn silage by 2 to 4 percentage units in this study but lower than alfalfa silage. As a forage ingredient, Kowalska et al. (2020) indicated that cup plant can be an alternative forage option providing various amino acids and high CP. The CP in the current cup plant silage ranged from 98 to 118 g/kg, and it was somewhat low, perhaps due to the leaf loss during the wilting process.

Greater mobilization of energy within the body likely occurred at the early lactation stage regardless of the nutrient intakes. Therefore, cows fed either the control or test diet in trial 1 lost body weight (Table 3). However, the replacement with cup plant reduced DM and nutrient intake and resulted in more weight loss. The linear increase ($P < 0.05$) of cup plant silage portion up to two-thirds of the forage portion in the test diet also caused increased suppression of DM intakes at the mid-lactation stage. However, milk production was maintained up to replacement of one-third of the alfalfa and corn silage with cup plant silage. According to the life cycle assessment on silages by Bernas et al. (2021), cultivation of cup plant targeting a replacement for corn-alfalfa silage at 33% level could maintain similar dairy production levels as monoculture silages of the alfalfa or corn silage while yielding a lower environmental load. Moreover, the lower milk yield without significant concentration changes in milk fat and milk protein resulted in a linear decrease of milk protein yield and 4% fat corrected milk production in cows fed increasing cup plant silage proportions in the diet (Table 4). This was due to a depressed intake of DM with the inclusion of cup plant silage. The low DM intake of cup plant silage is probably related to weak fermentation and some secondary compounds. There should be some improvement in cup plant preservation to enhance the palatability of the silage.

Cup plant is also known to contain a range of secondary compounds, including sesqui-, di-, and tri-terpenoids (Pcolinski et al., 1994). Reed (1997) reported that volatile terpenoids, even if non-toxic, can lower palatability due to a strong aroma. Thus, in addition to improving the fermentation of cup plant through wilting, the impact of secondary compounds on the utilization of cup plant should be clarified. Perhaps longer adjustment periods could overcome some of the reluctance of cows to consume cup plant silage. In trial 3, the intake and milk yield of cows given a diet containing regrowth cup plant silage at one-fourth of the forage portion were equivalent to those of cows fed a low fiber diet (Table 5). A beef cattle feeding trial with cup plant silage by Lehmkuhler et al. (2007) demonstrated that the average daily gain did not differ between the cattle fed 30% cup plant silage and those fed a 100% corn silage diet; however, DM intake and NDF digestibility decreased as cup plant silage was increased in the diet. In addition to the weak fermentation and potential secondary compound issues, longer lag time for ruminal digestion than alfalfa (Han et al., 2000a) may cause slower rumen passage of cup plant and contribute to the lower DM intake. The ash concentration in cup plant silage may also require another consideration for the lower intake.

Considering its feeding value, cup plant should replace no more than 30% of the conventional forage portion in diets of milking cows to avoid loss of milk production. Although there is potential for increased feed value with the improvement of ensiling quality, replacing conventional alfalfa-corn silage with cup plant silage should be carefully applied so as not to sacrifice the performance or body condition of milking cows.

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Abbreviations

aNDF: neutral detergent fiber after amylase treatment; ADF: acid detergent fiber; DIM: days in milk; Lignin (sa): sulfuric acid detergent lignin; CONT: control diet containing alfalfa-corn silage in the forage portion of total DM; $^{1/2}$CUPS: diet with one-half of the forage portion replaced with cup plant silage; $^{1/3}$CUPS: diet with one-third of the forage portion replaced with cup plant silage; $^{2/3}$CUPS: diet with two-thirds of the forage portion replaced with cup plant silage; HF: diet with high forage proportion; LF: diet with low forage proportion; $^{1/4}$CUPS: diet with one-fourth of the forage portion replaced with cup plant silage.

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Has Continued Exposure to Banana Xanthomonas Wilt Worsened Farmers' Welfare Over Time? Evidence From Banana-Producing Households in Uganda

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Abstract

The livelihoods of millions of banana-farming households have been affected by Banana Xanthomonas Wilt (BXW) in Uganda for nearly two decades. The disease has no known cure, all banana cultivars grown are susceptible to it and it is endemic in all banana-producing regions in the country. This study analysed the long-term impact of the disease on the livelihoods of banana-producing households. Using a balanced panel dataset of 1,056 households, which were visited in 2015 and revisited in 2018, provides the opportunity to empirically measure the long-term consequences of the disease on farmers' economic wellbeing in the four major banana growing regions in Uganda. We find striking results pertinent to disease incidence, success in disease management, household income and poverty when deploying BXW control practices. Although the disease has remained present in farmers' fields, there is a significant reduction in household poverty levels. Results show that some farmers expanded the production of beans and coffee without encroaching on their banana plantations. Increase in bean production was largely through intercropping. Investment in coffee was constrained by land ownership, hence only a viable venture for the wealthy farmers who own bigger pieces of land. Land-poor farmers continued to rely on bananas for their livelihoods. Consistent participation in disease management training significantly influenced adoption of the cultural control practices. Consequently, farming households that systematically adopted these practices were able to maintain low levels of disease incidence, improve productivity by 438 kg/ha/year and increased their daily and annual household income by US$1.75 and UGX 2.383 million (US$648), respectively. The findings suggest that banana is an important crop to smallholder farmers and expansion into other cash crops requires bigger resource outlays, and that despite continued exposure to BXW, farmers' income increased over time. Farmers should continuously and systematically use the recommended control practices to avoid BXW resurgence and, consequently, a reduction in their income.

Keywords: Banana Xanthomonas Wilt, coping strategies, household income, systematic adoption

1. Introduction

About a third of the global banana production is conducted in Sub-Saharan Africa (SSA) where the crop is a very important staple and a cheap source of minerals and vitamins, particularly vitamins A, C and B6 (Karamura et al., 2008). In East and Central Africa (ECA), the banana crop significantly contributes to household food security and income of the farming communities (Muchuruza & Melchior, 2013; Nkuba et al., 2015; Ocimati et al., 2019). Besides being the leading banana producer in ECA, Uganda has the highest per capita consumption of bananas in the world (Kabahenda & Kapiriri, 2010; Kikulwe, Okurut, Ajambo, Nowakunda, et al., 2018; Kilimo Trust, 2012). The crop accounts for 17% of daily caloric needs in the country (Fiedler, Kikulwe, & Birol, 2013) and is the major income source for millions of farmers (Jogo, Karamura, Tinzaara, Kubiriba, & Rietveld, 2013; Karamura et al., 2010; Kikulwe et al., 2018). Furthermore, it has great environmental value in most farming systems as it reduces soil erosion on steep slopes, conserves soil fertility and provides cover to other crops such as beans, groundnuts and coffee with which it is often intercropped (Geberewold, 2019; Uwamahoro, Berlin, Bylund, Bucagu, & Yuen, 2019).

Despite its importance, the banana crop is affected by Banana Xanthomonas Wilt (BXW) disease—the leading impediment to its production in ECA (Nkuba et al., 2015). It has no cure and all the cultivars grown in SSA are susceptible to it (Kubiriba & Tushemereirwe, 2014). In 2001, banana farms in Uganda were hit by the BXW epidemic (Tushemereirwe et al., 2004). The disease spread at such an alarming rate that, by 2005, more than 76% of the fields in the key growing regions were affected (Tushemereiwe et al., 2006). The epidemic resulted in drastic losses, since every mat with a stem that exhibited symptoms had to be uprooted and buried (Tripathi et al., 2009). Kalyebara et al. (2006) estimated that, if the disease was left to spread uncontrolled, Uganda stood to lose US$295 million per annum in revenue from banana output, valued at farm gate prices. Similarly, Kayobyo, Aliguma, Omiat, Mugisha, and Benin (2005) noted that uncontrolled BXW can spread at an infection rate of 8% per annum in cooking bananas, resulting in a total production loss of 56% over a 10-year period. Karamura et al. (2010) found that the total yield loss due to BXW at the peak of the epidemic between 2001 and 2004 was between 30-52% leading to a decline in banana harvests, sales, farm-gate prices and total household income.

Farmers resorted to different coping strategies to deal with the BXW outbreak, some of which negatively affected banana production and productivity. For instance, farmers in the Kagera basin of Rwanda, Tanzania and Burundi were prompted to diversify into other food crops such as maize, cassava and sweet potato, which led to a reduction in the area under banana (Nkuba et al., 2015). Similarly, after losing about 80% of their bananas within a two-year period, farmers in Katana village around Lake Kivu in the Democratic Republic of Congo cleared their plantations to make way for other crops (Vezina, 2014). In Uganda, the households whose plantations were infected with BXW reduced consumption of own-produced bananas and resorted to consuming and trading more of the other food crops (Karamura et al., 2010). A few of these households (9%) reported clearing the plots and planting similar or different banana cultivars, or different crops, whereas only 5% reported complete abandonment of banana (Karamura et al., 2010).

To curtail the spread of BXW, a set of cultural control practices are recommended. These practices include planting healthy suckers, breaking of male buds with a forked stick, disinfecting farm tools after use on each plant and removal of each infected plant also referred to as single diseased stem removal (Blomme et al., 2017; Jogo et al., 2013; Kikulwe et al., 2019). These cultural practices reduce the inoculum's density and limit the spread of the pathogens but must be applied as a package for effective disease control (Blomme et al., 2017; Kubiriba & Tushemereirwe, 2014). However, since more than 90% of the farmers in ECA use suckers from their own fields or rely on farmer- to-farmer exchanges, as opposed to pathogen-free tissue culture plantlets that are expensive and not readily accessible, they have no means to ascertain whether the suckers used are disease-free (Jogo et al., 2013; Smith, Jones, Karamura, Blomme, & Turyagyenda, 2008). Based on this evidence, the recommended package of three practices abbreviated as BCC (Breaking the male bud using a forked stick; Cleaning tools through disinfection, and Cutting down diseased stems) has been widely promoted and adopted (Blomme et al., 2017; Jogo et al., 2013). Adoption of this package has been found to significantly increase banana productivity and sales (Kikulwe et al., 2019). Male buds are the primary site for insect-mediated infection, hence their removal using a forked stick helps to control the disease (Biruma et al., 2007). Disinfecting farm tools using sodium hypochlorite or flaming them over a fire kills any pathogens that may remain on the tools after working on a diseased plant, preventing transmission from one plant to another (Mwangi, Nakato, & Muthoni, 2007). On the other hand, single diseased stem removal (SDSR) reduces disease inoculum and its correct implementation has been found to reduce BXW incidence from 80% to less than 2% within 3-4 months (Blomme et al., 2017). SDSR is also highly recommended because it is a labour-saving and cost-effective practice (Blomme et al., 2019; Kubiriba & Tushemereirwe, 2014).

Literature is replete on the adoption of these cultural control practices and the management of BXW in the region. Some scholars have assessed the role of specific control practices in curbing the disease (Blomme et al., 2014; Blomme et al., 2017; Mwangi et al., 2007; Nakakawa et al., 2017). Kikulwe et al. (2018) examined the role of gender in management of the disease, whereas Ocimati et al. (2019) cited the key factors responsible for disease spread and mapped the current hotspots and vulnerable landscapes, which is crucial for disease early warning and management to curtail further spread. Some studies have focused on the determinants of adoption of the control practices (Jogo et al., 2013; Kikulwe et al., 2019) and the approaches used to promote these practices (Kubiriba & Tushemereirwe, 2014). A handful of studies (Karamura et al., 2010; Kikulwe et al., 2019) have assessed the effects of BXW and adoption of control practices on the livelihoods of farmers in Uganda. Kikulwe et al. (2019) analysed the impacts of adoption on banana productivity and sales using augmented inverse probability weighting based on cross-sectional data. Yet, Karamura et al. (2010) assessed the impact of BXW on banana yield and livelihoods of farming households using data for two distinct periods (2001 and 2004) from four districts in central Uganda, which is not representative of the four banana growing regions in Uganda. We contribute to this literature by measuring the impact of systematic adoption of the control practices on banana productivity and household welfare. According to Nakakawa et al. (2017), effective management of BXW requires systematic adoption of control practices to avoid a possible resurgence of the disease. We used a balanced panel dataset of 1,056 farming households from four major banana-producing regions in Uganda and employed an Ordinary Least Squares (OLS) method to provide in-depth evidence on the long-term impact of BXW, and systematic adoption of the control practices on farmers' income and the coping strategies they employed amidst continued exposure to the disease.

The remainder of the paper is arranged as follows: the next section describes the methods and data used for the study. Section three presents and discusses empirical findings. The final section draws the concluding remarks.

2. Materials and Methods

2.1 Sampling and Sample Size

The data used was collected from the four major banana producing regions in Uganda namely: south-western, central, mid-western and eastern. Two rounds of household surveys were conducted, one in 2015 and the other in 2018 from 1,056 randomly selected banana-farming households constituting a balanced panel with 2,112 observations. Details of sampling and sample size determination are found in Kikulwe et al. (2019). The 2015 data served as the baseline. In both survey rounds (2015 and 2018), face-to-face interviews were conducted using a structured questionnaire to collect data on: socio-demographic characteristics of the farmer; status of BXW at individual farm level; BXW control practices adopted by the farmer to manage the disease; number of years since BXW was first observed on the farm; production details of banana and the other major crops grown; income from livestock; and income from off-farm activities.

2.2 Analytical Methods

2.2.1 Empirical Approach to Determine the Drivers of Households' Choice of Coping Strategy in the Face of Continued BXW Exposure

Exposure to BXW significantly affects households' welfare (Karamura et al., 2010; Kikulwe et al., 2019). Like any other shock, the level of impact of the shock depends on the level of exposure (in this case incidence of BXW) and level of household endowments such as land owned, skills and knowledge of how to deal with the shocks (Chapoto & Jayne, 2008; Mehar, Mittal, & Prasad, 2016). These factors determine the type of coping strategy adopted by the household and overall household welfare. We therefore first examine the drivers of the type of coping strategy employed by households amidst continued presence of BXW on banana farms. Six coping strategies are evaluated. Three of the six coping strategies (*i.e.*, change in maize, beans, and coffee acreages) focus on expected changes in crop mix land reallocation strategies to deal with the disease shock. Two other strategies (*i.e.*, livestock income and off-farm income) focus on diversification into other income sources to deal with the disease shock. The last coping strategy is the change in per capita consumption of banana. Similar coping strategies have been examined in the face of various shocks such as climate change (Mehar et al., 2016), human diseases (Chapoto & Jayne, 2008), and plant diseases (Karamura et al., 2010; T.-T. Nguyen, T. T. Nguyen, & Grote, 2020; Nkuba et al., 2015).

To determine the choice of coping strategies that households employ to mitigate the effects of BXW disease on household welfare, we used the Ordinary Least Squares (OLS) method. The OLS method has been widely used to examine welfare impacts (Chapoto & Jayne, 2008; Hartje, Bühler, & Grote, 2018; Jena, Chichaibelu, Stellmacher, & Grote, 2012; T.-T. Nguyen, T. T. Nguyen, & Grote, 2020) including those resulting from plant pests and diseases (T.-T. Nguyen, T. T. Nguyen, & Grote, 2020). We specified the OLS regressions for each year as follows:

$$Y_{it} = \alpha_1 + \beta_1 D_{it} + \theta_1 H_{it} + \varepsilon_{it} i \quad (1)$$

$$Y_{i(t+3)} = \alpha_2 + \beta_2 D_{i(t+3)} + \theta_2 H_{i(t+3)} + \varepsilon_{i(t+3)} \quad (2)$$

Equations 1 and 2 correspond to 2015 (time *t*) and 2018, (time *t*+3) respectively. Y_i denotes the outcomes for each household i; D_{it} and $D_{i(t+3)}$ denote the incidence of BXW for household i in 2015 and 2018, respectively, whereas H_i is a vector of household socioeconomic characteristics and ε_i is a random error term.

To obtain the changes in the outcomes resulting from the changes in disease incidence over time, we take the difference in the variables in the two time periods as shown in Equation 3:

$$\Delta Y_i = Y_{i(t+3)} - Y_{it} \quad (3)$$

The socioeconomic characteristics considered, such as gender and education level of the household head, membership in savings groups, and farm location and size, hardly changed in the study period while others like the household's age are changing by a constant. Therefore, to avoid endogeneity issues, we take these variables at baseline as in 2015 (Wooldridge, 2002). Thus, the regression equation is specified as follows:

$$\Delta Y_{ij} = \alpha + \beta\Delta D_i + \emptyset_1\Delta W_{it} + \partial_1 R_i + \theta_1 H_{it} + \Delta\varepsilon_i \quad (4)$$

Where, ΔY_{ij} is the 2018-2015 difference for a coping strategy j, for each household i. ΔY_{i1} is the difference in acreage allocated to maize, ΔY_{i2} is the difference in acreage allocated to beans, ΔY_{i3} is the difference in acreage allocated to coffee, ΔY_{i4} is the difference in per capita banana consumption, ΔY_{i5} is the difference in household livestock income, and ΔY_{i6} is the difference in household off-farm income. D_i is the disease incidence indicator, β is the effect of the disease incidence on the coping strategy adopted, α is a constant, H_{it} is a vector of socioeconomic household characteristics and location variables as captured in 2015 with their corresponding effects captured as θ_1. The socioeconomic household characteristics included in the model estimations were age, sex, and education level of the household head, household size, land owned and membership to a SACCO. ΔW_{it} is the difference in number of disease control practices adopted and R_i is a location variable captured by the region of the household. $\Delta\varepsilon_i$ is the difference between errors in 2018 and 2015.

2.2.2 Empirical Approach for the Impact of BXW on Farmers' Welfare

The OLS method was also used to estimate the impact of BXW on farmer' welfare as shown in Equation 5.

$$\Delta Z_{ij} = \alpha + \beta\Delta D_i + \emptyset_1\Delta W_{it} + \partial_1 R_i + \theta_1 H_{it} + \partial_1 E_i + \partial_1 P_i + \partial_1 T_i + \partial_1 S_i + \Delta\varepsilon_i \quad (5)$$

Where, ΔZ_{ij} is the 2018-2015 difference for a welfare variable *j*, for each household i. ΔZ_{i1} is the difference in banana productivity (ton/ha/year), ΔZ_{i2} is the difference in daily household Welfare, ΔZ_{i3} is the difference in Annual Household welfare. ΔD_i, ΔW_{it}, R_i, H_{it} and $\Delta\varepsilon_i$ are as already defined in Equation 4. E_i is the number of years the household has been exposed to BXW, P_i is a dummy variable that captures whether BXW was present on the farm by the time of the survey in 2018, T_i is a dummy variable for households that received training in management of BXW in both panels, and S_i is a dummy variable for households that adopted sanitation practices in both panels.

2.3 Variables Used

In this study, disease incidence was measured as the difference between the number of mats infected with BXW per acre on each farm in 2018 and 2015 at the point of data collection. This measure is used as the treatment variable in all models. We therefore hypothesize that, with increased incidence, farmers are more likely to employ coping mechanisms such as increasing the production of other crops, reducing consumption of bananas and/or engaging in other income-generating projects such as livestock production or off-farm activities.

Household welfare is measured as the aggregated value in Uganda shillings (UGX) of the value of production of four major crops (banana, maize, beans and coffee) grown, income from livestock sales and household off-farm income obtained over a period of one year. Off-farm income includes the wages, salaries and pensions of all household members, land rents and capital earnings, as well as any net profit (revenue minus cost) from non-agricultural businesses. A description of the welfare variable is shown in Table 1. Banana productivity affects food security and it influences the overall wellbeing of the household. BXW is expected to have a negative effect on banana productivity (Blomme et al., 2014; Karamura et al., 2010). Per capita consumption of bananas is another outcome variable that was considered, and it is measured as the total kilograms of bananas consumed per household member per year. As a result of the disease, per capita consumption of bananas is also expected to decline as the farming households feed more on other foods and less on bananas (Karamura et al., 2010).

Table 1. Description of the welfare variable

Component of Household Welfare	Detailed Description
Annual value of crop production*	• Value of banana produced • Value of maize produced • Value of beans produced • Value of coffee produced
Annual livestock sales	• Income from livestock sales
Annual household off-farm income	• Wages, salaries, and pensions • Land rents and capital earnings • Net profit from non-agricultural businesses
Annual household welfare	Summation of all the above
Daily household welfare	Annual household welfare/365

Note. * The value of crop production is obtained by multiplying the volume of crop produced by the average prevailing price per unit.

Most monetary values are expressed in Uganda shillings (UGX), except for daily household welfare that is expressed in US$ for ease of comparison with global poverty levels (1 US$ = 3,677 UGX). To account for inflation and make monetary values comparable for the two survey rounds, 2015 data were adjusted to 2018 using the official consumer price index as quoted by the Uganda Bureau of Statistics (UBOS, 2019).

For most of the regression models, the same vector of covariates is used, although other explanatory variables are sometimes added depending on the particular outcome. The vector of covariates includes household characteristics, such as age, education and gender of the household head, as well as the size of the household, social aspects such as membership in a SACCO (Savings and Credit Co-operative), farm characteristics such as total land owned, and the level of adoption of the BXW control practices. In this paper, we are not measuring adoption per se because it has been extensively explored in various studies (Bagamba et al., 2006; Jogo et al., 2013; Karamura et al., 2010; Kikulwe et al., 2018, 2019; Kubiriba & Tushemereirwe, 2014). We are focusing on the systematic adoption of these practices as opposed to conducting them sporadically. Recent research has revealed that for effective BXW management, adoption needs to be systematic and consistent, even when the disease is undetectable, to eliminate possible resurgence (Nakakawa et al., 2017).

3. Results and Discussion

3.1 Changes and Trends in Area Allocated to Each Crop, Production and Poverty Between 2015 and 2018

In this section, we present the descriptive statistics, changes, and trends in the area planted to each crop, production, and poverty between 2015 and 2018 (Table 2).

Table 2. Descriptive statistics of dependent and explanatory variables

Variable	2015		2018		t value
	n	mean	n	mean	
Age of the household head	1056	53.8 (14.5)	1056	56.7 (14.6)	-4.65***
Gender of the household head (1=Male)	1056	0.75 (0.43)	1056	0.74 (0.44)	0.75
Education level of household head (years)	1056	5.95 (3.91)	1056	5.95 (3.91)	0
Household size	1056	6.37 (3.13)	1056	6.37 (3.14)	0.05
Membership in a SACCO (1=Yes)	1056	0.23 (0.42)	1056	0.29 (0.45)	-2.75***
Presence of BXW (1=Yes)	1056	0.63 (0.48)	1056	0.56 (0.50)	3.45***
Number of adopted practices	1056	1.46 (1.06)	1056	1.90 (1.03)	9.70***
Sanitation practices employed (1=Yes)	1056	0.84 (0.37)	1056	0.76 (0.43)	4.5***
Banana acreage	1020	2.64 (3.33)	1051	2.52 (2.95)	0.931
Maize acreage	441	1.21 (1.48)	809	1.50 (1.88)	-2.787***
Beans acreage	616	0.65 (0.74)	933	1.10 (1.25)	-7.999***
Coffee acreage	458	2.27 (2.41)	745	1.85 (2.55)	2.826***
Disease incidence (mats infected per acre)	625	4.14 (5.22)	587	4.32 (5.59)	-0.577
Value of banana production to value of crop production	886	0.70 (0.32)	965	0.83 (0.20)	-10.550***
Value of banana production to household income	886	0.49 (0.34)	965	0.72 (0.25)	-16.849***
Poor (1=Less than $1.90 per day, 0=otherwise)	1056	0.55 (0.50)	1056	0.19 (0.39)	18.331***
Extremely poor (1=Less than $1.00 per day, 0=otherwise)	1056	0.36 (0.48)	1056	0.11 (0.31)	14.176***

Note. Values in parentheses are standard deviations. *** $p < 0.01$, ** $p < 0.05$, *$p < 0.1$.

Descriptive statistics of changes in the key variables in 2015 and 2018 are summarised in Table 2. Despite a significant increase in the land allocated to maize, beans and coffee, there was no significant change in the land allocated to bananas. However, the contribution of bananas to the total value of crop production and to household welfare increased significantly in 2018, showing a high concentration of earnings among the interviewed farmers. The number of farms that had traces of BXW significantly reduced from 63% in 2015 to 56% by 2018 and on average, there were approximately 4 mats infected per acre in both time periods. There was a significant increase in the average number of practices adopted from one to two. It is evident that disease incidence was maintained at low levels through continued adoption of the control practices. BXW has been found to resurge where the levels of adoption of the control practices are low (Jogo et al., 2013; Kikulwe et al., 2019). Globally, households surviving on less than US$1.90 per day are categorised as poor, whereas the official absolute poverty line for Uganda—as for other low-income countries—is at US$1.0 per day (Ssewanyana & Kasirye, 2013; UBOS, 2014; World Bank, 2008). For our analysis, we categorised those living below US$1.00 per day as extremely poor. We observed a significant reduction in the poverty levels, which implies that the farming households have not been thrown into abject poverty despite continued exposure to BXW.

3.2 Empirical Results

3.2.1 Coping Strategies

This section summarises the coping strategies that banana-producing households employed in the face of BXW. Table 3 shows the empirical results for the models used to evaluate the impact of BXW on land allocated to maize, beans and coffee; per capita consumption of banana, livestock sales and involvement in off-farm activities following Equation 4.

Table 3. How changes in BXW incidence influenced changes in maize, bean and coffee acreages; per capita consumption of banana; income from livestock and off-farm activities

Parameters measured	Maize acreage	Beans acreage	Coffee acreage	Per capita consumption	Livestock income	Off-farm income
Change in No. mats infected	0.004 (0.004)	0.012** (0.006)	0.041** (0.019)	0.037 (0.238)	0.001 (0.005)	-0.003 (0.015)
South-western vs Central	-0.292 (0.335)	-0.729 (0.573)	0.879 (0.633)	2.471 (9.518)	0.476 (0.533)	1.164 (1.055)
Mid-west vs Central	0.036 (0.207)	-0.626 (0.572)	-0.658 (0.621)	-25.596** (10.129)	0.381 (0.391)	0.487 (1.202)
Eastern vs Central	0.064 (0.220)	-0.488 (0.508)	0.491 (0.445)	-39.591*** (10.896)	-0.052 (0.402)	1.664 (1.188)
SACCO Membership (1=yes; 0=no)	0.003 (0.252)	0.311* (0.174)	0.453 (0.580)	9.111 (7.728)	0.079 (0.406)	-0.234 (0.589)
Age of household head	-0.003 (0.004)	-0.016 (0.014)	-0.004 (0.011)	-0.043 (0.241)	0.013 (0.014)	0.033* (0.019)
Male household head	0.259* (0.135)	-0.729 (0.476)	0.244 (0.396)	8.071 (8.020)	0.105 (0.208)	-0.991** (0.502)
Education of household head (years)	0.022 (0.014)	-0.011 (0.049)	-0.015 (0.062)	1.006 (0.908)	0.046 (0.056)	0.014 (0.068)
Household size	0.084*** (0.021)	0.064 (0.041)	0.003 (0.112)	1.023 (1.064)	0.120** (0.060)	0.177 (0.154)
Total land owned (acres)	0.015 (0.008)	0.000 (0.005)	0.038** (0.018)	0.381 (0.322)	-0.016 (0.020)	-0.049 (0.041)
No. of adopted practices	0.133** (0.058)	0.228*** (0.084)	0.029 (0.367)	-22.594*** (3.393)	0.255*** (0. 092)	0.290 (0.248)
Constant	-0.171 (0.331)	1.895 (1.972)	-0.583 (1.200)	30.538 (19.764)	-2.036* (1.212)	-3.830** (1.807)
No. of observations	1002	1002	448	949	1002	1002
R-squared	0.016	0.018	0.033	0.065	0.012	0.012
F-value	4.80***	2.09**	1.98**	5.88***	2.30***	1.48

Note. Estimation coefficients are shown with standard errors in parentheses. *** $p < 0.01$, ** $p < 0.05$, *$p < 0.1$. SACCO = Savings and Credit Cooperative.

Farmers employ different strategies in response to BXW, but resource reallocation and crop choices are key coping strategies (Karamura et al., 2010; Nkuba et al., 2015). Our results show that with continued exposure to BXW, farmers significantly increased the production of beans and coffee by 0.01 and 0.04 acres, respectively. However, only those with vast pieces of land were able to expand into coffee production. Land size is a measure of wealth, especially among rural households (Elias, Nohmi, Yasunobu, & Ishida, 2016; Jogo et al., 2013), and ownership of a large farm enables the farmer to engage in other income-generating crop enterprises such as coffee (Jogo et al., 2013). Thus, for the resource-poor farmers to invest in coffee they would have to give up banana cultivation, which is unlikely since they rely on the crop for both food and income (Bagamba et al., 2006). Furthermore, we did not observe a significant decline in banana acreage (Table 2), therefore expanding production of other crops did not necessarily involve displacement of bananas. Previous research indicates similar findings, whereby some of the households affected by BXW cleared their plots and planted similar or different banana cultivars while diversifying into other crops, whereas only five percent reported abandonment of banana production (Karamura et al., 2010).

Implementation of control practices is labour intensive. Although consistent participation in disease management training significantly influenced adoption of the cultural control practices, we also find that the control practices are more frequently applied in larger households that have more family members.

Survey data indicate that the larger families expanded into maize production and adopted the control practices, both of which are labour-intensive ventures, indicating that these households possibly utilize readily available family labour. On the other hand, households that allocated more land to the production of beans also adopted the control practices because beans and bananas are complementary crops and they are usually grown together (Uwamahoro et al., 2019). Households in the central region consume more bananas per capita compared to those in other regions. This is because bananas are largely consumed in the urban and peri-urban areas that are concentrated in the central region. Our findings also show that adoption of the control practices does not translate into increased per capita consumption of bananas. This may be so because the crop is a major income source for these farmers, so they increase sales instead. Our findings align with Kikulwe et al. (2019) who observed that the annual value of banana sales increased significantly with the adoption of more disease control practices.

The households that diversified into livestock production were mostly those with larger families. It is likely that they also adopted the disease control practices because they had family labour available to implement the practices as well as attend to their flock. Generally, gender of the household head did not have a significant effect on the coping strategies that households adopted, except for off-farm activities. Female-headed households ventured more into these activities as a coping mechanism than the male-headed households. These results concur with

Kikulwe, Okurut, Ajambo, Gotor, et al. (2018) who asserted that female-headed households have limited resource endowments to enable them cope with shocks like BXW outbreaks therefore they may opt for income generating activities off-farm as a coping strategy.

3.2.2 Impact of BXW on Farmers' Welfare

The results of the impact of BXW on farmers' livelihoods are shown in Table 4. Although the disease did not have a significant impact on farmers' welfare over time, its presence on the farm led to a significant decline in productivity amounting to 799 kg/ha/year, which is about a third of the decline (2,317.8 kg/ha/year) observed in the first four years after the onset of the disease in 2001 (Karamura et al., 2010). The yield losses at the onset of the epidemic in the early 2000s were much higher partly because there was limited understanding of the epidemiology of the disease (Blomme et al., 2014) and a fairly low rate of adoption of the control practices (Bagamba et al., 2006). Preventing resurgence and the drastic effects of the disease is therefore critical and strongly depends on sustained adoption of the control practices that keep the disease incidence at manageable levels (Nakakawa et al., 2017). Our results show that systematic adoption of the control practices had a positive and significant impact on productivity and household welfare. Each additional practice adopted systematically increased productivity by 438 kg/ha/year, as well as daily and annual household welfare by US$1.75 and UGX 2.383 million (US$648), respectively ceteris paribus. Therefore, through consistent adoption of the control practices, the farming households' economic wellbeing has improved despite the continued exposure to BXW.

Table 4. Impact of Banana Xanthomonas Wilt (BXW) on banana productivity, daily and annual household welfare

	Banana productivity (ton/ha/year)	Daily Household Welfare (USD)†	Annual Household Welfare (million UGX)
Change in No. mats infected	0.012 (0.011)	0.024 (0.031)	0.034 (0.043)
SACCO Membership 1=yes; 0=otherwise)	0.200 (0.526)	1.547 (1.106)	2.104 (1.504)
Age of household head (years)	0.029 (0.029)	-0.015 (0.033)	-0.020 (0.045)
Male household head	0.490 (0.816)	0.966 (0.969)	1.316 (1.317)
Education of household head (years)	0.036 (0.099)	0.421*** (0.124)	0.573*** (0.169)
Household size	-0.039 (0.083)	0.364*** (0.135)	0.495*** (0.180)
Total land owned (acres)	0.042** (0.020)	0.169* (0.092)	0.230 (0.104)
No. of practices adopted systematically	0.653** (0.315)	1.753*** (0.469)	2.383*** (0.637)
BXW exposure (years)	-0.081 (0.064)		
BXW present (1=Yes, 0=otherwise)	-1.309** (0.653)		
Consistent BXW training (1=yes, 0=otherwise)	2.614*** (0.770)	9.494*** (1.992)	12.912*** (2.709)
Sanitation practices (1=yes, 0=otherwise)	-2.526*** (1.048)		
Constant	-1.054 (1.271)	2.190 (2.543)	2.977 (3.458)
No. of observations	727	1002	1002
R-squared	0.055	0.107	0.107
F-value	2.49***	9.72***	9.72***

Note. The figures in parentheses are standard errors. *** $p < 0.01$, ** $p < 0.05$, *$p < 0.1$. † 1US$ = 3,677 UGX.

Households that consistently participated in training focused on BXW management received significantly higher yields and were better off in terms of welfare than their counterparts who did not receive training. Blomme et al. (2019) asserted that the application of the BXW control package is knowledge intensive. Previous research has shown the importance of these trainings in enhancing adoption of recommended practices (Jogo et al., 2013; Kikulwe et al., 2019) and improving farmers' welfare (Kikulwe et al., 2019). Ocimati et al. (2018) also found that households that had accessed BXW management training were the ones that had effectively controlled the disease on-farm.

The sanitation practices in the banana-productivity model were a combination of de-suckering, de-trashing and weeding and these had a significant negative effect on yield. These findings concur with previous studies, which showed that these practices play a crucial role in influencing disease presence and incidence on farm, especially if they are conducted without cleaning the tools between plants, thus transmitting the pathogens to other plants and resulting into low yields (Blomme et al., 2019; Ocimati, Ssekiwoko, Karamura, Tinzaara, & Blomme, 2011). To prevent this, hand weeding and use of herbicides have been recommended to minimize the use of tools, as well as sterilizing tools between plants whenever they are used (Blomme et al., 2019; Ocimati et al., 2011; Ocimati et al., 2018). On the other hand, productivity increased with bigger farm sizes. These results are consistent with Kikulwe et al. (2019) who found that large farms' value per acre for banana production increased, especially when they adopted all the BXW control practices.

Level of education of the household head and the size of the household also significantly influenced household welfare. This may be because more educated farmers can make better decisions regarding their farm portfolios (Jena et al., 2012) and larger households have more labour available to work on the farms thereby increasing support to farm and disease control activities, which ultimately improves their welfare.

4. Conclusion

Banana is an essential crop for the livelihoods of smallholder farmers in ECA yet BXW remains the major constraint to its production. The devastating effects it has caused to farm households in the region have been widely documented. In this article, we have contributed to the literature by analysing the long-term impact of the disease on the welfare of banana-producing households in Uganda, as well as the alternative strategies they pursue to cope with BXW, in addition to adopting the established BXW control practices. Previous adoption studies have overlooked the importance of consistency of application of the control practices. Time and experience have shown that the disease resurged in areas where adoption was sporadic. Our study therefore focused on the systematic adoption of control practices and how it has influenced suppression of disease incidence, and its effect on banana productivity and farmer welfare over time. Empirical analysis was based on a longitudinal study of farming households in the four main banana-growing regions of Uganda that are grappling with BXW. Results reveal that households expanded production of beans and coffee but not at the expense of their banana plantations. Investment in coffee was constrained by land ownership hence only the wealthy farmers could venture into it. Resource-poor farmers continued to capitalize on bananas for their livelihoods. Larger households were more likely to improve their welfare possibly due to availability of labour that they would utilise in conducting the control practices and working in their plantations. The use of tools for de-trashing, de-suckering and weeding in banana plantations needs to be minimal or avoided where possible since these transmit pathogens from one plant to another, unless they are thoroughly disinfected between plants. Consistent participation in training pertinent to disease management significantly influenced adoption of the control practices. Consequently, through systematic adoption of these practices, farming households were able to maintain disease incidence at low levels, improve productivity by 438 kg/ha/year, as well as daily and annual household welfare by US $1.75 and $648, respectively.

Based on these findings, we conclude that banana is an important crop for the farming households in the region, especially among the resource-poor households who depend on it for their livelihood. We noted that technologies that focus on reducing disease incidence levels are likely to have more benefits when targeted towards farmers that consider the crop under context as their main priority. Only wealthy farmers could expand into production of cash crops such as coffee. Availability of labour and consistent farmer training are essential to sustainably increase adoption of the recommended cultural control practices in countries where the disease exists or where it is expected to spread and emerge. Our results confirm that systematic adoption of these practices significantly boosts banana yields and improves the welfare of farming households despite continued exposure to the disease.

We recommend that efforts by research and development institutions focus on developing innovations that are pro-poor and applicable by smallholder farmers. There is need to adapt the technology package to better suit the needs and socio-economic conditions of smallholder farmers through a participatory technology development approach that takes into account research findings, farmers' indigenous knowledge and resource outlays to enhance wider adoption. Farmer outreach through extension and advisory services need to be continuous and consistent. Refresher training courses need to be conducted to keep farmers abreast with the recommended practices.

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Essential Oil Content and Chemical Composition in 14 Selected Species From a Stretch of Restinga in Southern Brazil

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Abstract

The restinga is an Atlantic Forest ecosystem characterized by tree, shrub, and herb species that are rich sources of essential oils. In this study, we aim to quantify the essential oil content and determine the chemical constituents of fresh leaves of 14 plant species in a restinga stretch in southern Brazil. Essential oils were obtained by hydrodistillation in a Clevenger-type apparatus and analyzed by gas chromatography coupled to mass spectrometry. *Campomanesia reitziana*, *Cortaderia selloana*, and *Sophora tomentosa* had no essential oils. Total essential oil content ranged from 0.01% (*Mikania involucrata*) to 1.56% (*Varronia curassavica*). In total, 60 chemical constituents were identified, representing between 46.2% and 96.5% of the chemical composition of the essential oils. Limonene was the common constituent in all species in which the essential oils were present. The major constituents were ar-curcumene (15.1%) and cis-chrysanthenol (14.2%) in *Ambrosia elatior*; benzyl benzoate (43.5%) and benzyl salicylate (23.7%) in *Aniba firmula*; caryophyllene oxide (35.7%) and spathulenol (10.6%) in *Austroeupatorium inulaefolium*; spathulenol (19.8%) and caryophyllene oxide (14.0%) in *Baccharis spicata*; caryophyllene oxide (16.3%) in *Eugenia astringens*; curzerene (30.0%), limonene (13.0%), and germacrone (11.9%) in *Eugenia uniflora*; caryophyllene oxide (17.1%) and ledol (11.3%) in *Lantana camara*; caryophyllene oxide (27.7%) and limonene (12.7%) in *M. involucrata*; 1,8-cineole (19.8%) in *Psidium cattleianum*; limonene (10.2%) in *Schinus terebinthifolius*, and allo-aromadendrene (15.2%) in *V. curassavica*. We expect that our results can assist in selecting species of potential interest for herbal, phytotherapeutic, and cosmetic products.

Keywords: bioprospecting, native species, phytochemicals, aromatic plants

1. Introduction

The restinga is an ecosystem type that originated from Quaternary marine deposits and is part of the Atlantic Forest biome. Restingas are characterized by dunes and sandy coastal plains, with vegetation growing in open and/or inaccessible places such as lagoons, lakes, and marshes. These communities include a mosaic of plants with physiognomic and xeromorphic variations that respond to the numerous constraints imposed by nutrient-poor sandy soils, drought, salinity, solar radiation, constant winds, and high air and soil temperatures (Reinert et al., 1997). The unique character of the restinga comes from a plant community with high ecological plasticity. Many restinga species colonize, grow, and survive in inhospitable situations despite their origin in forest environments.

The ecological balance of species in the restinga is largely maintained through the propagation of specific plants, including the abundant aromatic herbs, shrubs, and trees. The botanical families of Asteraceae, Fabaceae, Myrtaceae, and Poaceae are the most representative of this habitat (Melo Junior & Boeger, 2015). Other common families include Anacardiaceae, Boraginaceae, Lauraceae, and Verbenaceae. Species of this ecosystem are characterized by adaptations to its adverse conditions. Plants use various strategies to deal with their difficult environmental conditions (Amorim & Melo Júnior, 2017). These include changes in secondary metabolism, resulting in the production of a wide variety of compounds, including essential oils.

Essential oils are complex mixtures of volatile, lipophilic, generally aromatic, and liquid substances, the characteristics of which change depending on environmental conditions. Researchers have recently devoted considerable attention to their applications in herbal medicine, including antioxidant, antimicrobial, antifungal, antiviral, antinociceptive, and antitumor activities (Ali et al., 2015). Essential oils have been noted for their agricultural uses as acaricides, insecticides, fungicides, and herbicides (Ootani et al., 2013). They are also widely used in cosmetics and perfumes (Sarkic & Stappen, 2018). Despite the wealth of applications, the bioprospecting of essential oil-producing restinga plants has been limited to certain Myrtaceae species (Ramos et al., 2010; Albuquerque et al., 2012). Research into species of other families may uncover the potential of the Brazilian restinga as a source of secondary metabolites of potential interest.

Here, two hypotheses about the prospection of essential oils from native species can then be presented: the hypothesis of 'commercial potential' and the hypothesis of 'species conservation strategy'. The 'commercial potential' hypothesis suggests that the collected species could be commercially inserted, as they resemble existing species on the market. The hypothesis of a 'species conservation strategy' implies that the choice of these species would serve as an alternative for the sustainable management of the Atlantic Forest biome, which is highly degraded and in need of conservation. In this context, given the considerable interest in finding new sources of essential oils, the chemical richness of restinga plants, and the growing demand for phytotherapeutic, phytosanitary and cosmetic products, we selected a variety of herbaceous, shrub, and tree species from a restinga stretch in southern Brazil. This study, which is part of a larger effort to investigate the aromatic flora of the Atlantic Forest systematically, aims to (i) quantify the essential oil content and (ii) determine the chemical constituents of the essential oils in the fresh leaves of 14 plant species.

2. Method

2.1 Plant Material

Leaves of 14 plant species were collected in February 2014 in Penha, Santa Catarina, Brazil (between 26°47′57.9″S, 48°35′39.3″W and 26°48′39.7″S, 48°35′52.4″W). The sampling site is comprised of a restinga ecosystem with herbaceous, shrub, and tree communities. The study area has approximately 3.51 ha of coastline (Figure 1). The region's climate is subtropical, with hot and rainy summers and mostly dry winters. During the collection period, the mean monthly temperature was 26.1 °C, the mean monthly precipitation was 113.0 mm, and the mean monthly relative humidity was 85.0%.

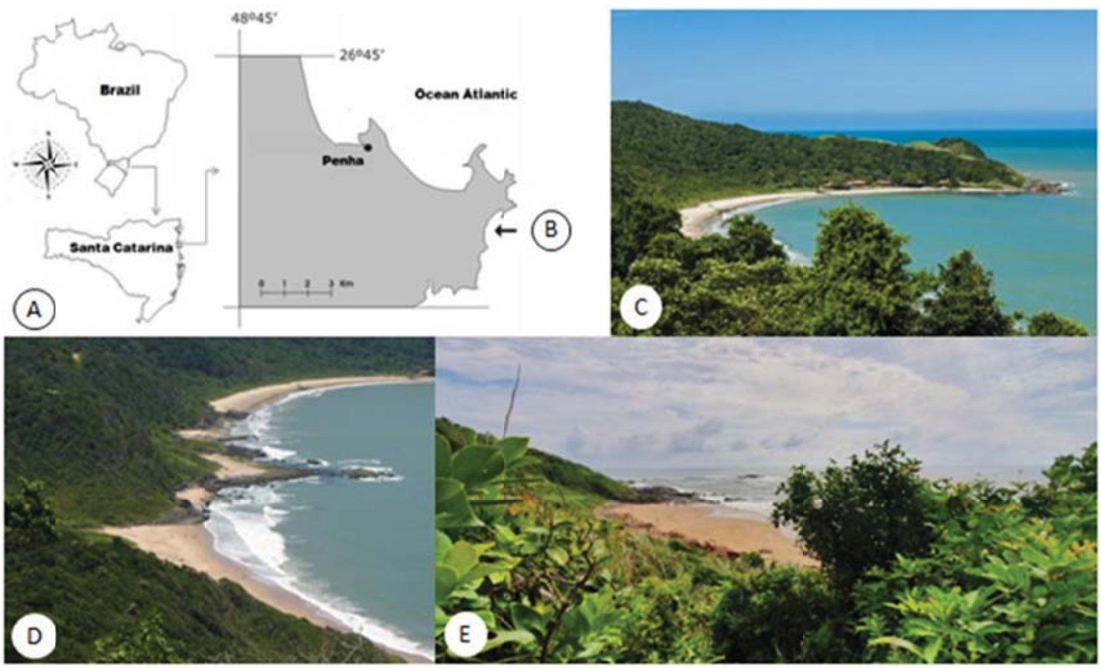

Figure 1. Location of the study area and its vegetation aspects. (A) Location map of the study area. (B) Detail of the collection area (arrow). (C-D). Panoramic views with vegetation formed by a restinga ecosystem covering the communities herbaceous, shrub and tree. (E) Shrub vegetation in the foreground and herbaceous vegetation in the background

The species analyzed were *Ambrosia elatior* L. (Asteraceae), *Aniba firmula* (Nees & Mart.) Mez. (Lauraceae), *Austroeupatorium inulaefolium* (Kunth) R.M.King & H.Rob. (Asteraceae), *Baccharis spicata* (Lam.) Baill. (Asteraceae), *Campomanesia reitziana* D. Legrand (Myrtaceae), *Cortaderia selloana* (Schult. & Schult. f. Asch. & Graebn. (Poaceae), *Eugenia astringens* Cambess. (Myrtaceae), *Eugenia uniflora* L. (Myrtaceae), *Lantana camara* Linn. (Verbenaceae), *Mikania involucrata* Hook. & Arn. (Asteraceae), *Psidium cattleianum* Sabine (Myrtaceae), *Schinus terebinthifolius* Raddi (Anacardiaceae), *Sophora tomentosa* L. (Fabaceae), and *Varronia curassavica* Jacq. (Boraginaceae) (Figure 2).

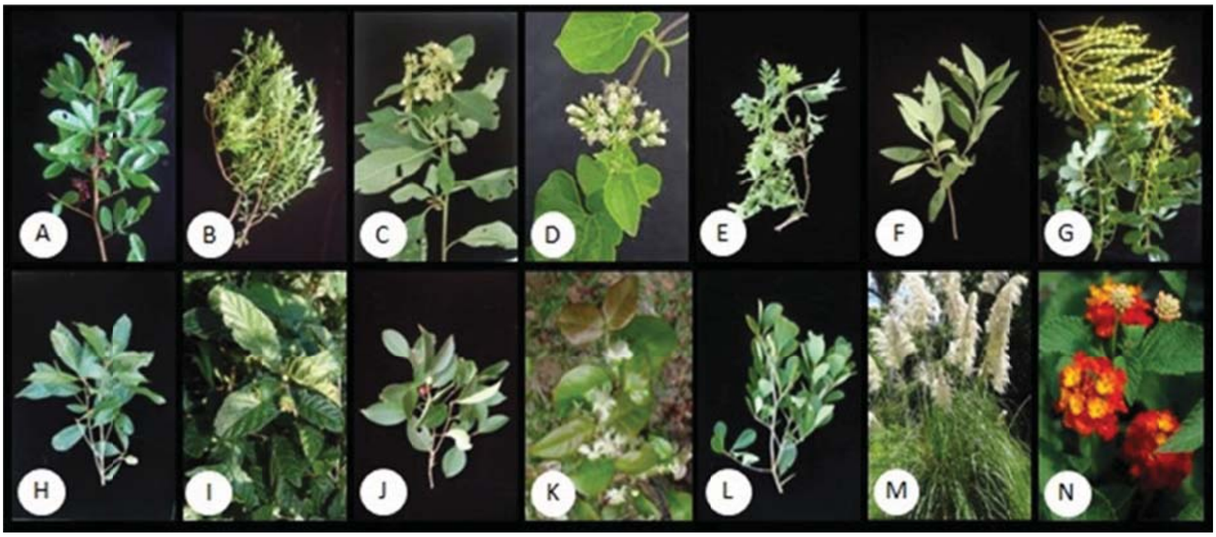

Figure 2. Species collected off the coast of Penha, Santa Catarina, Brazil, for essential oil extraction. *Schinus terebinthifolius* (A); *Baccharis spicata* (B); *Austroeupatorium inulaefolium* (C); *Mikania involucrata* (D); *Ambrosia elatior* (E); *Varronia curassavica* (F); *Sophora tomentosa* (G); *Aniba firmula* (H); *Campomanesia reitziana* (I); *Eugenia astringens* (J); *Eugenia uniflora* (K); *Psidium cattleianum* (L); *Cortaderia selloana* (M); *Lantana camara* (N)

The plants were selected based on their aroma and botanical groups based on aromatic characteristics, as reported in the literature (Trombin-Souza et al., 2017; de Souza et al., 2020; de Souza et al., 2021). Three leaf samples were collected from the terminal portion of a branch of each species during its vegetative period. Samples were collected from at least ten plants per species to provide sufficient quantity for essential oil extraction.

2.2 Extraction and Quantification of Essential Oil Content

Three 100g samples of fresh leaves per species were prepared by cutting the leaves into segments of approximately 2 cm in length. The plant material then underwent hydrodistillation in a Clevenger-type apparatus for 4.5 h. After obtaining the essential oil, it was dried over anhydrous sodium sulfate and then stored at 4°C until analysis was performed. Essential oil yield (%) was calculated as a percentage of dry matter using the following formula:

$$\textit{Essential oil yield}\ (\%) = \frac{\textit{Mass of essential oil obtained}\ (\mathrm{g})}{\textit{Mass of dry matter}\ (\mathrm{g})} \times 100 \quad (1)$$

2.3 Analysis and Quantification of Essential Oils

The analysis of the chemical composition of the essential oils was performed in a gas chromatograph (Agilent 6890) coupled to a mass selective detector (Agilent 5973N). The gas chromatograph was equipped with a fused HP-5MS capillary column (film thickness 30 m × 0.25 mm × 0.25 µm) coated with a stationary phase of 5% phenyl-95% dimethylpolysiloxane. Helium was used as carrier gas at a flow rate of 1.0 mL/min. The temperature programming was set from 60 °C to 240 °C at a rate of 3 °C/min and heated at 240 °C for 10 min. The injector temperature was maintained at 250 °C. The essential oil samples were diluted to 1% in dichloromethane, and 1.0 µL of the solution was injected with a separation ratio of 1:20. The mass detector was operated in electron ionization mode (70 eV) at a speed of 3.15 scans/min and a scanning range of 40-450 Da. The transfer line was kept at 260 °C, the ion source at 230 °C, and the analyte (in four replicates) at 150 °C.

For quantification, the essential oils were injected into a gas chromatograph (Agilent 7890) equipped with a flame ionization detector operated at 280 °C. Hydrogen was used as a support gas at a flow rate of 1.5 mL/min, using the same column and conditions described above. The quantification of each constituent was estimated by electronic integration of the flame ionization detector with the corresponding peak area, which was determined using the average of three injections.

2.4 Identification of the Chemical Constituents of the Essential Oil

Identification of the chemical constituents of the essential oil was performed by comparing Kovats indices (KIs) obtained from a correlation of the homologous series of alkanes (C_8-C_{26}) and matching their mass spectra with those of libraries, and comparing KIs from the literature (Adams, 2007).

2.5 Statistical Analyses

Essential oil content data were tested for homogeneity using Bartlett's test. An analysis of variance (ANOVA) was performed using ASSISTAT® software, version 7.7 (Silva & Azevedo, 2016), and a Tukey test was used to determine significance at the $p > 0.05$ level.

3. Results and Discussion

The essential oil was obtained through the hydrodistillation process from 11 of the 14 species sampled. Although these species are recorded in other coastal regions of the Atlantic Forest biome (Silva et al., 2021), to our knowledge, there is no information about the chemical compounds of essential oil found in these populations. *Sophora tomentosa*, *Campomanesia reitziana,* and *Cortaderia selloana* did not have essential oil in their leaves (Table 1). Though the plants exhibited presumed aromatic potential at the time of collection, these may be attributed to the presence of other compounds. Many water-soluble substances have odors that can be confused with the presence of essential oils, such as free amino acids, soluble carbohydrates, and aliphatic oxygenated compounds (Eisenreich et al., 1997).

Table 1. Description of essential oil content from collected herbs, shrubs, and trees from a patch of restinga in Penha, Santa Catarina, Brazil

Family	Species	Growth habit	Essential oil content*
Anacardiaceae	*Schinus terebinthifolius* Raddi	Tree	1.04 b**
Asteraceae	*Baccharis spicata* (Lam.) Baill.	Bush	0.48 c
Asteraceae	*Austroeupatorium inulaefolium* (Kunth) R.M.King & H.Rob.	Herbaceous	0.14 e
Asteraceae	*Mikania involucrata* Hook. & Arn.	Herbaceous	0.01 f
Asteraceae	*Ambrosia elatior* L.	Herbaceous	0.14 e
Boraginaceae	*Varronia curassavica* Jacq.	Bush	1.56 a
Fabaceae	*Sophora tomentosa* L.	Tree	-***
Lauraceae	*Aniba firmula* (Nees & Mart.) Mez	Tree	0.33 d
Myrtaceae	*Campomanesia reitziana* D.Legrand	Tree	-***
Myrtaceae	*Eugenia astringens* Cambess.	Bush	0.29 d
Myrtaceae	*Eugenia uniflora* L.	Bush	0.11 e
Myrtaceae	*Psidium cattleianum* Sabine	Tree	0.56 c
Poaceae	*Cortaderia selloana* (Schult. & Schult. f.) Asch. & Graebn.	Herbaceous	-***
Verbenaceae	*Lantana camara* Linn.	Herbaceous	0.08 ef
C.V. (%) = 8.61			

Note. * Content expressed as % of essential oil extracted from fresh leaves by hydrodistillation. ** Means followed by the same letter are not significantly different from each other according to the Tukey test at the 5% probability level. *** No essential oil present in their leaves.

The highest essential oil content was observed in *Varronia curassavica* (1.56%), while essential oil content ranged between 0.01% and 1.04% in the remaining plants (Table 1). Although phytochemical studies have been carried out for the selected species, comparisons of essential oil content are not an easy task due to their heterogeneous profiles. For example, the essential oil content of *Lantana camara* reported in the literature ranges from 0.004% (Zhu et al., 2013) to 0.09% (Sousa et al., 2010). These differences may be attributed to several reasons, including the duration and method of extraction, population genetics of each species (Nizio et al., 2015), the plant part used (Cole et al., 2014), collection time (Sousa et al., 2010), exposure to sunlight (Feijó et al., 2014), seasonality, temperature, and precipitation (Matias et al., 2016).

A total of 60 chemical constituents were identified in the essential oils extracted, comprising between 46.2% and 96.5% of their chemical compositions (Table 2). Of these constituents, 7.5-18.7% were from the hydrocarbon monoterpene class, 0.3-27.2% oxygenated monoterpenes, 2.5-32.1% hydrocarbon sesquiterpenes, 3.9-69.8% oxygenated sesquiterpenes, 1.4% phenylpropanoids, and 1.8-68.9% were esters. Limonene was the only common constituent in all the species analyzed, with a concentration ranging between 4.9% and 13.0% (Table 2). This similarity may be associated with the role of limonene as a precursor of monoterpene biosynthesis (Trombin-Souza et al., 2017; de Souza et al., 2021).

Table 2. Chemical constituents of essential oils from the fresh leaves of herbs, shrubs, and trees from a stretch of restinga in Penha, Santa Catarina, Brazil

Constituent	KI^{lit}	KI^{cal}	Species										
			S 01	S 02	S 03	S 04	S 05	S 06	S 07	S 08	S 09	S 10	S 11
1. α-pinene	933	932	4.0[1]	-	2.9	1.6	0.2	0.6	0.6	5.3	0.4	1.8	-
2. camphene	949	946	-	-	-	-	-	6.7	0.1	-	-	-	-
3. β-pinene	976	974	1.5	1.9	4.8	2.1	0.3	0.6	0.7	3.7	0.7	0.4	-
4. myrcene	992	988	-	-	-	-	-	0.2	0.2	-	-	2.5	-
5. p-cymene	1024	1025	1.6	-	0.4	0.5	0.4	0.6	0.8	2.2	0.4	1.1	0.9
6. limonene	1028	1029	10.2	5.8	4.9	12.7	6.6	8.3	6.9	7.5	13.0	8.8	7.8
Monoterpene hydrocarbon			17.3	7.7	13.0	16.9	7.5	17.0	9.3	18.7	14.5	14.6	8.7
7. 1,8-cineole	1031	1026	-	-	-	-	-	-	0.3	-	-	19.8	-
8. α-camphonelal	1127	1129	-	-	0.3	-	-	-	-	1.7	-	-	-
9. trans-pinocarveol	1138	1142	-	1.7	1.7	0.7	-	0.3	0.1	2.5	-	0.2	-
10. cis-chrysanthenol	1163	1163	-	-	-	-	14.2	-	-	-	-	-	-
11. borneol	1165	1169	-	-	-	0.2	4.2	-	-	-	-	-	-
12. terpinen-4-ol	1177	1174	4.6	0.9	0.6	0.4	-	-	0.2	1.6	-	0.6	-
13. ρ-cymen-8-ol	1187	1187	-	-	1.6	-	-	-	-	8.2	-	-	-
14. cryptone	1188	1189	5.7	-	-	-	-	-	0.1		-	-	-
15. α-terpineol	1191	1190	4.5	1.7	0.5	0.8	-	-	0.3	4.6	-	2.9	-
16. myrtenol	1197	1198	-	2.7	1.5	0.7	-	-	-	1.5	-	-	-
17. cis-piperitenone epoxide	1253	1254	-	-	-	-	-	-	-	3.5	-	-	-
18. thymol acetate	1344	1355	-	-	-	-	-	-	-	3.6	-	-	-
Oxygenated monoterpene			14.8	7.0	6.2	2.8	18.4	0.3	1.0	27.2	0.0	23.5	0.0
19. α-copaene	1374	1374	-	-	0.4	0.7	-	0.4	0.6	-	-	3.2	1.0
20. β-elemene	1392	1391	0.8	-	0.5	1.5	-	1.2	-	-	2.6	0.1	-
21. (E)-caryophyllene	1418	1417	0.7	-	1.8	6.8	0.8	6.3	0.4	-	-	0.9	4.3
22. aromadendrene	1438	1439	0.6	-	0.6	-	-	0.2	0.3	1.6	-	0.2	3.0
23. α-humulene	1452	1452	0.3	-	0.8	1.6	0.5	2.4	0.1	-	-	0.3	0.4
24. (E)-β-farnesene	1457	1459	1.4[1]	-	-	-	-		0.7	-	-	-	-
25. *allo*-aromadendrene	1459	1461	-	-	-	-	-	15.2	0.8	0.4	-	-	1.7
26. γ-muurolene	1476	1478	2.1	0.6	0.9	1.0	-	-	0.1	-	-	1.5	3.5
27. ar-curcumene	1483	1482	-	0.6	-	-	15.1	-	-	-	-	-	-
28. β-selinene	1484	1486	-	-	0.5	2.9	-	0.8	0.6	-	0.8	2.0	0.3
29. germacrene D	1485	1484	2.5	-	-	-	-	0.2	-	-	-	-	-
30. α-muurolene	1500	1500	1.4	0.8	0.3	-	-	0.3	-	-	-	0.3	0.8
31. γ-cadinene	1514	1513	-	0.8	1.0	-	-	-	0.1	-	-	0.2	2.5
32. trans-calamenene	1523	1525	2.7	1.0	-	-	-	-	0.1	0.5	-	0.6	2.2
33. zonarene	1534	1533	3.7	-	-	-	-	-	-	-	-	5.0	-
34. α-cadinene	1514	1517	-	-	-		-	5.1	-	-	-	-	0.6
35. selina-3,7(11)-diene	1541	1543	1.8	-	-	-	-	-	-	-	-	2.0	-
36. germacrene B	1557	1558	1.2	-	-	-	-	-	-	-	5.0	-	-

Sesquiterpene hydrocarbon			19.2	3.8	6.8	14.5	16.4	32.1	3.8	2.5	8.4	16.3	19.7
37. curzerene	1498	1497	-	-	-	-	-	-	-	-	30.0	-	-
38. (E)-nerolidol	1564	1561	-	-	-	-	-	-	5.9	-	-	0.8	-
39. spathulenol	1576	1576	3.1	19.8	10.6	8.2	-	-	3.7	1.5	1.6		-
40. caryophyllene oxide	1582	1581	4.7	14.0	35.7	27.7	-	4.7	2.5	16.3	-	7.9	17.1
41. globulol	1584	1583	-	-	-	-	-	-	-	-	-	-	2.4
42. viridiflorene	1592	1591	2.3	0.9	0.6	-	-	-	0.2	1.1	3.0	0.6	-
43. ledol	1602	1602	-	2.9	-	1.1	-	2.4	0.4	-	-	0.5	11.3
44. humulene epoxide II	1608	1608	-	4.4	4.4	2.6	-	2.5	0.3	-	-	1.2	0.9
45. 1-epi-cubenol	1628	1627	2.6	2.6	-	5.4	-	-	0.2	-	-	4.0	0.3
46. *epi*-α-muurolol	1641	1640	2.5	10.4	3.2	3.3	-	2.0	-	-	-	-	2.7
47. demethoxyencecaline	1643	1642	-	-	-	-	-	-	-	-	-	4.2	-
48. α-muurolol	1646	1644	0.7	2.8	0.6	0.6	-	0.8	-	-	-	2.5	0.7
49. α-cadinol	1654	1657	2.8	12.0	6.9	4.9	0.6	2.6	-	1.6	4.8	3.0	2.4
50. epi-α-cadinol	1661	1662	-	-	-	-	-	-	0.3	2.0	-	-	-
51. atractilone	1662	1660	-	-	-	-	-	-	-	-	4.0[1]	-	-
52. 14-hydroxy-9-epi-caryophyllene	1671	1662	-	-	-	-	-	-	-	-	-	-	4.0
53. α-bisabolol	1684	1687	-	-	-	-	3.3	-	-	-	-	-	-
54. germacrone	1697	1699	-	-	-	-	-	-	-	-	11.9	-	-
Oxygenated sesquiterpene			18.8	69.8	62.0	53.8	3.9	20.9	13.5	22.5	58.4	24.7	49.6
55. (E)-methyl-isoeugenol	1488	1489	-	-	-	-	-	-	-	-	-	1.4	-
Phenylpropanoid			0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0
56. α-terpinyl acetate	1349	1347	-	-	-	-	-	-	-	-	-	1.8	-
57. geranyl butyrate	1561	1663	-	-	-	-	-	-	-	5.9	-	-	-
58. benzyl benzoate	1769	1767	-	-	-	-	-	-	43.5	-	-	-	-
59. 2-phenylethyl benzoate	1879	1880	-	-	-	-	-	-	1.7	-	-	-	-
60. benzyl salicylate	1887	1889	-	-	-	-	-	-	23.7	-	-	-	-
Ester			0.0	0.0	0.0	0.0	0.0	0.0	68.9	5.9	0.0	1.8	0.0
Total constituents (%)			70.1	88.3	88.0	88.0	46.2	70.3	96.5	76.8	81.3	82.3	78.0

Note. Species: Kl^{it} = Kovats literature index; KI^{cal} = Kovats experimental index; S 01: *Schinus terebinthifolius*; S 02: *Baccharis spicata*; S 03: *Austroeupatorium inulaefolium*; S 04: *Mikania involucrata*; S 05: *Ambrosia elatior*; S 06: *Varronia curassavica*; S 07: *Aniba firmula*; S 08: *Eugenia astringens*; S 09: *Eugenia uniflora*; S 10: *Psidium cattleianum*; S 11: *Lantana camara*. -: trace element < 0.1%. [1]: Content expressed in %.

Limonene was only the most abundant constituent in *Schinus terebinthifolius*, accounting for 10.2% of the essential oil. The presence of 9-epi-(*E*)-caryophyllene (10.1%) and p-cymen-7-ol (22.5%) have been reported in fresh leaves of the species (Silva et al., 2010), as well as germacrene D (23.8%), bicyclogermacrene (15.0%) (Santana et al., 2012), and δ-3-carene (68.78%) (Uliana et al., 2016). However, the concentrations of these constituents measured in this study were lower or absent (Table 2). Quantitative and qualitative variations in the species' essential oil may be related to the metabolic plasticity of *S. terebinthifolius*. The production of secondary metabolites is likely influenced by the peculiarities of each environment, including abiotic and edaphic conditions, as well as herbivores, pollinators, and seed dispersers. Furthermore, alterations in essential oil biosynthesis may also reflect a possible deviation in metabolic pathways to help the plant survive in particular environments.

The most abundant constituents in the Asteraceae species were spathulenol (19.8%), caryophyllene oxide (14.0%), α-cadinol (12.0%), and epi-α-muurolol (10.4%) in *Baccharis spicata*; caryophyllene oxide (35.7%) and spathulenol (10.6%) in *Austroeupatorium inulaefolium*; caryophyllene oxide (27.7%) and limonene (12.7%) in *Mikania involucrate*, and ar-curcumene (15.1%) and cis-chrysanthenol (14.2%) in *Ambrosia elatior* (Table 2). The chemical profiles of these oils indicated a predominance of sesquiterpenes (3.8-69.8%) over monoterpenes (2.8-18.4%). These findings can be interpreted as a competition between two pathways for the same precursor. It is known that the concentrations of monoterpenes and sesquiterpenes are negatively correlated (Ghaffari et al., 2011). Thus, the highest flux of isopentenyl diphosphate (IPP) among the species studied tended to be in the cytosol (the site of sesquiterpene biosynthesis) in the restinga environment. Higher proportions of sesquiterpenes may also indicate the stressful conditions that plants undergo in this ecosystem since high temperatures, strong winds, and solar radiation contribute to the volatilization of smaller molecules such as monoterpenes. In contrast to our results, the essential oils of other Asteraceae species collected in non-coastal areas of the Atlantic Forest had roughly equal proportions of monoterpenes and sesquiterpenes (Amaral et al., 2017). This suggests that site-specific characteristics (*i.e.*, environmental differences) are determining factors in terpene variation.

The sesquiterpene hydrocarbon allo-aromadendrene was the most common constituent in *V. curassavica* (15.2%; Table 2). The chemical constituents most commonly found in the species' essential oil are trans-caryophyllene (14.4%), caryophyllene oxide (15.8%) (Feijó et al., 2014), α-pinene (16.2%), β-phellandrene (11.0%), sabinene (69.7%), γ-elemene (12.6%), δ-elemene (12.6%), β-caryophyllene (11.5%), γ-caryophyllene (15.6%), and germacrene B (13.8%) (Matias et al., 2016). Variations in essential oil composition have often been associated with plant growth conditions, seasonality (Matias et al, 2016), and solar radiation (Feijó et al., 2014). Recently, sampling from 59 *V. curassavica* accessions showed that the genetic composition of the plants and/or the genotype × environment interaction is probably the most influential factor on the diversity chemical constituents in the essential oil (Nizio et al., 2015). Thus, plants collected in the same locality have been classified into different chemical groups.

In *Aniba firmula*, the main constituents were benzyl benzoate (43.5%) and benzyl salicylate (23.7%; Table 2). The essential oils of Brazilian species of Lauraceae are generally divided into groups of chemotypes based on their main constituents. *Aniba firmula* belongs to the benzoate group. Species in this family can also belong to the linalool and allylbenzene chemotypes, depending on the principal constituents, which remain consistent across each species (Morais et al., 1972). Similarly, *Aniba firmula* exhibited low variation in the main constituents of its essential oil and lower sensitivity to environmental characteristics. These findings are interesting because they reveal that the restinga conditions did not result in significant changes in the essential oil composition.

The main constituents found in species of Myrtaceae were caryophyllene oxide (16.3%) in *Eugenia astringens*; limonene (13.0%), curzerene (30.0%), and germacrone (11.9%) in *Eugenia uniflora*, and 1,8-cineol (19.8%) in *Psidium cattleianum* (Table 2). The chemical constituents of Myrtaceae essential oils belong predominantly to the hydrocarbons (14.5-18.7%), oxygenated monoterpenes (0-27.2%), and oxygenated sesquiterpenes (22.5-58.4%). This finding contrasts with earlier results for Myrtaceae plants in the Atlantic Forest, which showed that sesquiterpenes generally predominated (Nakamura et al., 2010; Albuquerque et al., 2012). In the restinga, an increase in hydrocarbon and oxygenated monoterpenes has been observed (Ramos et al., 2010; Defaveri et al., 2011). Although monoterpenes volatilize easily under conditions of high temperature and solar intensity (Arruda and Victório, 2011), the abundance of these compounds in species of this family can be explained by their thick and wax-covered leaves, especially in plants from the restinga (Donato and Morretes, 2007). Thus, the functional traits of the leaves indicate the existence of mechanisms to reflect incident light and protect against the loss of water and volatile substances.

In *L. camara*, the most abundant constituents were caryophyllene oxide (17.1%) and ledol (11.3%; Table 2). The predominance of sesquiterpenes in this study reveals their importance for the species (Sousa et al., 2010; Medeiros et al., 2012; Zhu et al., 2013). The qualitative and quantitative presence of this class of compounds has been shown to vary in various organs of *L. camara* (Medeiros et al., 2012; Zhu et al., 2013). In leaves, the major essential oil constituents are germacrene D (24.5%), bicyclogermacrene (33.3%), spathulenol (25.0%), eremophilene (20.6%), valencene (33.7%), viridiflorene (19.5%), and 1,10-di-epi-cubenol (21.3%) (Sousa et al., 2010). The variation in the chemical composition is also due to the numerous varieties of the species, such as *L. camara var. aculeata*, *L. camara var. ava*, *L. camara var. hybrida*, *L. camara var. mista*, and *L. camara var. nivea* (Da Silva et al., 1999).

This study reports the chemical diversity present in the essential oils of plant species collected in the restinga ecosystem of southern Brazil. Although *E. uniflora* and *V. curassavica* are commercially exploited, in this work we report that these species have a high content of the substance of economic interest such as curzurene (30.0%) and α-humulene (2.4%), which may represent a potential commercial. Likewise, the selection of matrices with economic value can be subsidized with sustainable use practices of the species, since they are distributed in Biome highly threatened by anthropogenic disturbance (de Souza et al., 2021). This information is critical when selecting species with economic potential for phytotherapeutic products, as well as for the phytosanitary and cosmetic industries.

4. Conclusions

In conclusion, our study reports the yield and chemical composition of essential oils from 14 species distributed on the coast of Santa Catarina, Brazil. The EO content ranges from 0.01% (*M. involucrata*) to 1.56% (*V. curassavica*). The major constituents are ar-curcumene (15.1%) and cis-chrysanthenol (14.2%) in *A.elatior*;

benzyl benzoate (43.5%) and benzyl salicylate (23.7%) in *A. firmula*; caryophyllene oxide (35.7%) and spathulenol (10.6%) in *A. inulaefolium*; spathulenol (19.8%) and caryophyllene oxide (14.0%) in *B. spicata*; caryophyllene oxide (16.3%) in *E. astringens*; curzerene (30.0%), limonene (13.0%), and germacrone (11.9%) in *E. uniflora*; caryophyllene oxide (17.1%) and ledol (11.3%) in *L. camara*; caryophyllene oxide (27.7%) and limonene (12.7%) in *M. involucrata*; 1,8-cineole (19.8%) in *P. cattleianum*; limonene (10.2%) in *S. terebinthifolius*, and allo-aromadendrene (15.2%) in *V. curassavica*.

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Correlation and Path Coefficient Analysis for Seed Yield and Agronomic Traits of Newly Introduced Pigeon Pea Genotypes in South Africa

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Abstract

Pigeon pea is an important source of protein for smallholder farmers in South Africa. The average seed yield per hectare ranges from 0.5 to 1 tonne due to the use of un-improved varieties that succumb to both biotic and abiotic stresses. Understanding the relationship between seed yield and agronomic traits in a genetically diverse germplasm collection is a prerequisite for developing an efficient selection and breeding program. This study determined the relationship between seed yield and agronomic traits to identify key traits for selection. Two separate experiments involving seven short and six medium duration pigeon pea genotypes were planted in a randomized complete block design (RCBD), each with three replications. Agronomic traits, including yield and its components' genotype mean data was subjected to correlation, path coefficient and stepwise regression analyses. The results showed a strong positive and significant correlation between number of pods per plant and seed yield in both medium and short duration genotypes. The number of pods per plant had a high direct effect on seed yield in both medium and short duration with values of (0.43) and (0.63) respectively. Number of branches and pod length exhibited high direct effect (0.30-0.99) for medium duration. However, seed yield per plant was an important predictor of seed yield in short duration while seed yield per plant, 100 seed weight and number of seeds per pod were key predictors of seed yield in medium duration genotypes. Therefore, these traits should be targeted for seed yield improvement in Pigeon pea.

Keywords: crop improvement, direct effect, ICRISAT, legumes, plant breeding

1. Introduction

Pigeon pea [*Cajanus cajun* (L.) Mills sp.] is a multipurpose leguminous crop cultivated in the tropics and sub-tropics of Asia, Latin America and Africa. In South Africa, pigeon pea is an important source of income and nutrition to smallholder farmers. The crop has 20.5% crude protein which is available in the whole seed, although there are high-protein genotypes specially bred with up to 30% protein (Changaya, 2010). Despite its nutritional benefits, the average seed yield production in a South African farmer's field ranges from 0.5 to 1 tonne per hectare lower than those attained by most pigeon pea growing regions in sub-Saharan Africa (SSA) (Gwata & Shimelis, 2013). The lower yield production is attributed to the use of un-improved landraces that succumb to both biotic and abiotic stresses (Hluyako et al., 2017).

Tremendous milestones in Pigeon pea crop improvement have been attained in other countries with the help of the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT). For instance, specific agronomic traits for Pigeon pea yield improvement have been identified in Kenya and India (Cheboi et al., 2016). Since yield is a quantitative trait that results from an interplay of several inter-related traits and highly influenced by the environment with a low heritability (Rao et al., 2013). Selection based on seed yield alone is less efficient for improving Pigeon pea productivity. Nonetheless, selection efficiency for yield improvement can be attained by exploiting the interrelationships between yield and its associated traits. Correlation and path coefficient analyses are important in unravelling the relationships between key traits and designing effective breeding strategies for yield improvement (Rao et al., 2013; Kesha et al., 2014). However, their estimates are moderated by the environment and type of genotypes used (Kiranmai et al., 2016). Several studies in other countries have utilized correlation and path analyses to identify traits for selection in pigeon pea breeding programmes (Narayanan, Manivannan, & Mahalingam, 2018; Zavinon et al., 2019; Nyirenda et al., 2020; Behera et al., 2020). Such studies, however, are limited in South Africa especially for the recently received short and medium duration Pigeon pea genotypes from ICRISAT, Kenya. Understanding the nature, magnitude, direction and strength of the relationship between yield and its components in these newly introduced Pigeon pea genotypes is key to improving selection efficiency and productivity. The adaptability of these uniquely bred pigeon pea genotypes across different agro-ecological zones in South Africa remains un-exploited. This study, therefore, was undertaken to determine the relationship between seed yield and agronomic traits in newly introduced pigeon pea genotypes under rain-fed conditions in KwaZulu-Natal, South Africa.

2. Materials and Methods

2.1 Description of Experimental Site

The study was conducted at the University of KwaZulu-Natal Ukulinga Research Farm, Pietermaritzburg, South Africa with latitude 29.66° S, longitude 30.41° E, and 775m altitude (Mengistu et al., 2016). The study was conducted during 2018/2019 growing season under rainfed conditions. The Ukulinga Research Farm receives mean rainfall of 750 mm/year. The location has warm air temperatures during the months of December, January, February, and March with average temperatures of 26 °C. Mean temperatures are lower (8 °C) in the months of June, July and August (Figure 1).

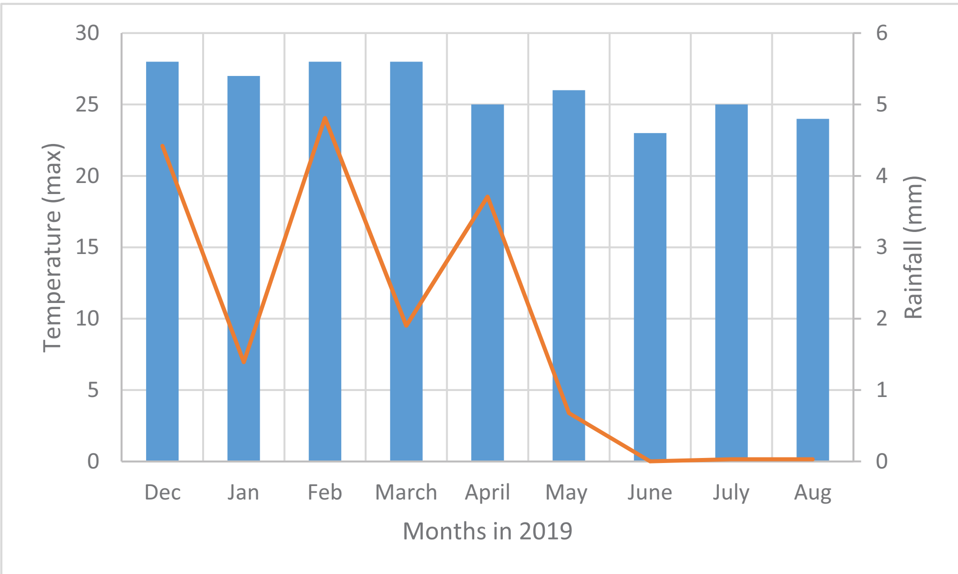

Figure 1. Monthly rainfall (mm) and temperature (°C) from December 2018 to August 2019 in Ukulinga Research Farm Source: University of Kwazulu-Natal agro meteorology weather station mast

2.2 Plant Genetic Materials

The pigeon pea genotypes used in this study are presented in Table 1. The germplasm comprised of six medium and seven short duration pigeon pea genotypes from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Kenya, selected for their unique yield characteristics.

Table 1. Short and medium duration pigeon pea genotypes from ICRISAT

Short Duration	Medium Duration
ICPL 87091	ICEAP 00540
ICPL 86012	ICEAP 00557
ICEAP 00660/3	CEAP 00902
ICEAP 01284	ICEAP 00911
ICEAP 01130/3	ICEAP 00850
ICEAP 00612	ICEAP 00068
ICEAP 01107/1	

2.3 Experimental Design

The short and medium duration pigeon pea genotypes were planted in two separate experiments arranged in a Randomized Complete Block Design (RCBD) with three replications. Each short duration genotype was planted in a single row plot. Each row was 2.5 m long and 2 m wide, giving a plot size of 5 m^2. Seeds were planted at 0.5 m apart within the row. Each medium duration genotype was planted in a single row plot. Each row was 3m long and 2.5m wide, giving a plot size of 7.5 m^2. Seeds were planted at 0.3 m apart within the row.

2.4 Agronomic Practices and Experimental Management

Two seeds of pigeon pea genotypes were planted and later thinned to one plant two weeks after germination. Experimental plots were weeded when required using a hand hoe. No fertilizer was applied to the pigeon pea. Pests such as pod borers (*Maruca testulalis* and *Helicoverpa armigera*), pod suckers (*Riptortus dentipes* and *Clavigralla* sp.), and pod flies (*Melanagromyza obtusa*) were managed by applying cypermethrin, a broad-spectrum pesticide. The pesticide was applied uniformly by using a 20-L knapsack sprayer.

2.5 Data Collection

Six sample plants were randomly selected and marked from each plot per genotype for data collection throughout the entire experiment. The following traits such plant height (measured in centimetres at harvest time), and number of branches were collected bi-weekly. Days to 50% flowering, days to 75% maturity, number of pods per plant, number of seeds per pod, pod length (measured in centimetres using a ruler), 100 seed weight, and seed yield per plant were determined from each plot according to the pigeon pea International Board for Plant Genetic Resources (IBPGR) guide (IBPGR and ICRISAT 1993). Environmental data such as rainfall and temperature were continuously monitored and recorded by UKZN Agrometeorology weather station mast.

2.6 Data Analysis

Agronomic traits, yield and yield components genotype mean data was subjected to correlation analysis in GenStat software 18th edition (VSN International Ltd., Hertfordshire, United Kingdom) while path coefficient analysis was done in Microsoft Excel package as suggested by Dewey and Lu (1959). Path analysis was performed using a genotypic matrix which was set up as $A = B \times C$ for seed yield, where, "A" represents the genotypic correlation coefficients of seed yield vs other agronomic traits in the matrix vector. Matrix vector "B" is the value of genotypic correlation for all possible combinations among the traits and vector C is the path coefficients. The inverse of matrix B was calculated using the Matrix Inverse function (MINVERSE) of Microsoft Excel 2016. The path coefficients were calculated as the product of vector A and each row of B^{-1} using the matrix multiplication (MMULT) function of Microsoft Excel 2016. Direct and indirect path coefficients were calculated according to Dewey and Lu (1959). Seed yield was regarded as the response variable, whereas other traits were causal variables. Path coefficients were classified as suggested by Lenka and Mishra (1973), where 0.00-0.09 is negligible association effects, 0.10-0.19 is low, 0.20-0.29 is moderate, 0.30-0.99 is high and > 1.0 is very high. The significance tests at 5% and 1% for the correlation coefficients of seed yield and other yield related traits was determined using a student's t-test (Snedecor & Cochran, 1989). A stepwise regression analysis was employed to identify key first order predictors of seed yield in Statistical Product and Service Solutions (SPSS) version 25 (SPSS Inc., Chicago, IL, USA). Tolerance (TOL) and variance inflation factor (VIF) were utilized to measure the level of multi-collinearity for each predictor trait. A VIF of greater than five indicated high multicollinearity (Akinwande et al., 2015).

3. Results

3.1 Phenotypic Correlation of Seed Yield and Agronomic Traits of Short and Medium Duration Genotypes

The correlation analysis results for seed yield and agronomic traits are presented in Table 2 and Table 3. In the short duration genotypes (Table 2), seed yield showed a strong positive significant correlation with number of pods per plant ($r = 0.86$; $P < 0.01$) and plant height ($r = 0.66$; $P < 0.01$). Seed yield exhibited a moderate positive significant correlation with 100 seed weight ($r = 0.47$; $P < 0.05$) and number of branches ($r = 0.38$; $P < 0.05$). In medium duration pigeon pea (Table 3), seed yield showed a strong positive significant correlation with days to 50% flowering ($r = 0.71$; $P < 0.001$), days to maturity ($r = 0.73$; $P < 0.001$), number of pods per plant ($r = 0.72$; $P < 0.001$), pod length ($r = 0.75$; $P < 0.001$), and number of branches ($r = 0.81$; $P < 0.001$). Seed yield showed a moderate positive significant correlation with 100 seed weight ($r = 0.48$; $P < 0.05$).

Table 2. Phenotypic correlation coefficients of traits that contribute to yield in short duration pigeon pea genotype

Trait	Yield/Plant	50% DTF	75% DTM	PH	100 SW	POD L.	NSP	NPP	Branches
Yield/Plant	1								
50% DTF	-0.21^{ns}	1							
75% DTM	-0.02^{ns}	0.69^{ns}	1						
PH	0.66*	-0.21^{ns}	-0.18^{ns}	1					
100 SW	0.47*	0.00^{ns}	0.14^{ns}	0.14^{ns}	1				
POD L.	0.36^{ns}	-0.16^{ns}	-0.04^{ns}	-0.25^{ns}	0.34^{ns}	1			
NSP	0.04^{ns}	0.22^{ns}	0.35^{ns}	-0.22^{ns}	-0.16^{ns}	0.02^{ns}	1		
NPP	0.86*	-0.1^{ns}	0.16^{ns}	0.56^{ns}	0.47^{ns}	0.27^{ns}	0.25^{ns}	1	
Branches	0.38*	0.03^{ns}	0.00^{ns}	0.08^{ns}	0.45^{ns}	0.39^{ns}	0.29^{ns}	0.23^{ns}	1

Note. DTF = 50% Days to Flowering; DTM = 75% Days to Maturity; NSP = Number of Seeds per Pod; 100 SW = 100 Seed Weight (g); NPP = Number of Pods per Plant; POD L. = Pod Length, PH = Plant Height.

* = Significant at $P \leq 0.05$; ^{ns} = Non-significant.

Table 3. Phenotypic correlations coefficients of traits that contribute to yield in medium duration pigeon pea genotypes

Trait	Yield/Plant	50% DTF	75% DTM	PH	NPP	POD L.	100 SW	NSP	Branches
Yield/Plant	1								
50% DTF	0.72*	1							
75% DTM	0.73*	0.99*	1						
PH	0.34^{ns}	-0.09^{ns}	-0.09^{ns}	1					
NPP	0.72*	0.86*	0.88*	0.11^{ns}	1				
POD L.	0.75*	0.55*	0.61*	0.10^{ns}	0.49^{ns}	1			
100 SW	0.48*	0.33^{ns}	0.36^{ns}	0.12^{ns}	0.35^{ns}	0.83*	1		
NSP	0.23	0.14^{ns}	0.26^{ns}	-0.17^{ns}	0.15^{ns}	0.46^{ns}	0.19^{ns}	1	
Branches	0.81*	0.36^{ns}	0.34^{ns}	0.75*	0.40^{ns}	0.52*	0.35^{ns}	0.02^{ns}	1

Note. DTF = 50% Days to Flowering; DTM = 75% Days to Maturity; NSP = Number of Seeds per Pod; 100 SW = 100 Seed Weight (g); NPP = Number of Pods per Plant; POD L. = Pod Length, PH = Plant Height.

* = Significant at $P \leq 0.05$; ^{ns} = Non-significant.

3.2 Path Coefficient Analysis of Seed Yield and Agronomic Traits of Short and Medium Duration Genotypes

Path coefficients were classified as suggested by Lenka and Mishra (1973), where, 0.00-0.09 is negligible association effects, 0.10-0.19 is low, 0.20-0.29 is moderate, 0.30-0.99 is high and > 1.0 is very high. In short duration pigeon pea genotypes (Table 4), number of pods per plant exhibited high positive direct effect on seed yield (0.60), followed by plant height (0.36) and pod length (0.25). The branches had a low direct effect on seed yield (0.13). Days to 50% flowering, days to 75% physiological maturity, 100 seed weight and the number of seeds per pod had negligible effect on the seed yield.

Table 4. Genotypic path coefficient analysis direct effects on main diagonal (bold & diagonal) and indirect effects (off diagonal) of different agronomic traits on seed yield of short duration pigeon pea

Trait	50% DTF	75% DTM	Ph	100 SW	POD L.	NSP	NPP	Branches	Correlations with Yield/Plant
50% DTF	**0.05**	-0.03	-0.08	0	-0.04	-0.01	-0.1	0	-0.21ns
75% DTM	0.03	**-0.04**	-0.07	0	-0.01	-0.02	0.1	0	-0.02ns
PH	-0.01	0.01	**0.36**	0	-0.06	0.01	0.34	0.01	0.66*
100SW	0	-0.01	0.05	**-0.02**	0.08	0.01	0.28	0.06	0.47*
POD L.	-0.01	0	-0.09	-0.01	**0.25**	0	0.16	0.05	0.36ns
NSP	0.01	-0.01	-0.08	0	0	**-0.07**	0.15	0.04	0.04ns
NPP	-0.01	-0.01	0.2	-0.01	0.07	-0.02	**0.6**	0.03	0.86*
Branches	0	0	0.03	-0.01	0.1	-0.02	0.15	**0.13**	0.38*

Note. DTF = 50% Days to Flowering; DTM = 75% Days to Maturity; NSP = Number of Seeds per Pod; 100 SW = 100 Seed Weight (g); NPP = Number of Pods per Plant; POD L. = Pod Length, PH = Plant Height.

* = Significant at P ≤ 0.05; ^{ns} = Non-significant.

Table 5. Genotypic path coefficient analysis direct effects on main diagonal (bold & diagonal) and indirect effects (off diagonal) of different agronomic traits on seed yield of medium duration pigeon pea

Trait	%50 DTF	75% DTM	PH	NPP	POD L.	100 SW	NSP	Branches	Correlation with Seed Yield/Plant
50% DTF	**0.18**	-0.3	0.03	0.37	0.24	-0.07	0	0.27	0.72*
75% DTM	0.18	**-0.31**	0.03	0.38	0.27	-0.08	0	0.26	0.73*
PH	-0.02	0.03	**-0.3**	0.05	0.04	-0.03	0	0.56	0.34^{ns}
NPP	0.16	-0.27	-0.03	**0.43**	0.22	-0.07	0	0.17	0.72*
POD L.	0.1	-0.19	-0.03	0.21	**0.44**	-0.18	0	0.39	0.75*
100 SW	0.06	-0.11	-0.04	0.15	0.37	**-0.21**	0	0.27	0.48*
NSP	0.03	-0.08	0.05	0.06	0.21	-0.04	**-0.01**	0.01	0.23^{ns}
Branches	0.07	-0.11	-0.23	0.17	0.23	-0.08	0.01	**0.75**	0.81*

Note. DTF = 50% Days to Flowering; DTM = 75% Days to Maturity; NSP = Number of Seeds per Pod; 100 SW = 100 Seed Weight (g); NPP = Number of Pods per Plant; POD L. = Pod Length, PH = Plant Height.

* = Significant at P ≤ 0.05; ^{ns} = Non-significant.

For the medium duration genotypes, the number of branches had the highest positive direct effect on seed yield (0.75) (Table 5). The genotypic path analysis also showed that number of pods per plant and pod length had high positive direct effect on seed yield of 0.43 and 0.44, respectively. Days to 75% physiological maturity and days to 50% flowering exhibited a high negative direct effect to seed yield of 0.30 and 0.31 respectively. However, when subjected to a step wise regression analysis that isolates multicollinear traits, seed yield per plant was an important predictor of seed yield in short duration while Seed yield per plant, 100 seed weight and number of seeds per pod were key predictors of seed yield in medium duration genotypes (Tables 6 and 7).

Table 6. Relationship between seed yield and first order predictors in short-duration pigeon pea genotypes

Response Trait	Predictor trait	Standardized B	t	Significance	Adjusted R2	Tolerance	VIF
Seed Yield	Seed yield per plant	0.95	10.87	0.001	91	1.00	1.00

Note. VIF = Variance inflation factor, t = t-Calculated, Tol = Tolerance.

Table 7. Relationship between seed yield and first order predictors in medium-duration pigeon pea genotypes

Response Trait	Predictor trait	Standardized B	t	Significance	Adjusted R2	Tolerance	VIF
Seed Yield	Seed yield per plant	1.32	10.9	0.001	96	0.29	3.45
	Number of seeds/pods	0.19	4.49	0.002	98	0.73	1.37
	100 seed weight	-0.36	-2.92	0.02	99	0.28	3.57

Note. VIF = Variance inflation factor, t = t-Calculated, Tol = Tolerance.

4. Discussion

Seed yield is a culmination of its seed yield components, agronomic and environmental factors and their interaction. As such, understanding the nature of the relationship that exists among key traits is essential for designing efficient and effective selection and crop improvement programs (Mashilo, Shimelis and Odindo, 2016). In the short duration genotypes, the number of pods per plant ($r = 0.86$; $P < 0.001$) and plant height ($r = 0.66$; $P < 0.01$) exhibited a strong positive significant correlation to seed yield. Implying the possibility of utilizing simultaneous selection for these traits to improve seed yield in short duration genotypes though testing at different agro-ecological zones is required to ascertain their usefulness. Similar results were reported by Khakhi (2014), and Nyirenda et al. (2020). Traits such as number of branches per plant ($r = 0.38$; $P < 0.05$) and seed weight ($r = 0.47$; $P < 0.05$) showed a moderate significant positive correlation with seed yield per plant. Studies by Thanki and Sawargaonkar (2010), Rao et al. (2013), and Kesha et al. (2014) reported similar findings. Days to 50% flowering, days to 75% maturity, pod length and number of seeds per pod traits were not significant in short duration genotypes contrary to Cheboi et al. (2016) findings. This could possibly be due to differences in genotypes used and testing environment conditions.

The medium duration genotypes exhibited a strong positive significant correlation between seed yield and number of days to physiological maturity ($r = 0.73$; $P < 0.001$), number of branches per plant ($r = 0.81$; $P < 0.001$), days to flowering ($r = 0.71$; $P < 0.001$), pods per plant ($r = 0.72$; $P < 0.001$), pod length ($r = 0.75$; $P < 0.001$), implying simultaneous selection for these traits would result in increase in seed yield in medium duration genotypes, though more location testing is required to ascertain their usefulness and stability. There was a moderate correlation between 100 seed weight ($r = 0.48$; $P < 0.05$) with seed yield. Kesha et al. (2014), Cheboi et al. (2016) and Narayanan et al. (2018) reported similar findings in pigeon pea crops. Days to 75% maturity showed positive and significant correlation with days to 50% flowering ($r = 0.99$, $P < 0.001$). Further, number of pods per plant showed positive and significant correlation with days to flowering ($r = 0.86$; $P < 0.001$) and days to maturity ($r = 0.88$; $P < 0.001$). Implying selection for earliness can be based on number of pods per plant. The results correspond the findings of Prasad et al. (2013). Saxena et al. (2019) highlighted the importance of selecting for earliness in pigeon pea genotypes for increased productivity of the crop and production of protein-rich legumes to meet the nutritional needs of small-holder farming communities.

4.1 Direct and Indirect Effects of Traits to Grain Yield

In crop improvement studies, it is challenging to determine which traits contributed to the increase in seed yield when multiple variables are included in the correlation analysis. Path coefficient analysis is a technique that overcomes this challenge by partitioning associations; it examines the relative contribution of direct and indirect effects of each trait independently and its contribution to yield (Thanki & Sawargaonkar, 2010; Kesha et al., 2014). In this study, the eight traits were considered as variables that determined seed yield.

Generally, number of pods per plant showed high and positive direct effect on seed yield for both medium (0.43) and short (0.60) duration genotypes. Thanki and Sawargaonkar (2010), Rao et al. (2013), Kesha et al. (2014) and Narayanan et al. (2018) reported similar findings of a high positive direct effect of number pods per plant. In medium duration genotypes there is relatively high and positive direct effect between 75% DTM to number of pods/plant (0.38), number of branches and pod length (0.56) and 50% DTF to number of pods/plant (0.37). Mashilo et al. (2016) reported that simultaneous selection of two traits that have high direct effect of each other may improve yield. These traits can, therefore, be used for direct selection for seed yield improvement in pigeon pea genotypes, though more testing is required to account for the effect of the environment and season on the phenotypic expression of these traits. Additionally, selection in medium duration genotypes should target pod length and number of branches as these showed a high direct effect on seed yield of (0.44) and (0.75) respectively. In short duration genotypes it is equally important to consider plant height (0.36), pod length (0.25) as they had moderate to high effect on seed yield.

Precise contribution of first order predictors such as number of pods per plant, seed yield per plant, 100 seed weight and number of seeds per pod to seed yield was explored further through a stepwise regression analysis, that automatically removes traits with negligible contribution to yield and/or with high multi-collinearity. Seed yield per plant was an important predictor of seed yield in short duration while Seed yield per plant, 100 seed weight and number of seeds per pod were key predictors of seed yield in medium duration genotypes. Implying that selection for seed yield improvement in Pigeon pea genotypes should target these traits.

5. Conclusion

The study determined that there was a relationship between seed yield and agronomic traits in short and medium pigeon pea genotypes. The path coefficient analysis results for medium duration pigeon pea genotypes revealed that number of branches, number of pods per plant and pod length were good seed yield contributors. While in short duration pigeon pea genotypes, number of pods per plant, plant height and the pod length contributed to increase in seed yield. However, for efficient selection for seed yield improvement in Pigeon pea, traits such as seed yield per plant in short duration genotypes and seed yield per plant, 100 seed weight and number of seeds per pod in medium duration genotypes should be emphasized with more multiple location testing.

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Mycorrhizal Association in Wheat Genotypes Submitted to Variable Irrigation in the Brazilian Cerrado

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Abstract

Mycorrhizal association contributes to plant growth, influencing tolerance to abiotic stresses such as water deficit. There is considerable variation in infection by arbuscular mycorrhizal fungi (AMF) in cultivars of the same crop, but there is little information regarding these differences in wheat. The objective of this work was to evaluate the influence of water deficit on the arbuscular mycorrhizal association in wheat genotypes in the Cerrado region and the association between soil attributes and mycorrhizal colonization. The experiment was conducted in a no-till system, using different water regimes. The experimental design was a randomized block with subdivided plots scheme, with 12 treatments and 3 repetitions. The plots consisted of 4 wheat genotypes and the subplots included 3 water regimes. Mycorrhizal colonization, soil microbial biomass carbon, total soil organic carbon, easily extractable glomalin-related soil protein, spore number and AMF species diversity were evaluated. Mycorrhizal colonization was not influenced by wheat genotypes, but it was favored by the higher water regime, being 44.8% higher when compared to the lower water regime. The soil moisture was positively correlated with the soil attributes with the exception of the number of AMF spores. The community of AMF associated with wheat genotypes was similar, comprising of 12 species, predominantly *Claroideoglomus etunicatum* and *Glomus macrocarpum*. The low variation among wheat genotypes for AMF diversity suggests no selective influence of the plants on the AMF community in the area of the study. Water regime was shown to be a dominant factor in mycorrhizal association.

Keywords: soil attributes, Cerrado, water deficit, arbuscular mycorrhizal fungi, no-till, *Triticum aestivum*

1. Introduction

Water availability is one of the environmental variables that are crucial for agricultural production. This resource is at the center of the concerns of farmers, since climate change projections point to an increase in the number of consecutive days of drought, with possible effects in the regions with the highest water demand for agriculture, such as the Brazilian Cerrado (Avila-Diaz et al., 2020). This region is home to 43% of the area of grain crops in Brazil (CONAB, 2021), but developing strategies capable of overcoming the cultivation limits in the winter period due to low rainfall represents an important possibility to increase production without expanding the area already cultivated. In non-irrigated systems, these strategies can collaborate to the implementation of the so-called “safrinha” crop, which occurs exactly in periods of lower water availability.

Wheat (*Triticum aestivum* L.) is one of the most cultivated and consumed cereals in the world (Takeiti, 2015; Wang et al., 2012) and has been managed in non-irrigated systems during the period of greater rainfall availability in the Brazilian Cerrado. In the 2021 crop season, the total area planted in this region was of 106,600 hectares, which represented an increase of 84.7% from the previous harvest (CONAB, 2021). The occurrence of high rainfall in the summer period associated with favorable edaphic conditions (*e.g.*, porous and deep soils, smooth topography and improved fertility), as well as the favorable geographical location for distribution and commercialization, has driven wheat cultivation in the region (Condé et al., 2009; Galindo et al., 2015). Breeding programs have been developed in the region seeking to adapt cultivars with lower water demand, with the purpose of enabling cultivation also in the period of greater irregularity in the amount and distribution of rainfall (Soares et al., 2021).

Associated with breending programs, the selection of cultivars capable of symbiosis with arbuscular mycorrhizal fungi (AMF) may be important due to the recognized contribution of these fungi in the ability of cultivated plants to tolerate periods of reduced rainfall (Bernardo et al., 2019). This contribution is related to the greater water uptake area by the extraradicular hyphae of AMF, with facilitated access to soil micropores that results in greater water flux in the apoplasm (Bárzana et al., 2012a; Chitarra et al., 2016). AMF also stimulate root exudation, especially from the release of glomalin, resulting in increased soil aggregation in the rhizosphere, minimizing the negative effects promoted by rainfall shortages (Cheng et al., 2021). Arbuscular mycorrhizal association also favors microbial biomass and soil carbon accumulation (Vlček & Pohanka, 2020). The composition of the AMF community influences the functionality of symbiosis in different ecosystems, such as agroecosystems (Castillo et al., 2016), with limited knowledge on the relationship between the AMF community and the plants' response to symbiosis by assessing mycorrhizal colonization (Léon et al., 2020).

The benefits arising from mycorrhizal association for cultivated plants are of great interest for sustainable agricultural production and are directly regulated by environmental conditions, with agricultural management practices being considered as the main regulator (Barea, 2015). Variations in AMF infection among cultivars have been reported in the literature (Lehnert et al., 2017; Nahar et al., 2020). The selection of wheat cultivars with higher capacity of association with AMF may be important to determine the most suitable materials for cultivation in the Brazilian Cerrado during the period of lower rainfall occurrence. Keeping this in view, the objective of the present work was to evaluate the influence of dryland wheat genotypes submitted to water stress on the dynamics of arbuscular mycorrhizal symbiosis under field conditions in the Brazilian Cerrado and the association between soil attributes and mycorrhizal colonization.

2. Material and Methods

2.1 Characterization and History of the Experimental Area

The experiment was conducted between June and September of 2016, in the experimental field of Embrapa Cerrados, in Planaltina, DF. The experimental field was located in the following geographical coordinates: latitude 15°35′30″ South and longitude 47°42′30″ West, altitude 1000 m, on a typical dystrophic Red Latosol with clayey texture (Soil Survey Staff, 2010).

The climate of the region, according to the Köppen classification, is seasonal tropical Aw type with two well-defined seasons: a dry period in the fall and winter; a rainy period in the spring and summer with possible occurrences of "veranicos"[1]. The average annual temperature is 20.5 °C with precipitation of 1538 mm (Figure 1).

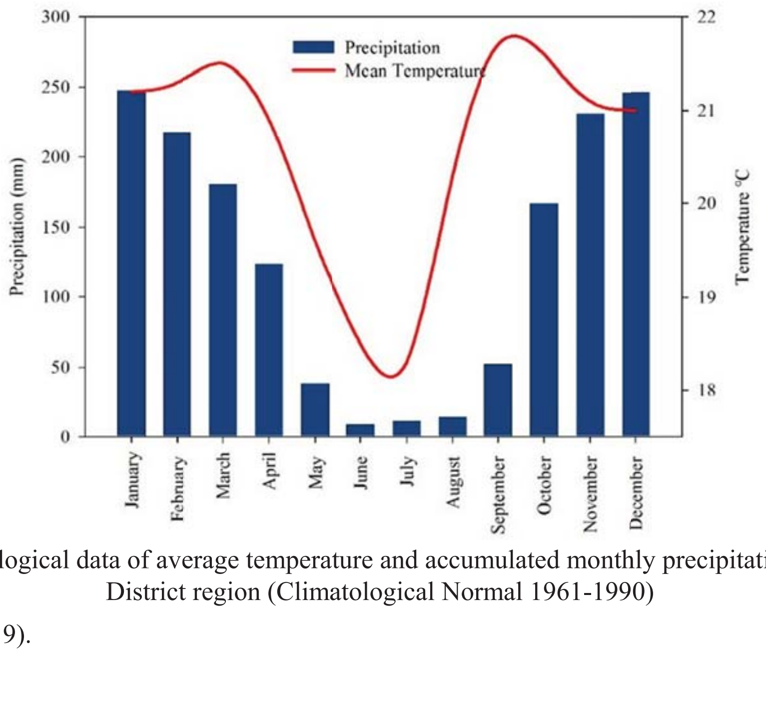

Figure 1. Climatological data of average temperature and accumulated monthly precipitation of the Federal District region (Climatological Normal 1961-1990)

Source: INMET (2019).

The area was cultivated with wheat in a no-till system for 10 years with the same cultivars selected for this study in winter (May to September), and in summer (October to April), soybean-bean rotation was performed. The chemical characterization of the soil in the 0-20 cm layer, sampled before the experiment was set up was: pH (H_2O) = 6.36; Organic matter = 21.6 g dm^{-3}, P (Melich-1) = 7.23 mg dm^{-3}; K, Ca, Mg, H + Al and CTC: 0.35; 3.01; 1.72; 3.47 and 8.55 $cmol_c$ dm^{-3}, respectively.

2.2 Experimental Design and Execution of the Experiment

The experimental design was a randomized block with subdivided plots scheme, with 12 treatments and 3 repetitions. The plots were composed of four rainfed wheat genotypes and the subplots were composed of three water regimes.

The wheat genotypes used were: (1) BRS 404: Dryland material, tolerant to drought, heat, and toxic soil aluminum. Bread-type commercial grade. Obtained from crossing the Aliança cultivar and the WT 99172 strain (Silva et al., 2015); (2) Brilhante: dryland material, tolerant to drought, bread-type commercial class. It was obtained from the PF 8640 strain and the BR 24 cultivar. It presents abundant root system, a desirable characteristic for the efficient use of water under conditions of hydric deficiency (Franco & Evangelista, 2018); (3) PF 020037: strain selected under dryland conditions. It presents intense waxiness in the leaves and stems, a natural mechanism for drought tolerance; 4) PF 080492: dryland material from the southern region of the country, however it adapted well in the Midwest region.

The subplots consisted of variable water regimes, lower than the replenishment of crop evapotranspiration (ETc). Irrigations were performed as described in the Brazilian Cerrado irrigation monitoring program (Embrapa, 2011), by replacing evapotranspiration, using agrometeorological indicators of the region, the soil type and the date of full emergence of the plants. Three water regimes corresponding to 96, 68 and 6% of ETc replacement were considered (L96, L68 and L6). The variable water regimes were obtained by adapting an irrigation bar with a set of sprinklers with different flow rates, in a declining pattern. It was coupled with a reel with adjustable speed in order to apply the desired volumes (Hanks et al., 1976).

The sowing occurred on June 1, 2016, and the useful area of each plot (wheat genotypes) was of 18 m^2 (18 m long by 1 m wide). They were installed perpendicular to the irrigation bar. The subplots consisted of 1 m^2 (1 m long by 1 m wide) located in the position of the water regime adopted within each plot. The plant density was of 350 plants m^{-2}.

Prior to the experiment setup, the soil was treated with glyphosate herbicide to control weeds. According to the chemical analysis of the soil, the fertilizer recommendation at planting was of 441 kg ha^{-1} of mixed mineral fertilizer 04-30-16. At the beginning of the crop tillering, nitrogen fertilization was performed with 100 kg ha^{-1} of nitrogen in the form of urea. Thirty days after emergence, the treatments received an application of 0.5 L ha^{-1} of trinexapaque-ethyl as a growth regulator.

Until 30 days after planting, the plots received the same water volume, accumulating 134.1mm (including precipitation). After this time, on the 35th day after planting, irrigation began with variable water regimes, using irrigation bars with different flow rates. Fourteen irrigations were applied at different volumes according to the corresponding treatments (L96, L68 and L6) until the end of the crop cycle (Figure 2).

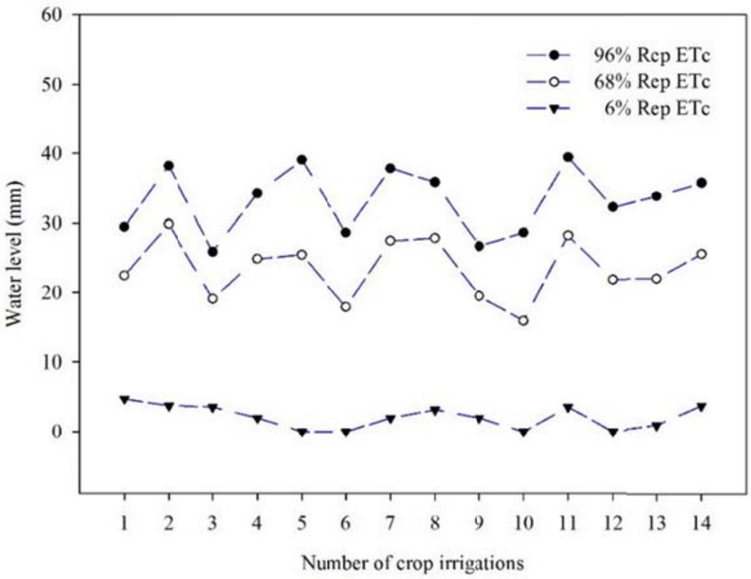

Figure 2. Water regimes applied during the period between days 35 and 71 after planting, based on the crop evapotranspiration replenishment (Rep ETc) in different hydric regimes and different wheat genotypes in a Red Latosol in the Brazilian Cerrado

On average, the water regimes applied during this period were of 33, 23 and 2 mm for L96, L68 and L6 respectively, accumulating a total volume of 599, 462 and 164 mm for the total crop cycle (Figure 3).

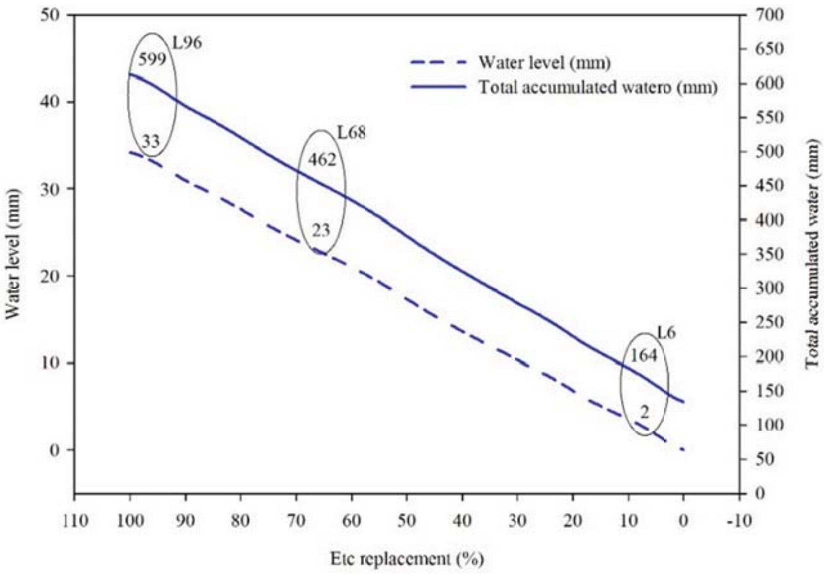

Figure 3. Diagram representing the average applied volumes and total accumulated volume during the cycle of different wheat genotypes on a Red Latosol in the Brazilian Cerrado

The monitoring of the volumes applied after each irrigation was performed by distributing plastic collectors installed perpendicular to the irrigation bar. With this, we determined the actual volumes (mm) applied in the treatments at each irrigation.

2.3 Collection and Analyzed Variables

Approximately 72 days after seedling emergence, during the flowering period, soil and root samples were collected. Soil was collected in the rows of the planting, in the 0-10 cm layer, in a composite sample of five sub-samples. The wheat roots were collected from 5 random plants within each experimental plot. The materials were immediately sent to the laboratory, the soil samples were kept in a cold chamber at 4°C and the roots were kept in a 70% alcohol solution. A portion of the soil samples was used immediately after collection for moisture determination using the gravimetric method (Black, 1965).

Soil microbial biomass carbon (MBC) was determined using the fumigation-extraction method proposed by Vance et al. (1987). For this, the samples were incubated at 80% of field capacity for 7 days and, after this period, half of the samples were fumigated with $CHCl_3$ for 24 h. A solution of K_2SO_4 0.5 mol L^{-1} was used as extraction solution and the oxidation of organic carbon employed $K_2Cr_2O_7$ 0.4 0.166 mol L^{-1} solution in acidic medium. The amount of MBC was determined by the difference between the carbon extracted from fumigated and non-fumigated soil, using the correction factor kec = 0.379, according to Vance et al. (1987).

Total soil organic carbon (TOC) was determined according to the methodology described by Walkley and Black (1934), by oxidation with $K_2Cr_2O_7$ 0.166 mol L^{-1} and titration of the remaining Cr^{6-} with Fe $(NH_4)_2(SO_4)_2{\cdot}6H_2O$ approximately 0.5 mol L^{-1}.

The determination of easily extractable glomalin-related soil protein concentration (GRSP-EE) was performed according to the methodology described by Wright and Upadhyaya (1996), from extraction of 1 g of TFSA (air-dried fine soil) with 8 mL of $Na_3C_6H_5O_7$ 20 mmol L^{-1}, at pH 7.0, at 121 ºC, for 30 minutes. The samples were centrifuged (5000 rpm, 10 min) and the concentration of GRSP-EE in the supernatant was determined by the Bradford essay (1976) on optical density of 595 nm using bovine serum albumin (BSA) as the standard protein.

The rate of mycorrhizal colonization was evaluated by bleaching the roots and staining the fungal structures according to Phillps and Hayman (1970). The bleaching was performed with KOH 0.1 mol L^{-1}, in water bath, followed by the staining of the AMF structures with trypan blue solution. To determine the colonization rate, a grid plate and stereoscopic microscope were used, according to Giovannetti and Mosse (1980).

The number of AMF spores in the soil was determined by the wet sieving method proposed by Gerdemann and Nicolson (1963), with a few adaptations. To recover the largest number of spores retained in the soil aggregates, the soil sample (50 cm^3) was shaken in a blender with tap water for 30 seconds and, after soil decantation, the suspension was disposed on sieves with 1000 and 45 µm mesh. The material retained on the 45 µm sieve was placed in centrifuge tubes with water and centrifuged (3000 rpm, 3 min). The supernatant was discarded, and a new centrifugation (2000 rpm, 3 min) was performed with 50% sucrose solution. The supernatant was recovered for spore counting using a channel plate and stereoscopic microscope.

Taxonomic identification of AMF species was performed through morphological characterization (color, shape, size, among other characteristics) of the healthy spores extracted from the spore density analysis. Permanent slides were made in PVLG (Polyvinyl-Lactoglycerol) and PVLG + Melzer's reagent medium. The identification of AMF species was conducted with the help of the database provided by the International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungal Cultures of West Virginia University (INVAM, 2021) and the Department of Plant Pathology of the Agricultural University of Szczcin Poland (Janusz, 2019), both available on their corresponding websites.

2.4 Statistical Analysis

The data were submitted to variance analysis and the means were compared using Tukey's test ($p \leq 0.05$) with the Sisvar 5.6 statistics software (Ferreira, 2010). The spore number and mycorrhizal colonization data were transformed in log X + 1 and (arc sen %/100) × 0.5, respectively.

3. Results

3.1 Soil Microbiological Attributes and Mycorrhizal Symbiosis

The cultivation of the different wheat genotypes resulted in similar effect on MBC, TOC, GRSP-EE contents, mycorrhizal colonization and AMF spore density (Table 1). However, the water regimes affected the contents of MBC, TOC, GRSP-EE and mycorrhizal colonization. In relation to the water regimes, the highest averages for MBC (284 mg kg^{-1}), TOC (21 mg kg^{-1}), GRSP-EE (6 mg g^{-1}) and colonization rate (42%) were observed in treatment L96.

Table 1. Microbial biomass carbon (MBC); total organic carbon (TOC); easily extractable soil protein related to glomalin (GRSP-EE); mycorrhizal colonization rate (MC) and number of spores (Spores) in a red Latosol with different wheat genotypes and water regimes, in the 0-10 cm layer

Wheat genotypes	MBC	TOC	GRSP-EE	MC	Spores
	mg kg^{-1} solo	g kg^{-1} solo	mg g^{-1} solo	%	n.º 50 cm^{-3}
Brilhante	236	19	4.9	32	190
BRS 404	223	20	4.6	35	241
PF 020037	261	20	5.0	33	281
PF 080492	208	19	4.6	37	296
L96	284 a	21^{a}	6.0a	42 a	224
L68	220 b	20b	4.6b	33 ab	239
L6	191 b	19b	3.6c	29 b	294
CV1 (%)	25	9	19	14	54
CV2 (%)	28	7	13	18	62

Note. The means followed by the same letter in the column do not statistically differ using Tukey's test ($p \leq 0.05$). CV1: coefficient of variation related to the wheat genotypes; CV2: coefficient of variation related to the water regimes. L96, L68, and L6 correspond to the volumes 96, 68, and 6% of ETc replenishment, respectively. Data transformed into (log X + 1) for spore density and (arc sen %/100) × 0.5 for mycorrhizal colonization rate.

On water regimes L68 and L6, a significant reduction in MBC contents on the order of 22.5 and 32.7%, respectively, compared to L96, was observed. For TOC, reductions of 5 and 9.5% were observed at L68 and L6, compared to L96. GRSP-EE contents were reduced by 23.3 and 40% in L68 and L6, respectively, while mycorrhizal colonization rates were reduced by 21.4 and 30.9%. Soil moisture positively influenced the increase in the contents of the soil attributes evaluated, except for the number of AMF spores (Table 2). GRSP-EE was the attribute that had the highest correlation with soil moisture (0.721**).

Table 2. Pearson's correlation in the attributes of a Red Latosol with different wheat genotypes and water regimes, in the 0-10 cm layer

	Soil Moisture	MBC	TOC	GRSP-EE	MC	Spores
Soil Mosture	1	0.470**	0.497**	0.721**	0.473**	-0.177
MBC		1	0.401**	0.380**	0.068	-0.160
TOC			1	0.524**	0.224	-0.019
GRSP-EE				1	0.453**	-0.185
MC					1	-0.277*
Spores						1

Note. MBC = Microbial biomass carbon; TOC = Total organic carbon; GRSP-EE = Glomalin-related soil protein-easily extractable; MC = Mycorrhizal colonization.

3.2 Occurrence and Distribution of Arbuscular Mycorrhizal Fungi Species

Twelve species of AMF were observed in the obtained samples, ten of which could be identified at species level and two at genus level. The species that occurred in all experimental plots were *Claroideoglomus etunicatum* and *Glomus macrocarpum* (Table 3), both with higher frequency of spores (100%). The species *Ambispora leptoticha*, *Scutellospora gregaria* and *Gigaspora* sp. had the lowest spore frequency values (42, 50 and 50%, respectively). The species *Acaulospora mellea*, *Acaulospora rehmii*, *Acaulospora scrobiculata*, *Glomus clavisporum*, *Glomus microagregatum*, *Glomus* sp. and *Scutellospora cerradencis* were also identified, showing varying occurrences in the studied areas.

In the plots cultivated with the genotype Brilhante, the species *Acaulospora rehmii, Glomus* sp. and *Gigaspora* sp. were observed only at the lowest water regime (L6), and the species *Acaulospora mellea* and *Ambispora leptoticha* only at the intermediate water regime (L68). Of the 12 species identified, 5 were present at all water regimes (*Claroideoglomus etunicatum*, *Glomus macrocarpum*, *Acaulospora scrobiculata*, *Glomus clavisporum* and *Scutellospora gregaria*).

Among the wheat genotypes, only in the genotype PF 020037, the influence of only one of the water regimes (L6) was found in the specific presence of the species *Ambispora leptoticha*, *Scutellospora gregaria* and *Scutellospora cerradencis*. On the other hand, for this same genotype, *Claroideoglomus etunicatum, Glomus clavisporum*, *Glomus macrocarpum* and *Gigaspora* sp. were found in all water regimes.

In the plots cultivated with the BRS 404 genotype, the species *Glomus clavisporum* was observed only at the lowest water regime (L6) and the species *Acaulospora mellea* only at the highest one (L96). Like the areas cultivated with the other genotypes, the species *Claroideoglomus etunicatum* and *Glomus macrocarpum* were observed in all the water regimes.

In the plots cultivated with the genotype PF 080492, the species *Glomus microagregatum* and *Gigaspora* sp. were observed only at the intermediate water regime (L68) and the species *Ambispora leptoticha* was observed only at the highest one (L96). Similar to the areas cultivated with the genotype Brilhante, five of the 12 identified species were present in all the water regimes (*Acaulospora mellea*, *Acaulospora scrobiculata*, *Claroideoglomus etunicatum* and *Glomus macrocarpum*).

Considering the influence of the water regimes on the distribution of AMF species, the presence of the 12 identified species was observed in at least one of the wheat genotypes. In treatment L6, the greatest number of the most frequent species was found, which were *Acaulospora scrobiculata*, *Claroideoglomus etunicatum*, *Glomus clavisporum* and *Glomus macrocarpum*. *Glomus microagregatum* was the species with the lowest frequency, found only in the subplot cultivated with the genotype BRS 404.

The species with higher frequency in the intermediate water regime (L68) were similar to those observed in treatment L6, except for *Glomus clavisporum*. Two species presented the lowest frequency: *Ambispora leptoticha* and *Scutellospora gregaria*, both occurred only in the subplot cultivated with the genotype Brilhante.

In treatment L96, only *Claroideoglomus etunicatum* and *Glomus macrocarpum* species presented higher frequency, also observed in the other water regimes. The species that presented the lowest frequency was *Gigaspora sp.* found only in the subplot cultivated with genotype PF020037.

Table 3. Diversity of morphologically identified AMF species of a red Latosol with different wheat genotypes and water regimes at a depth of 0-10 cm

FMA Species	Wheat genotypes/Water regime												Frequency (%)
	L6				L68				L96				
	Brilhante	PF020037	BRS404	PF080492	Brilhante	PF020037	BRS404	PF080492	Brilhante	PF020037	BRS404	PF080492	
Acaulospora mellea		+		+	+			+		+	+	+	58
Acaulospora rehmii	+		+	+		+		+		+	+		58
Acaulospora scrobiculata	+	+	+	+	+	+	+	+	+	+		+	92
Ambispora leptoticha		+	+		+						+	+	42
Claroideoglomus etunicatum	+	+	+	+	+	+	+	+	+	+	+	+	100
Glomus clavisporum	+	+	+	+	+	+		+	+	+		+	83
Glomus macrocarpum	+	+	+	+	+	+	+	+	+	+	+	+	100
Glomus microagregatum			+		+	+		+	+	+	+		58
Glomus sp.	+	+		+		+	+	+			+		58
Scutellospora gregaria	+	+	+		+				+		+		50
Scutellospora. cerradencis	+	+			+		+	+			+	+	58
Gigaspora sp.	+	+				+	+	+		+			50
Species richness	10	11	9	8	11	10	8	12	9	11	12	10	

Canonical correspondence analysis confirmed that the occurrence and spore frequency of the identified species were not influenced by wheat genotypes (Table 1), but by water regimes (Figure 4). The higher water regime favored the diversity of AMF. It is important to emphasize that the number of spores does not indicate the number of individuals of the identified species, since spores are only a reproduction structure of AMF. In this sense, although the number of recovered spores has an inverse relationship with mycorrhizal colonization, the increase in PSRG-FE contents and the greater diversity of AMF indicate that there is a greater activity in environments with greater soil moisture, as observed in the highest water regime.

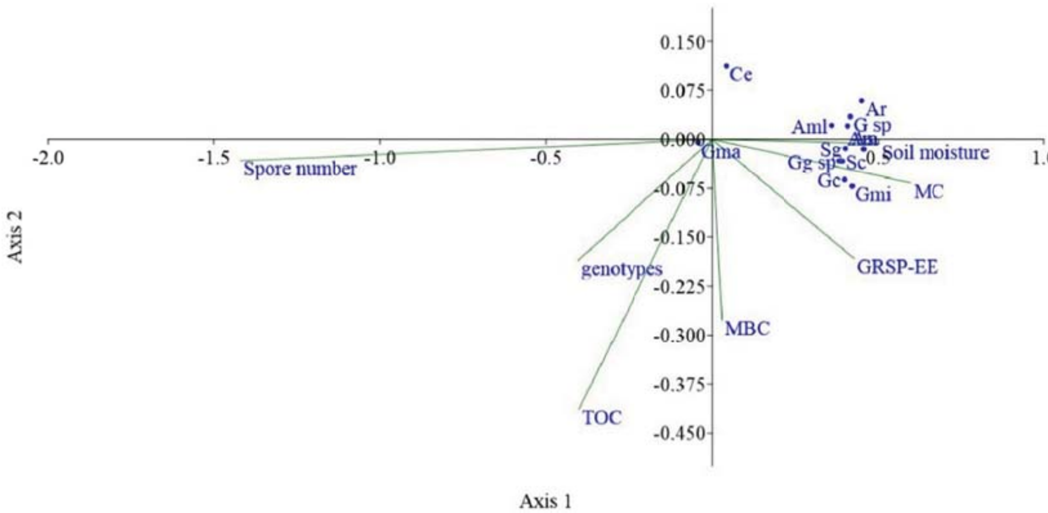

Figure 4. Canonical correspondence analysis of the frequency of spores of arbuscular mycorrhizal fungi species and soil attributes in a red Latosol with different wheat genotypes and water regimes, in the 0-10 cm layer. Am = *Acaulospora mellea*; Ar = *Acaulospora rehmii*; As = *Acaulospora scrobiculata*; Aml = *Ambispora leptoticha*; Ce = *Claroideoglomus etunicatum*; Gc = *Glomus clavisporum*; Gma = *Glomus macrocarpum*; Gmi = *Glomus microagregatum*; G sp. = *Glomus* sp.; Sg = *Scutellospora gregaria*; Sc = *Scutellospora cerradencis*; Gg sp = *Gigaspora* sp. TOC = total organic carbon; MBC = microbial biomass carbon; GRSP-EE = glomalin-related soil protein-easily extractable; Genotytes = Brilhante, PF020037. BRS404, PF080492; MC = mycorrhizal colonization; spore number = AMF spore number; soil moisture

4. Discussion

4.1 Soil Microbiological Attributes and Mycorrhizal Symbiosis

The survival of microorganisms and their activity are dependent on the availability of water in the soil. MBC responds to climatic conditions, and intensified water stress reduces MBC content (Geng et al., 2015). MBC contents below 245 mg kg^{-1} are considered low (Mendes et al., 2019) and associated with severe drought conditions (Geng et al., 2015), as was observed at water regimes L68 and L6.

Low soil water availability also affects the carbon flux between plants and soil by causing stomatal closure and subsequent decline in transpiration, as well as reduced photosynthesis and, consequently, decreased plant-derived TOC and rhizodeposition (Zhao et al., 2020). Other studies also show that water-deficit environments severely reduce soil carbon deposition, also affecting the contents of TOC (Hasibeder et al., 2015; Canarini & Dijkstra, 2015; Fuchslueger et al., 2016).

Wheat genotypes did not influence MBC and TOC contents. Morphological characteristics such as root to aerial part ratio and root thickness (data not evaluated) assist in understanding the contribution of plants to the allocation of soil carbon by rhizodeposition (Bakhshandeh et al., 2018). Unlike the findings in this study, wheat genotypes with higher root to aerial part ratio and thicker roots allocated more carbon in the soil through rhizodeposition at the expense of producing higher yield (Bakhshandeh et al., 2018), and these traits may play an important functional role in transferring more carbon to the soil TOC pool.

An essential component of TOC in terrestrial ecosystems (Jia et al., 2016) and a participant in soil stored carbon (Wilkes et al., 2021), GRSP-EE correlated positively with TOC and stands out as an essential component for TOC in terrestrial ecosystems (Jia et al., 2016). A positive correlation was also found for GRSP-EE and MBC in wheat areas cultivated under different management systems under temperate climate condition (Galazka et al., 2018).

The water restriction resulting from the application of the smallest water regime (L6) reduced the content of GRSP-EE in the soil and the CM, possibly due to unfavorable conditions for plant root growth (data not evaluated) and, consequently resulting in limitation in the development of mycorrhizal symbiosis, abundance and diversity of AMF. Different AMF species produce different amounts of glomalin and only these fungi produce PSRG; therefore, a diverse and abundant AMF community contributes to higher glomalin contents in the soil

(Hossain, 2021). This relationship is based on experiments with AMF colonized and non-colonized root samples, where glomalin was only detected in roots colonized by these fungi (Vlček & Pohanka, 2020).

In accordance with the results observed in this work, the CM at the highest water regimes (L96 and L68) is similar in wheat grown under different management systems, under temperate climate condition (Galazka et al., 2018) and is also within the average observed (41%) in a study that evaluated CM in 94 wheat genotypes (Lehnert et al., 2017).

Management practices that interfere with plant development, such as fertilization, use of pesticides, monocultivation, and constant soil disturbance, result in reduced presence of infective propagules of arbuscular mycorrhizae (Nyamwange et al., 2018), such as the number of AMF spores. In more conservationist systems, such as no-till, there is a favorable environmental condition for the development of symbiosis, due to the absence of soil disturbance (Schalamuk & Cabello, 2010). In the area of the study, the adoption of no-till farming as a cropping system, coupled with a crop rotation program with grasses and legumes (Silva et al., 2020), possibly created favorable conditions for the preservation of the AMF spore numbers. In the symbiosis development cycle, the production of spores among AMF species have different timing and quantity of sporulation and germination (Gomide et al., 2009; Nyamwange et al., 2018), thus, the absence of response observed for the number of AMF spores may be related to the evaluation period used in this study (70 days after wheat plants emergence), and this can be considered a short period for observing the response to changes in soil management and environmental factors.

4.2 Occurrence and Species Diversity of Arbuscular Mycorrhizal Fungi

The higher irrigation increased the diversity of AMF, according to the canonical correspondence analysis (Figure 4). Favorable environmental conditions such as the availability of water for the host plant and, consequently, for the symbiont, are factors that contribute to the abundance of AMF species, while adverse conditions such as water scarcity act as an environmental filter and a reduced number of AMF that are tolerant to the predominant habitat are able to remain in the environment (Deepika & Kothamasi, 2015). Studies have highlighted that changes in soil water availability can affect AMF community composition (Deveautour et al., 2018; Deepika & Kothamasi, 2015). The influence of soil management practices such as irrigation on the composition of the AMF community in areas of annual crops is apparently dominant when compared to the plant community present in the experimental area (Bainard et al., 2017).

The predominance of species that belong to the families *Glomeraceae*, *Acaulosporaceae* and *Gigasporaceae* is consistent with the assessment of the AMF community in no-till areas cultivated with wheat (Schalamuk & Cabello, 2010). The two species with the highest frequency in the study area, *Claroideoglomus etunicatum* and *Glomus macrocarpum* (Table 3), have already been identified with higher occurrence in agricultural areas, in conventional and conservationist systems, as well as in native Cerrado areas (Fernandes et al., 2016; Ferreira et al., 2012; Nunes et al., 2019; Pontes et al., 2017). The high frequency of a species of the genus *Claroideoglomus* (*Claroideoglomus claroideum*) was reported in an area cultivated with wheat in southern Chile (Castillo et al., 2016). Among the less frequent species observed in this work (*Ambispora leptoticha*, *Scutellospora gregaria* and *Gigaspor*a sp.), *Ambispora leptoticha* was also among the least frequent species in wheat-grown areas in southern Chile (Castillo et al., 2016).

The predominance of *Glomus* species (about 41% of the species identified in this work) in no-till areas is associated with the ability and evolutionary adaptation of AMF of this genus to use extraradicular hyphae and colonized root fragments as infective propagules, to the detriment of spore production (Schalamuk & Cabello, 2010). The contribution in the tolerance of wheat plants to water stress is described among the benefits promoted by inoculation with a species belonging to the genus *Glomus* (*Glomus mosseae*), improving the yield and growth of plants under low water availability and controlled conditions (Rani et al., 2018).

5. Conclusions

The wheat genotypes studied, intended for breeding programs aiming at tolerance to water deficit, did not promote changes in the association with arbuscular mycorrhizal fungi and in the parameters of microbial biomass carbon, total organic carbon, soil protein relative to glomalin-easily extracted.

The association between soil attributes microbial biomass carbon, total organic carbon, soil protein relative to glomalin-easily extracted and mycorrhizal colonization in wheat plants were confirmed, contributing to studies concerning mycorrhizal symbiosis.

The water regimes impacted the mycorrhizal colonization, as well as the soil parameters of microbial biomass carbon, total organic carbon, soil protein relative to glomalin-easily extractable, favored by the highest water regime, corresponding to 95% of the crop evapotranspiration.

The occurrence and frequency of arbuscular mycorrhizal fungal species, identified by morphological description, was favored by the water regimes and was not influenced by wheat genotypes.

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Notes

Note 1. A meteorological phenomenon common in the southern and middle-east regions of Brazil, consisting of a period of drought, accompanied by intense heat (25-35 ºC; 77-95 ºF), intense sunshine, and low relative humidity in the middle of the rainy season or in the middle of winter.

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Influence of Farmer Capacity Building in Financial Resource Mobilization on Performance of Smallholder Irrigation Projects in Migori County, Kenya

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Abstract

The study examined influence of farmer capacity building in financial resource mobilization on performance of smallholder irrigation projects in Migori County, Kenya. The study adopted pragmatism as its philosophy, and used cross sectional and correlation research design. The target population was 2,815, and comprised farmers from fifteen smallholder irrigation projects that receive water from River Kuja through Lower Kuja Project. The sample size was 341 farmers. The study used systematic random sampling to draw the sample, used questionnaire to collect data, and analyzed data using descriptive and inferential statistics. The results showed that farmer capacity building in financial resource mobilization has a significant influence on performance of smallholder irrigation projects ($r = 801$, $R^2 = 0.641$, $F (5, 331) = 118.405$, $0.000 < p < 0.05$). Therefore, the study concluded that financial resource mobilization is a critical factor in performance of smallholder irrigation projects in Migori County. Consequently, the study recommends that Migori County Government educate farmers in smallholder irrigation projects on loan facilities by financial institutions. Further, the study recommends that Migori County develop a framework to assist farmers in smallholder irrigation projects to qualify for loans facilities operated by financial institutions.

Keywords: financial resource mobilization, critical success factors, smallholder irrigation.

1. Introduction

Since early civilization, man has used irrigation to guarantee food production. Irrigation produces forty and seventeen percent, respectively, of food in the world and in developing nations (Asian Development Bank [ADB], 2015), and by 2027 irrigation could account for 80% of the world's food needs (Vermillion, 1997 as cited in Kahuro, 2012). Siebert et al. (2005) stated that the largest irrigation areas are found along Rivers Indus and Ganges in Northern India and Pakistan, respectively; Yangtaz, Huang He and Hai He Basins in China; Mississippi-Missouri River Basin in California USA; and River Nile in Sudan and Egypt. Despite offering a strategy for addressing hunger and poverty in rural areas, irrigation has not translated into project success in Kenya. For instance, studies in Migori County identified irrigation potential in the county from Rivers Kuja and Migori as being in excess of 16,500 hectares (GIBB Africa, 2011). However, Kenya National Bureau of Statistics [KNBS] (2018) described food poverty in Migori County as 32%, twice that in Nyeri and Meru Counties (15.5% each), and stated that poverty incidence in Migori County was 41.2%, higher than the national figure of 36.1%. The inability by Migori County to exploit its irrigation potential could be a pointer to existence of a gap in knowledge on project management between researchers and practitioners. Projects management practitioners consider planning, project implementation, cost and time overruns and quality as main concerns in project management (Alias et al., 2014). However, Golini et al. (2017) argued that project success entails use of project-specific approaches to development, and Alias et al. (2014) advised that project based research on critical success factors (CSFs) is more beneficial to project performance.

Studies have attempted to identify causes of poor performance in smallholder irrigation projects. For example, Salami et al. (2010) attributed poor performance in smallholder irrigation in SSA to amongst other factors, lack of finances. Similarly, Dlamini et al. (2014), and Mutambara and Munodawafa (2014) described lack of finances as the cause of poor performance of irrigation by smallholder farmers in Swaziland and Zimbabwe, respectively.

Simpson et al. (2003) stated that communities already over-stretched by daily financially needs are unable to contribute resources to project initiatives. Socio-economic factors influence the ability of farmers to utilize opportunities in smallholder irrigation (Omoregbee et al., 2013), and access to credit enables smallholder farmers to purchase farm inputs and to hire labor (Agwu & Edun, 2007). However, while Montaña at al. (2009) stated that failure to consider socioeconomic characteristics of farmers leads to flaws in project conceptualization, planning and implementation, Food and Agricultural Organization [FAO] (2008) stated that despite enormous funds dedicated to agriculture, rural agriculture had continued to perform poorly, and added that there was little to show for the funds spent in smallholder agriculture. Thus, FAO advised on review of agricultural financing to include savings by farmers, crop insurance, money transfer and leasing. The study found that, whereas other researchers identify lack of finances as causing poor performance in smallholder irrigation projects, there is a gap in knowledge on how capacity building in financial resource mobilization influence performance of smallholder irrigation projects. Thus, the study viewed financial resource mobilization as a critical success factor in performance of smallholder irrigation projects in Migori County, Kenya. New knowledge on financial resource mobilization could inform development of financial facilities for smallholder irrigation projects.

The study tested the following hypothesis; "H1: Farmer capacity building in financial resource mobilization has a significant influence on performance of smallholder irrigation projects". In the study, financial resource mobilization is an independent variable and refers to personal savings and assets, agricultural cooperative society loans, commercial bank loans, non-governmental organizations loans, and governmental organizations loans. Performance of smallholder irrigation projects is the dependent variable, and refers to irrigation water supply, farm labor demand, household food production, farm income generation, and environmental and social mitigation. The study used Resource Based View (RBV) Theory to ground the research. RBV theory dates back to Penrose's view of organization as a pool of resources in 1950. RBV theory considers resources of a firm as a fundamental predictor of a firm's competitive advantage and performance. Whereas there are different ways of categorizing resources, tangible resources are those that facilitate execution of business process, while intangible resources are ones that might result in competitive advantage by allowing organizations to incorporate unique and valuable practices (Ruivo, Oliveira, & Neto, 2015). As noted by Hitt, Carnes, and Xu (2016), RBV is based on two assumptions of resources; being heterogeneously distributed across organizations; and non-transferability of productive resources from one organization to another without incurring cost. Thus, RBV holds that only an intangible resource that is valuable, rare, hard to imitate and without strategically equivalent substitutes is critical in sustaining a firm's competitiveness (Ruivo, Oliveira, & Neto, 2015). The theory is relevant to the study as it notes that there is need for adequate financial resources to ensure better performance of smallholder irrigation projects in Migori County, and hence the need to mobilize financial resource. However, critics of the theory argue that it is not possible to test RBV due to lack of a methodology to measure intangible resources (Kozlenkova, Samaha, & Palmatier, 2014).

2. Material and Methods

2.1 Research Philosophy and Design

The study adopted pragmatism as its philosophy. The study used cross-sectional and correlational research design. Due to lack of funding, the study desired to use a method that would be cheap and cost effective. Thus, the study used cross-sectional research because of its advantage in enabling a one-time process of data collection from a population. Cross-sectional research is useful in describing a population and in determining prevalence of an outcome at a given time (Levin, 2006). Further, cross-sectional research is cheaper, compared to longitudinal design, and more appropriate for researchers working under budgetary constraints (Rindfleisch et al., 2008). Similarly, in order to understand the relationships between famer capacity building in financial resource mobilization and performance of smallholder irrigation projects, the study used correlational research. Correlational research is useful in determining relationships among variables and in forecasting events from existing information (Curtis et al., 2016).

2.2 Target Population

The target population comprised 2,815 farmers undertaking irrigation in fifteen smallholder irrigation projects in Migori County. The study selected the fifteen smallholder irrigation projects because National Irrigation Authority (NIA) has constructed the project to provide water from River Kuja for irrigation in Migori County, and the fifteen irrigation projects benefit from the project. The fifteen smallholder irrigation projects are members of the water user's association (Lower Kuja Irrigation Water Project [LOKIWAP]) and receive water through the Lower Kuja Project. The target population comprised farmers who are members of the fifteen irrigation projects in Migori County that receive water from River Kuja, as part of Lower Kuja Project.

2.3 Sample Size and Sampling Procedure

The study purposely selected all the fifteen smallholder irrigation projects in Migori County that are members of the water user's association (Lower Kuja Irrigation Water Project [LOKIWAP]), and that receive water from River Kuja through the Lower Kuja Project. Further, the researcher used systematic random sampling procedure to select the sample from the sampling frame. According to Krejcie and Morgan (1970), sample size for a population of 2800 is 338, while that for a population of 3000 is 341. The target population (N) was 2815, which falls in between 2800 and 3000. Hence, the study adopted the next highest value of 341 as its sample size (n). Using N = 2,815 and n = 341, the study sampling interval (R) was eight.

2.4 Data Collection Instruments

The study considered the questionnaire as a quick and cost effective method of collecting data. Thus, the study used a questionnaire to collect data from farmers. The study assumed that all farmers are well versed in project activities, and would base their response on experiences and insight in smallholder irrigation projects in Migori County.

2.4.1 Operationalization of Variables

Table 1 presents operationalization of the study variables.

Table 1. Operationalization of the study variables

Variable	Indicators	Measurement
Independent Variable: Financial resource mobilization.	Personal savings and assets.	Number of farmers who use personal savings and assets to finance farming.
	Agricultural cooperatives society loans.	Number of farmers who use agricultural cooperatives society loans to finance farming.
	Commercial bank loans.	Number of farmers who use commercial bank loans to finance farming.
	Non-governmental organization loans.	Number of farmers who use non-governmental organization loans to finance farming.
	Governmental organization loans.	Number of farmers who use governmental organization loans to finance farming.
Dependent Variable: Performance of smallholder irrigation projects.	Irrigation water supply.	Number of farmers who receive adequate irrigation water supply.
	Farm labor requirements.	Number of farmers who employ labor during an irrigation season.
	Household food production.	Number of farmers who meet household food needs from irrigation
	Farm income generation.	Number of farmers who meet financial needs from irrigation.
	Environmental and social mitigation.	Number of farmers who mitigate environmental and social concerns.

2.4.2 Pilot Testing

The study tested the questionnaire prior to use. Hilton (2015) described piloting as important in ensuring that respondents perceive and understand questions as intended by the researcher. The study tested the questionnaire in Lower Sio Project in Busia County, Kenya. This is because Lower Sio Project, also implemented under NIA, underwent similar development approach as Lower Kuja Project. Farmers in Lower Sio Project also share similar conditions to those in Lower Kuja Project, and would therefore most likely respond in the same manner. Lancaster et al. (2002) recommended use of 30 cases in a pilot study, while Mugenda and Mugenda (2003) recommended use of ten percent of the sample in a pilot study. The pilot study adopted 34 cases, translating to ten percent of the sample size, as recommended by Mugenda and Mugenda.

2.4.3 Validity of Research Instruments

Validity assesses how findings help to explain test scores of an investigation (Urbina, 2004). The study addressed content-related validity by peer review, criterion-related validity by correlating test scores with predicted criteria (predictive validity) and with existing criteria (concurrent validity), and construct validity by ensuring correlation of scores (convergence) and score differentiation based on variables (divergence).

2.4.4 Reliability of Research Instruments

Reliability concerns trustworthiness of scores provided by an instrument, and describes consistency of an instrument in providing consistent results over time (Caruth, 2013). Reliability also indicates the error in scores (Urbina, 2004). Urban described the Kuder-Richardson formula 20 (K-R 20) and Cronbach's alpha (coefficient alpha, α) as two most commonly used methods to determine reliability of instruments in social science. The researcher used Cronbach's alpha (coefficient alpha, α) given by Urbina (2004), shown below, to determine reliability.

$$\alpha = \frac{n}{n-1} \times \frac{S_t^2 - \Sigma (S_i^2)}{S_t^2} \quad (1)$$

Where, α = Cronbach's coefficient alpha; n = number of items in the test; S_t^2 = variance of total scores in the test; $\Sigma (S_i^2)$ = sum of the variance of item scores.

2.5 Data Collection Procedures

Before data collection, the researcher sought permission from the University of Nairobi (UON) and National Council for Science, Technology and Innovation (NACOSTI) to undertake the study. This is a requirement for undertaking research activities in Kenya. After securing the research permit, the researcher mobilized to site, and held introductory meetings with local county and national government officials in the project area, and farmers' leaders. Thereafter, the researcher recruited five research assistants to administer the questionnaire. The researcher trained the research assistants on ethics of social research to ensure that they understand how to administer the research instrument. Data collection took five days, comprising one day for mobilization and training of research assistants, three days for administering of the questionnaire, and one day for debriefing.

2.6 Data Analysis Techniques

The study used descriptive and inferential statistics to analyses the data. Descriptive statistics included central tendency (mean) and variability (standard deviation), while inferential statistics included Pearson's correlation analysis. The study tested the null hypothesis described below, and analyzed Pearson product moment correlation (r) and stepwise regression (r^2) based on $p < 0.05$ and 95% confidence level.

In the study, capacity building refers to inputs into project management that aim to improve the ability of individual farmers or groups of farmers in the smallholder irrigation projects to "produce, perform or achieve" their tasks and objectives effectively and efficiently in line with the objectives of Lower Kuja Project. Commercial banks listed in the study were Kenya Commercial Bank, Equity Bank, Cooperative Bank and ABSA. Non-governmental organizations listed in the study were CARE (K) and One Acre Fund, while governmental organizations listed in the study were Uwezo Fund, Women Development Fund, Youth Fund and Agricultural Finance Corporation.

Test for null hypothesis; H1: Farmer capacity building in financial resource mobilization has a significant influence on performance of smallholder irrigation projects.

The regression model took the form shown in Equation 2.

$$Y = a + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + \varepsilon \quad (2)$$

Where, Y = performance of smallholder irrigation projects; a = Y intercept; b_1, b_2, b_3, b_4, b_5 = regression coefficients; X_1 = Personal savings and assets; X_2 = Agricultural cooperatives society loans; X_3 = Commercial bank loans; X_4 = Non-governmental organization loans; X_5 = Governmental organization loans; ε = error term.

3. Results and Discussion

This section presents findings and discussion under the study. The study conducted Analysis of variance (ANOVA) using least significant difference test at $p = 0.05$, and compared the means using SPSS computer package.

3.1 Questionnaire Response Rate

The study targeted 341 respondents. Table 2, response rate, shows that 337 returned fully filled questionnaires, translating to a response rate of 98.8%. A response rate of 70% and above is significant for statistical analysis (Sheehan, 2001). The study concluded that the response rate of 98.8% was significant for statistical analysis.

Table 2. Questionnaire response rate

Name of Irrigation Scheme	Sample	Response	Percentage
Orango (Block M)	3	3	100.0
Owiro (Block 1.1)	19	19	100.0
Bala (Block 1.2)	5	5	100.0
Aego (Block 2.1)	17	16	94.1
East Kanyuor (Block 2.2)	37	37	100.0
Akala (Block 2.3)	27	27	100.0
Sagama (Block 3)	33	33	100.0
Obware (Block 4.1)	25	25	100.0
Magunga (Block 4.2)	40	39	97.5
Oguta (Block 4.3)	13	13	100.0
Angugo (Block 5.1)	21	21	100.0
Kaka (Block 5.2)	16	16	100.0
Kabuto (Block 6)	29	28	96.6
Kimai (Block 7)	22	22	100.0
Kanyarwanda (Block 8)	34	33	97.1
Overall response rate	341	337	98.8

3.2 Descriptive Statistics

The study asked respondents to indicate their level of agreement with various statements on financial resource mobilization (personal savings and assets, agricultural cooperatives society loans, commercial bank loans, non-governmental organization loans, and governmental organization loans).

Using SPSS computer package, the study determined the means and standard deviation based on the responses given by respondents on the various statement on financial resource mobilization. Tables 3-7 present means and standard deviation on responses given by respondents.

Table 3. Agreement with statements on personal savings and assets

	Mean	Std. Dev.
I do not know of any organization that gives loans to farmers.	2.395	1.033
I have never received a loan from any organization for farming.	2.872	1.241
I have never applied for a loan from any organization for farming.	2.932	1.288
I only use my personal savings and assets for farming.	3.086	1.341
I prefer to use my personal savings and assets for farming.	3.427	1.160

Table 4. Agreement with statements on agricultural cooperatives society loans

	Mean	Std. Dev.
I am a member of a cooperative society that gives loans to farmers.	1.825	0.674
I use loans from the cooperative society for farming.	1.792	0.630
Loan conditions by cooperative societies are easy to meet.	2.469	0.694
I repay loans from the cooperative society from sale of farm produce.	1.878	0.655
I prefer to use cooperative society loans for farming.	3.175	1.186

Table 5. Agreement with statements on commercial bank loans

	Mean	Std. Dev.
I have an account with a commercial bank that gives loans to farmers.	1.825	0.674
I use loans from the commercial bank for farming.	1.792	0.630
Loan conditions by commercial banks are easy to meet.	2.469	0.694
I repay loans from the commercial bank from sale of farm produce.	1.864	0.597
I prefer to use commercial bank loans for farming.	2.988	1.261

Table 6. Agreement with statements on non-governmental organizations loans

	Mean	Std. Dev.
I am a member of a non-governmental organization that gives loans to farmers.	1.994	0.852
I use loans from a non-governmental organization for farming.	1.991	0.836
Loan conditions by non-governmental organizations are easy to meet.	2.573	0.806
I repay loans from the non-governmental organization from sale of farm produce.	2.062	0.899
I prefer to use non-governmental organization loans for farming.	3.427	1.183

Table 7. Agreement with statements on governmental organizations loans

	Mean	Std. Dev.
I am a member of a governmental organization that gives loans to farmers.	1.727	0.531
I use loans from the governmental organization for farming.	1.766	0.513
Loan conditions by governmental organizations are easy to meet.	2.380	0.639
I repay loans from the governmental organization from sale of farm produce	1.816	0.465
I prefer to use governmental organization loans for farming.	3.279	1.217

The results in Table 3 show that whereby farmers are aware that there are organizations from where they could acquire loans for farming; farmers depend on their personal savings and assets for farming. Machethe et al. (2004) and Dlamini et al. (2014) identified lack of credit as a major contributor to poor performance in the smallholder irrigation sector. Further, from the findings in Table 3, farmers were non-committal on whether they preferred to use their personal savings and assets for farming, whether they had applied for a loan from any organization for farming, and whether they had received a loan from any organization for farming. The results suggest that farmers do not have a strategy to acquire financial resources for farming. Not surprising then, Omoregbee et al. (2013) stated that socio-economic factors influence the ability of farmers to use the opportunities in smallholder irrigation projects.

As per the findings in Table 4, respondents were non-committal on whether or not they preferred to use loan facilities from cooperative societies for farming. However, despite reporting that they were not members of cooperative societies that gives loans to farmers, respondents felt that loans conditions by cooperative societies were not easy to meet. This finding raises a question on why farmers would think that loan conditions by cooperative societies are difficult to meet, yet they are not members of any cooperative society. The finding suggests that there is misconception within the project area on the operations of cooperative societies. No wonder then, the respondents reported that they had not used loan facilities from cooperative societies to undertake farming. Likewise, the respondents reported that they had not used proceeds from the sale of farm produce to repay loans from cooperative societies. Ashraf et al. (2009) stated that poor farm practices and poor quality control inhibits commercialization of agriculture by smallholder farmers. Cooperative societies offer more to farmers than loan facilities.

According to the findings in Table 5, respondents were non-committal on whether or not they preferred to use loan facilities from commercial banks for farming. However, despite reporting that they did not have an account with a commercial bank that gives loans to farmers, respondents felt that loans conditions by commercial banks were not easy to meet. This finding raises a question on why farmers would think that loan conditions by commercial banks are difficult to meet, yet they did not have an account with a commercial bank that gives loans to farmers. The finding suggests that there is misconception within the project area on the operations of commercial banks. No surprising then, the respondents reported that they had not used loan facilities from commercial banks to undertake farming. Likewise, the respondents. Mutambara and Munodawafa (2014) reported that smallholder farmers in Zimbabwe lacked access to bank loans and that only 40 to 67% had access to fertilizers and pesticides, while only 17% had access to farm input loans.

Table 6 shows that respondents were non-committal on whether or not they preferred to use loan facilities from non-governmental organizations for farming. Likewise, respondents were non-committal on whether or not loan conditions by non-governmental organizations were easy to meet. Assuming that non-committal response represented a more favorable opinion than outward rejections, the finding would suggest that farmers have a more favorable opinion on loan facilities by non-governmental organizations compared to cooperative societies, commercial banks or government-based organizations. This means that non-governmental organizations are more likely to receive a more positive response or loans update in the project area compared to other organizations. GIBB Africa (2011) advised the NIA to enlist non-governmental organizations with experience in community financing to provide credit facilities to farmers in Lower Kuja Project.

From Table 7, the results show that respondents were non-committal on whether or not they preferred to use loan facilities from governmental agency loans for farming. However, despite reporting that they were not members of any governmental organization that gives loans to farmers, respondents felt that loans conditions by governmental organizations were not easy to meet. This finding raises a question on why farmers would think that loan conditions by governmental organizations are difficult to meet, yet they are not members of any governmental organization. The finding suggests that there is misconception within the project area on the operations of governmental organizations. In addition, the respondents reported that they had not used loan facilities from governmental organizations to undertake farming, and that they had not used proceeds from the sale of farm produce to repay loans from governmental organizations.

3.3 Inferential Statistics

The study used regression analysis to test the hypothesis: "H1: Farmer capacity building in financial resource mobilization has a significant influence on performance of smallholder irrigation projects". Tables 8-10 present data analyses and results of regression analysis for influence of farmer capacity building in financial resource mobilization on performance of smallholder irrigation projects.

Table 8. Model summary

Model	R	R Square	Adjusted R Square	Std. Error
1	.801[a]	.641	.636	.319

Note. [a] Predictors: (Constant), Personal savings and assets, Agricultural cooperatives society loans, Commercial bank loans, Non-governmental agency loans, Governmental agency loans.

Table 9. Analysis of Variance (ANOVA)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	61.041	5	12.208	118.405	.000[b]
	Residual	34.128	331	0.103		
	Total	95.169	336			

Note. [a] Dependent Variable: Performance of Smallholder Irrigation Projects.

[b] Predictors: (Constant), Personal savings and assets, Agricultural cooperatives society loans, Commercial bank loans, Non-governmental agency loans, Governmental agency loans.

Table 10. Regression coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.081	0.102		10.598	.000
	Personal savings and assets	0.678	0.143	0.772	4.741	.000
	Agricultural cooperatives society loans	0.816	0.105	0.851	7.771	.000
	Commercial bank loans	0.611	0.147	0.678	4.156	.000
	Non-governmental agency loans	0.719	0.144	0.814	4.993	.000
	Governmental agency loans	0.602	0.145	0.673	4.152	.000

Note. [a] Dependent Variable: Performance of Smallholder Irrigation Projects.

From the findings in Table 8, r = 0.801. This indicates that farmer capacity building in financial resource mobilization (personal savings and assets, agricultural cooperatives society loans, commercial bank loans, non-governmental organization loans and governmental organization loans) has a strong relationship with performance of smallholder irrigation projects in Migori County. From Table 8, R^2 = 0.641, indicating that farmer capacity building in financial resource mobilization (personal savings and assets, agricultural cooperatives society loans, commercial bank loans, non-governmental organization loans and governmental organization loans) explains 64.1% of variations in performance of smallholder irrigation projects in Migori County.

According to Table 9, ANOVA table, the regression model shows that p-value is 0.000 and F-calculated is 118.405. Since, p-value is less than 0.05 and F-calculated is greater than F-critical (2.2413), then the regression model was significant in determining how personal savings and assets, agricultural cooperatives society loans, commercial bank loans, non-governmental organization loans and governmental organization loans influence performance of smallholder irrigation projects in Migori County, Kenya.

Table 10 presents the following results: personal savings and assets ($\beta = 0.678$, $p = 0.000$), agricultural cooperatives society loans ($\beta = 0.816$, $p = 0.000$), commercial bank loans ($\beta = 0.611$, $p = 0.000$), non-governmental organization loans ($\beta = 0.719$, $p = 0.000$) and governmental organization loans ($\beta = 0.602$, $p = 0.000$). Hence, test of significance shows that personal savings and assets, agricultural cooperatives society loans, commercial bank loans, non-governmental organization loans and governmental organization loans are significant at $p < 0.05$ and 95% confidence level. This implies that farmer capacity building in financial resource mobilization has a significant influence on performance of smallholder irrigation projects in Migori County, Kenya. Therefore, the alternate hypothesis was accepted and a conclusion made that farmer capacity building in financial resource mobilization has a significant influence on performance of smallholder irrigation projects in Migori County, Kenya. Lack of financial resource inhibits commercialization of agriculture by smallholder farmers in Swaziland and Zimbabwe (Mutambara & Munodawafa, 2014). FAO (2008) advised on the need to review agricultural financing to include savings, crop insurance, money transfers and leasing.

5. Conclusion and Recommendations

The study concluded that while farmers in smallholder irrigation projects in Migori County are aware of financial institutions that offer financial loan facilities, farmers had a more favorable opinion on financial loan facilities by non-governmental organizations, but were hesitant to engage with cooperative societies, commercial banks or government based organizations. This was strengthen by the fact that farmers were not members of any cooperative society, nor did farmers have an account with any commercial bank or governmental organization, thus making it difficult for farmers to qualify for loan facilities by the financial institutions. Therefore, the study recommends that Migori County Government develop a framework to educate farmers in the smallholder irrigation projects in Migori County on benefits and demerits of using personal savings and assets, agricultural cooperatives society loans, commercial bank loans, non-governmental organization loans and governmental organization loans. Further, the study recommends that the county government develop a framework to assist smallholder irrigation projects in Lower Kuja Project to qualify for financial resource through loan facilities provided by agricultural cooperatives societies, commercial banks, non-governmental organizations and governmental organizations. In addition, the study recommends that NIA should enlist services of organizations that have experience in community financing to provide financial loan facilities to farmers in the smallholder irrigation projects in Migori County.

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Agronomical Performances of Angolan Natural Oil Palm Accessions and Interests for Oil Palm Selection in Côte d'Ivoire

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Abstract

In Côte d'Ivoire, Deli populations, descendants of four oil palms, constituted Group A of the recurrent reciprocal selection. Their genetic base was narrow, an obstacle to long-term genetic progress. Therefore, Angolan oil palm accessions were acquired to broaden Group A's genetic base. Angola selfed and Deli × Angola progenies were tested via Angola selfed × La Mé and (Deli × Angola) × La Mé intergroup hybrids for bunch and oil production, height growth, and tolerance to *Fusarium* in two progeny trials; one in La Mé (Côte d'Ivoire) and the other one in Bangun Bandar (Indonesia). On average, bunch yield (183 kg/palm/year) and oil yield (5.34 t/ha) were close to those of the control. The best 5 hybrids represented 104-112% of the control all traits put together. In addition, Angolan origin has transmitted tolerance to *Fusarium* to its progenies (*Fusarium* index = 80-90). [(Deli × Angola)'s LM 5448 T] × La Mé hybrids yielded 241.4 kg/palm/year of bunch and 7.30 t/ha of palm oil. Their mean height was comparable to that of the control and the *Fusarium* index low (82). Therefore, LM 5448 T was selected for further crop improvements. The modalities of its use were proposed.

Keywords: bunch production, genetic evaluation, genetic resources, oil palm, oil yield, Côte d'Ivoire

1. Introduction

Plant genetic resources, including those of oil palm, are a vital component of plant breeding without which plant improvement is impossible (Acquaah, 2007). At the oil palm research station of La Mé (Côte d'Ivoire), the improvement of productivity and other agronomic characteristics of oil palm uses the recurrent reciprocal selection scheme (RRS) (Meunier & Gascon, 1972). RRS exploits the complementarity of two groups A and B of oil palms: group A made up of oil palm populations with a small number of large bunches and group B of oil palm populations with a large number of small bunches.

Group B assembles a considerable number of palms from different African origins including La Mé (Côte d'Ivoire), Pobè (Benin), Lobé and Widikum (Cameroon), Yangambi (DR Congo), Sibiti (Congo), Calabar and Cowan (Nigeria) to name but a few. Group A is solely comprised of Deli provenance. It consists of *dura* type palms descended from four *dura* oil palms introduced into the Botanical Garden of Bogor (Indonesia) in 1848 (Hartley, 1977). The Deli *dura* used in oil palm breeding at La Mé are descendants of the third and fourth generations of selection.. The resulting populations are considered to have a narrow genetic base (Rosenquist, 1990). In fact, the frequency of genes related to traits of interest to the industry (bunch production) has been increased to the detriment of that of traits not directly related to production. However, the long-term effectiveness of a genetic improvement programme depends on the wide variability of available genetic resources (Gallais, 1990). Therefore, introductions of oil palm materials from new sources, including accessions from sub-spontaneous palm groves, are undertaken before each new selection cycle (Adon et al., 1993, Adon et al., 1998, Bakoume et al., 2001). It is in this context that Angolan natural oil palm accessions from the localities of Salazar and Novo Redondo Nhime were introduced in the Oil Palm Research Station of La Mé (Côte d'Ivoire) in 1971. A first stage of evaluation of the said palms was carried out by crossing them with genitors from Deli

(Asia), La Mé (Côte d'Ivoire), Yangambi (Democratic Republic of Congo) and by crossing them with each other as well. In the latter case, Angola × Angola populations were created (Adon, 1995, Adon et al., 1998). The first evaluation led to selecting Angolan genitors. Unfortunately, their mean total fresh bunch weight, average bunch weight, fruit to bunch ratio, and mesocarp to fruit ratio did not allow them to be directly introduced into the general oil palm improvement scheme despite their good tolerance to Fusarium wilt.

The present study that corresponds to the second stage of evaluation of Angola's oil palm materials, seeks to improve the frequency and the stability of characters of agronomic interest through selfing of candidate Angola genitors and combinations with well renowned Deli Dabou *dura*. Progeny trials were planted at La Mé (Côte d'Ivoire) in 1985 and Bangun Bandar (Indonesia) in 1986. The aim was to select Angola or Angola × Deli genitors with good general combining ability for bunch production, oil production, low height growth, and tolerance to *Fusarium oxysporum elaeidis*. In fact, the economic life of an oil palm plantation depends on the height growth rate. Slow growing oil palms have a long economic life. *Fusarium oxysporum elaeidis* is a causal agent of a fungal disease of the same name *Fusarium* which is endemic to African palm groves. Selected genotypes will be integrated into Group A to enrich it and increase its genetic variability.

2. Materials and Methods

2.1 Planting Material

Progenies evaluated were hybrids resulting from crosses between Deli × Angola or Angola selfed (Group A) and La Mé selfed and La Mé × La Mé partners (Group B). A total of 17 genitors of Deli × Angola or Angola selfed origin and 2 Deli *dura* (LM 5489 D, LM 5885 D) genitors (forming group A) were tested with 13 partners from La Mé selfs and 8 La Mé × La Mé intra-origin crosses within Group B. The ancestries of La Mé genitors and Angola-derived parents are shown in Figures 1 and 2.

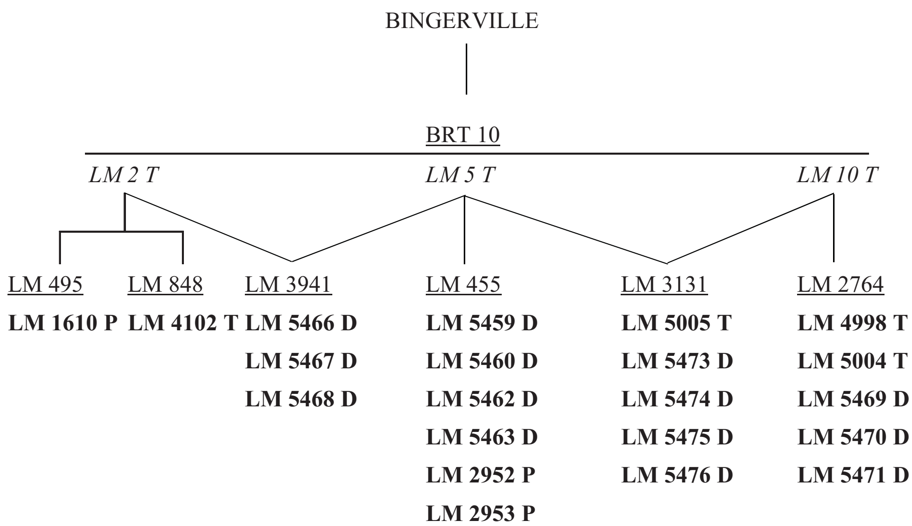

Figure 1. Genealogy of La Mé genitors

Note. Progenies are underlined. Genitors of first selection cycle are in italics. Genitors of the second cycle of selection tested are in bold.

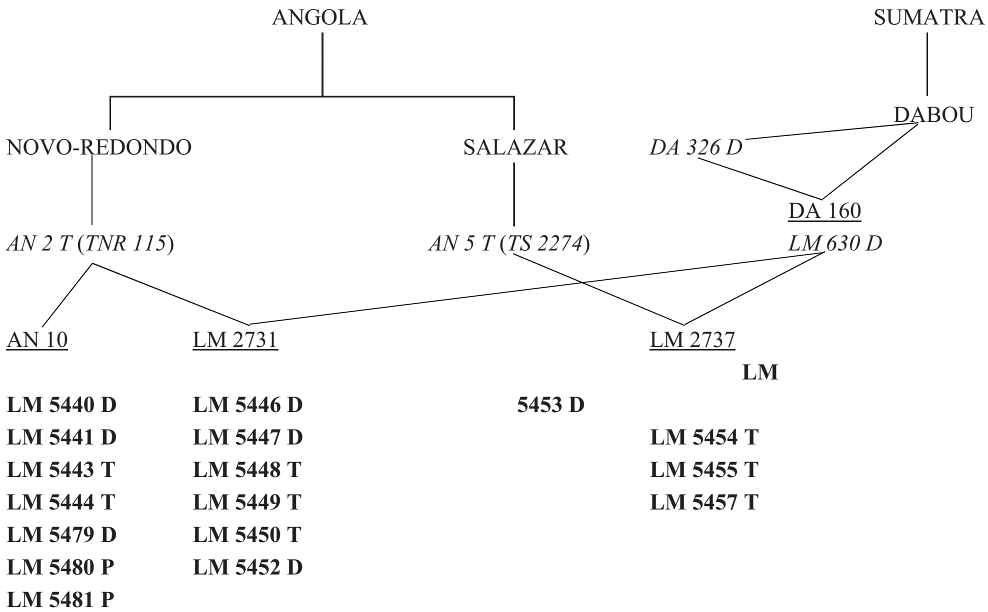

Figure 2. Ancestry of Angola-derived genitors of the second cycle of selection

Note. Progenies are underlined. Genitors of first selection cycle are in italics. Genitors of the second cycle of selection tested are in bold.

A total of 35 progenies were field tested in La Mé (24 progenies) and Bangun Bandar (12 progenies). Four progenies were common to the two trials notably the control (LM 2 T × DA 10 D) and progenies LM 11720, LM 12231, and LM 12281 derived from Angola × La Mé or (Angola × Deli) × La Mé crosses. The control cross (LM 2 T × DA 10 D)-derived progeny was labelled LM 11380 and LM 12649 in the La Mé trial and Bangun Bandar trial, respectively. In fact, the progeny from LM 2 T × DA 10 D cross was one of the best progenies of the first selection cycle. The second selection cycle used it as a control) (Table 1). The four common progenies subsequently served as a bridge (or link) between the two trials. The La Mé (Côte d'Ivoire) trial was laid in 1985 whereas the Bangun Bandar (Indonesia) progeny trial was established in 1986. La Mé oil palm research station is located in the retreat of the Gulf of Guinea, at 5°26′ N latitude and 3°50′ west longitude (Quencez, 1996). It is within an ombrophilic sector and benefits from a transitional tropical climate. The rainfall is abundant, very unevenly distributed throughout the year, and in decreasing trend with an average of 1740 mm/year (Quencez, 1996). The average annual water deficit is about 350 mm. The soil was formed on ferrallitic tertiary sediments and was considered poor in organic matter (Ollagnier et al., 1978). The average sunshine is 1,800 h/year. The Bangun Bandar oil palm research station is situated on volcanic soil (Tampubolo et al., 1990). Rainfall is abundant and well distributed throughout the year. Hence, there is no water deficit and the palm trees benefit from a shallow water table. Sunshine is more than 2,000 h/year. The experimental design was a Fisher block with 6 replicates and 12 palm trees per elementary plot, *i.e.*, 72 trees per progeny. The planting density was 143 palm trees per hectare.

Table 1. Crosses of provenance of hybrid progenies tested in trials LM GP56 and BB GT25

Progeny	Cross type	Progeny serial number in the trial
Trial LM GP 56		
Control: La Mé × Deli		
LM 11380	LM 2 T × DA 10 D	1
(Deli × Angola Novo-Redondo) × La Mé		
(LM 630 D × TNR 115) × La Mé		
LM 11622	LM 5447 D × LM 5005 T	2
LM 11413	LM 5460 D × LM 5449 T	3
LM 11407	LM 5468 D × LM 5449 T	4
LM 11603	LM 5470 D × LM 5450 T	5
LM 11772	LM 5446 D × LM 4102 T	6
(Deli × Angola Salazar) × La Mé		
(LM 630 D × TS 2274) × La Mé		
LM 12231	LM 5453 D × LM 4998 T	7
LM 11503	LM 5462 D × LM 5455 T	8
LM 11725	LM 5471 D × LM 5455 T	9
LM 11720	LM 5457 T × LM 5459 D	10
Angola Novo-Redondo selfed × La Mé		
TNR 115 selfed × La Mé		
LM 11839	LM 5440 D × LM 2953 P	11
LM 11353	LM 5441 D × LM 1610 P	12
LM 11821	LM 5441D × LM 2952 P	13
LM 11494	LM 5441 D × LM 5004 T	14
LM 12281	LM 5443 T × LM 5467 D	15
LM 11856	LM 5443 T × LM 5475 D	16
LM 11964	LM 5463 D × LM 5444 T	17
LM 11749	LM 5444 T × LM 5466 D	18
LM 11319	LM 5473 D × LM 5444 T	19
LM 11548	LM 5470 D × LM 5479 D	20
LM 11225	LM 5474 D × LM 5479 D	21
LM 11623	LM 5471 D × LM 5480 P	22
LM 11442	LM 5476 D × LM 5480 P	23
LM 11633	LM 5469 D × LM 5481 P	24
Trial BB GT25		
Control: La Mé × Deli		
LM 12649	LM 2 T × DA 10 D	1
(Deli × Angola Novo-Redondo) × La Mé		
(LM 630 D × TNR 115) × La Mé		
LM 12283	LM 5448 T × LM 5463 D	2
LM 12273	LM 5467 D × LM 5448 T	3
LM 12293	LM 5476 D × LM 5448 T	4
LM 12433	LM 5452 D × LM 5335 P	5
(Deli × Angola Salazar) × La Mé		
(LM 630 D × TS 2274) × La Mé		
LM 12231	LM 5453 D × LM 4998 T	6
LM 11406	LM 5473 D × LM 5454 T	7
LM 11720	LM 5457 T × LM 5459 D	8
LM 11337	LM 5468 D × LM 5454 T	9
Angola Novo-Redondo selfed × La Mé		
TNR 115 selfed × La Mé		
LM 12281	LM 5443 T × LM 5467 D	10
LM 12334	LM 5469 D × LM 5445 T	11
LM 11440	LM 5440 D × LM 2042 T	12

Note. LM GP 56: La Mé's 56^{th} genetic trial; LM: La Mé; T: tenera; DA: Dabou; D: dura; P: pisifera; BB GT25: Bangun Bandar's 25^{th} genetic trial.

2.2 Parameters Measured

Bunch production was recorded for each palm for seven years from 3 years after planting. The period 3-6 years after planting was the juvenile period and the period 9-10 years the adulthood.

The characteristics of the bunch and the fruit were determined by carrying out 2 series of physico-chemical analyses of bunches, at 5 and 6 years of age, on a sample of 30 to 40 bunches harvested from *tenera* palms from each progeny. Analyses led to determining (i) percentage of fruit per bunch (F/B), (ii) percentage of pulp (or mesocarp) per fruit (P/F), (iii) oil to pulp ratio (O/P), and (iv) palm oil extraction rate (OER). OER has been deducted from F/B, P/F and O/P using the following arithmetic formula:

$$OER = F/B \times P/F \times O/P \times 0.855 \quad (1)$$

Where, 0.855 is a coefficient that takes into account the various losses during the extraction of palm oil from the fresh fruit bunch.

Palm tree height was measured at 6 and 9 years of age according to the method described by Jacquemard (1979). It corresponded to the distance between the soil surface and the axil of the leaf 33, which generally bears a ripe and harvestable fresh fruit bunch or a rotten male inflorescence. Data recorded were used in the estimates of the vertical growth rate.

Progenies were tested for tolerance (or resistance) to *Fusarium* according to the method described by Renard et al. (1972). The Fusarium index (FI) of a progeny was the ratio of the number of *Fusarium*-infected individuals in the progeny to the average number of plants attacked in the test multiplied by 100. The Fusarium index 100 was attributed to the mean number of Fusarium-infected palms in the test. Any value lower than the index corresponds to a more tolerant (or resistant) progeny.

2.3 Data Analysis

An analysis of variance was carried out for total bunch weight, oil yield, and height growth for the periods of 3-6 years and 9-10 years after planting. Emphasis was put on progeny effect and progeny × station interaction. The analysis of variance model used was as follows:

$$\mu = m + D + S \quad (2)$$

where, μ is the mean of the station progeny, m is the overall mean, D is the downfall effect, S is the station effect.

An analysis of variance was also carried out for total bunch weight, oil yield and height growth for the periods of 3-6 years and 9-10 years after planting in a search of Angolan origin, La Mé origin and Angolan genitors effects. The statistical model used was as follows:

$$\mu = m + \text{Origin La Mé} + \text{Parent Angola} + \text{Origin Angola} \quad (3)$$

where, μ is the progeny mean, m is the overall mean, La Mé origin is La Mé origin effect, Parent Angola is the Angola parent effect, origin Angola is the Angola origin effect.

The comparison of mean values of the different progenies for each of the parameters measured was carried out using Tukey’s test However, only mean values of height of progenies from the genetic trial planted in La Mé (Côte d’Ivoire) whose heights were measured were compared.

3. Results

3.1 Progeny Effect and Progeny × Station Interaction

The analysis of variance detected a highly significant difference between the progenies tested, for bunch production and its components at 3-6 years after planting as well as at 9-10 years’ period, and oil yield. Difference was also found for tree height between 6 and 9 years. On the other hand, the analysis detected an absence of progeny × station interaction (Table 2). In the absence of progeny × station interaction, means were adjusted for each of the progenies studied for the components of bunch production and oil yield, over the periods of 3-6 years and 9-10 years after planting.

Table 2. Analysis of variance for bunch production and its components and height

	NR 3-6 years	TBW 3-6 years	ABW 3-6 years	NR 9-10 years	TBW 9-10 years	ABW 9-10 years	GR 6-9 years	Oil yield
Station	58.6**	69.21 **	O.4O ns	2.23 ns	10.98 **	9.43*	55.5 **	6.99*
Progeny	8.14**	2.42 **	18.5 **	5.30 **	1.59 *	10.45 **	7.35 **	4.65 **
Station × Progeny	0.89 ns	1.21 ns	1.07 ns	0.49 ns	1.19 ns	0.61 ns	-2.09 ns	

Note. BN: bunch number; TBW: total bunch weight; ABW: average bunch weight; GR: height growth rate.

3.2 Effects Angola Origin, La Mé Origin, and Angola Parents

No difference was observed between La Mé origin (selfs and crosses), Angola origin (Angola selfed and Angola × Deli combinations) and Angola parents as well, for all the variables studied viz BN, TBW, and ABW at 3-6 years and 9-10 years and vertical growth rate at 9 years after planting (Table 3).

Table 3. Analysis of variance of origins and Angola parent for bunch production and its components and height

Source of variation	NR 3-6 years	TBW 3-6 years	ABW 3-6 years	NR 9-10 years	TBW 9-10 years	ABW 9-10 years	GR
La Mé Origin	0.74 ns	1.61 ns	2.27 ns	0.00 ns	0.44 ns	0.42 ns	11.07 ns
Angola Parent	4.36 ns	1.28 ns	20.30 ns	1.28 ns	0.83 ns	2.53 ns	8.51
Angola Origin	0.59 ns	1.49 ns	6.35 ns	0.19 ns	1.02 ns	0.67 ns	5.08

Note. BN: bunch number; TBW: total bunch weight; ABW: average bunch weight; GR: height growth rate.

3.3 Mean Values of Hybrids Tested

On average, hybrids tested produced 130 kg/tree (17 t/ha/year) of fresh fruit bunches at 3-6 years of age and 190 kg/tree (26 t/ha/year) at 9-10 years after planting. The fresh fruit bunches production of the different types of hybrids tested was comparable to that of the control (LM 2 T × DA 10 D) (Table 4). The number of bunches and average bunch weight were also comparable to that of the control. Within each of the hybrids (LM 630 D × TNR 115) × La Mé, (LM 630 D × TS 2274) × La Mé, and TNR 115 AF × La Mé the variability was moderate for the bunch number (8-12%) and average bunch weight (5-15%) and relatively low for total bunch weight of (4-6%).

Table 4. Mean values of bunch production and its components at young age and maturity of different types of hybrids tested

	Types of hybrids tested					
	(LM 630 D × TNR 115) × La Mé (8)		(LM 630 D × TS 2274) × La Mé (7)		TNR 115 AF × La Mé (16)	
	Mean	CV	Mean	CV	Mean	CV
BN (3-6 years)	23.4	10 %	23.5	4 %	23.1	7 %
TBW (3-6 years)*	128	6 %	126	5 %	128	5 %
ABW (3-6 years)*	5.6	9 %	5.3	5 %	5.7	15 %
BN (9-10 years)	14.7	12 %	14.8	8 %	14.5	10 %
TBW (9-10 years)*	191	6 %	187	4 %	183	6 %
ABW (9-10 years)*	13.5	11 %	13.1	8 %	13.0	10

Note. ( ): number of hybrids' progenies; BN: bunch number; TBW: total bunch weight; ABW: average bunch weight; *: mean expressed in kg/year.

Oil extraction rates (OER) ranged from 20.8 for hybrids (LM 630 D × TS 2274) × La Mé to 21.9% for hybrids (LM 630 D × TNR 115) × La Mé (Table 5). Extraction rates were comparable to that recorded for the control (20.5%). CV value, an indication of the variability existing within each of these different types of hybrids, ranged from 5% to 7%.

Table 5. Mean values of oil extraction rates and oil yields of the different types of hybrids tested at young and mature periods

	Types of hybrids tested					
	(LM 630 D × TNR 115) × La Mé (8)		(LM 630 D × TS 2274) × La Mé (7)		TNR 115 AF × La Mé (13)	
	Mean	CV	Mean	CV	Mean	CV
OER *	21.9	7 %	20.8	7 %	20.8	5 %
Oil (3-6 years)**	3.78	10 %	3.50	9 %	3.57	5 %
Oil (9-10 years) **	5.64	10 %	5.26	9 %	5.13	7 %

Note. OER: oil extraction rate; ( ): number of hybrids' progenies; CV: coefficient of variation; *: mean expressed in %; **: mean given t/ha/year.

Hybrids' oil yields at 3-6 years and 9-10 years were close to those of the control cross (Table 5). The variability in the hybrids (LM 630 D × TS 2274) × La Mé and (LM 630 D × TNR 115) × La Mé was 10%. Vertical growth rates of the different hybrids were around 50 cm/year (Table 6). They were similar to the control in height. In addition, hybrid types tested showed a coefficient of 10% for this vegetative trait. The best 5 progenies' bunch number, fresh fruit bunch yield, average bunch weight and palm oil yield represented 104-to 112% of the trial control and 100-109% of the trial mean all parameters put together at maturity (6-9 years after planting). Three of the 5 best progenies share a common Angola-derived parent LM 5448 T (Table 7).

Table 6. Average growth rate of the different types of hybrids tested at 6-9 years of age

	Types of hybrids tested					
	(LM 630 D × TNR 115) × La Mé (8)		(LM 630 D × TS 2274) × La Mé (7)		TNR 115 AF × La Mé (13)	
	Mean	CV	Mean	CV	Mean	CV
Height growth rate 6-9 years	51	11 %	48	7 %	53	9 %

Note. ( ): number of hybrids' progenies; CV: coefficient of variation; *: mean expressed in cm/year.

Table 7. Performances of 5 best progenies in BB GP25

Progeny	Cross type	BN	TBW (kg/palm/year)	ABW (kg)	Oil (t/ha)	Height (m)
LM 12283	**LM 5448 T** × LM 5463 D	22.7	233.28	10.40	7.31	1.92
LM 12273	LM 5467 D × **LM 5448 T**	22.6	245.73	11.00	6.80	1.93
LM 12293	LM 5476 D × **LM 5448 T**	22.2	245.15	11.33	7.78	1.82
LM 12433	LM 5452 D × LM 5335 P	21.6	238.90	11.25	6.87	1.98
LM 12334	LM 5469 D × LM 5445 T	23.0	248.68	10.95	6.92	1.98
5 best progenies' mean		22.4	242.35	10.99	7.14	1.93
Trial mean		21.7	234.16	11.00	6.55	1.90
Control		21.6	232.38	10.93	6.35	1.80

Note. BN: bunch number; TBW: total bunch weight; ABW: average bunch weight.

3.4 Resistance to Fusarium Wilt

The average Fusarium indices recorded were less than 100 on average (Table 8). Hybrids (LM 630 D × TS 2274) × La Mé revealed the lowest index for this trait (IF = 83).

Table 8. Average Fusarium wilt index of different types of hybrids tested

	Types of hybrids tested		
	(LM 630 D × TNR 115) × La Mé (8)	(LM 630 D × TS 2274) × La Mé (7)	TNR 115 AF × La Mé (13)
Mean Fusarium index	97 (14: 15)	83 (22: 10)	90 (20: 14)

Note. ( ): number of hybrids' progenies; (14: 15) 14 tests with FI < 100 against 15 tests with FI > 100.

4. Discussion

4.1 Comparison of Progenies and Value of Progeny of Angolan Origin

Analysis of variance revealed a highly significant difference between progenies for bunch production and its components both at juvenile period (3-6 years) and at mature stage (9-10 years) as well as for height at 9 years. It also showed the absence progeny × station interaction. In the absence of progeny × station interaction. The two genetic trials were merged into one trial. Data were adjusted with reference to the trial planted in Bangun Bandar (Indonesia) whose mean values of the different parameters were relatively high. In fact, the four progenies common to two trials served as bridges and were used in the estimates of the adjustment factors. The 5 progenies that ranked first in the comparison of mean values (Table not shown) have produced 8.16 to 21% more oil than the control at 9-10 years after planting. Their oil yields were comparable to those of oil palm commercial fields planted Deli × La Mé materials of the second cycle of RRS represented by the control. However, they can be selected for improvement for future utilization as seed trees. They should be reproduced for seed supply to farmers. Four (4) progenies have recorded a lower height growth rate (44 cm/year, on average) than the control at 9 years after planting-period. In fact, the period is considered that of the exponential growth phase in oil palm (Baudouin & Jacquemard, 1987).

The second analysis of variance revealed the absence of an effect of each of the La Mé origin, Angola origin, and Angola parent. However, the 3 progenies that ranked among the 5 best in oil production per unit area had one common parent, *i.e.*, LM 5448 T a descendant of (LM 630 D × TNR 115) cross. In fact, LM 5448 T has demonstrated a good GCA for this trait. Therefore, it is possible to disseminate planting material derived from Angola origin with moderate height growth rate than the Deli × La Mé seeds that are currently supplied to oil palm growers. It should be considered a genetic progress on the trait since Angolan origin was characterised in the 'wild' state as high vertical growth rate material (Adon et al., 1998).

4.2 Mean Values of Hybrids From Angola origin-Contributed Crosses

Fresh fruit bunches production of Angola origin-derived hybrids have 130 kg/tree at 3-6 years of age (17 t/ha) and 190 kg/tree (26 t/ha) at 9-10 years. The values are very close to the potential yield of planting materials of the second cycle of RRS currently supplied to oil palm growers by the French PalmElit considered the producer of high potential bunches and oil yields planting materials for the African oil palm climates and soils. The relatively high bunch production of the Angolan origin-contributed progenies was due to their high bunch number (23 bunches per tree at 3-6 years and 15 at 9-10 years) of medium size (6 kg/bunch and 13 kg/bunch, respectively). The performances obtained were comparable to those the control LM 2 T × DA 10 D which is one of the best crosses of the first selection cycle in bunch production. In fact, it is not common to identify Deli × La Mé seeds from the second cycle of RRS that are currently more productive than the LM 2 T × DA 10 D control. It can be argued that the hybrids (Deli × Angola) × La Mé and Angola selfed × La Mé had a satisfactory bunch production per hectare. The performance obtained indicated the efficiency of the selection made in the accessions from Angola natural oil palm groves. Indeed, a 10% improvement in bunch production was recorded in the second stage of evaluation of this material compared to the one obtained in the first stage (Adon, 1995).

Oil extraction rates varied from 20.8% to 21.9% for hybrids contributed by the Angolan origin. OER values were comparable to that of the control (20.5%) which was recognised as poor performer for this trait. This characteristic of Angolan oil palm genotypes, which was already detected in the first stage of their evaluation by Adon et al. (1998), would be a consequence of a low percentage of pulp on fruit and a low oil content of the pulp. Okwagwu (1985) explained the low percentage of pulp to fruit by the presence of a large kernel. In fact, this trait is said to be governed by a gene related to the sh^-(*tenera*) gene of the 2 ancestors TNR 115 and TS 2274 of Angolan origin.

Mean oil yields of the hybrids derived from Angola origin were around 3.5 t/ha at 3-6 years and 5.3 t/ha at 9-10 years. Yields were comparable to those of the control, which were 3.5 t/ha and 5.5 t/ha, respectively, for the same periods. However, yields were relatively lower than those of the Deli × La Me seeds supplied to oil palm growers, which are producing 15-25% more oil than the LM 2 T × DA 10 D control. Angolan origin-based hybrids can be considered less productive in oil. However, the existence of considerable variability for this trait especially in the hybrids (LM 630 D × TNR 115) × La Me (CV = 10%) would allow the selection of progenies with a performance comparable to that of the current Deli × La Mé planting materials. They showed a vertical growth rate of about 50 cm/year recorded from two measures made one at 6 years and the other one at 9 years. The value was close to that of the control. Some of the progenies grew slower than the control, which was considered as a low vertical growth rate progeny (about 50 cm/year) in La Mé (Jacquemard & Baudouin, 1987). This characteristic was a pitfall in using Angolan materials in the first stage of evaluation, where Angola ×

Angola progenies averaged 117% of the control (Adon, 1995). In fact, oil palm planting materials must represent at most 100% of the control in terms of plant height. There was a genetic progress in reducing the vertical growth rate.

The Fusarium index, which is an indicator of oil palm tolerance, was less than 100 showing that Angolan origin-based hybrids were tolerant to Fusarium wilt. The tolerance to Fusarium observed in the Angolan origin in the first stage of evaluation was preserved. This character is an asset for the material of Angolan origin (Durand-Gasselin et al., 2000). Thus, the Angolan source material provides a new source of tolerance to Fusarium that would eventually lead to the distribution of seeds to susceptible areas.

4.3 Introgression of Angola Origin Genitors in Main Breeding Programme

In the long term, there is the possibility of genetic progress with selected oil palms of Angola origin in combination with the Deli origin. It would be possible, on the one hand, to increase the genetic basis of Group A of the reciprocal recurrent selection, which until now has consisted solely of Deli *dura* (sh^+ gene) materials, and on the other hand, to introduce there the *tenera* (sh^-gene present in the Angola origin). In addition, intra-group A recombinations between Angolan genitors selected in this work and Indonesian genitors acquired from an oil palm materials exchange programme between the Centre National de Recherches Agronomiques (CNRA) of Côte d'Ivoire and the Indonesian Oil palm Research Institute (IOPRI) would constitute a phase of the third cycle of RRS.

A genitor of Angola origin (LM 5448 T) has distinguished itself by its good general combining ability for agronomic performance, notably the tolerance to Fusarium, low vertical growth rate, and a decumbent architecture. Selected genitor deserves to be integrated as a priority in recurrent populations. There are two ways to use it. The first aims to test certain trees from LM 5448 T AF with partners from Group B. The production of Angola × La Mé seeds' objective is to identify better genotypes than their ancestors. The second is to integrate LM 5448 T into the Group A's populations, which will be broaden and enriched with the Angola origin.

5. Conclusion

The main objectives of introducing Angolan oil palm materials in Group A were, on the one hand, to enrich the genetic base of Group A for the selection scheme and, on the other hand, to introduce the sh^-(*tenera*) gene. The genitor of Angola origin LM 5448 T and the descendants from its selfing offered new genes for bunch production and tolerance to Fusarium to Group A. Recombinations involving Angola's *tenera* and *pisifera* of Deli × Angola crosses are means of transferring the sh^-gene into Group A (which initially consisted only of *dura* (sh^+ gene). Eventually, it will be possible to make crosses of the *pisifera* type of Group A × Group B *dura*. This inversion of the traditional direction of crosses, *i.e.*, Group A *dura* × Group B *pisifera* would make it possible to avoid problems of insufficient pollen on the *pisifera* of Group B which are rather too female. However, the low average bunch weight of Group B materials consisting of *tenera* and *pisifera* would result in the production of small bunches which leads to a reduced number of fruits per bunch. Therefore, seed producers will expect less seeds for supply to growers. Therefore, seed production should take the average bunch weight of Group B material into account.

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Abbreviations

AN 2 T: Second genitor of *tenera* type and of Angolan origin selected at La Mé; BB: Bangun Bandar (Indonesia); D: *Dura* palm; DA 10 D: 10th genitor of *dura* type selected at Dabou; LM: La Mé (Côte d'Ivoire); T: *tenera* palm; P: *pisifera* palm.

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Morphological Characterization of *Corynespora cassiicola* Isolates in Culture Media

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Abstract

Corynespora cassiicola threatens soybean and cotton production in Brazil. The objective of this study was to evaluate cultural and morphological aspects of *C. cassiicola* isolated from soybean and cotton of different Brazilian regions, in culture media. The isolates were grown in PDA (Potato Dextrose Agar) and V8 juice agar media. The characteristics evaluated were: color, aspect, and growth rate of mycelia, as well as production and dimension of conidia, and number of septa per conidium. Culture media and isolates were compared using the Kruskal-Wallis or Tukey's test at 5% significance level. The mycelia of the isolates were predominantly dark gray and light brown. *C. cassiicola* isolates grew better in V8 juice agar medium, presenting a higher mycelial growth rate. In PDA medium, the production of conidia was higher in isolates from cotton, compared with soybean isolates. There was great variation in the production of conidia in V8 juice agar medium, regardless of the host origin. Conidia length and width varied for isolate and culture medium. The isolates of *C. cassiicola* coming from cotton presented a higher number of septa per conidium when grown in PDA medium. The morphological aspects of *C. cassiicola* vary depending on the host of origin and the culture medium.

Keywords: conidia production, mycelial growth, target spot, V8 juice agar medium, PDA medium

1. Introduction

The fungus *Corynespora cassiicola* Berk. & M. A. Curtis (Wei, 1950), present in countries with tropical and subtropical climates, causes diseases in more than 530 plant species belonging to 400 different genera (Farr et al., 2019). This fungus has a wide range of economically important hosts including soybean (*Glycine max*) (Seaman et al., 1965) cotton (*Gossypium hirsutum*) (Fulmer et al., 2012), common bean (*Phaseolus vulgaris*) (Mendes et al., 1998), papaya (*Carica papaya*) (Qi et al., 2011), cucumber (*Cucumis sativum*) (Cutrim & Silva, 2003), pepper (*Capsicum annuum*) (Shimomoto, 2008) rubber tree (*Hevea brasiliensis*) (Qi et al., 2011), and tomato (*Lycopersicon esculentum*) (Lopes & Ávila, 2005).

Severe epidemics of *Corynespora cassiicola* have been recorded in the USA, Brazil, and Argentina, where soybean and cotton are grown in crop rotation (Galbieri et al., 2014), leading to an increasing incidence of target spot in the last harvests of these crops (Sumabat et al., 2018; Godoy et al., 2020).

C. cassiicola causes a disease in soybean known as target spot, affecting leaves, stems, pods, and seeds (Almeida et al., 2005). In the leaves, the lesions appear as brown spots with yellow halos, evolving from light to dark brown necrotic patches of up to 2 centimeters in diameter surrounded by chlorotic halos (Snow & Berggren, 1989). The severely affected leaves fall prematurely (Sinclair & Backman, 1989).

The fungus survives in debris, soybean seeds, and fallow fields for up to two years. It forms survival structures called chlamydospores (Almeida et al., 2001; Godoy et al., 2014) that are specialized structures resistant to adverse environmental conditions, acting as resting spores (Gould, 2010; Kimati et al., 1995).

In culture media, *C. cassiicola* initially presents white mycelium, later becoming light brown to dark gray (Snow & Berggren, 1989). Conidiophores are formed isolated or in small groups, branched, erect or slightly curved, whose conidia occur without stroma formation (Ellis, 1971b). Formed isolated or in chains, conidia are cylindrical or oblavate, straight or slightly curved, from hyaline to brown or light olivaceous, and may have from zero to 28 septa, and a pronounced hilum at the base (Snow & Berggren, 1989; Koenning & Creswell, 2006).

Isolates of *C. cassiicola* from soybean have an optimum temperature for mycelial growth between 18 to 21 ºC, with a minimum that allows in vitro growth from 5 to 7 ºC and a maximum from 34 to 39 ºC (Sinclair & Backman, 1989).

Studies on morphology characteristics of *C. cassiicola* isolates and how they develop in different culture media are useful to define which culture medium favors the growth and sporulation of the isolates. In the literature, morphological variations among *C. cassiicola* isolates from different Brazilian regions are reported (Nghia et al., 2008; Qi et al., 2011; Kurre et al., 2019).

In addition, information about the pathogen's physiology and variations among isolates from different hosts and regions may guide which management strategies best control the disease (De Azevedo, 1997). These data allow choosing the more suitable technique to multiple the pathogen and to obtain inoculum for the selection of host plant lines that are less susceptible to the disease regarding different isolates.

In light of the above, this study aimed to determine the mycelial growth capacity and morphological characteristics of eight *C. cassiicola* isolates from soybean and cotton of different regions of Brazil, grown in PDA and V8 juice agar culture media.

2. Method

2.1 Collection Soybean and Cotton Leaf Samples

Samples of soybean and cotton leaves with typical target spot symptoms were collected in the 2018/19 harvest, in different areas of cultivation covering three Brazilian states (Figure 1). These samples were sent to the Seed Pathology laboratory at the Rural Development Institute IAPAR-EMATER, IDR-Paraná for analysis of symptoms in a microscope and subsequent pathogen isolation.

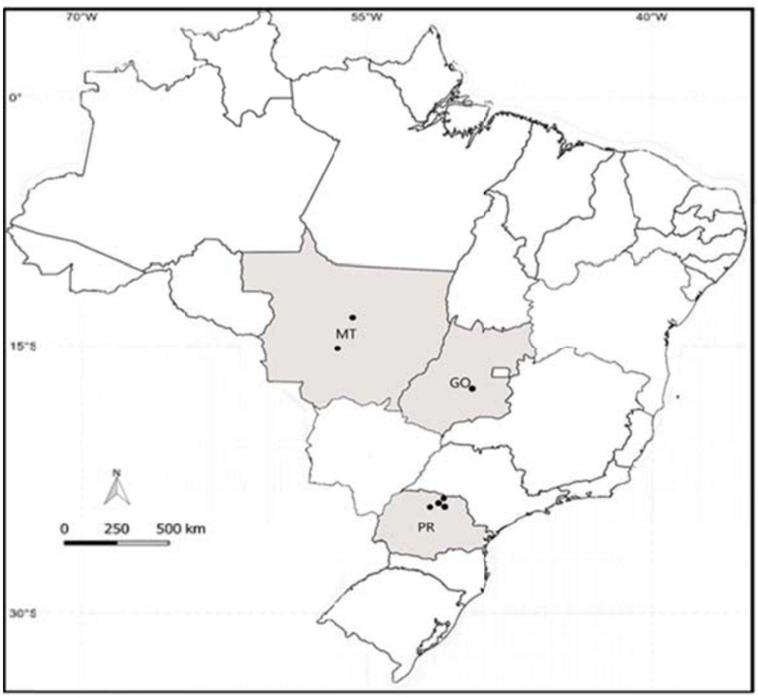

Figure 1. Map of Brazil showing the locations in three states where samples of soybean and cotton leaves with symptoms of target spot were collected to obtain isolates of *Corynespora cassiicola*

2.2 Pathogen Isolation, Purification, Identification, and Cultivation

Soybean and cotton leaf segments (3 mm in diameter) containing lesions were subjected to superficial disinfestation in 70% alcohol for 30 seconds and 2% sodium hypochlorite for 1 minute, followed by washes in sterile distilled water. The tissues were distributed in V8 juice agar culture medium and maintained at 25±1 ºC and 12/12 h light-dark photoperiod for pathogen isolation.

After four days, the mycelial growth of the fungus was observed and identified using the key of the genus *C. cassiicola* proposed by (Ellis, 1957). Then, the mycelium of each isolate was subcultured in V8 juice agar, and, subsequently, a monosporic culture was performed in agar-water medium (Fernandez, 1993). Each isolate was stored at ±4 ºC using the method proposed by Castellani (Figueiredo, 1967).

Eight isolates of *C. cassiicola* species were obtained from soybean plants, named ISO S-soybean and cotton plants as ISO C-cotton, from the states of Paraná (PR), Mato Grosso (MT) and Goiás (GO) (Table 1). For a more detailed identification of the isolates, in addition to morphological characterization described here, a pathogenicity test was also performed. All isolates studied here underwent pathogenicity tests in different crops, and soybean and cotton (data not yet published) were pathogenic.

Table 1. Code, year, and location of collection and plant host species of *Corynespora cassiicola* isolates used in cultural and morphological analyses

Code	**Year**	**Location**	**Host plant species**
ISO 1 S	2018	Goiânia-GO	
ISO 2 S	2018	Arapongas-PR	
ISO 3 S	2018	Sorriso-MT	*Glycine max* L.
ISO 4 S	2019	Londrina-PR	
ISO 11 S	2018	Diamantino-MT	
ISO 1 C	2018	Sertanópolis-PR	
ISO 2 C	2019	Jataizinho-PR	*Gossypium hirsutum* L.
ISO 3 C	2019	Porecatu-PR	

Mycelial discs of 5 mm in diameter were removed from the edges of the colonies after five days of growth and transferred to the center of Petri dishes (Ø = 9 cm) containing Potato Dextrose Agar (PDA: 200 g L^{-1} potato extract, 20 g L^{-1} dextrose, 17 g L^{-1} agar) and V8 juice agar (V8: 5 g L^{-1} Calcium carbonate, 340 mL V8 juice, 34 g L^{-1} agar). The dishes were sealed with Parafilm® and maintained at 25±1 ºC and 12/12 h light-dark photoperiod for 72 hours. After this period, the evaluations were performed.

2.3 Evaluations

The variables evaluated were: color, aspect, and growth rate of mycelia, as well as production, length, width of conidia, and number of septa per conidium. The necessary evaluations to calculate the mycelial growth rate were performed from three to eleven days after incubation. The other evaluations were carried out 12 days after incubation.

Colony color was characterized following the color annotation system of Munsell Color Company (1975). The dishes containing PDA and V8 juice agar culture media were analyzed for colony pigmentation hue at both sides (front and back). The isolates were also evaluated for mycelium type: plane, aerial, or aerial cottony (Snow & Berggren, 1989).

The daily rate of isolates mycelial growth was established by measuring the colony in two orthogonal directions, at 3, 5, 7, 9, and 11 days after incubation. The measurements were completed when the colony grew all over the Petri dish in one of the treatments (11 D.A.I.). However, the 9 D.A.I. evaluation was used to calculate the daily growth rate, since, at 11 D.A.I., mycelial growth may have been limited and could have interfered with the results. The daily rate (mm d^{-1}) was calculated by the average diameter of the two diametrically opposed measurements subtracted from Ø 0.5 mm (initial disc) and divided by 9 (evaluation days).

For conidia production, 10 mL of distilled water was added to each plate and the fungal colonies were scraped with a soft bristle brush. The concentration of the conidia suspension (conidia mL^{-1}) was determined in a Neubauer chamber under a microscope at 100x magnification. The counting was performed five times for each repetition, and the average number was used to define the final amount of conidia.

Conidia width and length (µm) and number of septa were determined by evaluating 50 conidia of the fungal suspension described above. The structures were observed under optical microscopy (100×), photographed, and subjected to the digital image processing and analysis software ImageJ version 1.32j (Wayne Rasband National Institute of Health, USA) (Rasband, 2012).

2.4 Experimental Design and Statistical Analysis

The experimente was conducted using a completely randomized design in a 2 × 8 factorial scheme (two culture media x eight *C. cassiicola* isolates) with five repetitions. The experimental unit consisted of a plate.

For the variables mycelial growth rate, conidia production, and average number of septa per conidium, the comparison between culture media within each isolate of *C. cassiicola* was performed by the non-parametric Mann-Whitney test. The comparison of isolates within each culture medium was performed using the Kruskal-Wallis test, at a significance level of 5% using the BioEstat 5.0 software.

The variables conidia length and width were subjected to analysis of variance (ANOVA), followed by the mean comparison Tukey's test at 5% significance level, comparing culture media and *C. cassiicola* isolates in a factorial scheme. The software used was SISVAR.

3. Results

3.1 Colony Pigmentation and Appearance

Great variation was observed in the pigmentation of the eight *C. cassiicola* isolates grown in PDA and V8 juice agar culture media (Table 2 and Figure 2).

At the superior part (front) of the dishes containing V8 juice agar medium, the mycelial pigmentation of the isolates varied from gray to olivaceous, while at the inferior part (back) of the dishes, light brown was the predominant color for all isolates (Figure 2). In PDA medium, mycelial pigmentation varied from gray to white at the front of the dishes and from brown to black at the back of the dishes (Figure 2).

In general, the mycelial color of *C. cassiicola* isolates was predominantly dark gray and light brown, at the front and back of the plates, respectively.

Regardless of the culture medium, the mycelial aspect of the isolates from cotton was plane, while, for soybean isolates, the mycelial aspect was aerial cottony. The only exception was the ISO 1S isolate, which proved to be plane when grown in both culture media (Table 2).

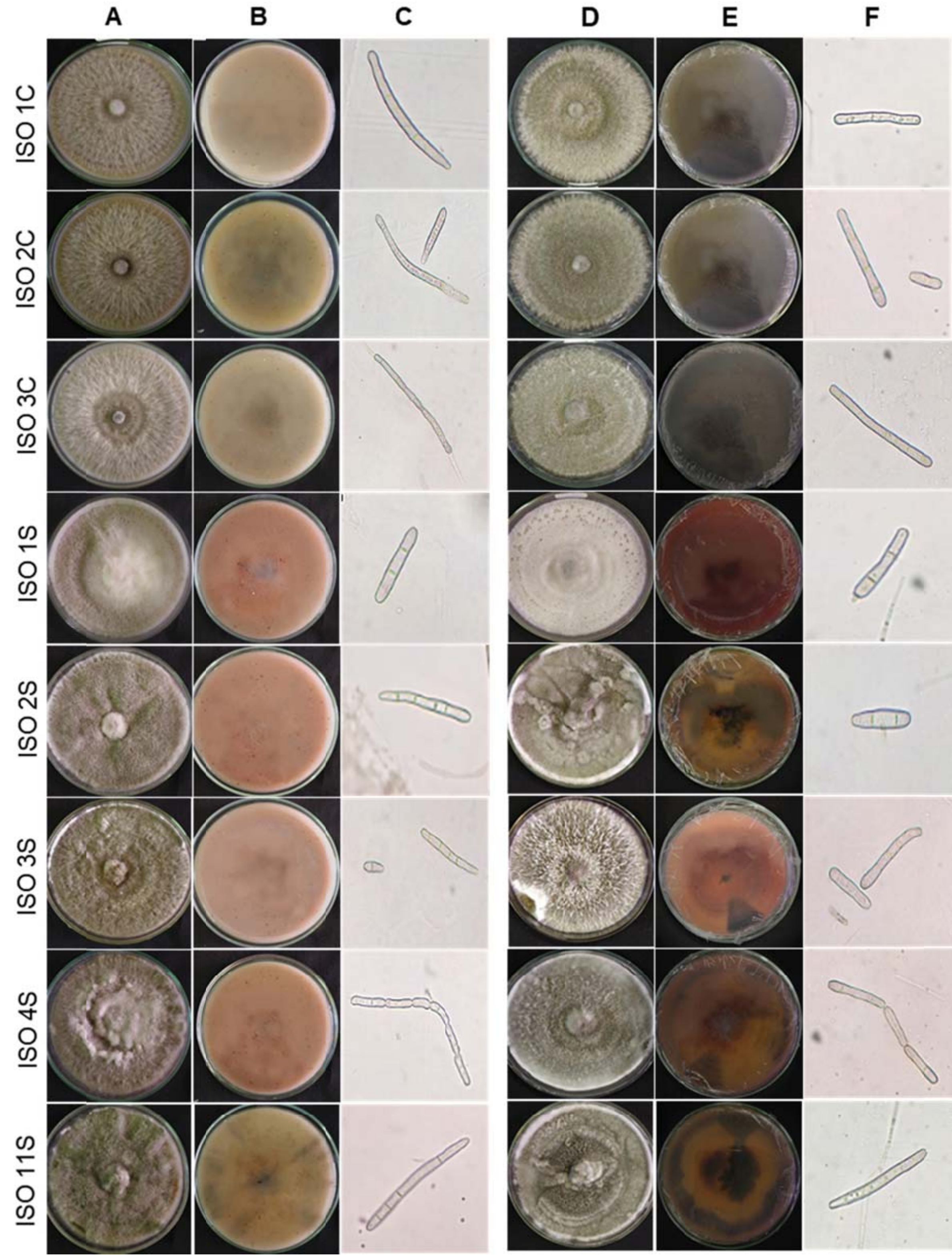

Figure 2. Morphological characteristics of *Corynespora cassiicola* isolates at 12 days of incubation in V8 juice agar and PDA culture media, Tº = 25±1 ºC and 12-hour photoperiod. (A) Front view of the dishes in Juice V8 agar medium; (B) Back view of the dishes with V8 juice agar medium; (C and F) Microscopic images of different shapes, septa and conidia sizes of *C. cassiicola* visualized in juice V8 agar and PDA; (D) Front view of the dishes with PDA medium; (E) Back view of the dishes with PDA medium

3.2 Mycelial Growth Rate

The mycelial growth rate (mm d^{-1}) varied from 5.40 to 8.82 mm d^{-1} among the eight isolates of *C. cassiicola* (Table 2). The highest mycelial growth rate occurred for isolates grown in V8 juice agar produc, with 8.40 to 8.82 mm d^{-1}, while the values ranged from 5.40 to 7.30 mm d^{-1} in PDA produc.

Mycelium of *C. cassiicola* isolates grew at production rates in PDA, with the highest growth rates observed for ISO 3C and ISO 1C, presenting 7.30 and 7.14 mm d^{-1}, respectively. The lowest growth rates occurred for the isolates ISO 4S, ISO 11S, and ISO 3S, with 6.35, 6.00, and 5.40 mm d^{-1}, respectively. Whereas for the V8 juice agar culture produc, no significant differences were detected regarding the mycelial growth rate of *C. cassiicola* isolates (Table 2).

3.3 Conidia Production

The performance of conidia production in the different culture media varied according to the plant host origin of the isolates (Table 2). No difference was observed in the production of conidia between the culture media when the isolates came from cotton plants (ISO 1C, ISO 2C, and ISO 3C). Whereas regarding *C. cassiicola* isolates from soybean plants, conidia production was higher for the ISO 2S, ISO 4S, and ISO 11S isolates in V8 juice agar medium (64.00, 99.13, and 107.38, respectively), compared with the PDA medium (23.88, 36.00, and 38.00, respectively) (Table 2).

In general, the production of conidia in PDA medium was higher in isolates from cotton, with 88.50 to 90.63 conidia mL^{-1}, compared to soybean isolates, ranging from 19.13 to 43.13 conidia mL^{-1}.

When the isolates were cultivated in V8 juice agar medium, the highest number of conidia was produced by the isolates ISO 11S, ISO 4S, ISO 2C, and ISO 1C, producing from 107.38 to 85.00 conidia mL^{-1}, in relation to ISO 3S and ISO 1S, with 49.38 and 22.88 conidia mL^{-1}, respectively (Table 2).

Table 2. Colony cultural and morphological aspects, growth rate, conidia production, conidia length and width, and number of septa in eight isolates of *Corynespora cassiicola* (C. c.) obtained from cotton and soybean and grown in PDA and V8 juice agar (V8) culture media after 12 days of incubation at 25±1 ºC and 12/12 h photoperiod.

Culture media	Host plant	Isolate *C. c.*	Colony color		Mycelium aspect	Growth rate (mm/day)	Conidia production mL^{-1}	Conidia		Septa number		
			Front	Back				Length	Width	Minimum	Maximum	Mean
PDA	*Gossypium hirsutum*	ISO 1C	Dark gray	Black	Plane	7.14 B[1] a[2]	88.50 A ab	52.36 A a	6.88 A b	0	7	2.62 A ab
		ISO 2C	Dark gray	Black	Plane	7.04 B ab	89.00 A ab	53.43 A a	7.04 A b	0	11	2.52 A ab
		ISO 3C	Dark gray	Black	Plane	7.30 B a	90.63 A a	47.70 A ab	6.65 A b	0	10	2.14 A ab
	Glycine max	ISO 1S	White	Brown	Plane	6.95 B abc	19.13 A d	49.78 A a	7.32 A ab	0	9	2.60 A a
		ISO 2S	Dark gray	Black	Aerial cottony	7.01 B abc	24.88 B cd	30.78 A c	8.06 A ab	0	5	1.12 A c
		ISO 3S	Light gray	Light brown	Aerial cottony	5.40 B d	43.13 A bc	31.42 B c	7.05 A ab	0	6	1.26 A c
		ISO 4S	Gray/Brown	Light brown	Aerial cottony	6.35 B bcd	36.00 B cd	33.65 A c	6.32 B b	0	3	1.22 B c
		ISO 11S	Dark gray	Light brown	Aerial cottony	6.00 B cd	38.00 B cd	35.19 A bc	6.69 B b	0	7	1.78 A bc
V8	*Gossypium hirsutum*	ISO 1C	Light gray	Light brown	Plane	8.40 A[1] a[2]	85.00 A ab	41.31 B a	6.86 A b	0	8	2.10 A bc
		ISO 2C	Light gray	Light brown	Plane	8.48 A a	89.88 A ab	49.88 A a	7.55 A ab	0	8	3.04 A a
		ISO 3C	Light gray	Light brown	Plane	8.73 A a	75.00 A bc	44.71 A a	6.82 A b	0	7	2.16 A ab
	Glycine max	ISO 1S	Gray	Light brown	Aerial cottony	8.82 A a	22.88 A d	40.05 B a	7.24 A ab	0	4	1.24 B cd
		ISO 2S	Dark gray	Light brown	Aerial cottony	8.67 A a	64.00 A bcd	26.35 A b	6.75 B b	0	4	0.92 A d
		ISO 3S	Olivaceous	Light brown	Aerial cottony	8.40 A a	49.38 A cd	43.92 A a	7.28 A ab	0	10	2.06 A ab
		ISO 4S	Dark gray	Light brown	Aerial cottony	8.47 A a	99.13 A ab	37.89 A ab	7.99 A a	0	9	2.14 A ab
		ISO 11S	Dark gray	Light brown	Aerial cottony	8.52 A a	107.38 A a	38.98 A ab	7.65 A ab	0	7	2.14 A ab

Note. [1] Means followed by capital letters in the column compare culture media within each isolate of *C. cassiicola*, different letters indicate significant difference (α = 5%) by the Mann-Whitney test.

[2] Means followed by lowercase letters in the column compare isolates of *C. cassiicola* within each culture medium, indicating significant difference (α = 5%) by the Kruskal-Wallis test.

3.4 Conidia Dimensions

In the two culture media tested, all isolates produced conidia and their length and width were determined. However, there was an interaction between culture medium and isolates of *C. cassiicola* (Table 2). The isolates ISO 1C (52.36 µm) and ISO 1S (49.78 µm) showed greater lengths of conidia produced in the PDA medium, while the ISO 3S isolate had greater conidium length (43.92 µm) in V8 juice agar. The other isolates did not differ from each other (Table 2).

The highest length of conidia produced in PDA medium was observed for the isolates ISO 3C, ISO 1S, ISO 1C, and ISO 2C, ranging from 47.70 to 53.43 µm. The shortest length was observed for ISO 2S, ISO 3S, and ISO 4S, presenting 30.78 to 33.65 µm. The longest conidia length obtained in V8 juice agar medium was found for ISO 1S, ISO 1C, ISO 3S, ISO 3C, and ISO 2C, showing from 40.05 to 49.88 µm and differing from the ISO 2S isolate (26.35 µm).

No difference was observed in the width of conidia grown in PDA or V8 juice agar when the isolates came from cotton (Table 2). However, for isolates from soybean plants, the ISO 2S isolate had a greater conidial width in the PDA medium (8.06 µm) compared with those produced in V8 juice agar (6.75 µm). The opposite occurred when the ISO 4S and ISO 11S isolates were grown in V8 juice agar medium, with 7.99 and 7.65 µm, compared with the values of 6.32 and 6.69 µm obtained in the PDA medium. The other isolates did not differ from each other (Table 2).

When grown in PDA medium, the largest conidia width was observed for ISO 2S, with 8.06 µm, differing from ISO 4S, ISO 11S, ISO 3C, ISO 1C, and ISO 2C, whose dimensions varied from 6.32 to 7.04 µm. Regarding the V8 juice agar culture medium, the largest conidium width was observed for the ISO 4S isolate, with 7.99 µm, while the ISO 2S, ISO 3C, and ISO 1C isolates had the smallest widths ranging from 6.75 to 6.86 µm (Table 2).

3.5 Number of Septa

For most isolates of *C. cassiicola*, the number of septa per conidium varied greatly, regardless of the culture medium (Table 2 and Figure 3). In all isolates, the presence of conidia without septa was observed. The maximum number of septa varied from 3 for the ISO 4S isolate to 11 in ISO 2C.

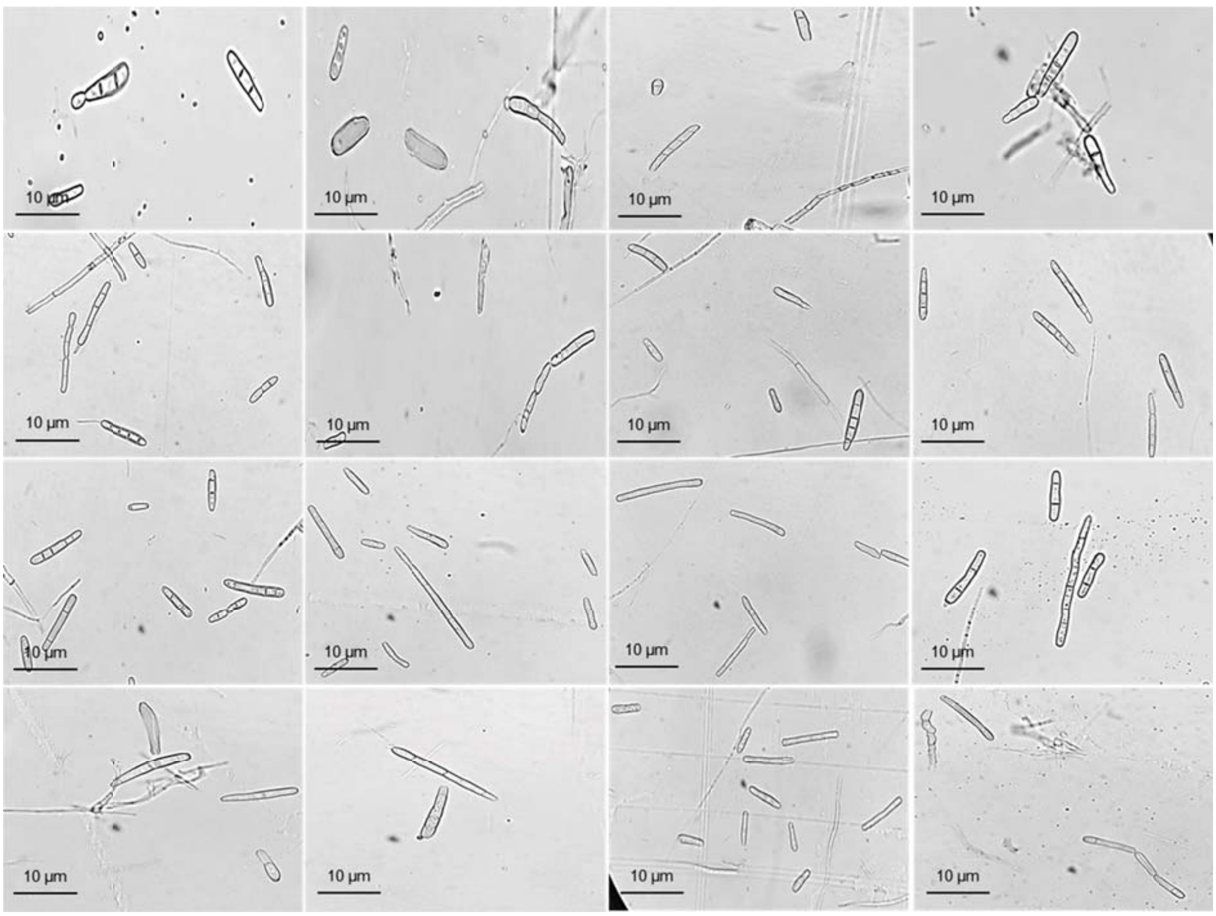

Figure 3. Morphocultural variability observed under a microscope produced by eight isolates of *Corynespora cassiicola* from different regions

No difference was found between the culture media tested regarding the average number of septa per conidium of *C. cassiicola* isolates from cotton plants (Table 2). However, differences were observed for isolates from soybean. ISO 1S had the highest average number of septa per conidium when grown in PDA medium, with 2.60 septa per conidium, compared with 1.24 septa per conidium in the V8 juice agar medium.

The opposite occurred for ISO 4S, with the highest average number of 2.14 septa per conidium in V8 juice agar medium, compared with PDA medium, where it produced 1.22 septa per conidium. The other isolates did not differ in terms of culture medium and average number of septa per conidium.

When grown in PDA medium, the average number of septa per conidium was higher for the ISO 3C, ISO 2C, ISO 1S, and ISO 1C isolates, ranging from 2.14 to 2.62. Whereas ISO 2S, ISO 4S, and ISO 3S presented 1.12 to 1.26 septa per conidium.

When isolates were cultivated in V8 juice agar medium, the highest average number of septa per conidium was observed for the isolates ISO 4S, ISO 11S, ISO 3C, and ISO 2C, with 2.14 to 3.04 septa per conidium, while the lowest average values were found for ISO 1S and ISO 2S, which presented 1.24 and 0.92 septa per conidium, respectively (Table 2).

4. Discussion

Similarities were observed in the characteristics of the eight isolates by the description of (Ellis & Holliday, 1971a), indicating that they belong to the species *C. cassiicola*. The study of fungi characteristics and morphological variations plays an important role in taxonomy and can assist in the identification of many pathogenic species (Mushrif, 2006).

In the present study, no variation was observed for characteristics related to the development of isolates from cotton; however, a great variation occurred for isolates from soybean. These results indicate that the host plant of the pathogen influences its developmental characteristics. These data corroborate previous studies, which described variations in color, texture, and dimensions of conidia between isolates of *C. cassiicola* from the same or different hosts and regions (Darmono et al., 1996; Spencer & Walters, 1969).

Qi et al. (2011) and Sousa et al. (2014) studied *C. cassiicola* isolates from soybean grown in PDA medium and also found variations in texture, ranging from fine to thick, and colony color, being white to light brown, red-brown, dark brown, dark gray, or black.

Probably, the variability among isolates from soybean can be attributed to the older interaction between *C. cassiicola* and soybean plants compared with cotton plants (Almeida et al., 1976; Fulmer et al., 2012). This longer time of interaction, together with the presence of diverse soybean cultivars in different regions of Brazil, may have resulted in greater selection pressure for *C. cassiicola* pathotypes. However, further studies should be carried out to elucidate this hypothesis, using a greater number of isolates from soybean and cotton plants. It is worth mentioning that the variability of phytopathogens is related to the occurrence of some mechanisms of genetic recombination (Kaufmann, Weidemann, & Peres, 2003).

The mycelial growth rate of *C. cassiicola* in other culture media was recorded from 5.7 to 10.0 mm d^{-1} for different isolates (Oliveira et al., 2007; Nghia et al., 2008). In this study, a lower mycelial growth rate was found for all isolates grown in PDA medium, ranging from 5.40 to 7.30 mm d^{-1}, when compared with the values of 8.40 to 8.82 mm d^{-1} in V8 juice agar medium.

Almeida et al. (1994) and Muliterno de Melo (2009) also found that different isolates of *C. cassiicola* develop slowly in PDA culture medium, forming dark shaded mycelium with color from gray to olive black. The authors relate the slower mycelial growth with the source of nutrients to which the isolate has access and the use of nutrients in each culture medium.

Although the PDA medium is frequently used for studies with different fungi (Diener, 1952) warns that the development of some fungi may be impaired by it, proposing the use of a culture medium with industrialized juice (Juice V8) composed of eight vegetables, rich in vitamins A, C, and E, fiber, and minerals. This culture medium provided higher rates of mycelial growth in the present study.

For fungi cultivation in vitro, the choice of the medium must be based on nutrients and physico-chemical conditions that are indispensable for the development of the pathogen. In addition to temperature and brightness, the composition of the culture medium determines the quality and quantity of mycelial growth (Carnaúba et al., 2007).

Griffin (1993) reported that each isolate has a metabolizes nutrients at different speeds depending on the culture medium, leading to the synthesis of other molecules or products that influence the fungus growth in the medium.

There is no ideal culture medium that is suitable for all pathogens, and it is necessary to compare media for growth and spore formation for each pathogen studied (Gava, 2002).

No differences were found in the production of conidia between the culture media when the isolates came from cotton plants. However, soybean-derived isolates reached the highest conidia production in the V8 juice agar medium, corroborating the results of (Almeida et al., 1976).

The higher production of spores in phytopathogens may be related to the medium in which it was grown (Carnaúba et al., 2007). For some phytopathogenic fungi, (Diener, 1952) recommends the use of a medium containing V8 juice in order to increase the production of conidia. As previously mentioned, this medium is rich in vitamins and minerals, which may partially justify the results found in this study since the PDA medium contains less concentration of vitamins and minerals.

Conidia length and width of the different isolates obtained in this study ranged from 26.35 to 53.36 µm and from 6.65 to 8.06 µm, respectively. The number of septa varied from 0 to 11 septa per conidium. These dimensions and number of septa are in accordance with the lower and upper limits proposed by Ellis (1971a) for the species description.

Qi et al. (2011) also observed a difference in the conidia dimensions of *C. cassiicola*, with length from 10 to 277 µm, width from 1.3 to 17.1 µm, and number of septa from 0 to 18 per conidium. For the same pathogen, (Teramoto, 2008) obtained dimensions from 46.1 to 110.9 µm for length, from 5.3 to 8.2 µm for width, and 1 to 15 for septa number. In studies with soybean isolates, (Ferreira, 2012) observed lengths of 20 to 300 µm, width of 7 to 15 µm, and number of septa varying from 1 to 22. Muliterno de Melo (2009) found conidia sizes from 8 at 280 µm, with an average of 10 to 150 µm.

The dimensions of conidia length and width as well as the number of septa obtained in this study showed less variation compared with the results brought in the literature. It can be seen that the values described by each author vary; these differences may be related to the origin of the isolates, such as host plant and geographical location, handling techniques, humidity of the environment in which they are grown, and the culture medium (Gasparotto & Pereira, 2012; Sousa & Bentes, 2014; Darmono et al., 1996; Spencer & Walters, 1969). Sousa and Bentes (2014) also reported that in natural conditions with high humidity, conidiophores and conidia are generally long and sharp.

The knowledge of how the chemical composition of the culture media physiologically influence the pathogen *C. cassiicola*, affecting the morphological, growth, and production characteristics of conidia is essential to determine which culture medium allows the multiplication of the pathogen more efficiently under in vitro conditions (Bogo et al., 2008).

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Seasonal and Regional Chemical Variability of the Wild Population of *Lantana camara* Leaf Essential Oil From Kenya

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Abstract

Studies examining the variability in wild plant metabolic expression propose that environmental factors significantly influence the essential oil (EO) quality and quantity in a plant. *Lantana camara is* a widely distributed invasive plant species worldwide. However, its immense metabolites can become a source of novel compounds to produce biopesticides in the agricultural industry. Although, the quality aspect has to be considered due to the environmental influence on the metabolites synthesised. Therefore, this research aimed to understand the influence of environmental factors and how it shapes the plant's metabolite profile in multiple populations of *L. camara*. Leaf samples were collected from six different geographic regions of Kenya and the corresponding monthly climatic data and soil samples. GC-MS data from leaf EO were analysed with environmental variables (climate and soil data) using unimodally unconstrained and constrained ordination methods for untargeted metabolomics analysis. Partial Least Squares-Discriminant Analysis (PLS-DA) and Random Forests (RF) were used to confirm the variability further. Seasonal and regional variability was observed for secondary metabolites (SMs) in the leaf EO, which correlated to climatic factors and soil attributes. We highlight the season-al-geographic metabolism relationship for *L. camara* and the combined analytical approach to obtain data that contributes to understanding the influence of environmental factors on the synthesis and accumulation of SMs. This research will have all-embracing implications for maximising phytochemical uniformity.

Keywords: adaptation, environmental factors, *Lantana camara*, seasonality, secondary metabolites

1. Introduction

Lantana camara Linn. (Verbenaceae), commonly known as Lantana, is a plant species widely found growing in many parts of the world and produces essential oils (EOs). Lantana is described as an invasive weed in different ecosystems (Bhagwat, Breman, Thekaekara, Thornton, & Willis, 2012; Willis, 2017), which is related to problems of ecological imbalance in areas infested with this plant because of its ability to adapt to different climate and soil conditions (Aruna & Balasubramanian, 2015). The composition of the essential oils of *L. camara* collected from several parts of the world are characterised by the principle components being terpenes (monoterpenes and sesquiterpenes) and their oxygenated derivatives (Anjum et al., 2017; Patil, Kumbhar, & Ambhore, 2017). Cited among the common major constituents identified are the sesquiterpenes, caryophyllene, isocaryophyllene, germacrene D, bicyclogermacrene, caryophyllene oxide, and caryophyllene epoxide (Anjum et al., 2017; Khan, Mahmood, & Alkhathlan, 2016; Nea et al., 2020, 2017; Omoregie, Aliyu, Doris, Ehiabhi, & Folashade, 2016; Pereira et al., 2019; Semdé et al., 2018).

Environmental and edaphic factors may influence the production and accumulation of secondary metabolites in plants of the same species growing wild in different regions. The respective group of secondary metabolites plays a significant role in the plant's adaptation to the surrounding environment (Ncube, Finnie, & Van Staden, 2012; Niinemets, 2015; D. P. Pavarini, S. P. Pavarini, Niehues, & Lopes, 2012; Pereira et al., 2019; Ramakrishna & Ravishankar, 2011; Sampaio & Da Costa, 2018). The environment influences the biosynthesis of secondary metabolites (SMs), facilitating the chemical interaction between plants, leading to the variations in metabolite profile of a plant, therefore, exerting their biological roles as a plastic adaptive response mechanism to their environment (Ncube et al., 2012; Pavarini et al., 2012; Pereira et al., 2019; Sampaio & Da Costa, 2018; Sampaio, Edrada-Ebel, & Da Costa, 2016).

The metabolites reported from *L. camara* growing in different regions have shown marked differences in composition and concentration. Several chemotypes have been described, including a sabinene/cineole/b-caryophyllene chemotype from Algeria (Zoubiri & Baaliouamer, 2012); β-caryophyllene (9.8%), 1,8-cineole (9.4%), and β-pinene (8.2%) from Egypt; β-caryophyllene (23.3%), α-humulene (11.5%), germacrene D (10.9%) or davanone β-caryophyllene/bicyclogermacrene from India (Rana, Prasad, & Blazquez, 2005) and bicyclogermacrene (19.4%), isocaryophyllene (16.7%), valencene (12.9%), and germacrene D (12.3%) from Brazil (Costa et al., 2010). These results have demonstrated that the environmental and edaphic factors influence plays a significant role in producing and accumulating secondary metabolites.

The variation in secondary metabolites' production influenced by environmental conditions can characterise one species' plant populations. In this context, the metabolites may be used as a chemical marker to differentiate species found in specific geographical zones and seasons (De Souza, Ferri, Fiuza, Borges, & Paula, 2018; Khan et al., 2016; Pereira et al., 2019). Plants that produce essential oils vary considerably in their quality and quantity (composition and concentration of their constituents) due to their interaction with the natural environment.

The vast array of compound synthesis in the *L. camara* plant points out the adaptive significance for such a diversity of compounds. The variability for the essential oil (EO) composition of *L. camara* can be related to geographical distribution and pressures (Agil & Hosseinian, 2012; Benites et al., 2009; Javier, Ocampo, Ceballo, & Javier, 2017; Khan et al., 2016; Murugesan, Senthilkumar, Suresh Babu, & Rajasugunasekar, 2016; Pereira et al., 2019; Zoubiri & Baaliouamer, 2012). The metabolism and accumulation of secondary metabolites reflect the integrated influences of multiple ecological factors on the plant during their developmental and growth periods in addition to genetic factors (Liu et al., 2016). Some metabolites are synthesised only under specific environments, or their contents significantly increase under specific environments. Moreover, previous studies have demonstrated that medicinal plants growing in different regions and environments produce different SMs resulting in differences in their qualities (W. Liu, J. Liu, Yin, & Zhao, 2015). Therefore, studies examining the diversity in the production of SMs of *L. camara* will explain the influence of abiotic and biotic pressures on the EO quality found in a species.

Many studies investigating the influence of environmental factors on plant SM biosynthesis consider these factors' effects on individual compounds. However, individual compounds rarely happen in isolation (Gershenzon, Fontana, Burow, Wittstock, & Degenhardt, 2012). Instead, any compound's influence depends on conditions within the prevailing environment since a single factor cannot be extrapolated from a combination of environmental factors in plants growing in the natural environment (Berini et al., 2018). Thus, understanding how environmental factors will influence a plant's metabolic profile is vital for interpreting how these changes influence the abundance of individual compounds.

Considering the wide distribution and adaptive capacity of *L. camara*, and the lack of studies covering this subject, as well as the pesticidal properties of this plant, we proposed to carry out a comparative study with samples of EO from leaves of *L. camara* obtained from samples collected from six different climatic zones of Kenya and collected in different seasons. The approach involves comparing the data obtained by chemical profiles of these oils from six regions. To assess the influence of seasonal and geographical location on the production of SMs and determine the underlying factors responsible for the variations in SMs in *L. camara*, feasibly suggest the best harvesting seasons and regions for this wild species and promote its reasonable exploitation for specific compounds for biopesticide production.

2. Method

2.1 Sampling Locations

Natural populations of *L. camara* plant, the first four leaves of the stem from the top (Figure 1A) were sampled from six representative climatic zones (Lower Highland-Njoro (LH-NJ), Upper Midland 1-Kakamega (UM1-KK), Upper Midland 2-Kandara (UM2-KA), Upper Midland 3-Embu (UM3-EM), Lower Midland-Kiboko (LM-KI) and Coastal Lowland-Mtwapa (CL-MT)) located in six counties of Kenya during the wet and dry seasons (2018, 2019 and 2020) (Figure 1B). Each region consisted of four collection sites (each population was separated geographically by at least 30 km and 5 m for adjacent individuals).

2.2 Plant and Soil Material Collections

The first four leaves of the stem from the top were picked up from four directions (north, south, east, and west), obtaining as many individuals as possible. The leaves from each sampling site were harvested and mixed to make a composite sample. The samples were transferred to the lab within two days of collection in nylon gunny bags. The plant samples were passed through running tap water to remove any foreign material and then air-dried immediately under room temperature (23-26 °C) in a well-ventilated room for two weeks until crispy.

Soil samples were collected using an alderman auger at 0-15 cm depth and 6 cm diameter directly under *L. camara* plant canopies. Soil samples were placed in labelled plastic bags (four replicates for each geographical location). Soil samples were used to determine the critical soil parameters, including nitrogen (N), phosphorus (P), potassium (K), Total organic carbon (TOC), electrical conductivity (EC) and pH using the standard soil analysis methods.

Nitrogen (% N) was determined using H_2SO_4 digestion and measured with the Kjeldahl method. Phosphorus (P) of soil samples was extracted with ammonium fluoride (NH_4F, 0.03 M) and hydrochloric acid (HCl, 0.025 M) (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) and measured by UV-Vis spectrophotometer (Thermo Fisher Scientific, San Jose, CA, USA). Potassium (K) of soil samples was determined by extraction with CH_3COONH_4 (1 M) (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) and quantified by Corning flame photometer (Sherwood Scientific Ltd, Cambridge, UK). Soil pH (soil reaction) was measured in 1:2.5 soil: water suspension solution using the Consort pH meter model C835. The Electrical Conductivity EC) was measured using the EC meter model 4510 in the soil to water ratio of 1:2.5. The analyses were undertaken at Jomo Kenyatta University of Agriculture and Technology Soil Chemistry Laboratory.

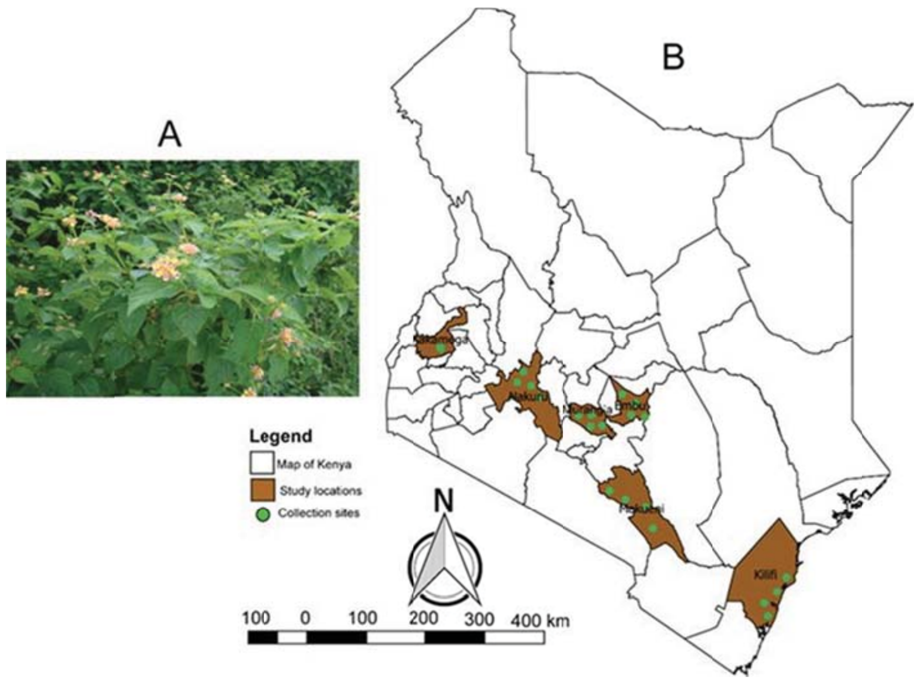

Figure 1. (A) Plant of *Lantana camara*, (B) Kenyan map showing the different sampling localities

2.3 Extraction of Volatile Oils

The volatile oils of *L. camara* were obtained from the dried leaves by steam distillation performed in a steam distiller apparatus (Deschem Science supply, China). 200 g of each sample was steam distilled using 600 mL distilled water for 3 hrs. The steam, which contains the essential oil, was then passed through a condenser. The condensing oils were separated with a separating funnel and collected in amber-coloured vials, labelled, and stored at 4 °C until use.

2.4 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The oil samples were diluted in 100% n-hexane and transferred to the auto-sampler vials for GC-MS analysis. Agilent Technology (GC, Agilent 6890) (Agilent Technologies Inc., Santa Clara, CA, USA) gas chromatograph with a split detector and Mass Spectrometer Detector (MSD) coupled with an autosampler was used for this study. Each sample (1 µL) was injected into the GC-MS with an autosampler (Agilent Technologies). The injections of the volatile oil were conducted with a splitless injector at 220 °C. The compounds were then separated on a nonpolar capillary HP column at an average linear flow rate of 35 cm s^{-1} with helium as the carrier gas. The oven temperature was held at 35 °C for 5 minutes and then increased to 280 °C by 10 °C/min and held for 10 minutes. The collected volatile compounds were then identified by comparing their mass spectra and retention times with the National Institute of Standards and Technology (NIST) 2017 library of mass spectra.

2.5 Related Data of Climatic Factors

The environmental data were divided into climate and soil data. The climate data consisted of Monthly average temperature (aT), maximum temperature (maxT) and minimum temperature (minT), average precipitation (P), sunshine duration (SD), and UV index for the collection month were collected from local meteorological stations for the six study sites and were pre-treated in MS Excel© and used for further analysis.

2.6 Data Processing and Analysis

The data for the study of seasonal and geographical variation in the composition and accumulation of SMs of the essential oil of *L. camara* (chemical profiles and environmental data) were divided into two sets of variables: chemical (secondary metabolites (SMs) and environmental (climate and soil data) variables. The data was obtained and used for further analysis after analysing the essential oils by GC-MS (chemical data).

The R software (version 3.6.3; R Core Team, 2020) and the RStudio graphical user interface (version 1.2.5033) were used to perform all the analyses. The total area of peak data was normally distributed (Shapiro-Wilk test: $p > 0.05$), and their variance was homogeneous (Barlet test: $p > 0.05$); therefore, we used an unpaired t-test to compare the amount SMs synthesised by *L. camara* between the rainy and dry seasons. For the same reason, we used the analysis of variance (ANOVA) followed by the Student-Neuman-Keuls (SNK) post hoc test to compare the amount of SM synthesised by *L. camara* across the different localities during a specific season using the R software package 'Agricolae' (de Mendiburu, 2020). We performed the one-way analysis of similarity (ANOSIM) using the Bray-Curtis dissimilarity matrix to compare the chemical profiles of the different compounds synthesised by *L. camara* between the seasons and across the different localities. Based on the similarity percentage (SIMPER) analysis, we identified the 10 most influential SMs contributing to *L. camara* EO's diversity between the seasons and across the different localities. To visualise this difference, we first used the non-metric multidimensional scaling (NMDS) plot, overlaid the physicochemical and environmental variables to the plot to see whether these parameters were related to SM profile diversities.

To better confirm the variation of *L. camara* EO SM composition between seasons and locations, we used two supervised machine learning algorithms, namely: Random Forest (RF) analysis (Breiman, 2001) and Partial Least Squares-Discriminant Analysis (PLS-DA) (Liland & Indahl, 2009). Helped by the R package called Random Forest (Liaw & Wiener, 2002), we ran the RF analysis using 10000 iterations (ntree) with 12 SM randomly selected at each split (mtry = $\sqrt{q}$, where q is the total number of SM (150)). Based on the function "importance ()", we generated the mean decrease in accuracy (MDA) for each compound selected. The compound with the highest MDA value was considered the most significant for *L. camara* EO's diversity between the seasons and regions. To visualise these differences, we generated the multidimensional scaling (MDS) ordination plot using the function "MDSplot ()" based on the proximity matrix from the RF analysis. Using the function "PLS-DA ()" embedded in the R package called mixOmics (Rohart, Gautier, Singh, & Kim-Anh, 2017), we performed the PLS-DA analysis. We visualised the difference using the function "plotIndiv ()". With this technic, we identified the most significant compounds for differentiating *L. camara* EO using the function "PLS-DA.VIP ()" found in RVAideMemoire R package (Maxime, 2020). All statistical results were considered significant when $P < 0.05$.

3. Results

3.1 Seasonal Variation in Essential Oil Composition of L. camara

The GC-MS chromatograms from *L. camara* essential oil showed that the plant synthesised many Secondary Metabolites (SMs) for both rainy and dry seasons (Figure 2). The number of SMs synthesised by *L. camara* varied significantly between the seasons, except for Kiboko ($p = 0.68$; Figure 2C) and Njoro ($p = 0.64$; Figure 1F). The number of compounds was lower in Mtwapa (Figure 2E) but higher in Embu (Figure 2A), Kandara

(Figure 1B) and Kakamega (Figure 2D) during the rainy season. In comparison, there was a tremendous increase in the number of compounds observed in the plants sampled from Embu (Figure 2A) and Mtwapa (Figure 2E) and a reduction in Kandara (Figure 2B) and Kakamega (Figure 2D) during the dry season. Independently to the site, when we ran the analysis of similarity based on the Bray-Curtis distance matrix, we consistently found a significant difference of *L. camara* EO SM composition between rainy and dry season (ANOSIM: $p < 0.0001$, $R = 0.465$). This difference was depicted by the Non-metric multidimensional Scaling (NMDS) plot (Figure 3A), with an excellent dissimilarity representation (Figure 3B; Stress: 0.199). This plot distinguished *L. camara* EO obtained during the rainy season from those obtained during the dry season. The similarity percentage (SIMPER) analysis identified trans-cadina-1(6),4-diene, (E-)-Caryophyllene, and 1,8-cineole as the three significant compounds responsible for the distinction of *L. camara* essential oil between the rainy and dry season (Figure 3C).

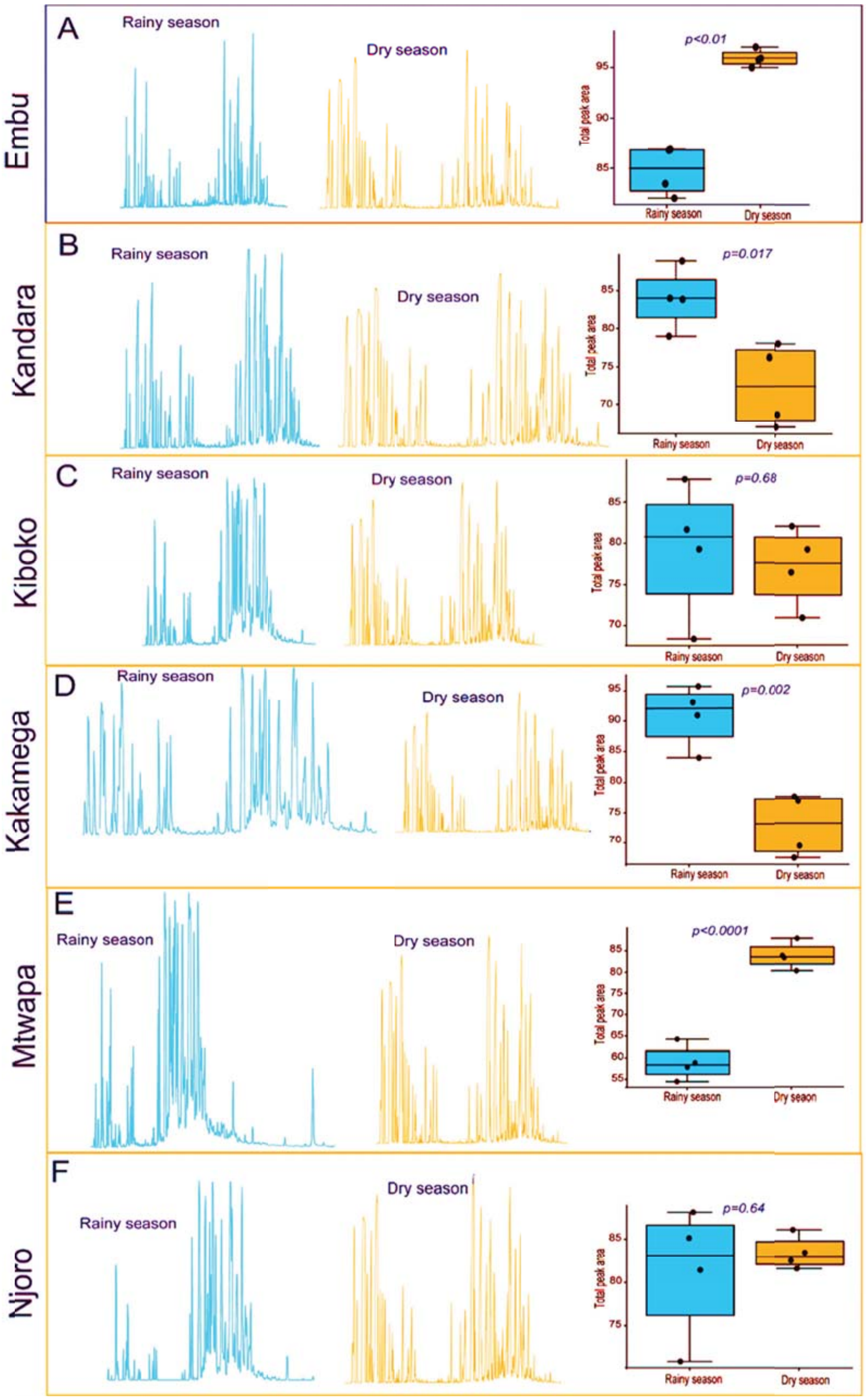

Figure 2. GC-MS chromatogram of *L. camara* SM synthesised during the rainy (in blue) and dry (in brown) seasons and across the different climatic zones of plant collections. Boxplot depicting the quantitative variation of the total amount of SM synthesised by *L. camara* between the rainy and dry season in each collection site. The ends of the boxplot whiskers represent the minimum and maximum of all the data

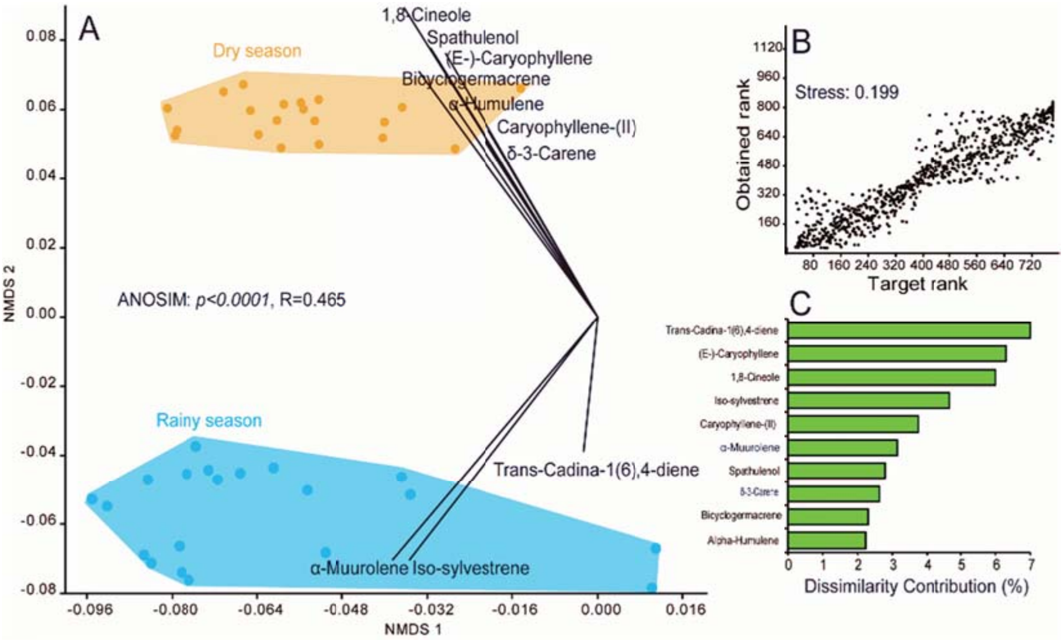

Figure 3. Secondary Metabolites synthesised by *Lantana camara* varied qualitatively and quantitatively between the rainy and dry seasons. (A) Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis dissimilarities index illustrates the SM's seasonal variation synthesised by *L. camara*. (B) Shepard plot showing the quality of the NMDS plot visualisation. (C) Histograms of the first ten most significant compounds contributing to the seasonal variation observed in *L. camara* essential oil

3.2 Regional Variation of L. camara Secondary Metabolites During the Rainy Season

Our precedent result revealed that *L. camara* oil SM composition varied with seasons. Here we aimed to determine whether this composition could also be affected by its location. We found that the total peak area of the SMs synthesised by *L. camara* significantly differed across the different localities where this plant was collected (Figure 4A). This difference was confirmed by the ANOSIM test and the NMDS plot that clustered *L. camara* SMs in their provenance (Figure 4B). Using the SIMPER analysis, we identified the trans-cadina-1(6),4-diene followed by Caryophyllene-(II), and Lavandulyl isovalerate as the most critical SMs contributing to this chemical variation across the locations (Figure 4D). To see if the observed *L. camara* EO changes across locations were related to each location's physicochemical and environmental parameters, we overlaid these parameters to the NMDS plot (Figure 4E). We found that the SM's synthesis by *L. camara* was closely associated with temperature, ultraviolet light (UV), and soil pH in Mtwapa. Simultaneously, in the localities such as Embu, Kiboko, and Kakamega, the synthesis of the SMs was related to the soil parameters such as phosphorous, potassium, and total organic carbon (TOC). While in Njoro, the synthesis of the SMs was more influenced by the humidity.

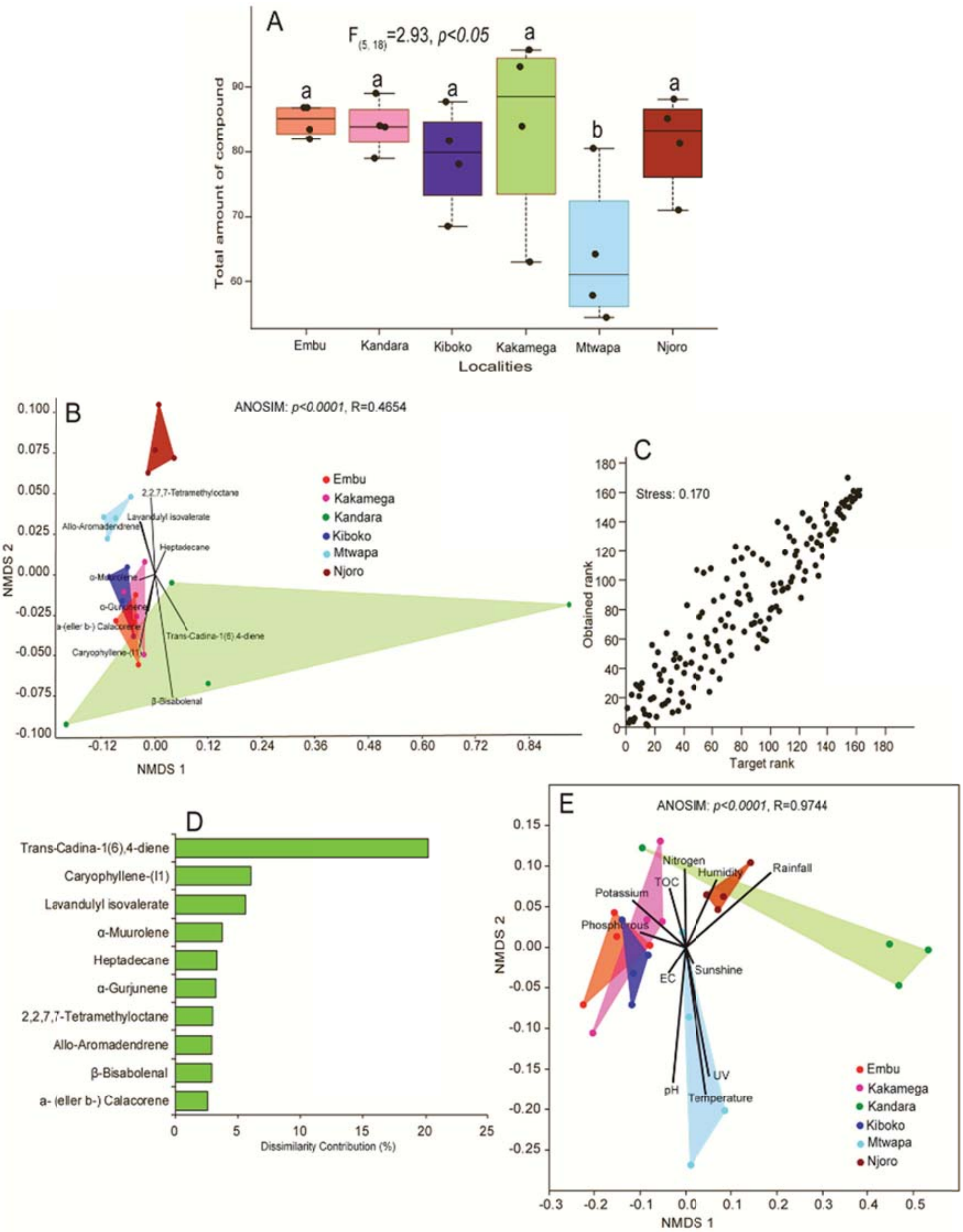

Figure 4. The variation of the SMs observed in *L. camara* during the rainy season is related to the plant location, the soil's physicochemical properties, and each location's climatic condition. (A) Boxplots showing the variation of the amount of SMs synthesised by *L. camara* across the different localities. Boxplot whiskers indicate ±1.5 interquartile range limits. Boxplots with different letters show significant differences as grouped by ANOVA tests followed by SNK *post-hoc* tests ($P < 0.05$). (B) Non-metric multidimensional scaling (NMDS) ordination plot illustrating the variation of the SMs synthesised by *L. camara* across the different localities. (C) Shepard plot assessing the quality of the NMDS plot representation. (D) Histogram depicting the SMs contributing to the overall dissimilarity of the synthesised SMs by *L. camara* across the different localities. (E) Non-metric multidimensional scaling (NMDS) ordination plot displaying the soil physicochemical and climatic variables that influence the *L. camara* SMs synthesis in each location

3.3 Regional Variation of L. camara EO Secondary Metabolites (SMs) During Dry Season

We also found qualitative and quantitative variations in *L. camara* EO across the locations during the dry season. The total SMs synthesised by *L. camara* significantly varied across the different localities (Figure 5A). *L. camara* plants sampled from Embu synthesised significantly more SMs than those sampled from Mtwapa, Njoro, Kandara, Kiboko, and Kakamega. According to their origin, there was a significant difference in *L. camara* EO SM composition as determined by the ANOSIM test and the NMDS plot (Figure 5B). Based on the SIMPER analysis, we identified (E)-Caryophyllene, δ-3-Carene, and 1,8-Cineole as the most critical compounds contributing to *L. camara* differentiation in the dry season across the different (Figure 5D). When we associated the physiochemical and the environmental parameters of each site to the NMDS plot (Figure 5E), we found that the synthesis of SMs by *L. camara* varied from region to region and was primarily related to sunshine, rainfall, humidity, temperature, soil pH, nitrogen, and phosphorous (Figure 5E).

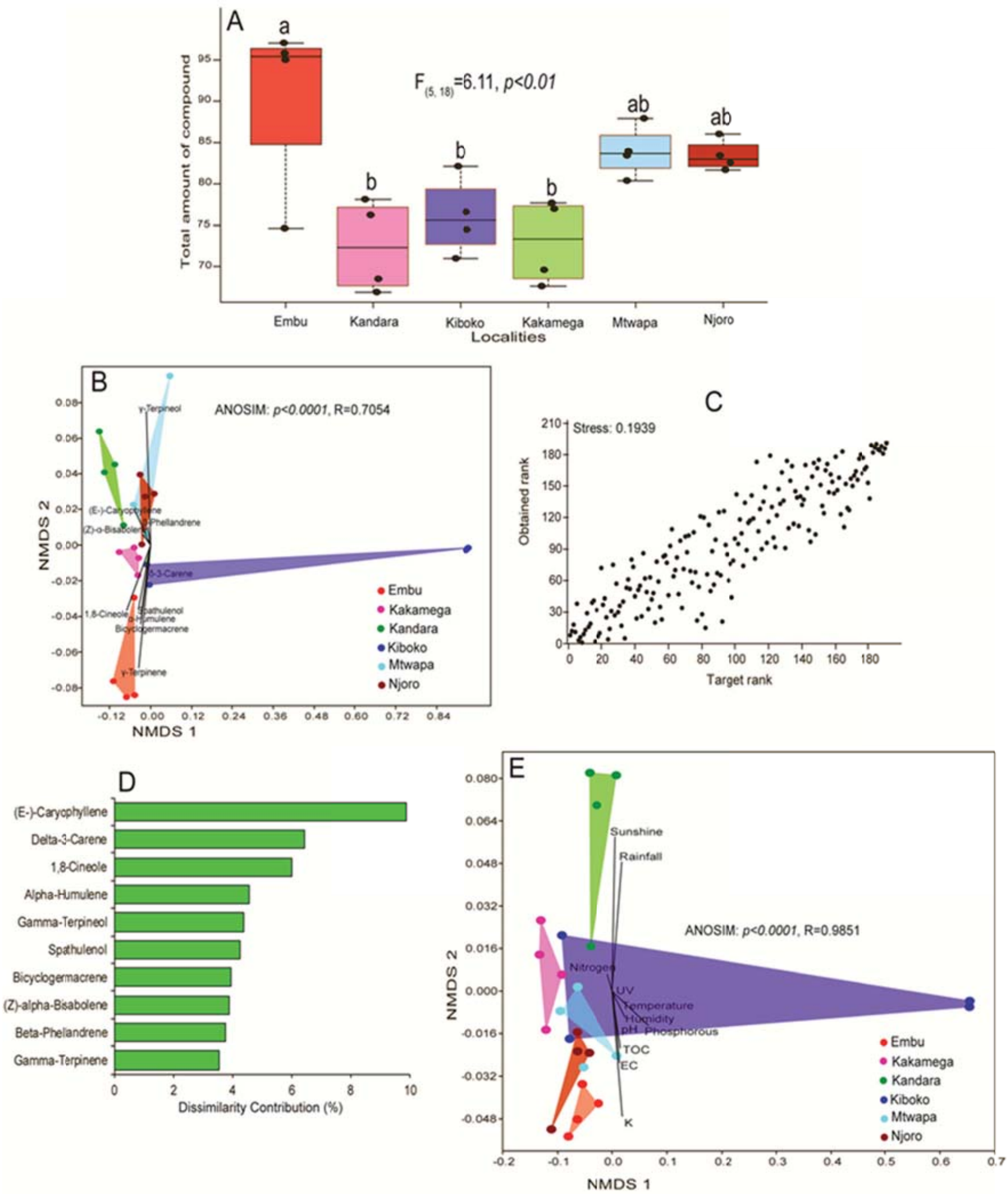

Figure 5. The change in SM observed in *L. camara* during the dry season is related to the plant location, the soil's physicochemical property, and each location's climatic condition. (A) Boxplots depicting the variation of the amount of SM synthesised by *L. camara* across the different localities. Each boxplot shows the median (horizontal line), 25th and 75th percentiles (upper and lower box limits). Boxplots with different letters show significant differences as grouped by ANOVA tests followed by SNK *post-hoc* tests ($P < 0.05$). (B) The non-metric multidimensional scaling (NMDS) ordination plot illustrates the SM synthesised variation by *L. camara* across the different localities. (C) Shepard plot assessing the quality of the NMDS plot representation. (D) Histogram showing SM that mainly contribute to the overall dissimilarity of *L. camara* EO SM synthesised. (E) Non-metric multidimensional scaling (NMDS) ordination plot displaying the soils physiochemical and the climatic variables that impact the *L. camara* SM synthesised in each location

3.4 Confirmation of L. camara SMs Profile Changes in the Function of Seasons and Locations Based on Supervised Machine Learning Algorithms

Results from Random Forest (RF) analysis and Partial Least Squares-Discriminant Analysis (PLS-DA) consistently confirmed the change of *L. camara* EO SMs in the function of seasons and localities. When we ran the multidimensional scaling analysis based on the RF analysis's proximity matrix, this technic differentiated *L. camara* oil SM based on their seasons (Figure 5Ai) and location (Figure 6Aii, 6Aiii) of collections. Based on the mean decrease in accuracy (MDA), this analysis identified Camphor (with a classification accuracy of 83.33%) as the most influential SM for differentiating *L. camara* oil between rainy and dry season (Figure 6Bi). Similarly, this analysis respectively identified 14-hydroxy-(Z)-Caryophyllene (with a classification accuracy of 65%) and Eugenol (with a classification accuracy of 80%) as the most significant SM for distinguishing *L. camara* oil across the different localities during rainy (Figure 6Bii) and dry (Figure 6Biii) seasons.

Also, the PLS-DA score plots separated *L. camara* EO in the function of seasons (Figure 7Ai) and locations (Figure 7Aii, iii). As with the RF analysis, the separation of *L. camara* SM synthesised during the rainy and dry seasons identified Camphor as the most influential compound (Figure 7Bi). The nPLS-DA analysis identified 2-Cyclopenten-1-one, 3-methyl-2-(1,3-pentadienyl)-,(E, Z)- as the most significant SMs responsible for the differentiation of *L. camara* EO across the localities during rainy (Figure 7Bii) and dry (Figure 7Biii) seasons, respectively.

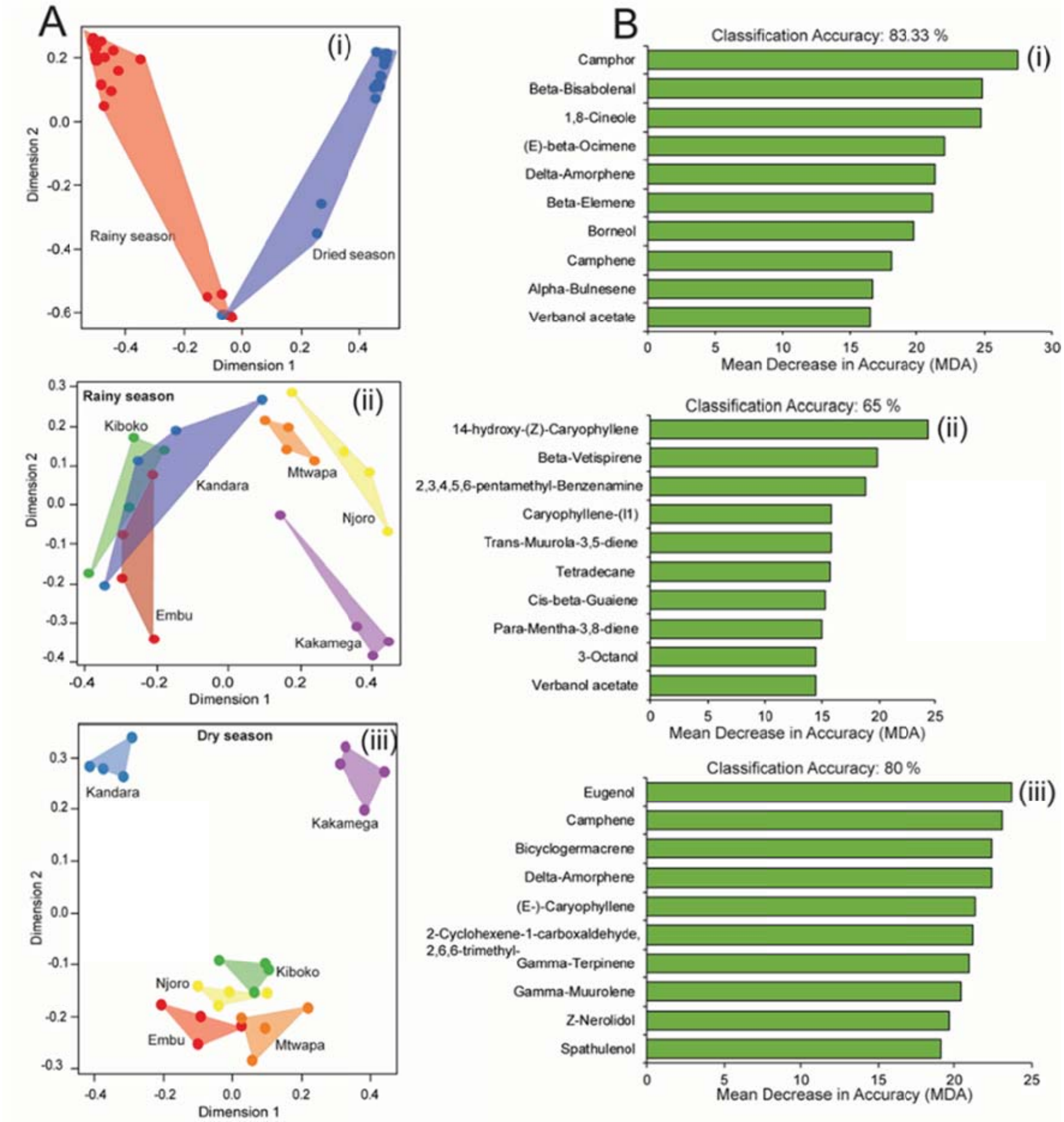

Figure 6. (A) Multidimensional Scaling (MDS) plot based on the proximity matrix coordinates from the Random Forest classification illustrating the change of *L. camara* EO SM synthesised between the seasons (i) and across the different localities during the rainy (ii) and dry (iii) seasons. (B) Histogram showing the most significant SMs contributing to the distinction of *L. camara* EO during rainy and dry seasons (i) and across the different localities during the rainy (ii) and dry seasons (iii)

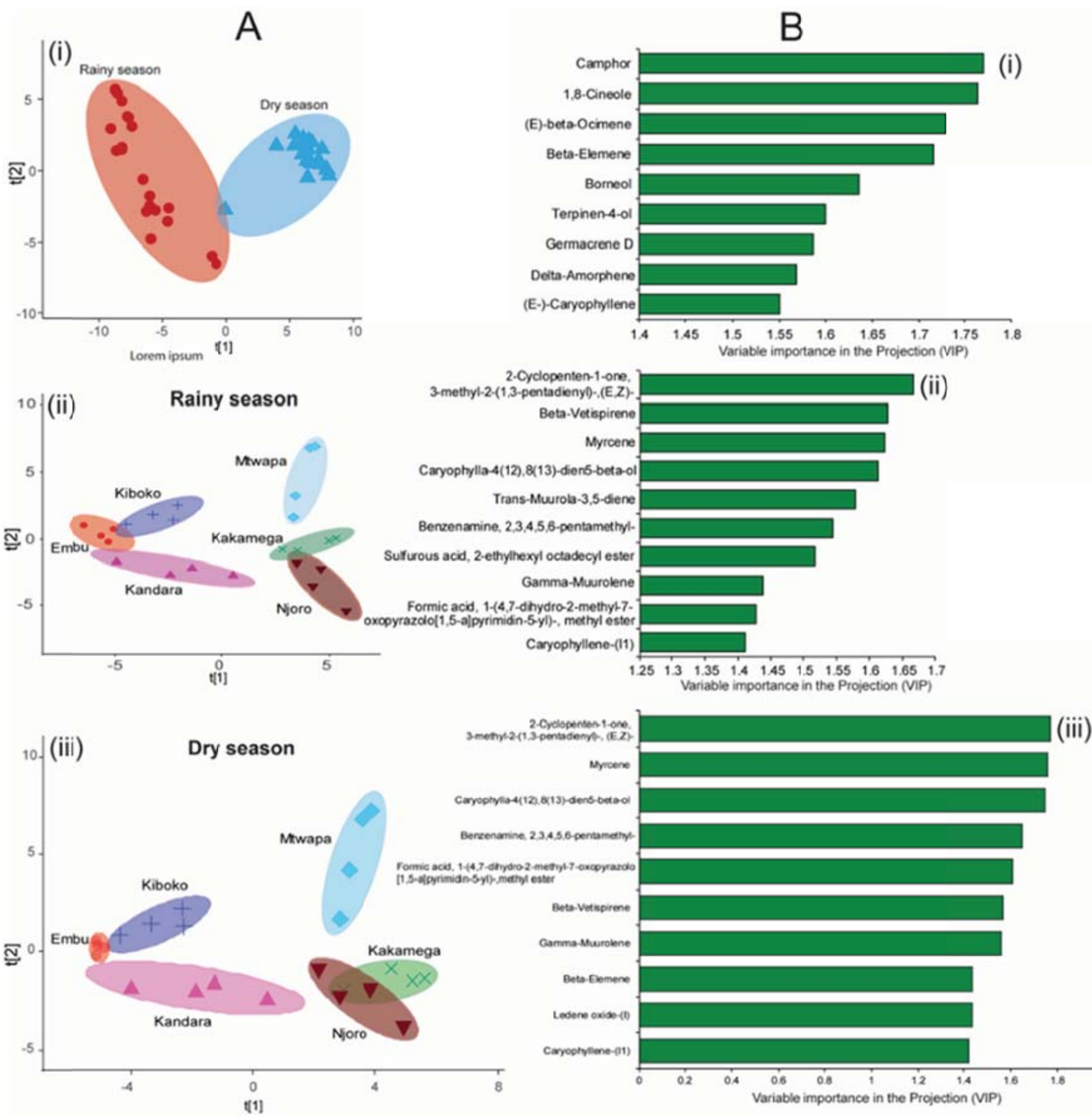

Figure 7. (A) Partial least squares discriminant analysis (PLS-DA) score plot separating the SM synthesised by *L. camara* in the function of seasons (i) and localities during rainy (ii) and dry (iii) seasons. (B) Histograms showing the ten most significant SMs from the PLS-DA model help differentiate SM synthesised by *L. camara* between the seasons and across the localities during the rainy (ii) and dry (iii) seasons

4. Discussion

The natural environment strongly influences the chemo diversity of medicinal plants by influencing the biosynthesis of secondary metabolites (SMs) in a plant population based on the environmental conditions where they are growing (Allevato, Kiyota, Mazzafera, & Nixon, 2019; Liu et al., 2015; Ncube & Van Staden, 2015; Pereira et al., 2019; Sampaio et al., 2016). Most plants can regulate SMs according to the growing environment (Isah, 2019; Li, Kong, Fu, Sussman, & Wu, 2020; Yang et al., 2018), causing changes in the plant's essential oil (EO). Secondary metabolite profiles of plants driven by environmental plasticity are poorly understood. This study established that the production of SMs of the *L. camara* plant growing in the wild differed significantly during the seasonal changes and geographical location. Therefore, heritable variations observed in the EO of the *L. camara* wild population across Kenya are strongly linked to climatic factors, geographical location, and soil properties while not disputing the strong influence of the plant species' genetic makeup. Here, we discuss the findings from the ecological point of view.

Striking variation in the EO of *L. camara* leaf metabolic profile was observed from the samples collected from six different climatic zones in Kenya, dry and rainy seasons. The variability between the *L. camara* plant populations was compared using the ANOSIM test based on differences in the abundance and composition of multiple metabolites. Seasonal specificity is observed in this study, with variability in the number of SMs observed for both dry and rainy season in each region. Kakamega, Kiboko, and Kandara showed an increased metabolites number compared to the other regions in the rainy season. This increase in metabolic variation can be attributed to the plant's interaction with the prevailing condition at that particular season. These regions received adequate rainfall and maximum sunshine hours and UV index compared to the other regions where sunshine was reduced during the rainy seasons. The results agree with other researchers that the metabolite synthesis of essential oil-producing plants is strongly influenced by the slightest changes and how they interact with the natural environment without detrimental effects leading to newly formed compounds that could be advantageous during the prevailing conditions.

Ten dominant variable compounds contributed to shaping the seasonal variation of the *L. camara* plant populations. For the samples of *L. camara* evaluated in this work, we propose two chemotypes according to the season: a chemotype for the samples collected in the dry season (chemotype I) and another for the rainy season (chemotype II). Based on the NMDS test results and SIMPER analysis, the chemotype I is discriminated by the higher proportion of (E-)-caryophyllene, 1,8-cineole, spathulenol bicyclogermacrene, α-humulene, caryophyllene (II), and δ-3-carene in the leaf EO. The chemotype II is discriminated by the higher proportion of trans-cadina-1(6),4-diene, α-muurolene, and Iso-sylvestrene, as the dominant compounds. Overall, three significant compounds, trans-cadina-1(6),4-diene, (E-)-Caryophyllene, and 1,8-cineole, contributed significantly to the seasonal variability in the SM profile of the *L. camara* EO.

The environmental factors are the mechanism involved in influencing the accumulation and biosynthesis of *L. camara* SMs, and it is related to seasonal induction stress factors in the plant, such as changes in temperatures, humidity, rainfall and soil conditions (Guo et al., 2013; Pavarini et al., 2012; Ramakrishna & Ravishankar, 2011; Selmar & Kleinwächter, 2013; Yang et al., 2018). For example, plants collected during the dry season may have encountered high temperatures influencing an increase in (E-)-caryophyllene production, while low temperatures in the rainy season influenced an increase in trans-cadina-1(6),4-diene. This result agrees with De Almeida et al. (2016), who reported an increase in (E-)-caryophyllene in the EO composition of *Copaifera langsdorffii* during the dry season as compared to the wet season. There is no official report in the literature regarding the influence of seasonal change on trans-cadina-1(6),4-diene accumulation. This variability can be attributed to the plants' relationship with the environmental conditions during plant growth and productivity at that particular season, changing the biosynthetic pathway of secondary metabolites toward metabolites' production. Therefore, different prevailing environmental pressures between the two seasons played a significant role in the diversity of the SMs of *L. camara* leaf EO. Our results corroborate with Nea et al. (2020), Pereira et al. (2019) and Dos Santos et al. (2019) reported fluctuation patterns of SMs produced by *L. camara* to correlate to seasonal changes. Collectively these results revealed that the seasonal variation predominantly influences the SM profile composition in wild plant populations. Therefore, this work provides new insight into understanding the response of *L. camara* plant populations SMs biosynthesis to the seasonal variation.

Our precedent results revealed seasonal variability in the synthesis of SM in the *L. camara* wild population plants. We further aimed to determine whether this variability is regional and what environmental factors drive the variation. This study observed significant variability in *L. camara* EO's chemical profile across the different regions where samples were collected during the dry and rainy season as determined by the ANOSIM test and NMDS plot. Three significant compounds trans-cadina-1(6),4-diene, caryophyllene (II) and lavandulyl isovalerate were identified that contribute significantly to the regional variability in the rainy season, while (E-)-Caryophyllene, δ-3-carene and 1,8-cineole contributed to the differentiation during the dry season across the regions. These results are consistent with previous reports (Murugesan et al., 2016; Pereira et al., 2019; Sena Filho et al., 2012). Recently, Nea et al. (2020), and Pereira et al. (2019) confirmed high variability in SM composition of the EO of *L. camara* from Bregbo South of Côte d'Ivoire and samples collected from 21 municipalities representing three regions in Brazil respectively. Geographical location is a crucial factor that affects plant growth's prevailing conditions, having significant effects on secondary metabolic processes in a plant species (Liu et al., 2015)—Consequently, the cause of the relationship between the SM composition and contents in their growing locations.

The number of synthesised compounds variability across the regions in both seasons were more or less the same in the region of Njoro and Kiboko with a p-value of 0.64 and 0.68 respectively but differed significantly in Mtwapa, Embu, Kandara, and Kakamega with a p-value of < 0.0001, < 0.01, 0.017 and 0.002 respectively. Overall, Mtwapa showed high variability in the number of compounds synthesised as compared to the other regions. The number of compounds synthesised was very high during the dry season as compared to the rainy season. The significant difference in SM content is climatic factors of temperature, rainfall, UV index, and pH property. These changes in the environmental conditions may explain the differentiation of SMs in the Mtwapa region, located in the coastal areas and dominated with a more considerable climatic seasonal variability, particularly temperature, compared to Njoro and Kiboko that had a reasonably stable climatic condition. Temperature change is known to substantially affect SM synthesis since areas with more considerable climatic changes are faced with more variation and could lead to more significant variability in their SM profile (Allevato et al., 2019). Furthermore, Molina-Montenegro and Naya (Molina-Montenegro & Naya, 2012) argue that locations with slight seasonal variations and constant warm temperatures lead to a low environmental plasticity capacity. Subsequently, stability in the environment would reduce the plant's overall pressures, therefore reducing metabolite variation.

The significant differences in climatic conditions and soil characteristics among the six regions and other explanations are the determinant factors for the variability in SM profile observed in *L. camara* EO of the same species growing in Kenya's diverse regions. It is observed that *L. camara* wild plants population responds differently to environmental variations, therefore variability in the production and accumulation of SMs. Ncube et al. (2012) explain that these variabilities are due to the physiological characteristics associated with genetic conditions, which arise probably with the prevailing climate in both seasons in the regions under study. Plant metabolism is influenced in many ways by those conditions. Factors such as temperature, humidity, rainfall, sunshine duration directly respond to these variations. These factors jointly influence the biosynthesis and accumulation of SMs and correlate with each other and do not act in isolation (Gobbo-Neto & Lopes, 2007).

Among the environmental conditions, soil characteristics represent a complicated biological system that strongly influences the plant's ability to produce SMs (Muscolo et al., 2019; Ramakrishna & Ravishankar, 2011). The nutritional elements (*e.g.*, N, P, K) of soil are required for medicinal plants' growth and are actively involved in plants' metabolic activities (Al-Humaid, 2005; Chrysargyris, Xylia, Botsaris, & Tzortzakis, 2017; Muscolo et al., 2019; Yadegari, 2015). Correctly, soil characteristics play a crucial role in diverse soil conditions that causes significant differences in biosynthesis and SM accumulation in plants of the same species. The SM profile variation response of *L. camara* to the soils with diverse characteristics was in agreement with the findings of Ormeño and Fernandez (2012), and Muscolo et al. (2019), showing that soil, with its intrinsic characteristics, is directly responsible for plant metabolite production.

This study established a relationship between soil properties and metabolic profile to be regionally specific. Extrapolating these results at the regional and seasonal scale suggests that any alteration of the soil properties leads to changes in SMs accumulation in the *L. camara* plant population, affecting its quality and quantity. The plant material analysed in this study was collected from plants growing under diverse natural conditions. Therefore, it was not easy to separate the effects of individual factors from the environment's multifactorial influence (Climatic and soil variables). Therefore, we conclude that different climatic factors (rainfall, temperature, UV, humidity) have different effects and intensities on the accumulation of SMs in the EO of *L. camara*. At the same time, different SMs are affected by different kinds of soil properties in the soil to a different extent, where the physicochemical properties including N, P, K, TOC, EC and pH in the soil all have a relatively significant strong effect on plant secondary metabolism. Thus the correlation differs significantly with SM composition from region to region.

Besides, further identifying the compounds through PLS-DA and RF confirmed SMs variability as a season and geographical location. The results indicated that SMs composition and contents were near related to the growing locations of *L. camara,* and environmental factors influenced the production of the SMs. The SMs synthesis and accumulation in medicinal plants are complex processes affected by many environmental factors comprising a multivariate system. The variability in chemical profile was expected because the soil factors were significantly different due to the different growing regions and the climatic factors changed with geographical conditions. Local adaptation depends on both genetic, soil properties and environmental factors; Thus, the high chemical variability reflects the need for the plant to adapt to such different environments (Ncube et al., 2012; Pereira et al., 2019; Sampaio & Da Costa, 2018; Sampaio et al., 2016).

This difference observed could lead to a more targeted analysis and understanding of biological pathways. Although determining the plasticity of a species is difficult without a conventional garden experiment. Our study used only ordination methods to associate climatic and soil variables with variation in chemical profiles of *L. camara*, and correlation does not imply a relationship. Many laboratory and greenhouse experiments are needed to confirm the effect of climatic and soil variables on the SM profile. Greenhouse experiments with the different species in the exact location could help detect whether chemical variation differences are due to environmental, soil, or genetic differences. The result would be beneficial for the production of EO with unique active ingredients. One could choose the location of plant growth for a particular compound, or one could add/omit nutrients to modify the EO quality and quantity. Therefore this exploratory analysis in wild populations of *L. camara* is advantageous and essential as it has reduced the environmental and soil variables and will allow for a more guided experimental analysis such as greenhouse experiments.

5. Conclusions

This study provides information on seasonal and regional variations of the quality and quantity of SMs of *L. camara* EO from the leaves. The results showed that environmental conditions in the drier season favour the production of more dominant compounds than in the rainy season. The optimum time to achieve the highest quantity of (E-) caryophyllene is during the drier period, while the trans-cadina-1(6), 4-diene is more abundant in rainy seasons. We can highlight the relationship between the environment and the metabolic profile of *L. camara,* and the variation in SMs is a direct response to fluctuations in conditions in the surrounding environment. Therefore, the knowledge gathered from this study on the influence of seasonal and regional variation on *L. camara* EO composition can help decide the best period to harvest the plant according to the desired compound for exploitation in the agricultural industry. The analysis of wild populations of *L. camara* has provided us with potential environmental variables that require more follow up with greenhouse experiments to determine their importance in SM biosynthesis.

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Calcium and Magnesium Dynamics in Litter in a Successional Forest Ecosystem, Under Hydroperiod

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Abstract

This study was part of the Manipulation of Moisture and Nutrient Availability in Young Regrowth Forests in Eastern Amazonia Project (MANFLORA). The experiment was designed in completely randomized blocks containing control and irrigated treatments during the dry period (5 mm of water/day), with four repetitions each. The monthly mean litter values ranged from 316.10 to 997.90 kg ha^{-1} $month^{-1}$. The magnitude of this phenomenon can be explained by the functional role of the floristic structure, represented by the species *Myrcia sylvatica* (G. mey) DC., *Myrcia bracteata* (Rich) DC., *Miconia ciliata* (Rich) DC., *Lacistema pubescens* Mart., *Lacistema aggregatum* (Berg.) Rusby, *Vismia guianensis* (Aubl.) Choisy, *Cupania scrobiculata* Rich. and *Ocotea guianensis* Aubl., which constituted the determinant factors, associated with the hydroperiodic effect and ecosystem manipulation. The monthly mean of the analytical results of mass treatments were significant ($P < 0.05$), however, when compared annually there was no significance, which indicates seasonal influence, since the period of greatest deposition is the dry one, regardless of the water manipulation along the period studied. Only in time the mass values of Ca and Mg were not significant for treatment ($P < 0.05$). The amount of Ca was significantly ($P < 0.05$) higher than that of Mg.

Keywords: biogeochemistry, eastern Amazon, irrigation, litter, secondary forest

1. Introduction

Tropical regions are characterized as locals of intense luminosity, abundant rainfall, and predominance of soils with clay minerals of low activity. However, it is precisely in these regions that it is observed a high level of plant biodiversity. Among these characteristics, the nutrient poverty of the soil caused most studies on nutrient cycling to focus on biotic compartments, mainly litter, of easier measurement (Klinge & Rodrigues, 1968; Clevelário Júnior, 1988; Summers, 1998). Therefore, litter production is considered the most important mean of transferring essential elements from vegetation to soil (Nascimento et al., 2018). Besides, it is fundamental for the knowledge of the structure and functioning of forest ecosystems.

Litter can be defined as all types of biogenic material in different stages of decomposition, which represents a potential source of energy for consumer species. Nonetheless, many biotic and abiotic factors influence in litter deposition, such as: vegetation type, latitude, altitude, relief, temperature, rainfall, light availability during growing season, photoperiod, evapotranspiration, deciduousness, successional stage, water availability and amount of nutrients in the soil. Litter deposition results from the interaction of these factors and, according to the peculiarities of each system, one factor may prevail over the others (Vitousek & Sanford, 1986; Odum, 1988; Golley et al., 1978; Mateus et al., 2013; Zhang et al., 2014; Silva et al., 2015).

Low water availability is considered one of the main conditions of environmental stress (Santos, 1996) and it is of extreme importance for metabolical activity and leaf survival, being the survival ability during hydric stress determinant for the distribution and productivity of plants (Pimentel et al., 1990). In regions prone to water deficit, due to low rainfall or irregular rainfall distribution, the growth of planted forests in height and diameter is limited (Santos et al., 2020). For this reason, water scarcity is a concern in tropical forests and may lead to an environmental collapse, in which several species may disappear if these ecosystems undergo consecutive droughts (Santos, 1996; Nepstad et al., 1998). Therefore, the necessary amount of water and nutrients are major factors, as their lack and/or excess may compromise the production and/or productivity of forest species (Sobrinho et al., 2020).

Studies on nutrient cycling are of fundamental importance for the knowledge of the structure and functioning of any ecosystem. Phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K) and nitrogen (N) are essential elements which are determinants in plant growth, because they have considerable importance in plant metabolism and function (Barroco Neta & Nishiwaki, 2018). Considering the importance of nutrient cycling for the soil, this is directly related to seasonality, as the amount and decomposition of litter varies according to rainfall.

The aim of this study was to quantify the monthly accumulated litter production and the concentration of Ca and Mg in successional forest, under water manipulation, with the following question: can water availability during the dry period alter the biogeochemical cycles of Ca and Mg in a successional ecosystem? If biogeochemical cycles vary according to the hydroperiodic phenomenon, then they have seasonal dynamics.

2. Material and Methods

2.1 Study Area

The study was developed in the Manipulation of Moisture and Nutrient Availability in Young Regrowth Forests in Eastern Amazonia Project (MANFLORA), which began in 1999, when forest regeneration was 12 years old. The experiment was carried out at the Fresh Water Fish Farming Station (EPAD), which belongs to the Federal Rural University of the Amazon (UFRA), in the region of the middle Apeú River, Castanhal, in the Praquiquara River basin (1º19′S, 47º57′ W), 80 km away from Belém.

The surrounding landscape is marked by secondary forests, agroecosystems and pastures. According to Falesi et al. (1980), in the Bragantina Zone there existed the humid tropical forest that, with colonization and agriculture practice, through successive cuts and burnings, was modified, which caused the formation of a mosaic of different successional stages, with predominance of several agroecosystems, mainly pasture. The settlement under study was modified due to shifting agriculture, which includes the cultivation cycle of corn, cassava and bean, for 1 to 2 years, followed by fallow, which was abandoned in 1987. The region's relief is slightly wavy to wavy, under forest vegetation, mostly constituted by a flattened surface, dissected in flat top hills, with small altimetric variation. It presents a dystrophic yellow latosol of clayey texture and concretionary laterites (Tenório et al., 1999).

The granulometric composition in the first 20 cm is 40% clay, 30% sand and 30% silt. Concretions represent 16% of the volume of superficial soil (0-10 cm), with organic pH of 5.0, organic C of 2.2 kg, organic C stock of 2.9 kg m^{-2}, total N of 0.15 %, C:N 14.4, and Mehlich-1 extractable phosphorus 1.58 kg mg^{-1} (Rangel-Vasconcelos, 2002).

The climate, according to the Köppen classification, is type Am3, with annual mean rainfall of 2000-2500 mm; 70-90% of the annual rainfall occurs between January and July, while the dry period occurs from August to December, with dry months being considered when rainfall was lower than 100 mm (Table 1).

Table 1. Rainfall distribution and intensity during experimental period at the site. The dry months were considered when rainfall was lower than 100 mm. The dry period included rainfall from August to December

	Year	
	2000	2006
Annual rainfall (mm)	2399	2756
Minimum monthly rainfall (mm)	66	63
Maximum monthly rainfall (mm)	291	434
Total rainfall in dry period (mm)	694	630

The analysis of the seasonal rainfall climatology confirmed previous results (Figuera & Nobre, 1990; Rocha, 2001; Vieira et al., 2004), which demonstrated that most part of the Amazon shows two distinct periods: dry, with monthly mean rainfall below 100 mm, and rainy, with mean rainfall above 200 mm/month. The daily mean air temperature ranges from 24.7 to 27.3 °C, with a maximum of 30.1 to 32.7 °C and a minimum of 19.2 to 24.2 °C. Relative air humidity has annual mean values ranging from 78 to 90% (Martorano & Pereira, 1993).

2.2 Sampling Procedures

For this, a floristic survey was performed in the experimental plots, at the beginning of the project, in 1999, that is, when the forest was 12 years old, and later another, in 2006. The tree vegetation was inventoried in four 10 × 10 m plots, where individuals of tree species with a diameter of 1.3 m in height (DBH) ≥ 1 cm were measured. Each measured tree was identified with a numbered metal plate and identified up to the species level. The botanical material was collected and herborized in the Herbarium of Embrapa Eastern Amazon.

The procedure of area selection for the collection of depositional flow samples from the biogeochemical matrix was performed at sites with full canopy cover and homogeneity of ecosystem characteristics to obtain a flow sample with greater accuracy. Sample collection was carried out following collectors' location, according to the experimental design of randomized blocks, with repeated measures through time.

The ecosystem was divided into four randomized blocks, with two treatments: control and irrigation (irrigation during the dry season). Each treatment plot measured 20 m × 20 m, with central plots of 10 m × 10 m, that contained three collectors of biogeochemical matrix (depositional litter). The experiment was evaluated during 2000 and 2006, while treatments remained during the interval of the evaluated years.

In the irrigated treatment, 5 mm of water/day were added for about 30 minutes during the dry season in the late afternoon with irrigation hoses (Santeno), systematically allocated in the central plots (Figure 1). The amount of daily irrigation applied corresponded to the estimation of regional evapotranspiration per day, in secondary forest in the Amazon (Lean et al., 1996; Jipp et al., 1998; Sommer et al., 2002).

Figure 1. Irrigation system implanted in irrigated treatment in the successional forest at the UFRA Station, Castanhal/PA

In 2001, irrigation tapes were spaced 4 m apart. In subsequent irrigations, the distance between the tapes was reduced to 2 m in order to obtain a more uniform water distribution. The total applied irrigation varied from 630 to 790 mm per dry season, which represented a 100-200% increase in water intake in the dry season, and an increase in annual rainfall of 21-34%.

The collectors measured 1 m^2 (1 m × 1 m) and 0.10 m in depth, suspended from the ground at 0.3 m. The sampling frequency was weekly in order to reduce the nutrient leaching process in samples which were still in the collector (Proctor, 1983) (Figure 2).

Figure 2. Litter collector in control treatment, installed 30 cm above the soil surface, in successional forest, Castanhal/PA

Samples of fractions of the biogeochemical matrix were classified as: i) non-woody (leaf, flower, seed, fruit and miscellaneous) and ii) woody > 3 cm (branches). The plant materials collected in each litter collector were dried in the laboratory at 60-70 °C for 48 hours and weighed. At four-week intervals, materials from the same collector were mixed and then separated into woody and non-woody fractions. Compounds from non-woody samples were grinded and stored in 60 ml glass bottles.

For Ca and Mg analyses, 0.5 g subsamples were taken from an extract for chemical analysis of the elements, which was performed by atomic absorption spectrophotometry (Miyazawa et al., 1999; Rothery, 1986). The analyses were carried out at the Embrapa Eastern Amazon Soils Laboratory. The nutrient mass values were determined by multiplying the mean concentrations (ppm) of Ca and Mg by the dry mass values (g m^{-2}) of the biogeochemical matrix of weekly collected litter.

2.3 Statistical Analysis

The analytical results were organized into sheets in the XLS format. MINITAB version 15 was used for all statistical analyses. We analyzed the treatment effects, time and the treatment x time interaction. When necessary, logarithmic and square root transformations were performed to meet the model requirements, such as normality tests and variance homogeneity, with significance criteria ($P < 0.05$).

3. Results

In the successional forest ecosystem, during the study period, there were found 2,744 individuals belonging to 24 families and 29 species with a mean DBH of 2.61 cm and a mean height of 4.86 m in 1999, while in 2006 the mean DBH was 3.31 cm and the mean height was 6.82 m.

The most representative families were Lacistemataceae and Myrtaceae, due to the large number of individuals of *Lacistema pubescens* Mart. (1,046) and *Myrcia sylvatica* Barb. Rodr. (595), respectively, totaling more than 60% of the floristic composition of the area (Table 2).

Table 2. List of dominant species identified in the experimental area of the Freshwater Fish Farming Station (EPAD), in the municipality of Castanhal, through a floristic survey

N.	Species	Family	Common name
1	*Lacistema pubescens* Mart.	Lacistemataceae	Passarinheira-H
2	*Myrcia sylvatica* (G. Mey.) DC.	Myrtaceae	Murtinha
3	*Vismia guianensis* (Aubl.) DC.	Clusiaceae	Lacre
4	*Cupania scrobiculata* Rich.	Sapindaceae	Pau de espeto/andorinha
5	*Inga rubiginosa* (Rich.) DC.	Mimosaceae	Ingá chato/Ingá peludo
6	*Myrcia bracteata* (Rich.) DC.	Myrtaceae	Murta
7	*Banara guianensis* Aubl.	Flacourtiaceae	Pau de picos/andorinha
8	*Miconia ciliata* (Rich.) DC.	Melastomataceae	Chumbinho
9	*Inga flagelliformis* (Vell.) Mart.	Mimosaceae	Ingá chato
10	*Inga thibaudiana* DC.	Mimosaceae	Ingá cipó
11	*Chimarris turbinata* DC.	Rubiaceae	Pau de remo
12	*Astrocaryum gynacanthum* Mart.	Arecaceae	Mumbaca
13	*Couratari guianensis* Aubl.	Lecythidaceae	Tauari
14	*Talisia* Aubl.	Sapindaceae	Pitomba
15	*Stryphnodendron pulcherrirum* (Willd.) Hochr.	Mimosaceae	Paricazinho

The monthly variation of total litter production during the periods from January to December 2000 and 2006 in the control and irrigated plots is shown in Figure 3b. The monthly litter production in 2000, in the control treatment, varied from 421.20 kg ha^{-1} (March) to 997.90 kg ha^{-1} (September), with a mean value of 644.40±225.90 kg ha^{-1}, representing a total of 7.73 Mg $ha^{-1}year^{-1}$ (Figure 3b). In this case, it is observed that the values are in the same period, but in this year the rainfall indices have been low since June (Figure 3a), indicating that the hydroperiodic phenomenon influences the litter dynamics in this ecosystem, evidencing the seasonality in the different periods studied (2000 and 2006).

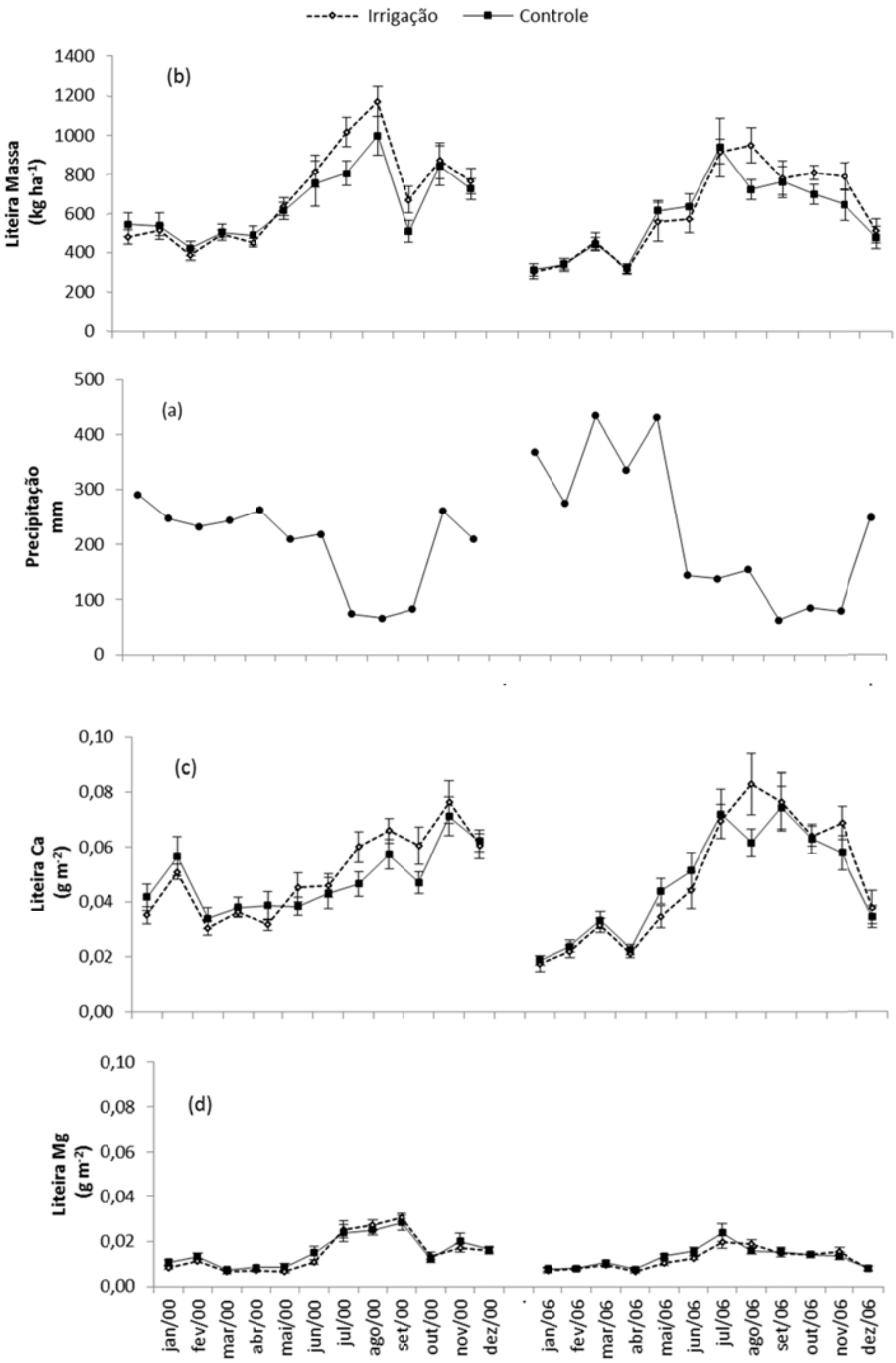

Figure 3. Dynamics of Ca and Mg masses in litter in control and irrigated treatments, in successional forest in Castanhal/PA. (a) Monthly rainfall, (b) non-woody litter mass, (c) Ca mass dynamics, (d) Mg mass dynamics

The monthly distribution of Ca and Mg masses are shown in Figures 3c and 3d, respectively, and were highly significant ($P < 0.01$) only in time (Table 3).

Table 3. Variance analyses with associated significance levels for the effects of treatments (control and irrigated), sampling time and their interaction on non-woody mass and nutrients in a secondary rain forest in eastern Amazon, Brazil. The significance level is indicated (*: P < 0.05, **: P < 0.01, ns: not significant)

Litter (non-woody)	Irrigated experiment		
	Treatment	Time	Treatment × Time
Monthly mass	0.043*	0.000**	0.613ns
Ca monthly mass	0.298ns	0.000**	0.400ns
Mg monthly mass	0.171ns	0.000**	0.933ns
Ca monthly concentration	0.012*	0.000**	0.997ns
Mg monthly concentration	0.000**	0.000**	0.970ns
Annual mass	0.126ns	0.003**	0.803ns
Ca annual mass	0.440ns	0.353ns	0.805ns
Mg annual mass	0.316ns	0.000**	0.951ns
Ca annual concentration	0.060ns	0.348ns	0.581ns
Mg annual concentration	0.000**	0.212ns	0.134ns

In the control treatment, Ca production in 2000 was 5.74 kg $ha^{-1}year^{-1}$, ranging from 0.034 g m^{-2} to 0.071 g m^{-2}, with a mean value of 0.048±0.017 g m^{-2}. In 2006, the mean production was 0.047±0.017 g m^{-2}, ranging from 0.019 g m^{-2} to 0.074 g m^{-2} with a total amount of 5.58 kg $ha^{-1}year^{-1}$ (Table 4, Figure 3c).

Table 4. Annual production of Ca and Mg via litter, under control and irrigated treatments, in 2000 and 2006 in kg ha^{-1} $year^{-1}$

Period	Treatments			
	Control		Irrigated	
	Ca	Mg	Ca	Mg
2000	5.74	1.88	5.99	1.79
2006	5.58	1.53	5.71	1.44

In the control treatment, the annual production of Mg in the litter flow was 1.88 kg ha^{-1} $year^{-1}$, with a monthly mean of 0.016±0.007 g m^{-2} in 2000, and 1.53 kg ha^{-1} $year^{-1}$ in 2006, with a monthly mean of 0.013±0.005 g m^{-2}. On the other hand, in the irrigated treatment the Mg production was 1.79 kg ha^{-1} in 2000, with a mean value of 0.015±0.005 and in 2006 the total production was 1.44 kg ha^{-1} $year^{-1}$, with a mean value of 0.012±0.005 g m^{-2} (Figure 4b).

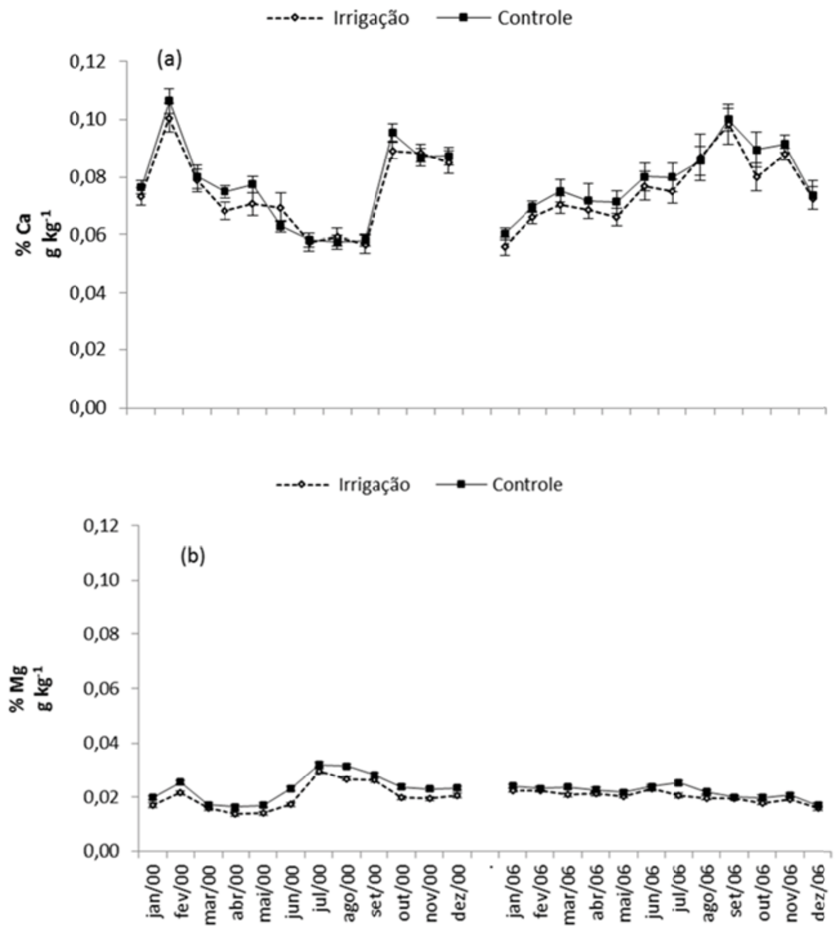

Figure 4. Dynamics of Ca and Mg concentration in control and irrigated treatments in successional forest in Castanhal/PA. (a) Ca concentration and (b) Mg concentration

4. Discussion

As for the species, the number found in this experiment was considered low, but it followed the pattern of the secondary forests of the Bragantina Zone, which have few species. The vegetation is the main responsible for the horizontal variability of the litter, that is, the more diverse the plant community, the more heterogeneous the litter (Correia & Andrade, 1999), as well as the amount of nutrients that will be available to be reused by the forest community.

The most abundant species in the area, besides the ones mentioned above, were *Vismia guianensis* (Aublet.) Pers. (134), *Cupania scrobiculata* Rich. (131) and *Inga* sp. (Lima, 2003), which were also very abundant in the upper stratum of this forest.

These species are considered pioneers in the secondary forests of Bragantina Zone (Oliveira, 1995; Vieira, 1996; Santana, 2000). The most peculiar habit of the vegetation was tree, which indicates that the forest is at a more advanced stage of succession (Smith et al., 1997). In addition, the species differ from each other in their absorption capacities, that is, it depends on some aspects such as porosity of the deposited material, decomposition rate, precipitation variation and environmental temperatures (Santos Junior, 2020).

Factors affecting nutrient form and cycling are closely linked to climatic and phenological conditions, as well as to environmental aspects and pollutants, varying from species to species (Schumacher, 1992; Poggiani & Schumacher, 2000).

On the forest litter, the minimum and maximum values are found in different periods, since March is a typical month of the rainy season and September one of the dry season, so that there is a seasonal control of hydroperiodicity. For Santos Junior (2020), among the factors that contribute to the variation in litter storage, climate is one of the biggest drivers, especially precipitation.

Leaf fall is caused by senescence, resulting from a series of metabolic processes linked to the physiology of each species, as well as by environmental stimuli such as photoperiod, temperature and water stress (Kramer & Kozlowski, 1960). However, in 2006, the annual litter quantity in the control treatment reduced to 6.91 Mg ha^{-1} $year^{-1}$ when compared to the irrigated treatment, ranging from 316.10 kg ha^{-1} (January) to 937.90 kg ha^{-1} (July), with monthly mean of 575.96±202.60 kg ha^{-1}. In addition, the litter flow varies according to the ecosystem considered and its successional stage (Delitti, 1989).

The annual production in successional ecosystems in plateau areas in the Amazon is in the range of 7-10 Mg ha^{-1} $year^{-1}$ (Klinge & Rodrigues, 1968; Stark, 1971; Herrera et al., 1978; Vitousek, 1984; Vasconcelos et al., 2008), but it can vary considerably from one year to the next, depending on the phenology of tree species and, mainly, on rainfall patterns, because there is a strong seasonal control of thin litter production: larger productions are measured in the driest periods of the year (Luizão, 1989). Therefore, the litter layer on the soil also presents a strong seasonal pattern, decreasing its thickness at the end of the rainy season and increasing it in the dry period (Luizão & Schubart, 1987).

The irrigated treatment presented an annual deposition of 8.26 Mg ha^{-1} $year^{-1}$ in 2000, with a mean monthly value of 688.76±183.00 kg ha^{-1}, ranging from 388.20 kg ha^{-1} (March) to 1171.60 kg ha^{-1} (September). In 2006, the annual deposition decreased to 7.29 Mg ha^{-1} $year^{-1}$, with a monthly mean value of 607.91±205.80 kg ha^{-1}, which varied from 305.70 kg ha^{-1} (January) to 949.20 kg ha^{-1} (August).

The irrigated treatment had a greater litter production than the control plot, that is, the artificial hydroperiodic pulses probably contributed to a higher phytomass deposition in this ecosystem, which may have caused an ecophysiological disturbance on the plants, in relation to water availability outside the rainy season. However, there was no significant interaction on treatment × time (Table 3, Figure 3b). For the annual mass, only for time it was highly significant ($P < 0.01$).

In forestry cultivation, irrigation has been used mainly in the first year of stomatal formation, presenting stomatal closure under conditions of high evaporative demand, which may have variable or gradual effects depending on the stage of development (Amaral, 2019). The variation of water in the soil directly influences transpiration and conductance.

For Delitti (1984), the increase in litter quantity with low rainfall is common in tropical regions and reflects a strategy of minimizing the effects of water scarcity. In tropical regions such as the Amazon, the influence of rainfall is of fundamental importance for the accumulation, decomposition rate and release of nutrients from the litter (Luizão, 1982). The monthly distribution of Ca and Mg masses are shown in Figures 3c and 3d, respectively, and were highly significant ($P < 0.01$) only in time (Table 3).

Ca dynamics were significant both in the dry season and in the rainy season. However, considering that it is proportionally associated to the pulses of monthly litter production, which are higher in the dry period, the dynamics of Ca become more pronounced in the latter. The monthly water pulses in the irrigated treatment, the Ca mass, in 2000, ranged from 0.031 g m^{-2} to 0.076 g $m^{-2,}$ with a mean value of 0.050±0.015 g m^{-2}, totaling 5.99 kg ha^{-1} $year^{-1}$. In 2006, the average monthly production was 0.048±0.018 g m^{-2}, corresponding to a total of 5.71 kg ha^{-1} $year^{-1}$.

Comparing the monthly distribution in the years studied (2000 and 2006) in the dry period (August to December), when rainfall is lower than 100 mm, it is observed that the deposited amount of Ca in both treatments was higher than the one of Mg. Calcium dynamics is high in most of the tropical forests studied (Vitousek, 1984), because it is a fixed element in plant tissues (leaf, bark, wood and branches), however, there was not a marked difference between the treatments.

The concentration and nutrient content of the litter varies according to soil type, vegetation, population density, species ability to absorb, utilize and redistribute nutrients, natural habitat and tree age. The monthly concentration of Ca (Figure 3) and Mg (Figure 3) varied significantly ($P < 0.05$) in treatment and in time, but only Mg concentration was highly significant ($P < 0.01$) and only by treatment (Table 3, Figures 4a and 4b).

According to Yavitt et al. (2004) irrigation did not have a significant effect on the concentration or fall of leaves or amount of nutrient return annually by leaf fall. The temporal patterns in nutrient concentrations tended to accompany those in the leaf fall. The biogeochemical cycle encompasses the processes of nutrient transferring within the soil-plant system. The nutrients cycling depends on several factors, including their mobility within the plant.

According to Mengel and Kirkby (1982), the biochemical cycle, which represents the movement of translocation of nutrients from old to new tissues of the plant, is of fundamental importance for high mobility nutrients such as

Mg. Nonetheless, it is of less significance to those of limited redistribution such as Ca. In addition, much of the nutrients is allocated in the trees. Among the components of the aerial part of the tree, the highest nutrient content is found in the leaves, then in the branches and the rest in the wood and trunk (Vieira, 1998). Therefore, Ca values are higher in relation to Mg due to the higher deposition of senescent tissues, such as leaves, and their low mobility.

5. Conclusion

The seasonality of total litter production was significative, being higher in dry season, regardless of irrigation performed in dry period, in the irrigated treatment. The annual litter production did not show significant differences between control and irrigated treatments. However, a longer observation period would be necessary to confirm the information more conclusively or to obtain more significant results for irrigated treatment.

The litter quality in the successional forest ecosystem with predominant species *Lacistema pubescens* Mart. (1,046) and *Myrcia sylvatica* Barb. Rodr. showed a significant mobilization of Ca, in relation to Mg, in quantity (production) and concentration, regardless of time and treatment, *i.e.*, those species mobilize nutrients fixed in the soil and provide for plants that are installed in secondary forest.

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Economic Viability of Cassava Residues in the Nile Tilapia Diet

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Abstract

Fish feed represents between 50% and 70% of intensive aquaculture production costs. In this context, the present study aimed to evaluate the economic viability of the production of Nile tilapia under a diet with cassava residues in it. To evaluate the economic efficiency in relation to the inclusion of residues in the tilapia feed, the approximate cost of feed per kilogram of live weight (CMR) gained during the experimental period was determined, and later the cash flow of the production was raised for analysis of indicators: NPV (Net Present Value), CBI (Cost Benefit Rate), and IRR (Internal Rate of Return) submitted to different discount rates from existing financing sources in the region. It was found that tilapia under the diet with the inclusion of tapioca sweep in the diet, showed greater economic efficiency, and consequently higher NPV (R $ 4,583.33), IRR (15%) and CBR (1.17). In this sense, the viability analysis showed that cassava residues in diets of tilapia diets, indicate to be a viable strategy to better develop aquaculture production, in a more sustainable way, enhancing the technical and economic viability and minimizing the environmental impacts.

Keywords: costs, animal nutrition, *Oreochromis niloticus*, economic profitability

1. Introduction

Aquaculture is an essential agricultural practice for food security worldwide (FAO, 2016; Fore et al., 2018) and the Nile tilapia is one of the most popular species (Cai et al., 2018). In general, the pisciculture is also widely considered an option to fight hunger and poverty in developing countries (Lithgow et al., 2017).

In turn, aquaculture is a fast-growing sector with a world fish production of 80 MT in 2016, which represents 88% of the world fish production (FAO, 2018). In this sector, the creation of tilapia is included, as the only aquaculture species with a consolidated improvement program in the country, which has contributed to the sharp increase in its production compared to other fish species (Silva et al., 2020).

Nile tilapia (*Orechromis niloticus*) was the most widely cultivated aquaculture species in Brazil in 2018, corresponding to 55.4% (400.3 thousand tons) of the total national fish production. From a social perspective, if practiced in a sustainable way, it can promote socioeconomic development by reducing inequalities and distributing in an equitable manner, income and assets (Costa-Pierce et al., 2010), and also in areas with natural resources abundant, can be a strategy for low-income communities to reconcile socioeconomic improvement and environmental conservation (Fonseca et al., 2017).

In Rio de Janeiro, commercial aquaculture developed from the 1980s onwards, following some sporadic incentives, but mainly driven by the private sector in search of new investment alternatives in the agricultural production sector (Scott et al., 2002). A relevant goal to keep this sector in constant growth involves the development of new researches with the objective of determining the benefits of using different feeding strategies and how these strategies influence economic and productive parameters (Gutiérrez et al., 2015; Janssen et al., 2017).

This activity has also been consolidated in Brazil as a result of available water resources, the favorable climate, relatively inexpensive labor and the growing domestic and foreign market (Garcia et al., 2013). Mostly, it has been practiced in semi-intensive systems, in excavated ponds with little water renewal, low or medium storage density and the use of balanced rations combined with the use of natural food (Brande, 2017).

Within this technological package, special attention should be given to the cost of food, which according to Perea-Román et al. (2018) represents 50% to 70% of the operating cost and, therefore, its change reflects a great impact on the final cost. For Nunes Souto (2015), the value of the feed should not exceed 25% of the market value of the cultivated species. This percentage can vary depending on the productivity obtained in the creation and on the value of the ingredients used in food, the latter being also greatly impacted by the cost of logistics for the purchase of these inputs in some regions.

Tilapia are organisms with protein requirements from lower food chains and eating habits that tend to be herbivores (Montoya-Camacho et al., 2018), however, intensive tilapia rearing models in close systems require protein/energy levels between 18 and 23 kg/MJ for maintaining optimal growth in the early stages, according to Kabir et al. (2018). Another important feature to consider is that depending on the type of fish, the nutritional needs for their food will be (Boyd, 2013).

In feeding fish farming systems, fishmeal, as well as meat, blood and soy pie, are the common sources of protein used to make concentrate today (Treviño et al., 2014). The use of fish evisceration residues through silage processes, for subsequent inclusion in animal feed diets, is a high quality nutritional alternative that allows obtaining a lower cost feed, but with high production rates and feed efficiency, due to the fact that they have an appreciable nutritional composition, whose proteins are of high biological value and high digestibility, which provides great benefit in animal feed (Garcez, 2015).

Considering the high costs of these raw materials and their possible low availability in some regions, it is assumed that there is a need to seek regional alternatives of low-cost proteins with high nutritional quality in order to obtain adequate production indicators that allow improving the profitability of production units.

According to Haygood and Jha (2018), several studies on the effects of alternative feed ingredients cite economic concerns as one of the important factors to be analyzed, such as the studies carried out by Dias et al. (2020), Sabbag (2014), Carvalho et al. (2020), among others.

Thus, research on the economic viability of raising tilapia under diet with different ingredients from agro-industrial waste becomes necessary and fundamental, since the focus of fish farming is to produce high quality products at the lowest possible cost. However, there are few economic studies that use analyzes with econometric equations to obtain profitability studies in Nile tilapia, based on analyzes of economic indicators that consider monetary values over time and their attractiveness margin against others financial markets.

Among the financial economic feasibility studies used to evaluate the production of Nile tilapia, we can point out the economic analyzes of this production in a greenhouse (Gutiérrez-Leyva et al., 2020), in earthen nurseries (Trombeta et al., 2017) and in net tanks installed in lakes and reservoirs (França et al., 2016; Brabo et al., 2017), as well as in integrated production with vegetables in an aquaponic system (Quagrainie et al., 2018), in addition to application for other species in different cropping systems.

In general, these studies have shown that fish farms periodically need new investments to increase the technological level of the production system. On the other hand, existing data cannot be widely used, since the production cost reflects the use of production technology under certain environmental and economic conditions, as pointed out by Ayroza et al. (2011). Given the above, this research aimed to evaluate the economic efficiency and economic feasibility of using cassava processing residues in Nile tilapia diet.

2. Method

2.1 Characterization of the Experiment

The experiment was conducted at the Federal Institute of Education, Science and Technology Fluminense Advanced Campus Cambuci (IFF-Cambuci), in the municipality of Cambuci/RJ, in partnership with the State University of North Fluminense Darcy Ribeiro (UENF), from January to May 2017, totaling 112 days. The simulated production system is a small fish farm carried out in a nursery (excavated tank) of 1,000 m² of water depth (50.0 × 20.0 × 1.0 m) and rearing in a single phase with a density of 3 fish/m³.

Three rations were manufactured to feed the fish, one with only traditional ingredients, without inclusion of cassava waste (control; T1) and two with alternative ingredients (waste obtained from agro-industries in the region): cassava peel flour 24% (T2) and tapioca dusting flour (T3). The formulations of the three diets, shown in Table 1, are isoprotein (36% crude protein), isocaloric (3.100 Kcal).

Table 1. Formulation in percentage of the ingredients of the experimental and control diets

Ingredients (%)	Treatments		
	T1 (WAR)	T2 (ARMPF)	T3 (ARTSF)
Corn meal	19.08	2.00	1.00
Wheat bran	8.00	2.00	2.00
Soybean meal	54.92	38.09	28.00
Fish's flour	15.00	30.91	42.00
Premix	2.00	2.00	2.00
Fish oil	1.00	1.00	1.00
Residue 1 (RAFCM)	0.00	24.00	0.00
Residue 2 (RAFVT)	0.00	0.00	24.00
Total	100.00	100.00	100.00

Note. T1 (WAR): without agro-industrial residue; T2 (ARMPF): agro-industrial residue from manioc peel flour; T3 (ARTSF): agroindustrial residue from tapioca sweeping flour.

All diets were formulated with 3,100 Kcal/Kg of digestible energy and 36% crude protein.

2.2 Economic Evaluation

Economic aspects are important in the planning, control and decision-making of fish farming, since costs play two important roles, managerial and business. The aim is to provide data for establishing standards, budgets and other forms of forecasting and, subsequently, comparing the values already reported by other researches in different production systems.

To evaluate the economic efficiency in relation to the inclusion of residues in the feeding of tilapia, the approximate cost of feed per kilogram of live weight (AFC) gained during the experimental period was determined, as recommended by Bellaver et al. (1985). The AFC correlates the cost of the feed provided to the zootechnical performance obtained.

$$AFC = \frac{(Q_i \times C_i)}{WG_i} \quad (1)$$

Where, AFC = average feed cost per kilogram gained in the i-th treatment; Q_i = average amount of feed used in the i-th treatment; C_i = average cost per kilogram of feed used in the i-th treatment; GP_i = mean weight gain of the i-th treatment.

Then, the economic efficiency index (EEI) and the cost index (CI) were calculated according to Barbosa et al. (1992) from the following equations:

$$EEI = \frac{LCe}{ACe_i} \times 100 \quad (2)$$

$$CI = \frac{ACe_i}{LCe} \times 100 \quad (3)$$

Where, EEI = economic efficiency index; CI = cost index; LCe = lowest average cost observed in feed per kilogram of live weight between treatments; ACe_i = average cost of treatment i considered.

2.3 Economic Feasibility Analysis

Productivity was calculated based on the survival rate of the fingerlings of 93% for the entire cycle and a slaughter weight of 800 grams at the end of a 12-month rearing period. The price of whole fish paid to the producer considered was R$ 6.00/Kg and the prices of inputs used to make up the cash flows were average prices obtained in the North and Northwest regions of Rio de Janeiro in 2016.

The cash flow values resulted from the inflows and outflows of resources and products throughout this period and, at the end of this investment horizon, the inputs that had not yet exhausted were recorded as revenues in this last year. In this way, the residual values of land, facilities and equipment entered as revenue at the end.

All prices used in the economic analysis, whether for sale of the product or purchase of inputs, were collected in the North Fluminense region to reflect the real economic potential of the alternatives tested.

The prices of the ingredients of the diet were obtained in Campos dos Goytacazes/RJ, in October 2016. Such prices (R$/Kg) used in the elaboration of the costs were: corn (R$ 1.30), wheat bran (R$ 1.18), soybean meal (R$ 2.16), fish meal (R$ 2.22), vitamin and mineral premix (R$ 13.50) and fish oil (R$ 2.00). Waste for reuse was considered as zero cost.

From the construction of the cash flow through the technical coefficients, the following profitability indicators were used: the Net Present Value (NPV), the Internal Rate of Return (IRR) and the Benefit-Cost Ratio (BCR), which have the fact that they consider the effect of the period of time on monetary values is an advantage.

The NPV consists of transferring to the current instant all expected cash flows, discounting them at a certain interest rate and adding them algebraically, being determined by the expression below. The NPV must be positive for the project to be accepted.

$$NPV = -I + \sum_{j=1}^{n} \frac{CF_t}{(1+k)^t} \tag{4}$$

Where, I = capital investment at zero date; FC_t = return on date t of the cash flow; n = project analysis period k = minimum rate to carry out the investment or capital cost of the investment project.

If the result is less than zero, it means that the return on investment was less than the desired minimum, so the project is considered rejected. If the result is greater than or equal to zero, the project presents an indication of feasibility (Ross et al., 2015; Assaf Neto, 2014).

$$0 = -I + \sum_{j=1}^{n} \frac{FC_t}{(1+IRR)^t} \tag{5}$$

The BCR of a project is the rate that nullifies the NPV of the investment's cash flow. It is the one that makes the present value of future profits equivalent to the expenses incurred with the project, thus characterizing the rate of return on invested capital. The higher the IRR, the more solid the project will be, which must be above the minimum attractiveness rate for the project to be accepted.

The BCR consists of transferring to the current instant all expected cash flows, discounting them at a certain interest rate and dividing them by the invested capital, being determined by the expression below. The BCR must be above 1 for the project to be accepted, indicating that the capital obtained (benefit) was greater than the capital invested (cost).

$$BCR = \sum_{j=1}^{n} \frac{CF_j}{(1+i)^t} \Big/ CF_0 \tag{6}$$

Where, CF_j = cash flow at time j; i = interest rate; CF_0 = capital invested at the start of the project. This index analyzes the "cost/benefit" of the project and provides a measure of the expected return per monetary unit (BRAGA, 2010).

3. Results and Discussion

It was observed that the cost of conventional feed is higher than the cost of feed with cassava waste, having as its main justification the assumption of zero cost, that is, the reuse of cassava waste (Table 2). However, it is worth noting that the average feed cost per kilogram of live weight with tapioca dusting flour residue is lower than the cost of feed with cassava husk, thus demonstrating a better economic/zootechnical balance, since this alternative significantly achieves the ratio of high quality products at the lowest possible cost.

It is possible, therefore, to point out that the economic efficiency index generated by the treatment with tapioca dusting flour enables greater economic efficiency than the others.

Table 2. Economic efficiency of the use of cassava processing residues in the Nile tilapia diet

Variables	Treatments		
	T1 (SRA)	T2 (RAFCM)	T3 (RAFVT)
Race cost (R$/Kg)	2.15	1.85	1.87
AFC (R$/Kg PVG)	7.57[ab]	7.78[a]	4.85[b]
Cost index	193.27	198.86	124.03
EEI	51.74	50.29	80.63

Note. T1 (WAR): without agro-industrial residue; T2 (ARMPF): agro-industrial residue from manioc peel flour; T3 (ARTSF): agroindustrial residue from tapioca sweeping flour.

[abc] Means followed by at least one equal letter on the same line do not differ at the 5% probability level by Tukey's test.

These data corroborate other studies that established comparisons between the sources of feed ingredients. Pereira Junior et al. (2013), observed that the use of cassava flour in different degrees of substitution to corn in the feeding of tambaqui contributed to a decrease of around 15% in the cost of feed production. As observed by Cruz et al. (2006), who identified a lower cost per kilogram of feed with the inclusion of cassava trimming flour to replace corn in feed for laying hens.

It is worth noting that although the treatments show statistically significant similarity, in terms of economic aspects, the inclusion of cassava residues in Nile tilapia farming may enable the identification of alternative sources of nutrients that are less costly at the total cost of production. In addition, the reuse of these residues as an alternative ingredient also contributes to the preservation of the environment and the sustainability of all agricultural activities.

Gutierrez-Leyva et al. (2020) explain that within the panorama of the development of new policies in world aquaculture, the application of new production models aimed at species with high commercial demand and low production costs, has clear objectives for economic development, employment and protection of the environment.

The simulation of cash flows obtained over 3 years from the use of feed was done only with traditional ingredients and alternative feed made with the inclusion of tapioca dusting flour (T3), considering that the latter presented superior zootechnical results to the diet with inclusion of cassava peel flour. Based on this observation, the production costs and the economic viability of tilapia production were comparatively analyzed with the two treatments that presented the highest economic efficiency index.

Although the revenue obtained is considered the same, considering that there is no price difference for the product offered, the lower feed cost with tapioca sweeping allows for greater NPV achievement over three years. For both conditions, the NPV > 0 is obtained, however in the rearing of tilapia with traditional feed, NPV equivalent to R$ 1,041.81 was obtained, and R$ 4,583.33 for tilapia fed with tapioca-scanned feed (Table 3).

Table 3. Summarized cash flows of Nile tilapia production with traditional diet feed and cassava sweeping residue insertion

	T1 (SRA)			T3 (RAFVT)		
	Year 1 (R$)	Year 2 (R$)	Year 3 (R$)	Year 1 (R$)	Year 2 (R$)	Year 3 (R$)
Revenue	13.392,00	13.392,00	13.392,00	13.392,00	13.392,00	13.392,00
Costs	42.367,60	10.587,60	10.587,60	41.117,68	9.337,68	9.337,68
Cash flow	(28.975,60)	2.804,40	30.754,90	(27.725,68)	4.054,32	32.004,82
NPV 6%	R$ 1.041,81			R$ 4.583,33		
IRR	7,98%			15,00%		
BCR	1.04			1.17		

Note. T1 (WAR): without agro-industrial residue; T3 (ARTSF): agroindustrial residue from tapioca sweeping flour.

In western Paraná, Queiroz (2015) analyzed the economic feasibility of raising tilapia in excavated tanks, and concluded that the NPV was positive, meaning that the capital invested in the project will be recovered. And just as in this research, in addition to the financial indicators, the researcher also emphasizes that agricultural and livestock activities are exposed to climate and price variations.

Regarding the IRR, it can be considered that the closer the IRR of the business is to the minimum attractiveness rate (MAR), the more subject the activity will be to inflationary fluctuations in the economy, which can be crucial for the family producer who does not have of financial reserve to get through periods of economic recession. Based on this principle, the T3 ration is observed as promising a greater safety margin for the activity, as only interest rates above 15% would make the project unfeasible, while interest rates above 8% already make the use of the ration unfeasible. T1.

Rocha et al. (2020) when analyzing the economic viability of the cultivation of Nile tilapia in Rio Grande do Sul also found both the NPV and the IRR, indicating that the cultivation of tilapia will pay more than the minimum rate expected by the rural property.

Following the assumption of the benefit-cost ratio, it indicates how much profitability there will be in that period, in adjusted values, based on the investment made. Thus, for every R$1.00 invested in raising tilapia using the T3 feed, there will be a cash flow of R$1.17. In the use of T1 feed, this flow would be only R$ 1.04, about 11% lower.

It is important to pay attention to this information as indexes that demonstrate the alternatives that could contribute to cost reduction, especially in food, since this is commonly identified as the largest composition of the total cost of fish farming, according to Oliveira et al. (2014); Janssen et al. (2017).

In this context, it is worth noting that this is a sustainable activity that can take advantage of different agricultural residues, in addition to providing the fish farmer with profitability, with significant gains for the regional economy, as shown by Sabbag (2014). However, like any other economic activity, it needs a strategy or basic planning to produce good results in its production units.

Finally, it is noteworthy that these observations must be added to the possibilities of marketing and the variation in product prices (Ayroza et al., 2011), as determining factors for its good cost-benefit. Since there are both zootechnical and market factors that interfere in the productivity and profitability of tilapia farming, as pointed out by Garcia et al. (2016).

4. Conclusion

The highest economic profitability index was achieved with the inclusion of tapioca sweeping in the diet, which is why it is highlighted that when feeding Nile tilapia with this food, a greater economic reward is achieved per kilogram of fish meat produced. Furthermore, the use of cassava residues in tilapia diets made it possible to establish a sustainable production strategy, with a better cost-benefit rate between the reduction in food costs and the environmental impact.

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Asian Rust Severity in Soybean Sown in December and February in Mato Grosso State

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Abstract

The objective of this work was to assess the effect of December sowing time with February on the Asian soybean rust severity. In on-farm trials two soybean treatments sowing in December (2020) (DSS.) and February (2021) (FSS) were assessed for Asian soybean rust severity in 24 sites, in three regions of Mato Grosso state. The DSS treatment was established in the growers commercial farms and the FSS in a 5 ha area sown specifically for this treatment. The DSS treatment was conducted in 16 sites and the FSS in eigth. For rust control fungicides with efficacy higher than 60% were sprayed consisting of DMIs, QoIs and SDHIs in double or triple mixtures, always added by multisites (chlorothalonil, mancozeb, or copper oxychloride). About eighty soybean leaflets from four plots repetitiond, demarcated at random in each field, were taken in each smpling. In laboratory leaflet severity was appraised and area under disease progress curve (AUDPC) calculated. Related to DSS, the AUDPC overall mean was 174 units and receiving 6.9 fungicide spraying and for FSS 26 units with 4.8 fungicide sprayings. Our results reinforce that the sowing time can be changed from the end of December to February to maintain soybean crop sustainability.

Keywords: *Glycine max*, *Phakopsora pachyrhizi*, site-specific and multisite fungicides

1. Introduction

The importance of soybean [*Glycine max* (L.) Merr.] in Mato Grosso, the country largest producer state, is reflected in the cultivated area in the last season, reaching 9.6 million hectares (IMEA, 2021).

In Mato Grosso, the main crop disease is Asian soybean rust (ASR) caused by the basidiomycete biotrophic fungus *Phakopsora pachyrhizi* H. Sydow & P. Sydow, 1914).

Although with little published scientific data, damage up to 90% have been cited. Nevertheless, scientifically crop damage can be precisely estimated with the mathematical functions developed by Danelli et al. (2015).

The ASR control has been based on the spray of only three site-specific fungicides (DMI, QoI, SDHI) in double or triple co-formulations, but in mixtures with multisites (chlorothalonil, mancozeb, or copper oxychloride) just in a restricted area.

Despite of available technology the decision-making of first spray has been based on empiricism. The use of site-specific mixtures at risk to the development of resistance is accelerated by their use in the state total grown area, in all sprayings, and an increase in the spraying number, such as in December fields (Reis et al., 2020, 2021a, 2021b). This practice has resulted in the evolution of *P.pachyrhizi* sensitivity reduction to such site-specific fungicides with cross and multiple resistance and with actual efficacy below 40% (Zambolim, 2019).

The state of Mato Grosso has set a limit on the sowing period for soybeans that may start on September 16th, depending on the soil water content, and must be completed on December 31th (INDEA, 2015). Even though, based on practical observations, this resolution has been indicated to reduce the inoculum potential in the state since 2006 and recently arguing that this measure may reduce fungicide directional selection by reducing the spraying number (CAF, 2014).

Soybean fields for the complementary commertial and own growers seed production have been established in December, as a result of IN 002/15. Despite being changed by IN 001/21, nothing has changed in relation to the seeding date and continues to lead to high rust severity as has been recorded, demanding a great number of site-specific fungicide applications in double or triple mixtures (DMI + QoI or DMI + QoI + SDHIs) (Reis et al., 2021b). This practice has reduced the growers profit and with high potencial for environmental damage. On the contrary, the generated technology aims to maximize the growers profit.

As far as 2015, farmers have observed ASR lower intensity, less fungicide applications and better seed quality in February sowing fields. For this reason they have requested, with the Aprosoja-MT support, from the government agencies, authorization to grow soybeans from February 15th with the objective of producing their complementary own seed. This authorization has been denied by state agencies without any scientific evidence for this impediment, but empirically deducing that soybean cultivation in February enters the soybean free-period (from June 15 to September 15) and increases the number of site-specific fungicide applications.

Recently the Ministry of Agriculture issued a rule allowing the seed production even within the soybean free period, which the producers do not agree with, but only in February without entering the soybean free-period.

To solve the impasse between legislators and growers, experiments were carried out during two seasons, showing the greatest rust severity in the DSS and demanding a great number of fungicide applications compared to the FSS (Reis et al., 2020, 2021a) and this situation persists since 2015 without any action by the government plant protection agencies. But, conducting these two experiments was not enough for the state plant sanitary authorities, based on scientific data, to release the FSS.

We hypothesized that, due to the high inoculum pressure, the high rain volume and mainly frequency, and longer plant cycle in the DSS, the rust severity is greater than in the one carried out in February.

The objective of the work was to reconfirm the stated hypothesis that ASR is severer in DSS than in FSS.

2. Material and Methods.

2.1 Experimental Sites

The on farm trials were conducted in the North, South, and West regions of the state under regional soil and climate differences.

2.2 Treatments

The reseach consisted of two treatments: (i) soybean sowing in December (2020); and (ii) sowing in February (2021) with four repetitions.

2.3 Experimental Plots

The DSS treatment was established in the growers commercial soybean fields and the FSS in a 5 ha area sown especifically for this treatment. The DSS treatment was established in 16 locations and that of FSS in eight (Table 1).

Table 1. Experimental areas, sites, and seeding time

Farms	Regions	Counties	Sowing month/day	
			December	February
(1) Farm 1	South	Primavera do Leste	12/14/2020	-
(2) Farm 2	South	Primavera do Leste	12/31/2020	-
(3) Farm 3 A-1	South	Primavera do Leste	12/29/2020	-
(4) Farm 3 A-2	South	Primavera do Leste	12/31/2020	-
(5) Farm 4	South	Primavera do Leste	12/31/2020	-
(6) Farm 5	South	Campo Verde	12/30/2020	-
(7) Farm 6 A-1	South	Campo Verde	12/28/2020	-
(8) Farm 6 A-2	South	Campo Verde	12/28/2020	-
(9) Farm 7 A-1	North	Cláudia	12/31/2020	-
(10) Farm 7 A-2	North	Cláudia	12/31/2020	-
(11) Farm 8 A-1	North	Lucas do Rio Verde	12/29/2020	-
(12) Farm 8 A-2	North	Lucas do Rio Verde	12/29/2020	-
(13) Farm 9 A-1	North	Vera	12/31/2020	-
(14) Farm 10 MB01	West	Campos de Júlio	12/28/2020	-
(15) Farm 10 M3P3	West	Campos de Júlio	12/28/2020	-
(16) Farm 10 M3P5	West	Campos de Júlio	12/17/2020	-
(17) Farm 11	South	Campo Verde	-	02/24/2021
(18) Farm 4	South	Primavera do Leste	-	02/22/2021
(19) Farm 3	South	Primavera do Leste	-	02/24/2021
(20) Farm 7	North	Cláudia	-	02/28/2021
(21) Farm 8	North	Lucas do Rio Verde	-	02/25/2021
(22) Farm 9	North	Vera	-	02/22/2021
(23) Farm 12	North	Marcelândia	-	02/19/2021
(24) Farm 13	West	Campos de Júlio	-	02/26/2021

In each experimental site, the distance between sampling plots ranged from 100 to 150 m to avoid interplots interference.

2.4 Farm Management

The cultural practices follow the farm management and for the fungicides, mixtures of site-specific (DMI, QoI, SDHI) + multi-site (chlorothalonil, mancozeb or copper oxychloride), with more than 60% efficacy were recommended. The first spraying was performed when detected rust symptoms/signs and the others considering a 14-16 days protection period as recommended by the fungicide companies. The chemicals within an efficacy list (> 60%) were freely chosen by the farmers since they were site-specic plus multissite. Fungicides timing and spraying numbers were defined by the growers considering the efficaccy of the last spray and new spray recommended as the rust progressed. In some cases, with Fundação Rio Verde-MT technical assistance, sprayings were recommended at shorter intervals for control reinforcement, mainly due to the rain volume and frequency. Although not scheduled, in some cases tate phytosanitary defense agency made recommendations for fungicde sprayings in the FSS, putting pressure on growers, interfering with the purpose of the research, and increasing unnecessarily the spraying numbers.

2.5 Rust Assessment

In each sampling time, central leaflets with the petiole inserted in the main stem of 10 plants per plot were detached (Ogle et al., 1979). The leafletes were taken to the laboratory to appraise the disease severity according to the diagrammatic scale proposed by Godoy et al. (2006). A total of four samplings were perform along the soybean cycle at R2, R4, R5.4, and R6 growth stages.

2.6 Rainfall Data

Data were obtained from NIMET (National Institute of Meteorology) weather stations located in Diamanino (West), Poxoréu (South) and Matupá (North) counties.

2.7 Experimental Design and Statistical Analysis

The experimental design was composed by two treatments at random with four repetitions and the AUDPC means compared by the Tukey test.

3. Results and Discussion

The work was conducted in 24 fields, 16 sown in DSS and eigth in FSS, located in 12 counties in the South, West and North regions, the main Mato Grosso soybean production areas, covering differences in soils and total and frequency of rainfall.

The evaluations were carried out from January 2021 to May 2021, covering the entire crop cycles.

On average, the rust severity was estimated in 80 leaflets sampling per plot totaling 1920 analysed.

Regarding the number of sprayings, in the DSS treatment, a general mean of six fungicide sprays were performed and in the FSS treatment just four, ane in the western region AUDPC severity ranged from three to four for DSS and five for FSS (Figure 1).

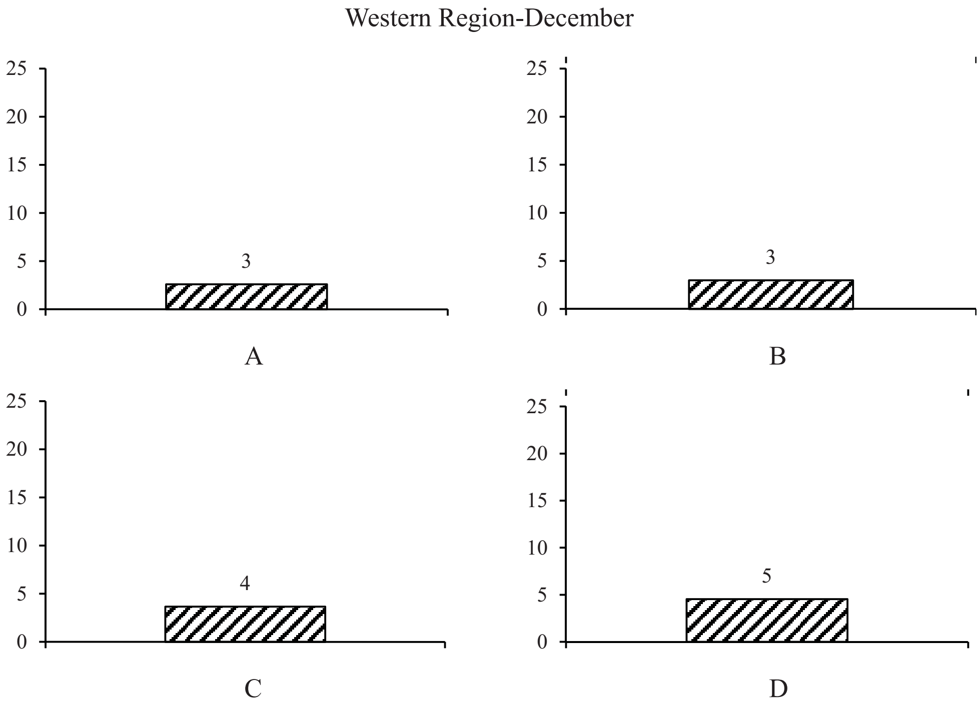


Figure 1. Area under the Asian soybean rust progress curves (AUDPC) rated by the leaflet severity in soybean plants sown in December and February in the western region [Campos de Julio, Farm 10 (A); Campos de Julio, Farm 10 (B); Campos de Julio, Farm 10 (C); Campos de Julio, Farm 14 (D)]

In the overall mean there was no difference for rust severity rated by AUDPC for DSS and FSS in the western region (Figure 2). The average number of fungicide applicationsspryings were seven in DSS and seven in FSS. This higher and desnecessary sprayings number in FSS was due to the pressure made by the state Plant Sanitary Defense Agency.

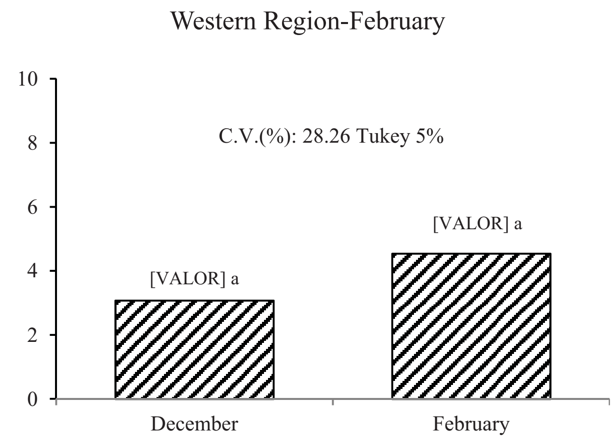
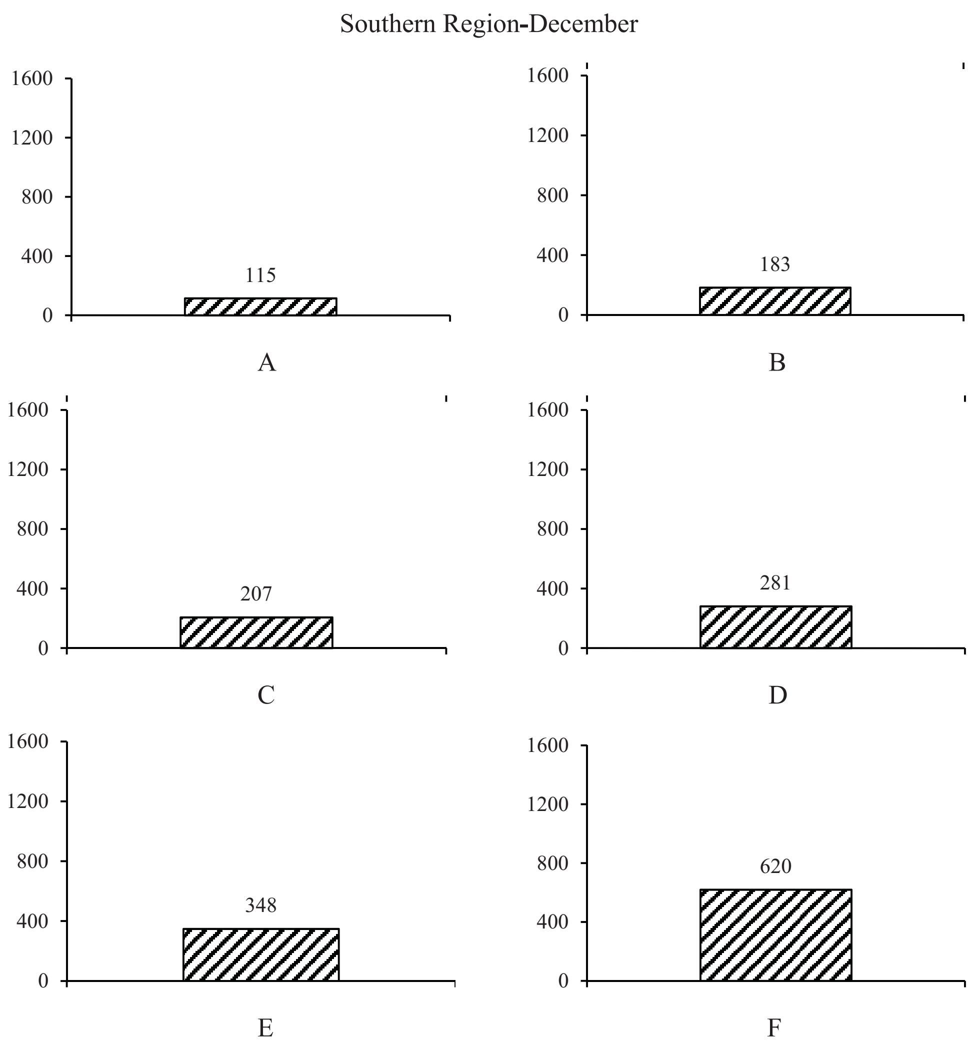


Figure 2. Area under the Asian soybean rust progress curves (AUDPC) rated by the leaflet severity in soybean plants sown in December and February means for the western region. Means in columns followed by the same letter do not differ by Tukey test at 5%

Considering the southern region, AUDPC ranged from 115 to 1,413 for DSS and from 4 to 118 for for FSS (Figure 3). The mean number of fungicide sprays were 7.6 in DSS and 5.3 in FSS. As in the preceding region there was pression for more sprayings than needed for FSS.



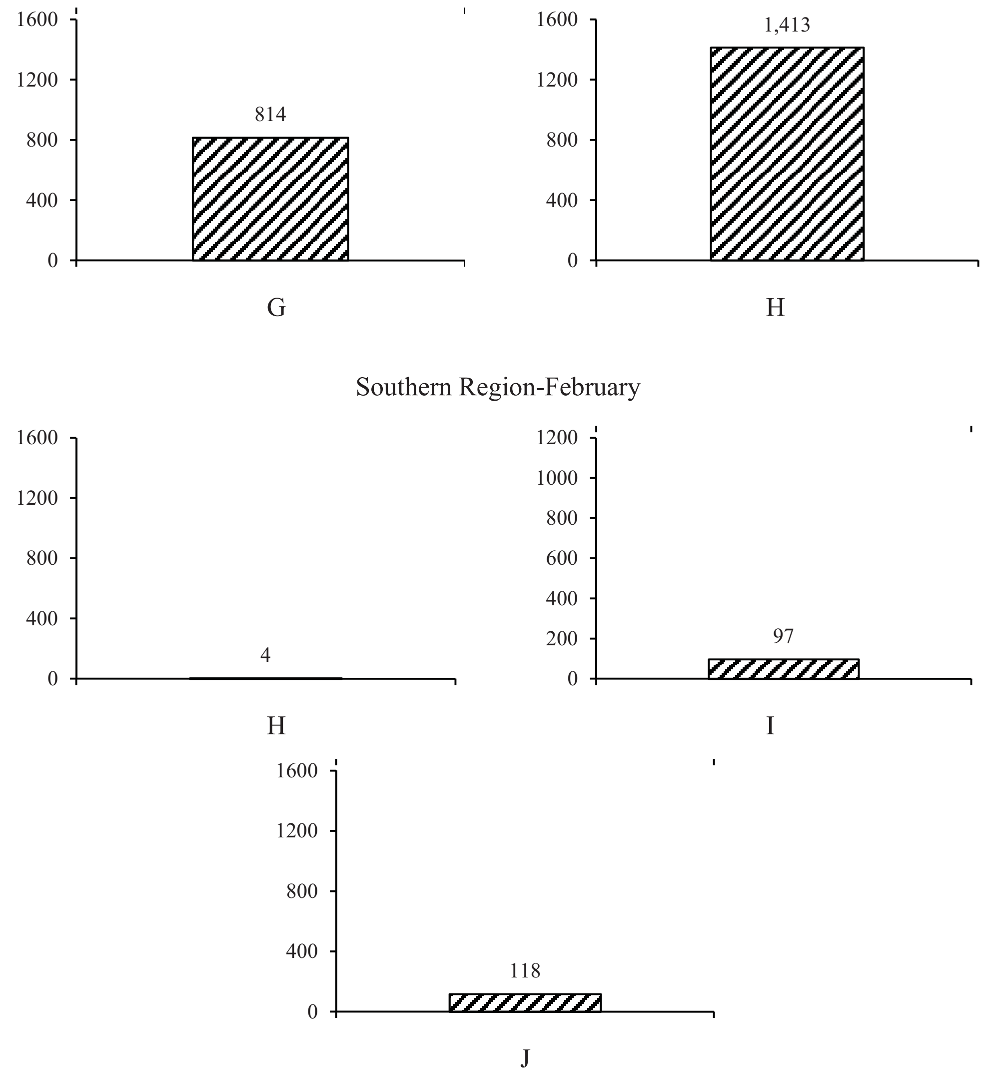


Figura 3. Area under the Asian soybean rust progress curves (AUDPC) rated by the leaflet severity in soybean plants sown in December and February in the southern region [Primavera do Leste, Farm 3 A-1 (A); Primavera do Leste, Farm 1 (B); Primavera do Leste, Farm 2 (C);; Primavera do Leste, Farm 3 A-2 (D); Primavera do Leste, Farm 4 (E); Campo Verde, Farm 5 (F); Campo Verde, Farm 6 A-2 (G); Campo Verde, Farm 6 A-1 (H); Primavera do Leste, Farm 3 (I); Campo Verde, Farm 11 (J); Primavera do Leste, Farm 4 (K).

Analysing the general means rated by the AUDPC for the DSS (498 units) and FSS (73 units), there was a statistical difference between the two treatments (Figure 4).

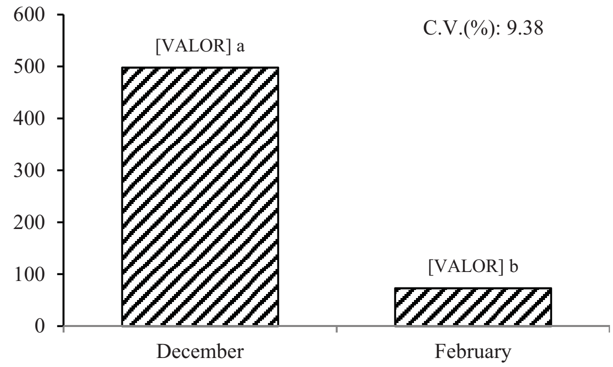
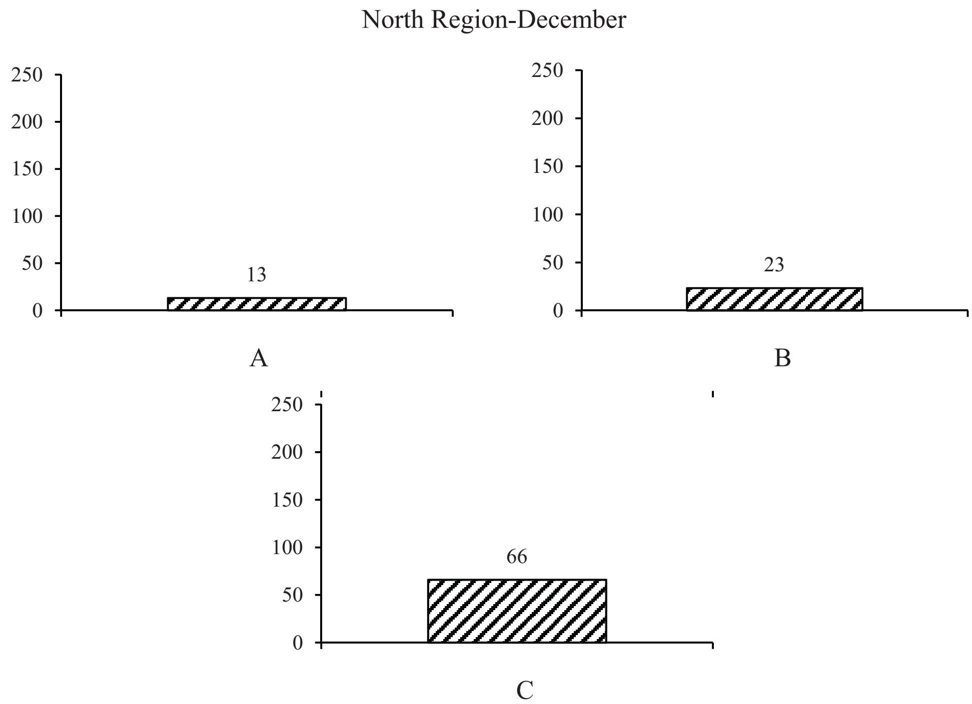


Figure 4. Area under the Asian soybean rust progress curves (AUDPC) rated by the leaflet severity in soybean plants sown in December and February means for the southern regions. Means in columns followed by the same letter do not differ by Tukey test at 5%

The larger AUDPC in DSSs may be due to the following facts: (i) receiving early rust inoculum from neighboring fields that were being harvested, and (ii) the rain in January was slightly above the average (Figure 10) but within the normal range for the region, therefore, it is a typical season according to data from the last 24 years.



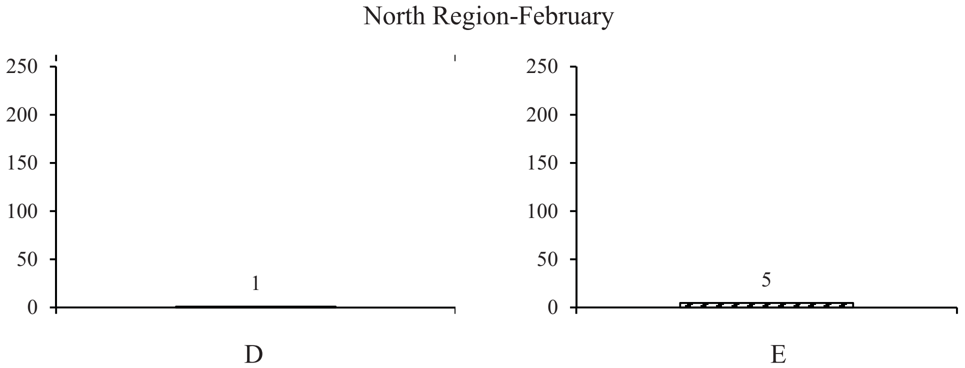


Figura 5. Area under the Asian soybean rust progress curves (AUDPC) rated by the leaflet severity in soybean plants sown in December and February in the northern region [Lucas do Rio Verde, Farm 8-A1.1 (A); Lucas do Rio Verde, Farm 8-A1 (B); Vera, Farm 9 (C); Vera, Farm 9 (D); Lucas do Rio Verde, Farm 8 (E)]

Regarding AUDPC general means comparison for the DSS (21 units) and FSS (2 units), there was a statistical difference between the two sowing times (Figure 6). DSSs plots received 5.8 fungicide sprays and FSS 3.75.

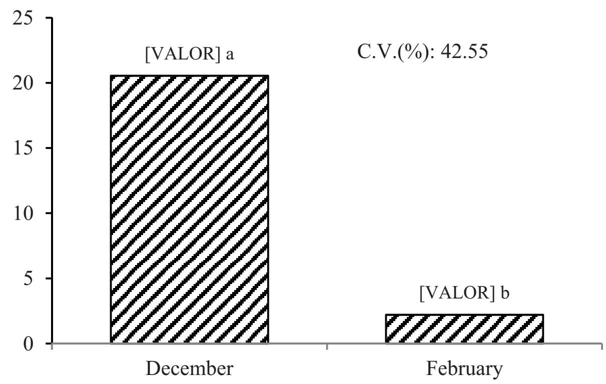


Figure 6. Area under disease progress curve (AUDPC) rated by the leaflet severity in soybean plants sown in December and February means for the northern region. Means in columns followed by the same letter do not differ by Tukey test at 5%

For the overall means there were statistical difference between treatments (DSS and FSS), on rust severity rated by the AUDPC. For DSS the area was 174 and for FSS 26 units (Figure 7). In the overall means DSS were saprayed 6.9 times while in FSS 4.8.

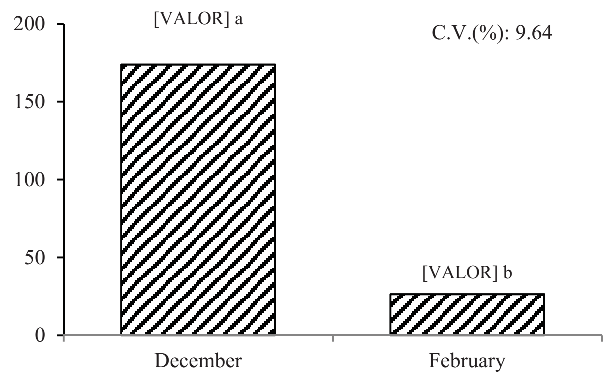


Figure 7. Overall means of the area under disease progress curve (AUDPC) rated by the leaflet severity in soybean plants sown in December and February in the northern, western, and southern regions. Means in columns followed by the same letter do not differ by Tukey test at 5%

It has been noticed in MT since 2015 growing season when was issued the State Normative Instruction 002/2015, that DSS showed greater severity than FSS and, therefore, receiving a great number of fungicide sprayings, as scientifically documented in previous reports (Reis et al., 2020, 2021) as well in the present work (Figure 7).

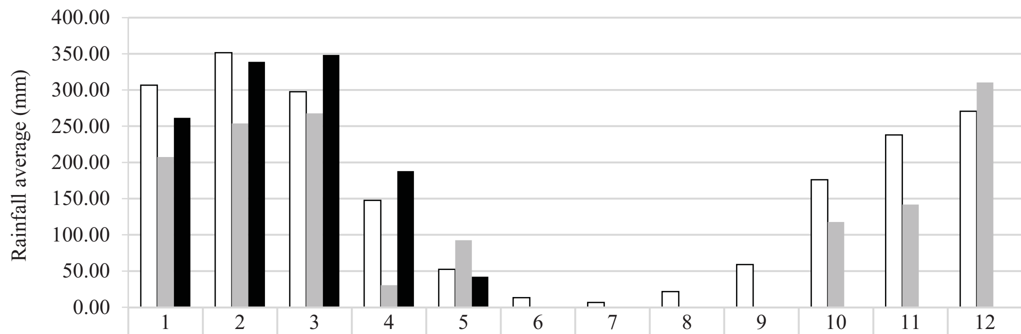
The difference in the rust severity between the two sowing times can be attributed to climate effect, such as rain volume (Del Ponte et al., 2006) but mainly to its frequency during the two crops (Melching et al., 1989). The mounthly average comparison of current rainfall showed great volume and frequency (Figures 8, 9 and 10) during the DSS compared to FSS which reinforces our hypothesis.

Both total rainfall and the number of rainy days for every region were computed according to the sowing date of each treatment.

3.1 Western Region

In this region there were three DSS areas and one in FSS. Similar to the other regions, this season was atypical, with lower historical rain volumes at the season begginning (Figure 8), in October and November, which resulted in sowing delay and on the rust onset in the region. Rust was first detected in the western region on February 9th, 2021 (Campo Novo do Parecis) and on February 17th, 2021 (Tangará da Serra), 30 and 15 days after rust occurrance in the last season.

On the other hand, as in other regions, there was an increase in the rain volume and frequency in March and April. The highest rainfall in February made difficult to control the rust in the areas DSS which, with 25 rainy days out of the 28 in the month, volume (431.8 mm). The high rainfall was also maintained during March increasing the rust pressure in FSS with greater need for sprayings, and both seeding times receiving six spraying. Despite the high rust inoculum coming from DSS, the highest number of sprayings in April and May, was due to pressure for applications by the state phytosanitary defense agency. There was a case where the application was repeated two days later, unnecessarily increasing the number of sprays that were extended until May. During a visit to the experimental area carried out by the researchers, it was observed that unnecessary applications that had been made and still programmed scheduled for the month of May at the FSS. This same situation occurred in the FSS areas of the southern region, which were informed to the researchers who did not authorize extra applications, but even so they wew pressured by the state plant sanitary defense agency, made extra sprayings, which increased the number in February, influencing the survey results.



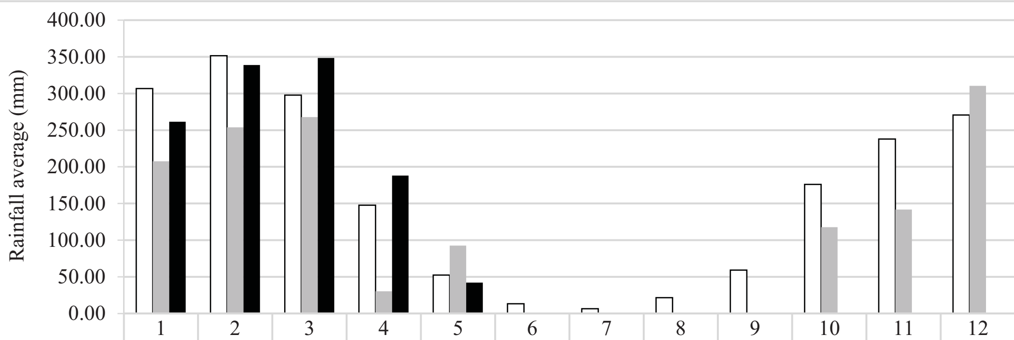
	1	2	3	4	5	6	7	8	9	10	11	12
□ Média 1995 a 2019	306.64	351.60	297.70	147.65	52.33	13.19	6.57	21.55	59.09	176.13	237.86	270.70
■ Ano 2020	207.5	253.8	267.8	30.4	92.5	0	0	0	0	117.7	141.8	310.4
■ Ano 2021	261.5	338.9	348.4	188.0	42.2	0.0						

Figure 8. Mean rainfall (mm) from January to May 2021, compared to 2020 and to 1995-2019 average at Diamantino, MT. Source INMET (2021). The white bars represent the mounthly hstorical average, from 1995 to 2019, greys to 2020 and black the rainfall occurred in 2021, between the months January to May.

3.2 Northern Region

Historically, rust has been less intense in this region due to low altitudes and high temperatures. The lower rust pressure has already been shown in the two comparative works previously published (Reis et al., 2020, 2021a).

In this season, just as in the south and west regions, this was the situation throughout Mato Grosso, with a lower rain volume than the historical average for the last 25 years. The rain deficit in October and November (Figure 9), only was recovered from February, remaining above average in the next months until April. Even with these rain volumes in April and May, the rust severity in the FSS was statistically lower than in the DSS, as the frequency was lower (Figure 6).

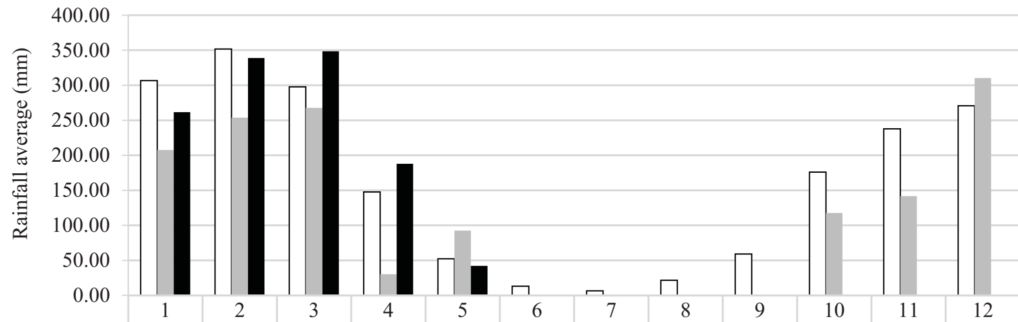


	1	2	3	4	5	6	7	8	9	10	11	12
□ Média 1995 a 2019	306.64	351.60	297.70	147.65	52.33	13.19	6.57	21.55	59.09	176.13	237.86	270.70
■ Ano 2020	207.5	253.8	267.8	30.4	92.5	0	0	0	0	117.7	141.8	310.4
■ Ano 2021	261.5	338.9	348.4	188.0	42.2	0.0						

Figure 9. Mean rainfall (mm) from January to May 2021, compared to 2020 and to 1995-2019 average at Matupa, MT. Source INMET (2021). The white bars represent the monthly hystorical average, from 1995 to 2019 and grey to 2020 and black the rainfall occurred in 2021, between the months January to May.

3.3 Southern Region

The rain event in the southern region, considering the total rainfall occurring during the DSS cycle was 719.0 mm and the 40 rainy days. On the other hand, for the FSS cycle rainfall was 579 mm and 31 rainy days (Figure 10). Total rainfall and mainly its frequency may explain the difference in the AUDPC between the two treatments (Figure 3).



	1	2	3	4	5	6	7	8	9	10	11	12
□Média 1995 a 2019	306.64	351.60	297.70	147.65	52.33	13.19	6.57	21.55	59.09	176.13	237.86	270.70
■Ano 2020	207.5	253.8	267.8	30.4	92.5	0	0	0	0	117.7	141.8	310.4
■Ano 2021	261.5	338.9	348.4	188.0	42.2	0.0						

Figure 10. Mean rainfall (mm) per ten days period from January to May 2021, compared to 2020 and to 1995-2019 average at Poxoréu, MT. Source INMET (2021). The white bars represent the mounthly decennial historical average, from 1995 to 2019 and grey to 2020 and black the rainfall in 2021, between the months January to May.

In this season, rust was detected in Primavera do Leste on December 30th, 2020 and the second detection on January 15th, 2021. Although in this season the rust was detected at the same time as in the previous season, January 1th, 2020, the high rain volumes and frequencies from the end of January to March were higher than the averages of the last 25 years registered in Poxoréu (Figure 10). It is likely that this high rainfall increased rust severity, especially in March for DSS. On the other hand, the FSS, although influenced by interplot interference, due to the proximity of the DSS treatment, even with this high inoculum in March, after harvesting the DSS areas and reduced rain volume and frequency in April and May (Figure 10), a better rust control efficacy was achieved.

These earlier rust detections in the region, in commercial crops, which are normally close to their end cycle, means that, at the harvesting, in early January, there is a great spore dispersion to neighboring crops and from there to the DSS, which justifies the high pressure in these later plantings.

Although 50% of the sprayings were performed in DSS, they were not enough for efficient disease control as shown by severity data. Sprayings were also with the shortest interval of seven days. We should emphasize that the FSS crop was located at 1,500 m far from the DSS and probably due to the spores migration the first two sprayings were at the shortest interval. Similar condition occurred for DSS, on April 13th, requiring three sprayings per week after harvesting DSS.

In the Primavera do Leste, southern region, similar fact happened in one experimental site, indicative of the interplot interference. It is likely that this situation led to an almost uncontrolled rust in DSS, where farm advisers recommended applying weekly or even with shorter intervals when rain occurred soon after the last application. Soybean crop in this month received six out of nine site-specific sprayings, even with multi-site fungicides.

This was the reason for a greater need for fungicide spraying in FSS made in this season in the southern region, a situation that would not have occurred in the absence of DSS, which have only been done because of the non-authorization of planting in February. This was also the trend in FSS in Primavera do Leste, due to interplot interference. To elucidate the importance of the interference among plots and sowing time, we exemplify with the Entre Rios farm case, which received a corn border to separate the FSS plots sown in the February last week. Despite the high rainfall and the disease having been detected a week later than at Canario farm, only two sprays in March and three in April were performed. This site was the one that resulted in the lowest rust severity of all areas in the southern region, indicating that the farther the plot and its isolation from inoculum sources, the better the efficacy of ASR control (Figure 3). This is the situation nearest to the reality of what FSS will be, as long as there are no soybean cultivation in December

In southern region, an increase in the number of sprayings in FSS compared to previous season experiments may be due two reasons, but even so the mean number of sprayings in FSS was lower than in DSS: (i) delay in the official authorization for the FSS, many farmers did not reserve the best areas, therefore, when authorized, they had to choose areas that were close to DSS; (ii) the non-authorization of growing larger areas in FSS, where experimental units were be installed, made the producers sowed most of their crops at the end of Decembrt; (iii) the high DSS inoculum pressure when near the FSS, increasing interplot interference. In one site the farmer had to destroyed part of the DSS to open a small area for the February experiment, which was literally surrounded by the DSS and resulting in the greatest rust pressure. Even in sites where the DSS and FSS were more distant, there was still a strong influence from the DSS inoculum on the FSS.

Soybean predisposition to rust infectons can also be attributed to great leaf area index with a closer canopy in DSS maintaining a longer duration of leaf wetness fulfilling the water requirement for infection of 6-7 h of continuous daily leaf wetness (Melching et al., 1989) and less predisposition in FSS.

Other important point is that the FSS is less subject to the inoculum amount from the surrounding soybean farms than DSS, when the majority have been harvested, and associated with the lower rain volume and mainly frequency as discussed.

Although, rainfall occurring in the DSS cycle was the highest and the lowest in FSS the rust severity should not be related to the rain volume, but rather to its frequency. However, Del Ponte et al. (2006.) have shown relation between rust final severity with total rainfall. But we may question their findings suggesting that the major effect was due to rain frequency.

Limiting the sowing time, as actually recommended, is not the most efficient strategy to make chemical control of soybean rust with more efficacy. The normative Instruction 001/21-INDEA (2021) deals with the regulation of soybean sowing date in order to reduce the number of fungicide sprayings, and so, to reduce the directional selection. But, to achieve this goal, as statiscally shown by our data, do not grow soybeans in DSS but rather in FSS.

The ASR showed the greatest severity in FSS in 2021 season compared to those occurring in the prevoius seasons. This can be explained by the extemporaneous rains that occurred in March, 2021 (Figures 8, 9 and 10) which increased the rust pressure on the DSS and, consequently, greater inoculum pressure for the FSS. Our hypothesis, was comfirmed due to the lowest rain in April and May, even with high inoculum pressure in DSS a situation that would not exist if FSS were authorized, still the efficiency of fungicides showed the feasibility of replacing DSS plantations for FSS.

It is important to discuss that an efficient strategy to fight directional selection has been the reduction of site-specific fungicide applications (Ishii & Hollomon, 2015). To fulfill this objective in Mato Grosso, has been imposed by legislation limiting the soybean sowing time. However, more importante than sowing time limitation is the highest frequency of sprayings reaching *P. pachyrhizi*, performed for several seasons and in a large area, on soybean weeds amid cotton crop where, so far, nothing has been done to be mitigated (Zambolim, 2019; Reis et al., 2021b). A second strategy is the application of co-formulation allways containing site-specific + multissite fungicides as performed in our work (Ishii & Hollomon, 2015).

In summary: DSS: (a) Soybeans receiving maximum inoculum amount from the lagest soybean cultivated surrounding areas; (b) Higher rain volume and frequency during the soybean cycle (Figure 2 and 3); (c) Longer soybean cycle; (d) Demanding larger number of fungicide sprayings; and (e) Decison making for the first spraying empirically taken. February grown soybean: (a) Lower inoculum potential considering that the most surrounding soybean areas have been harvested; (b) Lower rain volume and mainly frequency during the soybean cycle; (c) Fewer number of fungicide application; (d) Use of multiseites in all sprayings; (e) Shorter plant height and lower leaf area index, facilitating the plants coverage by fungicides; (f) Shorter soybean cycle; (g) Decision making to time the first spraying based on the economic damage threshold (EDT) (Danelli et al., 2015).

The results of our research confirm statistically that the sowing period can be changed from the end of December to February, but always with the use of multisites fungicides with the highest efficacy. This change, in accordance with the basic principle of disease control, escape, implies a significant reduction in risks, less environmental damage and a reduction in economic costs and is still in accordance with the principles of IN 002/2015, updated by IN 001/2021.

In relation to *P. pachyrhizi* survival in the state, another point must be discussed: when FSS treatment was in the field there were no other cultivated farms with soybeans and, at the time of its harvest, there were no more volunteer plants where the spores can be deposited. Even if there were voluntary plants, they should not be infected due to lack of leaf wetness required for the infection (Melching et al., 1989) and f *P. pachyrhizi* survivalo in the off-season. It is reinforced that FSS, unlike those of DSS, ensure a greater economic sustainability of the crop both for the farmer who make their own seed and for the others who did not suffer the inoculum pressure of these late sowings.

The seed quality, mainly vigor, is one of the main factor for agriculture success. Therefore, the best seed quality obtained in FSS also would require a shorter storage period resulting in still better quality. Seed producers in Mato Grosso state are encouraged to use the technology generated in our work to produce quality seeds, especially producing and storing them in refrigerated warehouses in regions far from the current producing region, for example along the BR 163 axis, where there is no tradition for seed production.

Regarding soybean crop sustainability, we should consider the economic and environmental aspects. In the DSS, compared to FSS, due to the reduction in the fungicides applications, the exchange of sowing times represents economic and environmental advantages for producers who conducted the experiment in their fields. They will have better seed quality, due to reduced rainfall at harvest and shorter storage period, which will already represent gains in productivity, in addition to the fact that they have at their disposal the materials they need at the seeding time. This possibility of seeding in other times can also be extended to commercial seed producers, as they have also seeding in December to complement seed amount that were not able to produce during the normal season. They are certainly looking for seed amount and quality, which will also result in better crops for their customers and a reduction in the number of complaints. It is feasible that, wheather the supervised seed producers use FSS as the better sowing time with better seed quality and reduced storage costs, the other farmers may reduce their need to produce their own seed considering the improving seed quality.

In relation to quality, data from samplings carried out by Aprosoja-MT, among its members, show that in recent seasons there has been a significant reduction in germination and vigor of commercialized seeds among soybean producers in MT.

Taking into account, the site-specific fungicides efficacy reduction, by having a smaller spraying number, and allways combined with associated multissites, this will result in less selection pressure on the fungus populations, which will also represent lower demand for fungicides in the following crops. Less selection pressure results in less chemical usage or maintenance of current levels thus reducing costs to producers and less chemical pressure on the environment and increasing farmers profitability through lower production cost and better control of ASR throughout the state and even in the country.

In relation to the efficacy reduction of site-specific fungicides, by having a smaller number of applications, and allied to the multissites use, this will result in less selection pressure on the fungal populations, which can represent lower use of fungicides for the following crops. Less selection pressure results in less chemical usage or maintenance of current levels thus increasing profit through lower production cost and better ASR control. It is regrettable that public research institutions, as well as the sanitary defense agencies, are not aware of this situation and, together with the research, seek an alternative to final season seeding.

Another question that deserves discussion, since the subject seems to have taken more political than scientific course is the discussion of royalty payment for the used technology when multiplied and used as seeds by the farmers. Both the growers and Aprosoja - MT agree with royalties payment for the used germoplasm.

Finally, no information was found in the consulted scientific literature to support the DSS considering the principles of integrated disease management. On the contrary, once again based in scientific method has been shown the advantages of soybean cultivation in February in Mato Grosso state.

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Fungi Resistance to Multissite Fungicides

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Abstract

Multisite fungicides have been used for many years in fruit and vegetable crops worldwide. Cases of the fungi resistance development to these fungicides have been rare. From the 2002 season onwards, with the outbreak of Asian soybean rust in Brazil, caused by *Phakopsora pachyrhizi*, site-specific fungicides became the main weapon for its control. From 2002 to 2011, penetrant mobile site-specific fungicides were used and until today in double (DMI + QoI) or triple (DMI + QoI + SDHI) co-formulatoons in an area of more than 30 million hectares and with three sprays per area. This resulted, as expected, in the fungus sensitivity reduction, today with cross and multiple resistance to those site-specific fungicides. From the 2011 season in an attempt to recover control that for some chemicals and mixtures reached < 30%, research was started with site-specific + multi-site mixtures, taking as example *Phytophthora infestans* resistance development to metalaxyl in Europe showinig long-lasting solution found by the addition of multisite mancozeb. It is expected that the effective life of site-specific + multi-site mixtures may be as long in controlling soybean rust as it has been for potato, tomato and grape downy mildews. This review presents the concepts involved in the sensitivity reduction to fungicides. Some fungal species and fungicides involved are listed. Considering the *P. pachyrhizi* sporulation potential, the great soybean area sprayed and the number of sprays per area mainly with site-specific co-formulations and the reduced area sprayed with multisites, we discuss the need for annual monitoring of *P. pachyrhizi* sensitivity to the these chemicals.

Keywords: arylaminopyridine, chloronitriles, dithiocarbamates, phthalimides, reduced fungal sensitivity

1. Introduction

Between 1940 and 1970, fungicidal organic compounds with a broad spectrum of activity were developed by the plant protection chemical industry. Most of them were multisite inhibitors, *i.e.*, captan, chlorothalonil, folpet, mancozeb whose fungicidal action is attributed to their reaction with proteins containing sulphydryl groups (*i.e.*, glutathione, glyceraldehyde 3-phosphoate dehydrogenase or alcohol dehydrogenase) or proteins not containing thiols (*e.g.*, α-chymotrypsin or lysozyme) in the cell (Klitthich, 2008).

Mobile-penetrating fungicides necessarily have an intimate association with plant biochemistry and physiology and their modes of action are specific and usually involve only one biochemical mode of action (Hollomon, 2015). The market introduction of these fungicides over 50 years ago (*i.e.*, benomyl in the 1970s) revolutionized the chemical plant protection, showing high efficacy and low toxicology for the control of diseases caused by fungi. However, it was soon found that plant pathogenic fungi can easily and quickly adapt to these fungicides through mutations (among other resistance mechanisms) that lead to reduced efficacy (Klittich, 2008; Mosbach et al., 2017).

Reducing sensitivity to fungicides involves a fundamental property of fungi, the ability to adapt to different environmental conditions, usually adverse, and thus to survive. Frequent use of fungicides creates an adverse environment for a fungus that was previously sensitive to a particular compound, leading to its adaptation and resistance to the new situation (Bardas et al., 2008; Kretschmer, et al., 2009).

From the 1970s, the resistance of phytopathogenic fungi became a problem with the predominant use of mobile-penetrating fungicides that were site-specific (Klittich, 2008).

On the other hand, the resistance of fungi to multisite fungicides (arylaminopyridine, chloronitriles, dithiocarbamates, copper, tin and mercury derivatives, phthalimides, sulfur, etc.) is still a rare event. The difficulty is due to the low probability of the occurrence of the minimum necessary number of mutations at different loci in the same fungus. On the contrary, with the introduction and repeated use of site-specific, acquired resistance has become common but incomparable to multisite (van den Boch & Gilligan, 2008).

2. Basic Concepts

2.1 Fungicides

Fungicides are synthetic or natural chemical compounds, or biological organisms capable of killing or inhibiting fungi, or the germination of fungi and oomycete spores (Mueller et al., 2013).

2.2 Fungitoxicity

Fungitoxicity is the property that a chemical substance has of being toxic to fungi and stramenopylae (pseudo fungi or chromists) in low concentration. This property is a molecule attribute.

2.3 Mode of Action, Mechanism of Action or Biochemical Mechanism of Action

The chemical structure of the fungicde active ingredient (a.i.) defines its mode of action by determining its uptake, movement in the plant, and its ability to reach and bind to the site of action—the physical location where the fungicide acts (Delp & Dekker, 1985). Mode of action is the process by which a chemically active substance produces an effect on a living organism or on a biochemical system. Or, the mechanism refers to the biochemical interaction through which the substance produces its toxic effect (Hewtt, 1988; Latin, 2017; Mueller et al., 2013).

2.4 Site of Action

Site of action, or target site, are specific enzymes in cellular processes to which the fungicide binds (Hewitt, 1988).

2.5 Sensitivity (of Sensitive, That Feels)

Property of the fungus to receive changes from the environment and to react to them. Sensitivity is an attribute of the fungal species (Reis et al., 2019).

2.6 Insensitivity

Not all fungi are sensitive to all fungicides (spectrum of action); some are always insensitive to certain molecules. For example, fungi of the genera *Alternaria*, *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* are insensitive to benzimidazole fungicides; on the other hand, benzimidazoles are not fungitoxic to these genera. Another example is the insensitivity of oomycetes, which cause mildews, to triazoles and benzimidazoles (Reis et al., 2019).

A fungus sensitive to a fungicidal molecule may have altered sensitivity, which is why it is said to have developed resistance. However, an insensitive fungus will never become sensitive.

2.7 Control Failure

The resistance of plant pathogenic fungi to fungicides is observed as a control failure or as a reduction in the performance of the fungicide; in this situation, farmers often react by increasing the dose and/or by reducing the interval between sprayings. In the next step, field experiments confirm the control failure. Situation in which the farmer observes that, when compared to previous crops, the fungicide efficiency was reduced. He says that there was a "failure of control" and starts to complain and seeks explanations for the fact (Reis et al., 2019).

2.8 Loss of Sensitivity

The word loss implies total insensitivity, which is not always true. Nevertheless, the concept of loss can be delimited following the Edgingnton & Klew (Edgington & Kew, 1971) criterium. Thus, it can be considered as sensitivity loss, or non-toxic, when the fungus presents inhibitory concentration, IC_{50}, > 50 mg/L to a fungicida, and when lower than 50%, sensitivity reduction.

2.9 Sensitivity Reduction

Reduction is a slow process, requiring the application of a site-specific fungicide for many seasons and over a large area such as *P. pachyrhizi* and the DMIs (FRAC group 3, demethylase inhibitors), QoIs (Group 11, quinone outside inhibitors) and SDHIs (Group 7, succinate dehydrogenase inhibitors) fungicides. The reduction is present when the inhibitory concentration (IC_{50}) increase over time for the mycelial growth, spore germination or disease control. Therefore, in most cases, what is happening is a slow reduction instead of sensitivy loss.

Molecular techniques are useful in proving the presence of reduced sensitivity after resistance has been quantified in laboratory bioassays (Hollomon, 2015).

2.10 Erosion of the Fungicide

Expression taken as a synonym for reduced sensitivity of a fungus to a given fungicide (Hahn, 2014).

2.11 Resistance

Fungicide resistance is the result of the adaptation of a fungus to a fungicide due to its stable hereditary genetic alteration leading to the emergence and spread of mutants with reduced sensitivity to the fungicide (Delp & Dekker, 1985). The term proposed by FRAC (2019) refers to a stable and hereditary adjustment of a fungus to a fungicide, resulting in a reduction in the pathogen sensitivity. This adjustment results in a 'considerable' reduction in the sensitivity of the pathogen to the chemical compound, which can be partial or total, always with an increase in the IC_{50} [sensitivity reduction factor (SRF)] > 1.0. This ability is gained through evolutionary processes (Mueller el al., 2013).

2.12 Cross-Resistance

Fungicides of the same chemical group, for example tebuconazole and cyproconazole, have different chemical structures. However, both have the same toxicity to fungi. Therefore, both are considered demethylation inhibitor fungicides (DMI), a name that expresses the same-shared mode of action. This fact means that even if you rotate two within the same fungicide group, the fungus detects them as being the same fungicide. It also means that if resistance develops for one member of the group, it will be present for all other members of that family. The resistance is called crossed reaching all group members (EPPO, 1998).

2.13 Multiple Resistance

When fungicides of different chemical groups, *i.e.*, carboxamides, strobilurins, triazoles) and with different mechanisms of action (DMI, QoI, SDHI) the reduction in sensitivity affects everyone equally (EPPO, 1988).

2.14 Multiple Drug Resistance (MDR)

It is the sensitivity reduction to various fungicides with different modes of action shown by a fungus specie. MDR is defined as the acquired sensitivity reduction of at least one fungus to at least three fungicides with a distinct mechanism of action. The main resistance mechanism involved here is the overexpression of the efflux transporter genes present in the plasma membrane. It results in increased cellular expulsion of the fungicide reducing the fungus sensitivity to several unrelated fungicides (Alekshun & Levy, 2007; Chapman et al., 2011; Chen et al., 2017; Hahn & Leroch, 2015; Leroux et al., 2002).

2.15 Acquired Resistance

It refers to a fungus that in the wild state was sensitive to the fungicide and that developed resistance after exposure to the chemical (Hollomon, 2015). This is what happens with fungicides applied to control diseases in the field.

2.16 Site-Specific, Monosite or Unisite Fungicide

Of the millions of biochemical reactions that take place in the fungus cell, the site-specific fungicide (monogenic resistance) interferes with only one biochemical site (an enzyme). This is a vital enzyme for the fngus physiology, so if it is blocked, the fungus will die. Fungicides with a site-specific mode of action are at high risk for the development of resistance compared to multiple-site fungicides (Mueller et al., 2013).

2.17 Multisite Fungicide

It refers to the fungicide that paralyzes at least five metabolic processes of the fungus (Mueller et al., 2013). For this reason, the development of resistance to them has not yet been frequently reported.

The main multisite fungicides in use in Brazil are: captan, chlorothalonil, cuprics (copper hydroxide, copper oxychloride, cuprous oxide, basic copper sulfate), dithianon, famoxadone, fluazinam, fludioxonil, mancozeb, pencicuron, pyrimethanil and thiram.

2.18 Site-Specific and Penetrant Mobile

Many use these two terms considering that all site-specific are penetrant mobile, however iprodione is a site-specific, signal transduction inhibitor, non-penetrating with protectant and some eradicant activity (PPDB, 2021).

2.19 Fungicide Effective Life

The effective life of a fungicide is the time from its introduction on the market for use in the control of a given fungus, until the moment when efficient control is no longer obtained due to the development of resistance of the target fungus (Hobbelen et al., 2011).

2.20 Mechanisms of Fungi Resistance to Fungicides

How do fungi defend theselves against fungicides? There are four main mechanisms by which fungi become resistant to fungicides.

For a better understanding of the mechanisms, the functions of cell organelles involved in the defense mechanisms of fungi are briefly reviewed.

2.21 Plasma Membrane

The cell, or cytoplasmic, membrane is a biological membrane that has selective permeability to organic molecules and ions. Controls the movement of substances into and out of the cell. This membrane is made up of a double layer of phospholipids and interspersed with proteins embedded in it. The membrane is said to be semi-permeable in that it can let a substance (molecule or ion) pass freely, pass through a limited form, or pass at all. Membranes also contain receptor proteins that allow cells to detect external signaling molecules such as hormones (Ishii & Hollomon, 2015).

The main mechanisms of fungi resistance to fungicides are:

(a) Substance transport across the plasma membrane: To reach the intracellular organelles, the fungicide has to cross the plasma membrane with a complex constitution.

There are three forms of transport across the cell membrane (Alekshun & Levy, 2007; Ishii & Hollomon, 2015; Ward et al., 2006). The movement of substances across the membrane can be passive by simple diffusion, or by diffusion facilitated by the transport proteins channel following a positive concentration gradient and active, with energy consumption (ATP) against a concentration gradient.

(b) Change in target site reducing sensitivity to fungicide: The most common mechanism of resistance is a change in target site (enzyme) in the fungus and occurs only with site-specific fungicides, which dominated the market after 1970. Multisite fungicides, most of those developed since 1969, are not prone to the development of resistance at the target or action site. As the fungus grows, its DNA is replicated when new cells are created. This replication process is imperfect and errors can occur. Such errors are known as mutations. DNA is the code used to produce enzymes in the cell, and some mutations result in a change in the target site's amino acid sequence which in turn alters the shape of the receptor site (lock) of the fungicide. Thus the (key) fungicide may not fit into the site (lock) resulting in a partial or total reduction of the fungus' sensitivity to the fungicide. Therefore, an alteration by mutation in the fungicide target of action reduces the drug fitness through this target site, resulting in reduced sensitivity (Ishii & Hollomon, 2015).

(c) Gene overexpression: Gene overexpression is the abnormal production of large amounts of a substance which is encoded by one or more genes. In the case of overexpression, the target enzyme does not undergo any change (mutation). Instead, the pathogen produces it in large quantities (Cools et al., 2012).

For example the overexpression of the Cyp51 gene. Azole fungicides (DMIs) inhibit the Cyp51 gene encoding the demethylase enzyme involved in the ergosterol biosynthesis process. The fungus to defend itself from the effect of the fungicide increases the production of the enzyme in order to produce much more enzyme (demethylase) so that ergosterol is still produced even in the presence of the fungicide. Due to the increased production of the enzyme, the amount of fungicide present in the cell is not enough to couple with all the available enzyme, completely blocking the production of ergosterol. This leaves an amount of free enzyme without the coupling of the fungicide, producing enough of the ergosterol to keep the cell alive. In this case, the amount of fungicide is not enough to completely inhibit ergosterol production. Gene overexpression results in greater production of demethylase beyond normal. Therefore, even though triazole inhibits part of its synthesis, there is still an amount of enzyme remaining, maintaining the cell's functional activity (Alekshun & Levy, 2007; Hahn & Leroch, 2015; Leroux et al., 2001; Price et al., 2015; Ward et al., 2006).

(d) Exclusion of the fungicide from the cell: The cell's efflux is the elimination of a certain substance from its interior to the outside. Active efflux is a condition where pathogen cells pump the fungicide out of the cell faster than it accumulates to a toxic concentration. However, the target site remains unchanged. Active efflux prevents the accumulation of sufficient concentration to stop cell function and fungus growth.

Unlike influx, entry to the interior of the cell, efflux pumps occur naturally in cells that exclude or expel foreign substances or import substances useful to their metabolism. In fungi, the most common efflux pumps are protein transport pumps. Occasionally, these transporters succeed in expelling sufficient amounts of the fungicide from within the cell. Transport or carrier proteins in the plasma membrane are responsible for the active efflux of foreign material, including fungicides (Price et al., 2015).

There are two types of efflux: (i) Passive efflux is the expulsion of a certain substance to the outside of a cell (movement, or passive diffusion). (ii) There is also the participation of an efflux pump, which consists of the active pumping of the fungicide from the intracellular to the extracellular environment, that is, the active efflux. Efflux pumps are transmembrane proteins that can act to expel fungicides against a concentration gradient. There may also be an oveexpression of efflux pumps consisting of an increase in the concentration of their number (Hahn & Leroch, 2015). Multiple drug resistance is related to the overexpression of transport proteins.

(e) Detoxification or molecule inactivation by thiols overproduction: Substances that inactivate molecules of fungicides, has been suggested as the most likely mechanism that confers fungal resistance to mancozeb (Barak & Edgingtom, 1984; Gilpatrick, 1982; Yang et al., 2019). However, the mechanisms that confer resistance to this fungicide are questionable and very complex to be clarified. The main genomic and molecular study was carried out with the yeast *Saccharomyces cerevisiae*, having determined 286 genes that would be involved with resistance to a xenobiotic (Dias et al., 2009).

Many papers have been published on the fungi resistance to multisite fungicides, some were selected as an example (Table 1).

Table 1. Reports of fungi resistance to multissite fungicides

Year	Plant specie	Fungus	Fungicide	Resistance mechanism	Reference
1965	-	*Aspergillus niger*	Mercury	-	Ashworth & Amin (1964)
1966	Oat	*Drechslera avenae*	Mercury	Inactivation by a group of thiols	Nobel et al. (1966); Ross & Old (1973)
1968	Oat	*D. avenae*	Mercury	Inactivation by a group of thiols	Malone (1966)
1971	Oat	*D. avenae*	Phenylmercury acetate	Mercury chelation by the production of red pigments	Greenaway (1971)
1973	Apple	*Venturia inaequalis*	Dodine	Unknown	Szkolnik & Gilpaterick (1973)
1976	Apple	*V. inaequais*	Dodine	Unknown	Jones & Walker (1976)
1976	Apple	*V. inaequalis*	Dodine	Unknown	Yoder & Kloss (1976)
1977	Apple	*V. inaequalis*	Dodine	Unknown	Ross & Newberry (61)
1978	Beet	*Cercospora beticola*	Triphenyl tin acetate and triphenyl tin hydroxide	Unknown	Giannopolitis (1977)
1980	Pimentão Pepper	*Colletotrichum capsici*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Thind & Jhooty (1980)
1980	-	*Rhizopus stolonifer*	Copper	Physiological adaptation to Cu	García-Toledo et al. (1980)
1980	Beet	*Cercospora beticola*	triphenyltin	-	Giannopolis (1978)
1984	-	*Botrytis cinerea*	Chlorothalonil, captafol, folpet, thiram	Inactivation of the fungicide molecule by overproduction of thiols	Barak & Edgington (1984)
1982	Apple	*V. inaequalis*	Dodine	Unknown	Gilpatrick (1982)
1989		*Botrytis cinerea*	Dichlofluanid	Unknown	Malathakis (2006)
1989	Apple	*V. inaequalis*	Dodine	Unknown	Sholberg et al. (1089)
1989	-	*Exserohilum rostrata*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Reddy & Anilkumar (1989)
1993	Pesseguiro	*Taphrina deformans*	Copper hydroxide	Unknown	Cheah et al. (1993)
1994	-	*Trichoderma viridae*	Cupric	Metallothione in chelation mechanism	Cervantes & Gutierez-Corona (1994)
1995	-	*Phytophthora infestans*	Chlorothalonil	Inactivation of the fungicide molecule by overproduction of thiols	Sujkowsky et al. (1995)

1998	Potato	*P. infestans*	Chlorohtalonil	Inactivation of the fungicide molecule by overproduction of thiols	Cooke et al. (1998)
1998	Beet	*Cercospora beticola*	Triphenyl tin hydroxide	Unknown	Campbell, et al. (1998)
1999	Apple	*V. inaequalis*	Dodine	Unknown	Köller et al. (1999)
2002	-	*Botryotinia fuckeliana* (*Botrytis cinerea*)	Fludioxonil	-	Vignutelli et al. (2006)
2003	-	*B. cinerea*	Fenilpirrole	-	Baraffio et al. (2003)
2003	Potato	*Alternaria solani*	Chlorothalonil	Inactivation of the fungicide molecule by overproduction of thiols	Holm et al. (2003)
2005		*A. brassicae*	Fenilpirrole	Unknown	Avenot et al. (2005
2007	Mango	*C. gloeosporiodes*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Kumar et al. (2007)
2006	Greenhouse vegtables	*Botrytis cynerea*	Dichlofluanid	-	Malathrakis (2006)
2008	-	*Penicillium digitatum*	Fludioxonil	Unknown	Kanetis et al. (2008)
2008	Rubber tree	*C. gloeosporiodes*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Cai et al. (2008)
2008	Rice	*Helminthosporium oryzae*	Maneb	Inactivation of the fungicide molecule by overproduction of thiols	Gupta & Kaiser (2008)
2008	Apple	*C. gloeosporiodes*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Tam et al. (2008)
2009	Banana	*Mycosphaerella fijensis*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Aguilar-Barragan et al. (2014)
2009	-	*C. gloeosporiodes*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Ferreira et al. (2009)
2010	Apple	*V. inaequalis*	Dodine	Unknown	Carisse & Jobin (2010)
2010	-	*C. acutatum*	Mancozeb	Inactivation of the fungicide molecule by overproduction of thiols	Gullino et al. (2010)
2010	Beet	*C. beticola*	Triphenyl tin hydroxide	Unknown	Secor et al. (2010)
2011	Wheat	*Septoria tritici* (= *Zymoseptoria tritici*)	Chlorothalonil & folpet	Overexpression of thiols that inactivate the fungicide	Beyer et al. (2011)
2011	-	*B. cinerea*	Fludioxonil	Unknown	Webber (2011)
2013	-	*B. cinerea*	Fludioxonil	Unknown	Leroux & Walker (46)
2013	Strawberry	*B. cinerea*	Fludioxonil	Unknown	Fernandez-ortunõ et al. (2013)
2013	-	*B. cinerea*	Fludioxonil	Unknown	Leroch et al. (45)
2013	Potato	*A. solani*	Chlorothalonil	Inactivation of the fungicide molecule by overexpression of thiols	Fairchild et al. (2013)
2014	-	*B. cinerea*	Fludioxonil	Unknown	Hahn (2014)
2015	-	*A. alternata*	Mancozeb	Inactivation of the fungicide molecule by overexpression of thiols	Malandrakis et al. (2015)
2016	-	*B. cinerea*	Fludioxonil	Unknown	Ren et al. (2016)
2017	Apple	*Colletotrichum* spp.	Mancozeb	Inactivation of the fungicide molecule by overexpression of thiols	Moreira et al. (2019)
2018	Potato	*P. infestans*	Fluazinam	Inactivation by conjugation of fluaziam with glutathione	Schepers et al. (2018)
2019	Apple	*C. acutatum*	Mancozeb	Inactivation of the fungicide molecule by overexpression of thiols	Moreira et al. (2019)
2019	-	*A. alternata*	Mancozeb	Inactivation of the fungicide molecule by overexpression of thiols	Yang et al. (2019)

3. Final Remmarks

Although, fungal resistance to iprodione, a nonpenetrant fungicide, has been reported, it was not included in this review due to its site-specific mode of action.

The number of site-specific fungicide molecules marketed is considerably higher than multisite. From the 1970s onwards, site-specifics dominate the world market, being used to control diseases in a greater number of plant species, in a larger area and with a greater number of sprayings per season, in addition to their high risk to resistance development. This has resulted in the largest number of citations of site-specific resistance.

It is likely that for all commercialized, both multisite and site-specific fungicides, regardless of their active principle and resistance mechanism, at least one fungus resistant to them has already been reported. However, as site-specifics dominate the market, the large volume published focuses on this group.

Based on the consulted literature, even new site-specific mechanisms of action developed in the future will have the potential to select, in a few seasons, fungi resistant to them, shortening their effective life.

Although, Bordeaux mixture is considered the oldest foliage protectant fungicide, developed in 1885, no resistance of *Phytophthora infestans* (Mont.) de Bary to this fungicide was found in the consulted literature. Perhaps it is the fungicide with the longest effective life in the history of downy mildew chemical control on potatoes, tomatoes and grape. Are the cuprics the hardest to be defeated by fungi? In this sense, in the consulted literature only two reports of reduced sensitivity of fungi to cupric fungicides were found, but to a large number of species of phytopathogenic bacteria (Lamichane et al., 2018).

In the available literature, no reports were found on rust fungi resistant to mutissites.

It would also be important to determine the time required since it first use to the emergence of resistance or the duration of their effective life. According to the FRAC (2019), fungal resistance to pencycuron (phenylurea; recommended for the control of *Rhizoctonia solani* in the treatment of potato tubers) and to tricyclazole (triazolobenzothiazole) penetrant-mobile for the control of *Pyricularia oryzae* Cav in rice has not yet been reported.

Should the chemical industry continue to synthesize site-specific fungicides, as it has been doing intensively, even with a relatively short effective life as is happening with the new carboxamides towards *Phakopsora pachyrhizi* Sydow & Sydow.

In Brazil, the greatest use of multi-site fungicides (chlorothalonil, mancozeb and copper oxychloride) has been in soybean crop, to control *P. pachyrhizi*, the causal agent of Asian rust. Its use began in 2010/11, therefore beeing used in the last 10 seasons. To give an idea of the selection pressure that multi-sites are subject in soybean crop, in the 2020/21 season, the area cultivated with soybeans was > 38 million hectares, with 2.6 sprayings/ha, but with multi-site in an area of only 12%. What can happen with these multi-sites in the control of soybean rust under this situation? Should multissítes be used alone for ASR control?

At the moment, in Brazil, the use of multisite is the main weapon to face the development of *P. pachyrhizi* resistance to mobile penetrant site-specicif fungicides.

Multiple resistance is present in *P. pachyrhizi* to DMIs, QoIs and SDHIs and even so, these fungicides are applied in the largest area of soybean, without the multisite mixture, and thus, their efficacy has been reduced season after season. If the efficacy, which is already low, but has been reduced season after season, reaches < 30%, could the addition of multi-site revert the situation? Would multi-sites be used solo because they have superior control than site-specific?

Considering the chemical control of ASR in Brazil, with the well-defined presence of cross and multiple resistance to site-specific, reflected in a constant sensitivity reduction evolution of *P. pachyrhizi*, season after season, we will reach a situation in which the most efficient control would be achieved with multisites solo? Therefore, would multisites withstand the enormous selection pressure for resistance?

Let us remmember the development of *P. infestans* resistance to metalaxyl (in the 1977) and the solution given by the ready-made commercial mixture with mancozeb (in the 1980) would not be an indication that this would be the practice to be pursued in Brazil for economically sustainable control and fungicide with long effective life in controlling soybean rust? What has been the effective life of the metalaxyl + mancozeb mixture in controlling mildews and whether cases of mildew resistance to this mixture or similar ones has been reported? In the same direction, and similarly to ensure a long effective life in the control of *P. pachyrhizi,* the use of ready-made, liquid commercial mixture, containing IDM (prothioconazole and/or tebuconazole) and IQe (picoxystrobin and/or trifloxystrobin) + multisite (chlorothalonil), or mancozeb, or copper oxychloride) would be a solution?

The exposure time of *P. pachyrhizi* to multi-site fungicide is still too short to make a judgment about their effective life.

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Effect of Coronavirus on Aquaculture in Oyo state, Nigeria

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Abstract

Coronavirus 2019 is a global health concern that has left most countries in a state of severe economic meltdown. Scientific research has been down on the virus and its impact on various sectors but that of the Nigerian aquaculture industry has been missing. This paves the way for this research to aim at bridging this gap by looking at the perception of fish farmers on the influence of coronavirus on their activities, the challenges they face during the period of the virus, and the coping strategies adopted to mitigate the impact of the virus. The research used cross sectional survey design with the sample size being 11 fish farmers living in Oyo state, Nigeria. Homogeneous purposive sampling was used and primary data collected through the use of google form. The data collected was analysis using SPSS version 25.0. The result of the analysed data showed that: on socioeconomic characteristics; the majority of the respondent reported that Coronavirus has had an effect on their fishing activity and they were mostly small scale farmers with catfish being the predominate fish farmed. The majority of fish farmers perceived demand decline, high cost of production, fish being more expensive, and reduction of manpower on the farm due to lockdown measures. Reduction in walk-in customers to the farm was revealed as the major challenge posed by the pandemic, while the inability to get technical support as least. On coping strategies adopted, it was revealed that farmers have resorted to the development of their own feed.

Keywords: effect, Coronavirus, aquaculture, Nigeria

1. Introduction

The novel Coronavirus disease (COVID-19) from the family coronaviridae (subfamily Orthocoronavirinae), a single-stranded RNA virus measuring 80 to 120nm in diameter is broadly distributed in mammals (Sharma et al., 2020). The virus was first reported in December 2019 in Wuhan, China (Wu et al., 2020). It was declared a public health emergency of international concern on 30 January 2020, and as a pandemic on 11 March 2020 by World Health Organisation. The virus primarily spread through close contact and small droplets produced when infected patients cough, sneeze, or talk (World Health Organisation, 2020; Chinese Center for Disease Control and Prevention, 2020). The virus has spread to 110 countries, with an estimated 11 million people infected as of 2 June 2020. Africa recorded its first case on 14 February 2020 in Egypt and Nigeria on the 27 February 2020 (Gilbert & Gubar, 2020). The advent of the virus has led to a global health crisis, which has disrupted the economic system, security, and health of many countries. As some countries try to return to normality, the full economic impact of the virus is yet to be ascertained.

Aquaculture is a fast-growing agro-industrial activity with the potential to outpace population growth (Van der Merwe, 2015). The sector production has expanded by 12 times at an annual average of 8.8% (FAO, 2009). In 2012, FAO estimated that the total production of farmed fish stood at $119 billion from the over 300 farmed fish species worldwide. The world distribution of aquaculture shows that Asia, mainly China, contributes about 90% of the total aquaculture production output, with only 1% from Africa. In Nigeria, aquaculture has been considered as an alternative to the declining capture fisheries, accounting for 85,000 metric tons in 2010 (Akinrotimi, Abu, & Aranyo, 2011), and with demand for its output growing with increasing population.

Although COVID-19 does not directly affect fishes. It has however an indirect impact on the aquaculture sector through altering demand and supply patterns, logistical problem, reduction in the level of livelihood of fish farmers, and the serious health and food security consequence for people who depend on fish for their animal protein and essential micronutrients (Grema et al., 2020). COVID-19 which emerged from China, the major fish producer in the world will be the most hard-hit, hence all countries it export fish product to including Nigeria. In

Nigeria, It is expected that on the supply side, the country will be hurt by the shortage of labour that will because due to lockdown restriction. Access to input and output market is also expected to be hindered by the restriction on movement and also the loss of food resulting from the disruption of the aquaculture value chain. Whiles, on the demand side, it is expected that demand for aquaculture products which is generally inelastic will cause price hikes. The resulting impact will be the shift of fish consumers to other protein products like beans, eggs, and meat.

Most literature on COVID-19 has paid attention to the socioeconomic, and economic impact of COVID-19 at the expense of the impact of COVID-19 on aquaculture in Africa and the world. This paper further contributes to our understanding on real effects COVID-19 has placed on aquaculture farmers and pathways that could help to mitigate such effects in foreseeable future.

1.1 Research Objectives

Hence, this research will seek to:

(1) identify the socioeconomic characteristics of fish farmers in Nigeria;

(2) examine the perception of fish farmers on the influence of COVID-19 on aquaculture;

(3) identify challenges faced by fish farmers during COVID-19, and;

(4) coping strategies adopted to mitigate the impact of COVID-19 on Nigeria aquaculture.

1.2 Research Hypothesis

H_0: There is no significant relationship between the socioeconomic characteristics of the respondent.

H_1: The paired challenges faced by farmers during COVID-19 are not different.

2. Literature Review

2.1 Aquaculture

Aquaculture, which is farming of seaweeds, crustaceans, bivalves, and fish, is a fast-growing sector of the food production industry in most economies around the world (Cai & Leung, 2017). The rapid growth has been captured to be between 10- 15% over the last decade (Brugère et al., 2019). Pauly and Zeller (2017) reported that aquaculture growth between 1996 and 2001 saw a 10.8million tons increase in production. The rapid growth of the sector according to Cisneros-Montemayor and Vincent (2016) is due to the growing population of the world, and the increasing demand for fish and fish products. The traditional catch system has failed to meet the growing demand, hence the improved growth of aquaculture. The growth pattern reported by Pauly and Zeller (2017) revealed that aquaculture was fast growing among developing countries at a rate of 10% as against 3.7% of developed countries.

In Nigeria, aquaculture after a series of failed attempts, has gain ground as the country moves to meet its projected annual 1.5 million tons of fish demand. Aquaculture accounted for 43,950 tons in 2004 of the total fish production of the country (Eriegha & Ekokotu, 2017). This has led to the realization of aquaculture as a potential to diversifying farmer livelihood and income. The system of aquaculture production practiced in the country uses mostly earthen ponds, while ponds, raceways, and tanks are also gradually gaining popularity. Urban and peri-urban areas of the country due to the scarcity of land and growing problem of urbanization have led to the development and usage of recirculatory aquaculture systems (Haldén, Lindberg, & Masembe, 2014). Small scale fish farms account for 70% of fish produce under aquaculture as the small scale farms vary from commercial subsistence to non-commercial subsistence farming (Kawarazuka, 2010). The most farmed fish species in Nigeria are the North African catfish, African bony tongue, and Nile Tilapia by small scale; O. niloticus, C. gariepinus, and H. bidorsalis by medium scale. Large scale combines the small scale and medium farmed fish with *Gymnarchus*, *Atlanticus*, *Heterobranchus* spp., Hybrid catfish, common carp, Aba, Flathead mullet among others.

2.2 Challenges Faced by Aquaculture Sector in Nigeria

Nigeria with its estimated 1.75 million hectares of suitable sites for the development of aquaculture is still far from achieving its full potential (Adewumi, 2015). A sector that was developed with the hope of making the country self-sufficient in its fish production, base on its high reliability in return on investment and low capital intensity relative to catch fisheries, is yet to be felt (Nchuchuwe & Adejuwon, 2012). The sector as it currently stands is under developed despite it being a large source of livelihood to fish farmers and people that derive their livelihoods in coastal areas of the country. Aquaculture's contribution to the total fish production in Nigeria is insignificant. Adeoye and Elegunde (2012) attributed this to the monoculture (farming only catfish) nature of the

Nigerian aquaculture sector. Therefore, the challenges of catfish tend to hinder the production capacity and aquaculture development of the country. The country has to find ways to overcome its annual 1.5 million tons of fish deficit. According to Morgan et al. (2017), the disruption of the production process has led to a large time of farms failing to attain profitability status. These disruptions in the production process may come as a result of the delay in the delivery of fish seeds, fingerlings, and even products like feed, and among others. Financial risk situation of farming makes loan acquisition difficult for farmers, forcing them to restore to small scale production. The lack of adequate technology and technical knowledge in fish farming production are some of the initial challenges fish farmers face. The genesis of these problems is the result of poor government policies toward the sector.

Secondly, Land which is the paramount resource to the start of fish farms is readily available. Though readily available, the land acquisition systems variation of the country varies and these lands sometimes are litigated (Adeogun et al., 2007). The location of the land also determines how the land will be used and the type of fish farming to be adopted by a farmer. Farmers who stay along swampy areas are more likely to adopt earthen ponds. According to Famakinwa et al. (2017), land availability for fish farming is entangled with variables such as population, the land tenure system, development of the country, and level of technology. Therefore, as population increases, land for agriculture are traded off for accommodation, meaning that the number of fish ponds will have to be limited to fit the land area. Land tenure systems ownership and rights, other than that which is based on outright purchase by a farmer or investor, will imply that after a farmer's leases or rent is over, the farmer will have to sell the ponds or destroy them. This is so because the land reverts to the owner per the status under which it was acquired by the farmer or investor. This in most cases, makes the use of earthen pond difficult as most rented and leased lands use plastic tanks to cultivate and produce fishes for targeted markets in Nigeria.

Furthermore water, which is the home and backbone of the aquaculture system is overly polluted by mining activities and industrial waste. Olowosegun et al. (2005) and Omitoyin and Tosan (2012) reported that water and water bodies in major part of Nigeria are highly toxic due to the release of waste into them by industries and the oil exploration, spillage and dredging of oil companies. The polluted nature of water and viable water bodies make the aquaculture production sector unattractive in these areas, especially in and around the Delta and River states of Nigeria. However, those who still practice aquaculture have to treat the water, especially shared water flows, before they can use it for production. This leads to high production costs to the fish farmer. Lastly, marketing and distribution channels are ineffective in the country. Oluwatayo and Adedeji (2019) noted that the transportation system for fish and fish products to be made available to markets (consumers) is bad, as output goes wasted in most cases. Due to the perishability of fish, the few that gets to the market sometimes becomes unsold or sold at a lower price, since consumers are not willing to pay a high price for produce that are near their shelf live. This makes the business of producing and venturing into aquaculture difficult and unattractive to the productive hands, farmers and investors in Nigeria.

Other challenges identified by other authors include the shortage of inputs (fingerlings and feed), lack of knowledge resulting in poor management practices, inadequate funding, theft, and direct involvement of government in production (Lam et al., 2012). Use of poor quality seeds, inadequate information, high cost of feeds, traditional techniques, small-size holdings, poor infrastructural facilities, and low capital investment are also factors reported to be limiting the growth of aquaculture sector in Nigeria (Ugwumba & Chukwuji, 2010; Adewumi, 2015; Adebayo & Daramola, 2013).

2.3 COVID-19 and Aquaculture

Coronavirus disease 2019 popularly known as COVID-19 is caused by severe acute respiratory syndrome coronavirus 2. A similar strain of the virus SARS-CoV-1, which was reported to have affected 8000 people in 2002-2003 (Surico & Galeotti, 2020). A study in February 2020 revealed that the virus is made of 96% DNA match between bat coronavirus and human through an intermediate host (Xu et al., 2020). The virus enters through the mouth, eyes, and nose, attaching itself to respiratory tracts through the production of a protein called ACE2. The virus has become a global pandemic with only a handful of countries exempted or without any cases. The actual magnitude of the spread is still unclear as global confirmed cases have already exceeded the 1million mark in less than a year. Although no research has proven that COVID-19 affects fish, it's the human component of aquaculture that raises concern for the sector. In Nigeria, the pandemic is expected to have serious consequences on the country's aquaculture directly and indirectly. Indirectly, Nigeria's underdeveloped health sector will mean an immense pressure on aquaculture. This will imply that the number of the labour force will drop as the infrastructure at the health sector cannot hold the likely number of infected persons. UNDP (2020) reports that the likely fall in labour will lead to a serious economic and fiscal crisis. GDP growth has already fallen to -1.58% mainly from oil prices decline by 55%. As the outbreak continues to intensify, the countries services, trade, and financial sectors will fall along with the falling GDP as the sector continues 30% to GDP.

For the aquaculture sector, this will mean that farms are likely to lose their workforces especially commercial farms that need a larger number of labour. This will also mean that farms will have to reduce their production capacity to hold the available workforce. Marketing of the end product of fish farming (fish at all stages of growth) cannot be done as the country has adopted policies of lockdown, the ban on interstate travel, and also a ban on import and export. With this means restricting marketing, it expected that sales volume will fall greatly as market sizes will be reduced to the local market. This may be good for small farms in the short run but in the long run, be overshadowed by big ones. On consumption, it's expected that the fall in production level will mean price increase to help cushion losses made by farmers. Directly, the sector faces huge competition from other protein sources as the relative cost of other protein source are likely to be low to fish. It will be expected that the consumption pattern of consumers will shift to a cheaper source.

3. Materials and Methods

3.1 Description of the Study Area

Oyo state is an inland state, which is located in the southwestern part of Nigeria with its capital being Ibadan. The state is located on longitude 8°10′N and Latitude 4°15′E. The state was created in 1976 from the western state which included Osun sate until it was spilled in 1991 (Makinde, 2014). The state is home to the Yoruba ethnic group, who are predominately agrarian. The state is bounded by Osun state (east), Ogun state (South and partly to the west), Kwara state (North). In 2006, the state had a total population of 5,580,894 (National Population Commission, 2006). The state covers an estimated land size of 10,986 sq. mi (28,454 square km) making it the 14th biggest state in the country (Umeokeke, Okoruwa, & Adeyemo, 2017). Its landscape is mostly made of old hard rocks and dome-shaped hills rising gently from 500 meters in the southern part to 1200 meters in the northern part of the state. The state has its climate being equatorial with dry (starting from November to March) and wet season (April to October) with relatively high humidity. The temperature of the state range between 25 °C and 35 °C yearly (Adeogun et al., 2017). The nature of the climate favours the development of fishing and fish farming activities as while as the growth of food crops and cash crops such as yam, maize, cassava, plantain, millet, rice, and banana.

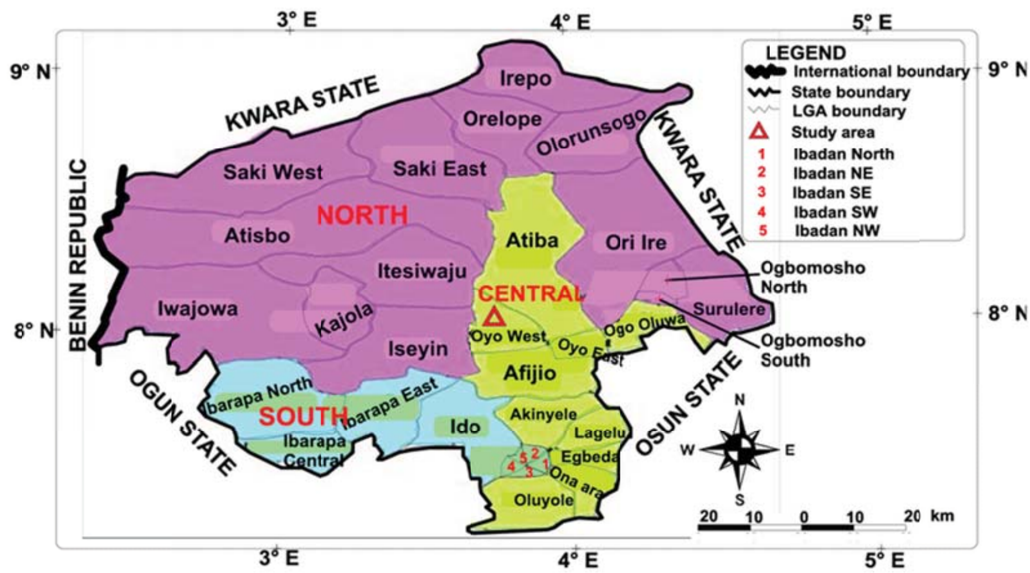

Figure 1. A map of Oyo state (Adopted from Adagunodo et al., 2018)

3.2 Research Design

The study adopted the cross sectional survey design. The cross sectional survey design was adopted to helps measure the outcome and the exposure of respondents (fish farmers) to the effect of COVID-19 on their activities within the period of the virus. This allows for generalization of the study to a large population-based on inference from the sample. The selection of respondents for the study was based on the respondent being in

Nigeria and a fish farmer. The advantage of this design is that it allows for the study to be conducted relatively faster and it is inexpensive.

3.3 Sample Procedure and Sample Size

The study used the purposive sampling technique to select fish farmers. Due to the homogenous nature of fish farming activities in Nigeria, the studies made use of the homogenous purposive sampling (Type of purposive sampling). The population of the study was all fish farmers in Nigeria during the period of COVID-19. However, the actual number of the population was unknown. As a general rule, selecting a larger sample size is better than a smaller sample size. This is because the larger the sample size the smaller the sampling error and the higher the representativeness of the sample to the population. The intended sample size of the study was obtained using Slovin's formula (1960) for the unknown population. The desire sample size for the study was 20 respondent, hence;

$$n = \frac{N}{1 + Ne^2} \quad (1)$$

Where, N = population size; e = margin of error; n = sample size.

However, the unwillingness of the respondent and the difficulties involved in getting respondents to answer the questionnaires made the desire sample size unachievable. At the end of the data collection, a total of 11 respondents answered the questionnaire.

3.4 Sample Collection Instrument

Primary data was used for the study, which was obtained through the use of a questionnaire (google form). The selection of primary data was due to the COVID-19 being a new phenomenon and the absence of existing data. Primary data has the advantage of ensuring reliable data is obtained. Face validity and content validity of the instrument was ensured through a review of an expert to remove ambiguous and misleading questions. The questionnaire was made up of both open and close-ended questions. The questionnaire was made up of four parts, namely:

(1) Socioeconomic characteristics: This looked at the socioeconomic characteristics of respondents in terms of age, educational qualification, sex, farm size and the number of ponds used, number of smoking kink, number of nurseries tanks, and COVID-19 status of the farm. Socioeconomic characteristics were included to find out the basic characteristics of fish farmers. This was meant to find out if these characteristics have any influence on the perception, challenge, and measures adopted by the respondent.

(2) Perception of fish farmers about COVID-19: This looked at how respondents perceived the effect of COVID-19 on their activities. This was mostly made up of questions looking at the agreement level of respondents on the perceived effect COVID-19 is having on their activities.

(3) Challenges of aquaculture caused by COVID-19: This section looked at the challenges faced by the respondent during COVID-19. This section of the questionnaire provided possible challenges likely to be faced by farmers from literature and also opportunity was made for them to add their challenges which were not captured.

(4) Measures adopted to reduce the effect of COVID-19: This section of the questionnaire looked at measures adopted by respondents to reduce the effect of the pandemic on the aquaculture. Respondents were provided some basic mitigation strategies and asked if applied and also provided the opportunity for them to mention their mitigation strategies adopted.

3.5 Data Analysis Techniques

Collected data was converted into Excel version 2013 for cleaning and adjusted per the protocol. The data was then transferred into SPSS version 25.0 for analysis. The analysis was based on the objectives of the studies. The analytical tools for each of the objectives are:

(1) Socioeconomic characteristics: The socioeconomic characteristics of the respondent were analyzed using descriptive statistics like the frequency, percentage, mean, Standard deviation, and t-test. Descriptive statistics were used to show the distribution of respondents across age, farm size, education, number of ponds, number of nursery tanks, number of smoking kinks, type of species, COVID-19 affected status of farm, and sex.

(2) Perception of fish farmers about COVID-19: Perception of the respondent was analyzed using descriptive statistics like the frequency, and percentage. The frequency and percentages were based on the level of agreement to the perception variables of the study.

(3) Challenges of aquaculture caused by COVID-19: This was analyzed using the Kendell's coefficient of concordance to rank the challenges caused by COVID-19. The challenge with the highest mean value was the most felt challenge by respondents. A further test was conducted using the Wilcoxon sign rank test to match the challenges. This was essential in calculating the difference in the match (pairs) and also reveal if there existed any significant differences. Bonferroni adjustment was also used to test the comparisons that exist between the challenges. The rule for the Bonferroni adjustment was that the p-value for each test must equal alpha divided by the number of tests.

(4) Measures adopted to reduce the effect of COVID-19: The measures adopted to reduce the effect of COVID-19 were analyzed using a bar chart to give an illustrative representation of which measures were most adopted.

4. Result

4.1 Socioeconomic Characteristics of Respondent

The results on socioeconomic characteristics of respondents as displayed in table 1 below showed that majority of fish farmers interviewed were aged 25-35 (10) and predominantly were females (8). Females (8) were more likely to engage in fish farming than males (3) despite the labour intensiveness of fish farming.

On the educational level, it was revealed that most of the respondents had tertiary education (6). Farm size under this study was mostly small, measuring 0.5-2.5 acres (7). 1-15 ponds (2) were used by the majority of the farmers for their activities. 10 of the respondent were using 0-10 nursery tanks and 2 of the total respondent had 0-5 smoking kinks for their operations. The most farmed fish species revealed by table 1, showed that Catfish was leading with 8 respondents

Lastly, the result on COVID-19 affecting farming activities, showed that 10 respondents out of the 11 said "yes" their operations have been affected by COVID-19.

Table 1. Socioeconomic characteristics of respondent

Variables	Freq.	%	Mean±SD	t
Age			1.091±.302	12.000***
25-35	10	90.9		
46-56	1	9.1		
Sex				
Male	3	27.3		
Female	8	72.7		
Education			3.273±1.009	10.757***
No formal Education	1	9.1		
Primary	1	9.1		
Secondary	3	27.3		
Tertiary	6	54.5		
Farm size			1.455±.688	7.016***
0.5-2.5	7	63.6		
2.6-5.6	3	27.3		
6.7-10.7	1	9.1		
Number of ponds			1.182±.405	9.690***
1-15	9	81.8		
16-30	2	18.2		
Number of nursery tanks			1.091±.302	12.000***
0-10	10	90.9		
11-21	1	9.1		
Number of smoking kinks			1.182±.405	9.690***
0-5	9	81.8		
6-11	2	18.2		
Type of species			1.909±.539	11.739***
Catfish	8	72.7		
Tilapia	2	18.2		
Both	1	9.1		
COVID-19 status of farm			1.182±.603	6.500***
Yes	10	90.9		
No	0	0.0		
Not yet	1	9.1		

Note. n = 11; ***: Significant at 5%.

H_0: There is no significant relationship between the socioeconomic characteristics of the respondent.

The hypothesis of significance between the socioeconomic characteristics of the respondent revealed that there exists no significant relationship between the various variables at 5% alpha value. Hence, we reject the null hypothesis. This implies that the socioeconomic characteristics of fish farmers played a significant role in the extent of impacted faced during the COVID-19 pandemic.

4.2 Perception of Fish Farmers on the Influence of COVID-19 on Farming Activities

The result on the perception of fish farmers on the influence of COVID-19 on their farming activities showed that the pandemic caused a decline in demand (72.7%), high cost of production (63.6%), making fish more expensive (54.5%) and reduction of manpower on the farm due to the lockdown (63.6). However, farmers disagreed that the pandemic led to improve sales volume (63.6%). On improved marketing strategies (63.6%) and increase feasibility in the market (45.5%), it was shown that farmers were indifferent as to the influence of the pandemic.

Table 2. Perception of fish farmers

Variables	Response (%)		
	Disagree	Neutral	Agree
Demand decline	2(18.2)	1(9.1)	8(72.7)
The high cost of production	1(9.1)	3(27.3)	7(63.6)
Fish is more expensive	2(18.2)	3(27.3)	6(54.5)
Improved marketing strategies	1(9.1)	7(63.6)	3(27.3)
Improved sales volume	7(63.6)	3(27.3)	1(9.1)
Increase feasibility in the market	4(36.4)	5(45.5)	2(18.2)
Reduction of manpower on the farm due to lockdown	1(9.1)	3(27.3)	7(63.6)

Note. n = 11.

4.3 Challenges Faced by Fish Farmers During COVID-19

Table 3, below showed the challenges faced by fish farmers during the period of the COVID-19 pandemic. The results showed that the major challenge faced by fish farmers was the reduction in walk-in customers to the farmers (4.50), this was followed by the high cost of inputs (4.29), inability to access raw materials (4.01), inability to access the fish market because of the lockdown (3.98), inability to access fingerlings (3.84), inability to get technical support (3.73) and the least challenge being employee's inability to come to work because of the lockdown (3.66).

A further probe into the results leads to the Wilcoxon sign rank test to find the relationship that exists between the variables and the significance of the relationship. The pairing of the variables revealed that all the variables had opposite direction, meaning that none of the variables was dependent on the other as shown by the Z-score. A look at the significance level revealed that; Inability to access fingerlings * Reduction in walk-in customers to farm, Inability to access fingerlings * High cost of input, Employees inability to come to work because of the lockdown * Reduction in walk-in customers to farm, Employees inability to come to work because of the lockdown * Inability to access fish market because of the lockdown, Employees inability to come to work because of the lockdown * High cost of inputs, Inability to get technical support * Reduction in walk-in customers to farm, Reduction in walk-in customers to farm * Inability to access fish market and Inability to get technical support * High cost of inputs were observed as significant. However, the Bonferroni adjustment (correction) was used to check for errors that may have occurred. The result of the Bonferroni adjustment showed that only; Employees' inability to come to work because of the lockdown * Reduction in walk-in customers to farm and Inability to get technical support * reduction in walk-in customers to the farm were significant. This implies that the reduction in walk-in customers to the farm, which was the major challenge faced by fish farmers was tied to the employees' inability to come to work because of the lockdown, and the absence of technical support.

Table 3. Kendall Coefficient of Concordance for Challenges faced by fish farmers during COVID-19

Challenges faced by fish farmers	Mean	SD	Mean Rank	Median
Inability to access fingerlings	1.50	.50	3.84	1.50
Inability to access raw materials	1.55	.50	4.01	2.00
Employees inability to come to work because of the lockdown	1.45	1.45	3.66	1.00
Reduction in walk-in customers to farm	1.69	1.69	4.50	2.00
Inability to get technical support	1.47	1.47	3.73	1.00
Inability to access the fish market because of lockdown	1.54	1.54	3.98	2.00
The high cost of inputs	1.63	1.63	4.29	2.00

Note. n = 11; σ^2 = 23.714 Asymp; Sig. = .001; Overall Median = 1.50.

Table 4. Wilcoxon signed-rank test for challenges faced by fish farmers during COVID-19

Challenges faced by fish farmers	**Z-score**	**Sig.**	**Bonferroni Adjustment (p < .002)**
Inability to access fingerlings * Inability to access raw materials	-.674	.500	Not significant
Inability to access fingerlings * Employees inability to come to work because of the lockdown	-.898	.369	Not significant
Inability to access fingerlings * Reduction in walk-in customers to farm	-3.042	.002***	Not significant
Inability to access fingerlings * Inability to get technical support	-.600	.549	Not significant
Inability to access fingerlings * Inability to access fish market because of lockdown	-.649	.516	Not significant
Inability to access fingerlings * High cost of input	-2.335	.020***	Not significant
Inability to access raw materials * Employees inability to come to work because of the lockdown	-1.508	.132	Not significant
Inability to access raw materials * Reduction in walk-in customers to farm	-1.941	.052	Not significant
Inability to access raw materials * Inability to get technical support	-1.109	.267	Not significant
Inability to access raw materials * Inability to access fish market because of lockdown	-.135	.893	Not significant
Inability to access raw materials * High cost of input	-1.206	.228	Not significant
Employees inability to come to work because of the lockdown * Reduction in walk-in customers to farm	-3.618	.000***	Significant
Employees inability to come to work because of the lockdown * Inability to get technical support	-.535	.593	Not significant
Employees inability to come to work because of the lockdown * Inability to access fish market because of the lockdown	-2.714	.007***	Not significant
Employees inability to come to work because of the lockdown * High cost of inputs	-3.087	.002***	Not significant
Inability to get technical support * Reduction in walk-in customers to farm	-3.395	.001***	Significant
Reduction in walk-in customers to farm * Inability to access fish market	-2.023	.043***	Not significant
Reduction in walk-in customers to farm * High cost of input	-1.095	.273	Not significant
Inability to get technical support * Inability to access fish market because of lockdown	-1.400	.162	Not significant
Inability to get technical support * High cost of inputs	-2.921	.003***	Not significant
inability to access fish market because of lockdown * High cost of input	-1.342	.180	Not significant

Note. n = 11; ***: $p < 0.05$.

H_1: The paired challenges faced by farmers during COVID-19 are not different.

The test of the paring for the challenges faced by fish farmers during the COID-19 revealed that not all the pairing were significant, hence, the null hypothesis of "The paired challenges faced by farmers during COVID-19 are not different" was accepted, implying that the challenges faced by fish farmers were not paired in occurrence, but rather a sequential occurrence (one challenge leads to another).

4.4 Coping Strategies Adopted to Mitigate the Effect of COVID-19

The coping strategies adopted to mitigate the effect of COVID-19 by fish farmers is shown in Figure 2. The results revealed that the most adopted strategy was the development of own feed (36.4%), this was followed by reduction in borrowing rate (27.3%), assistance from the government and organisations (18.2%) while, the least adopted was the reduction in production activities and starving fish for days (9.1%).

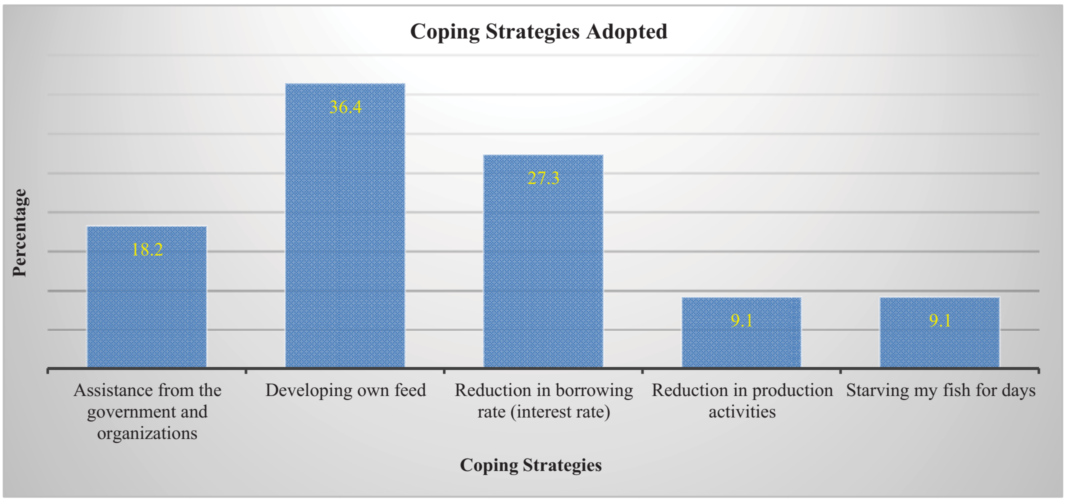

Figure 2. Coping Strategies adopted to mitigate the effect of COVID-1

5. Discussion

5.1 Socioeconomic Characteristics of Respondent

On age, the result found agrees with Pandey and Upadhayay (2012) and Salau et al. (2014) who found out that young people are more likely to engage in fish farming than older people due to the labour and capital intensive of fish farming. Whiles with gender, the results was inconsistent with Ifejika et al. (2018) and Olaoye et al. (2016) whose findings revealed that males are more likely to engage in fish farming activities due to the labour intensity. The hypothetical reason for our findings being at variance could be due to males taking the risk during the lockdown to find alternative income and food source to supplement the already depleted household income and food. Hence females are left with the responsibility of taking care of the fish farms.

Results on education is consistent with Olaoye et al. (2016) who found out that fish farmers were well educated and had obtained tertiary education. The results of Pandey and Upadhayay (2012) were consistent with findings of this research, which found out that fish farmers were mostly small in nature due to the issue of land litigation, low capital of farmers and subsistence nature of production.

The result on nursery tanks agrees with Abraham, Sil, and Vineetha (2010), who reported that fish farmers have less number of nursery tanks because of their small scale nature of operation. Olaoye et al. (2016) findings on smoking kinks number is consistent with this research, which revealed that farmers have low number (0-5) of smoking kinks because of the small nature of their production capacity and the high demand for fresh fish over smoked.

The result of this research is consistent with Adeoye and Elegunde (2012), who in their research reported that Nigeria's aquaculture sector is monoculture in nature, with catfish being the most farmed fish species. This agrees with Senten et al. (2020) studies in the US, which revealed that 90% of 537 fish farmers accepted that COVID-19 has affected their farm activities.

5.2 Perception of Fish Farmers on the Influence of COVID-19 on Farming Activities

The results agrees with Sunny et al. (2020) and Senten et al. (2020), which revealed that fish farmer's perception on COVID-19 has led lead to a reduction in their income due to the high cost of production, and decline in demand. This implies that the impact of COVID-19 on aquaculture was felt from both the demand and supply side. Also, the result implies that government policies like the inner state lockdown had negative impact on aquaculture in Oyo state. As cost of production increased within the period, input price rose and consumer restricted movement reduced demand for fish and fish products in the state.

5.3 Challenges Faced by Fish Farmers During COVID-19

The findings disagreed with Sunny et al. (2020), whose report showed that the major challenge faced by fish farmers was the low attendance of service providers. Morgan et al. (2017) noted that the disruption of the production process lead to a large failure of farms attaining profitability status.This implies that the major challenge to fish farming during the COVID-19 period was market for input and output, which could be attributed to the restriction on movement within the country. Also, Adebayo and Daramola (2013) and Lam et al.

(2012), revealed that Nigerian aquaculture sector, before the pandemic, was already faced with challenges such as the shortage of inputs (fingerlings and feed), lack of knowledge resulting in poor management practices, inadequate funding, use of poor quality seeds, direct involvement of government in production,inadequate information, high cost of feeds, traditional techniques, small-size holdings poor infrastructural facilities, and low capital investment. This implies that COVID-19 pandemic was not the actual cause of challenges faced by farmers, but they are already exposed to these challenges. Consultative Group on International Agricultural Research (CGIAR,2020) and the fisheries committee for the West Central Gulf of Guinea (FCWC, 2020) also reported that the major challenges of Nigeria aquaculture during the COVID-19 period was the decline in demand, inability to access to market (input and output markets), reduced technical assistance to far and high cost of production. It further reported that the difficulties faced by fish farmers is rooted in the government policy of movement restriction (Movement control order (MCO).

5.4 Coping Strategies Adopted to Mitigate the Effect of COVID-19

The result on development of own feed is consistent with van Beijnen and Yan (2020), who in their research reported that African fish farmers should focus on the production of their own product (input) to reduce the cost of production. Fish farmer's adaptation of developing their own feed could be attributed to the high cost of input and the restriction on movement, which hindered access to the input market. An FAO (2020) report in April, 2020 emphasised the need of governments to support the aquaculture sector through subsidies and other relief services. This support the finding of this study that assistance from government and stakeholders and the reduction of borrowing rate are principal coping strategies adopted by the Nigerian aquaculture sector.

6. Conclusion

The global health crisis caused by COVID-19 has led to an unexpected effect on world economies with economies struggling to mitigate the effect of the pandemic. The Nigerian aquaculture sector is no exception to these difficulties. The pandemic has led to increased cost of input, raw material, market inaccessibility and the reduction of walk-in customers to farms. This is as a result of some policies taken by the government, like measures restricting movement and the closure of the country's borders.

This study was conducted to find out the extent of the impact of COVID-19 on the Nigeria aquaculture, and the adaptation strategies farmers are undertaking to mitigate it impact. The study's core objective was to identify the socioeconomic characteristics of fish farmers in Nigeria, examine the perception of fish farmers on the influence of COVID-19 on aquaculture, identify challenges faced by fish farmers during COVID-19 and coping strategies adopted to mitigate the impact of COVID-19 in the sector. The study found that there is significant difference in the relationship between socioeconomic characteristics of fish farmers. It also revealed that fish farmers perceived that COVID-19 has led to the decline in the demand of fish purchase. Cost of fish input has increased and a reduction of manpower on the farm due to the lockdown protocol were further observed. Farmers also revealed that the cost of fish is more expensive during the COVID-19 period.

Furthermore, the study revealed that the major challenge faced by fish farmers during the COVID-19 period was the reduction in walk-in customers to the farmers. This has made farmers to develop their own feed and also taking advantage of the government reduced interest rate policy to reduce the impact on their activities.

The study therefore concludes that fish farming and its related activities are submerged in various degrees of shocks. These shocks are either from within the aquaculture sector or spill-overs from national shocks from to the emergence of the COVID-19 pandemic. Hence government and stakeholders in reducing such shocks, should consider a holistic approach to national policies on aquaculture.

7. Recommendation

The study recommends that fish farmers and other stakeholders engaged in aquaculture in Nigeria should begin considering multiple supply chains for aquaculture products in an attempt to reduce shocks such as those exposed by COVID-19.

Fish farmers should also consider other marketing approaches like online marketing and contract farming to create direct market avenues for their products.

The government and private sector stakeholders in aquaculture in Nigeria should invest in the local production of fish feed and other fish farming inputs to reduce the cost of fish farming and also create lucrative jobs in the sector.

Lastly, the government of Nigeria should continue with it reduced lending rate and subsidies to cushion fish farmers entering into the post-COVID-19 fish farming season.

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