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Disease incidence, severity and changes in morphological characteristics of some high value hybrid genotypes of oil palm (*Elaeis guineensis* Jacq.) seedlings as influenced by *Fusarium oxysporum* f. sp. *Elaeidis*

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ABSTRACT

Fusarium oxysporum f. sp. *elaeidis* (*F.o.e*) which is a causative agent of vascular wilt disease is the main threat to oil palm (*Elaeis guineensis*) cultivation in Africa. Its effects are more widely known on adult palms at production stage than on palm trees at the pre-nursery stage. The aim of his study was to evaluate the effects of *F.o.e* on the morphological parameters and the disease incidence and severity on seedlings during four months of pre-nursery stage. Ten high value hybrid genotypes of germinated seeds, including three tolerant to *F.o.e* were used. Seedlings were inoculated at four weeks of age with *F.o.e*. The morphological parameters (height and stem diameter of the seedlings, leaf length and width, leaf area) were recorded every two weeks and the disease incidence and severity on seedlings were determined. All the observed parameters were negatively influenced by *F.o.e*. Seedling height showed the highest difference (5.34%) and two genotypes (T1N and T3F) out of the ten assessed recorded no influence of *F.o.e*. As these results are not correlated with the internal symptoms of vascular wilt, it is not evident to deduce tolerance of the progeny solely based on *F.o.e* effects on the growth traits. There was a positive and significant correlation ($r = 0.67$, $P < 0.05$) between the index of *Fusarium* (IF) and the disease severity, between the IF and disease incidence ($r = 0.85$, $P < 0.01$). However, the correlation between the IF and seedling morphology was negative and not significant ($r = - 0.17$, $P > 0.05$). The morphological parameters of T3N and T5N genotypes initially known to be susceptible to *Fusarium* wilt were the most affected (19.05% and 13.33% respectively) of the inoculated seedlings compared to control seedlings. T1N initially known to be susceptible here manifested signs of tolerance confirming the observations made on internal symptoms. Moreover, T3F, T2F and T1F progenies recorded an IF < 100 and have also been morphologically more efficient thus confirming their *Fusarium* tolerance status. These results may serve as a pre-diagnostic index of *Fusarium* wilt in oil palm.

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Keywords: *Elaeis guineensis*, *Fusarium oxysporum* f. sp. *elaeidis*, growth parameters, hybrid genotypes, pre-nursery.

INTRODUCTION

Oil palm is the major source of vegetable oil in the world. Its cultivation is seriously threatened on the African continent

mainly by a soil-borne fungus, *Fusarium oxysporum* f. sp. *elaeidis* (*F.o.e*) and causal agent of vascular wilt (Ntsomboh-Ntsefong et al., 2012; 2015). Oil palm (*Elaeis guineensis*)

is a perennial, monocotyledonous and heliophyte plant with a fasciculate and densely branched root system, which ensures the mineral nutrition of the palm in the first 40 centimeters from the ground surface (Jourdan et al., 2000). It is mainly cultivated in the wet equatorial zone with temperatures between 18 °C and 32 °C, and an average rainfall of 2500 mm per year (Goh, 2000). The plant has an economic life span of 25 to 35 years. The fruits of this crop produce two types of oil: crude palm oil extracted from the mesocarp and palm kernel oil extracted from the kernel (Oil world, 2014). Palm oil is used for food purposes (cooking oil, margarine, vanaspati, and shortenings), oleochemicals (detergents, cosmetics) and pharmaceutical purposes (carotenes), health supplement and as a source of biofuel (Ntsomboh-Ntsefong et al., 2012; 2015).

Symptoms of *Fusarium* wilt disease occur when oil palm seedlings are inoculated with the fungus (Tengoua and Bakoumé, 2008). *Fusarium oxysporum* f. sp. *elaeidis* (*F.o.e*) is a soil-borne pathogenic fungus that invades intact roots then the xylem to cause water stress and hormonal imbalance, with consequent severe yield loss and even death of the palm tree (Cooper, 2011). In Africa, *Fusarium* vascular wilt is widespread and can cause up to 70% mortality of palm trees in plantations (Cochard et al., 2005). *F.o.e* exists in two forms; pathogenic and non-pathogenic, constituting more than half of the *Fusarium* population of the soil (Edel et al., 2001). The pathogenic form is substantially the most abundant in the rhizosphere of susceptible plant varieties and its virulence varies from one strain to another (Tengoua and Bakoumé, 2008). The visible symptoms of vascular wilt on oil palm are a function of the age of the plant, the stage of infection, the susceptibility of the plant and the environment (Flood, 2006; Ntsomboh-Ntsefong, 2012; Claudine et al., 2019). Isolates of *F.o.e* obtained from root tissues of herbaceous plant species (*Amaranthus spinosus*, *Eupatorium odoratum*, *Mariscus alternifolius* and *Imperata cylindrica*) from a plantation in Nigeria were pathogenic to oil palm seedlings (Oritsejafor,

1986). Even pathogenic *F.o.e* isolates from oil palm may cause vascular wilt on the date palm while isolates of *F. oxysporum* f. sp. *Albedinis* are pathogenic to oil palm (Paul, 1995).

The identification of vascular wilt symptoms on infected oil palm plants, has been well described by Claudine et al. (2019), Flood (2006) and Ntsomboh-Ntsefong (2012). In oil palm at production stage, *Fusarium* wilt occurs mainly in two forms; a typical form that leads to palm death two to three months after the onset of the first symptoms, and the chronic form where the palm survives for a few years (Cooper, 2011). In oil palm of 1 to 4 years of age, the disease manifests itself by yellowing and then browning of the middle leaves of the crown, followed by the lower leaves. In the nursery, *Fusarium* wilt appears only after inoculation of the parasite on the roots manifested by yellowing of the young leaves. Here, internal symptoms are most abundant and are observed on pseudo-bulbs after their dissection (de Franqueville and Diabaté, 1995). The manifestations of *Fusarium* wilt on the morphological parameters and the disease incidence and severity on seedlings in pre-nursery are still not well described. The aim of this study was to evaluate the effects of *Fusarium oxysporum* f. sp. *elaeidis* on the morphological parameters and the disease incidence and severity on seedlings during four months of pre-nursery stage.

MATERIALS AND METHODS

Study area

The trial was conducted at the Ekona Regional Center of the Institute of Agricultural Research for Development, located in the Fako Division, Southwest Region of Cameroon. The area lies between 3°54'22" and 6°29'52" N latitude and between 8°30'58" and 10°6'45" E longitude (Figure 1).

Plant and fungi material

Plant material used in this study was ten oil palm seed genotypes (Table 1). Seeds of hybrid genotypes were obtained from the Institute of Agricultural Research for

Development (IRAD), Specialized Centre for Oil Palm Research of La Dibamba (CEREPAH), Cameroon. These seeds of hybrid genotypes were obtained by controlled pollination at IRAD La Dibamba and pre-germinated following the dry heat technique (Ntsomboh-Ntsefong et al., 2015a). They included three tolerant hybrid genotypes [C1001IIF (T1F), C2301IIF (T2F) and C2501IIFX (T3F)] and seven susceptible hybrid genotypes [C1001II (T1N), C2301II (T2N), C2501II (T3N), C2001II (T4N), C2101II (T5N), C1501II (T6N) and C1901II (T7N)] (Table 1). Two strains of *Fusarium oxysporum* f. sp. *elaeidis* (*F.o.e*) known to be virulent (Tengoua and Bakoumé, 2008) were used for seedling inoculation. Biological materials used in this study were palm fronds and *F.o.e* isolates. The palm fronds were cut at the level of petioles from infected Dura, Pisifera and Tenera in three blocks of palm trees at the IRAD Ekona research site. *F.o.e* isolates were collected from brown fibers in the petioles of these palm fronds cut from 30 palm trees infected by fusarium wilt. Soil samples were also collected from around the infected trees (Ntsomboh-Ntsefong et al., 2015a).

Inoculum preparation

Inoculum preparation was carried out in the palm pathology laboratory at EKONA. Two strains of *F.o.e* earlier characterized as being aggressive (Tengoua and Bakoume, 2008) were used with Armstrong *Fusarium* culture medium. Using the procedure of Tengoua (1993), *F.o.e* strains were purified in an inclined test tube. Then a small fragment was deposited in a test tube containing distilled water that had been sterilized thrice with autoclave at 120°C for 45 min. Part of the conidial suspension obtained was collected with a Pasteur pipette and spread in a Petri dish containing NASH medium for 1 L (peptone 15 g, agar agar 20 g, monopotassium phosphate 1 g, magnesium sulfate 0,5 g, distilled water 1 L, dihydrostreptomycine 1 mL, pentachloronitrobenzene 1 g, autoclave at 120 °C for 50 min (pH = 6.5). After spore germination, a fragment was collected and

placed on mycelium medium in a test tube. Then a fragment of this medium containing the fungus was cut and introduced in 75 mL of sterile Armstrong medium in a conical flask. This was agitated every 10 min for four days to enhance multiplication of *Fusarium*. Then 2 mL of the solution obtained were put in 100 mL of sterile Armstrong medium in roux bottles which were inclined on a surface sterilized with alcohol and flaming. For rapid multiplication of *Fusarium*, this was agitated every 10 min for 8 days. The solution obtained was mixed and ground for 10 secs with the laboratory blender (Ntsomboh-Ntsefong et al., 2015b).

Inoculation of seedlings in the pre-nursery

The pre-nursery *Fusarium* wilt test was performed with a completely randomized block design involving inoculated seedlings and non-inoculated seedlings (as controls) using *Fusarium* inoculum previously prepared with sterilized distilled water in a Roux bottle (Figure 2). Inoculation was done at least one and a half months after transplanting the pre-germinated seeds in the nursery following the steps described by Renard et al. (1972).

Morphological characteristics

Seedlings growth (height, stem diameter, number of leaves, leaf area, length and width of leaf) were evaluated using 40 plants for each hybrid genotype in four replicates. The height was measured every week with a graduated ruler. Stem diameter was measured with a caliper and leaf area was measured every week and calculated using the method described by Tailliez and Ballo (1992).

Disease incidence on seedlings

The disease incidence (I) on seedlings were determined by Tucker and Chakraborty (1997) methods.

$$I = (Nm / Nt) \times 100$$

with I: disease incidence; Nm : number of seedlings infected ; Nt : number of seedlings infected and uninfected

Disease severity on seedlings

The disease severity (S) on seedlings were determined by Tucker and Chakraborty (1997) methods.

$$S = [\Sigma (a \times b) / n] \times 100$$

with S : Infection severity, $\Sigma (a \times b)$: sum of the products of the number of seedlings infected (a) and the corresponding degree of infection (b) ; n : number of seedlings infected.

Tolerance index of *Fusarium*

The index (I) of tolerance is attributed to each progeny according to the procedure described by Renard et al. (1972). Based on the results of the nursery test, the progenies used for the production of seeds tolerant to vascular wilt are classified according to their categories:

$$I = \frac{\% \text{ infected seedlings and wilthered of progeny A}}{\% \text{ infected seedlings and wilthered of all progeny}} \times 100$$

- I < 90: high tolerance
- 90 < I < 100: moderate tolerance
- 100 < I < 120: sensitive
- I > 120: highly sensitive

Statistical analysis

Data are presented in terms of mean (\pm standard deviation). The data collected on the various morphological parameters were entered in the Excel software and analyzed with SPSS Statistics 17.0 (2012) software. The data were analyzed for variance (ANOVA) to detect the difference in *Fusarium* wilt effect. Separation of the means was performed at the 5% threshold by the DUNCAN test. A correlation test between the different parameters and a hierarchical classification test between the seeds of hybrid genotypes were also carried out.

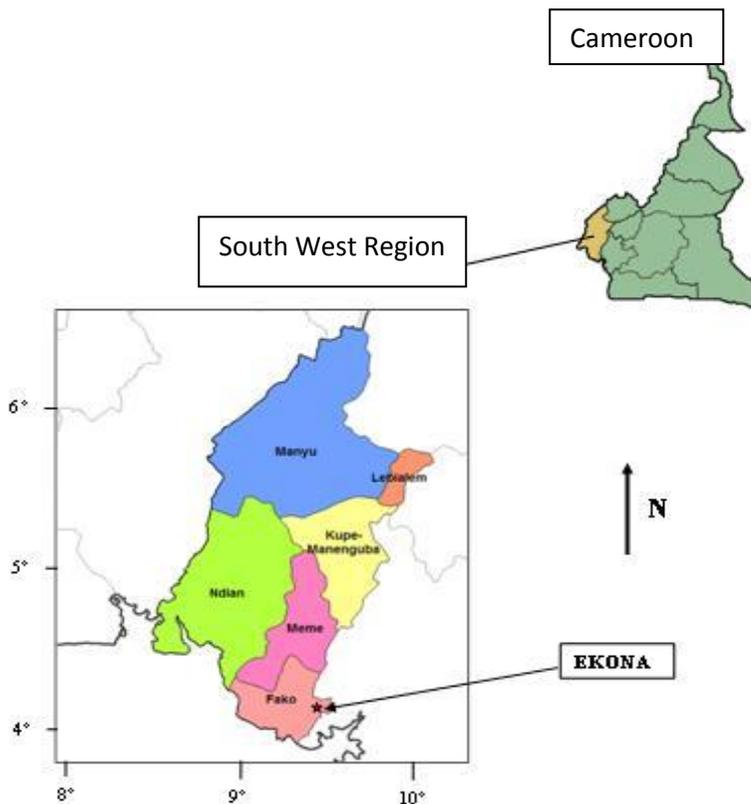


Figure 1: Study area.

Table 1: Seeds of hybrid genotypes oil palm used in this study.

Code of hybrid genotypes	Seed of hybrid genotypes	Female progeny code	Male progeny code
T3F	C2501IIFX	LM 13533	LM 19029
T3N	C2501 II	LM 17685	LM 17685
T6N	C1501 II	LM 17115	LM 18978
T1F	C1001 IIF	LM 19106	LM 19029
T1N	C1001 II	LM 18801	LM 18106
T2F	C2301 IIF	LM 18745	LM 19029
T4N	C2001 II	LM 17164	LM 18978
T7N	C1901 II	LM 19171	LM 18978
T5N	C2101 II	LM 17163	LM 18978

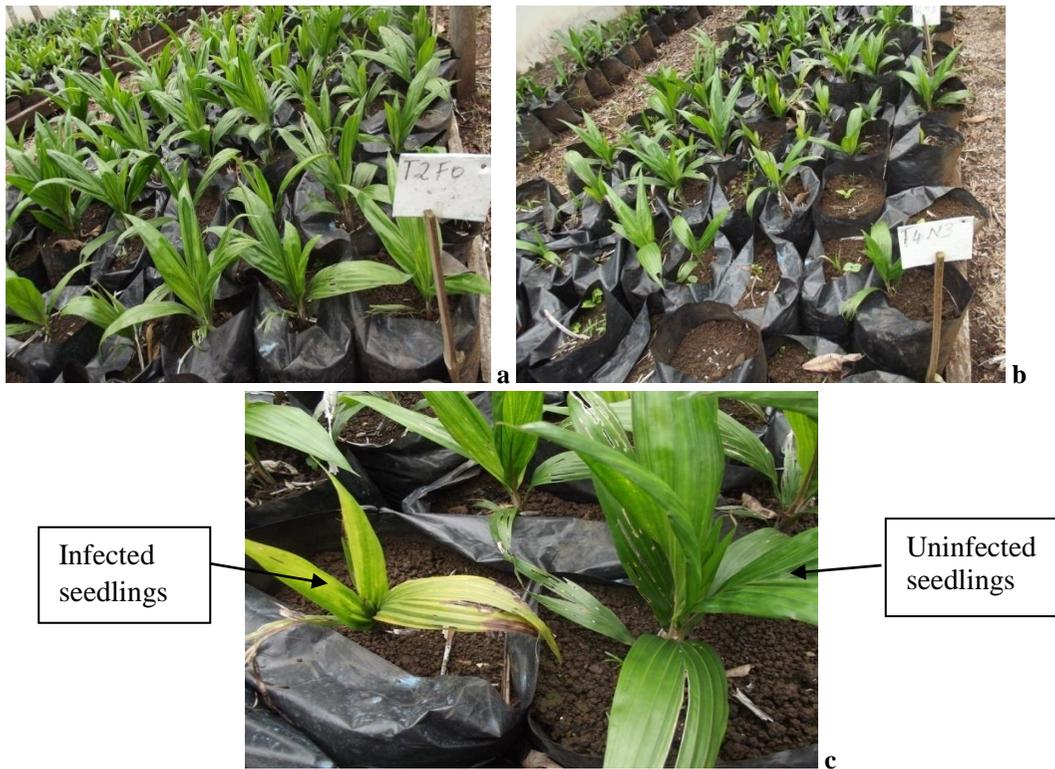


Figure 2: Inoculation of hybrid seedlings in the pre-nursery. **a:** non-inoculated seedlings (control); **b:** inoculated seedlings; **c:** samples of non-inoculated and inoculated seedlings.

RESULTS

Plant growth parameters

Seedlings height

The mean height of the un-inoculated seedlings (27.19 ± 0.51 cm) was generally higher than that of the inoculated seedlings (25.48 ± 0.46 cm) for all the hybrid genotypes except for (T3F, T1N) where the tendency was reversed (Figure 3). The observed difference between the inoculated seedlings and the controls were 5.95%, with the T3N hybrid genotype having the greatest difference (17.23%). The T1F and T6N hybrid genotypes were noted to be the best performers (28.45 ± 0.56 cm).

Stem diameter

Figure 4 shows a better performance of stem diameter of the control seedlings (0.86 ± 0.04 cm) compared to the inoculated seedlings (0.81 ± 0.03 cm) in seven hybrid genotypes with an overall difference of 5.34% and with the T3N genotypes having the greatest difference of 28.05%. The standard deviations for the stem diameter of the seedlings are very variable from one hybrid genotype to another and show a greater dispersion than that of the height of the seedlings. Seedlings of *Fusarium* wilt-resistant hybrid genotypes (T1F and T2F) recorded the diameters below their controls contrary to expectations.

Leaf length

Inoculated seedlings globally recorded leaf length (15.76 ± 0.04 cm) lower by 1.12% compared to control seedlings (15.91 ± 0.06 cm) (Figure 5). T3N hybrid genotype which is susceptible to *Fusarium* witnessed the largest difference (16.72%). On the other hand, hybrid genotypes T1N and T3F had a reversed tendency with respect to the majority. The standard deviations for the lengths of the leaves being small and very variable (3.1 to 4.4) imply a weak dispersion of values around the mean.

Width of leaves

In a leaf width, the results obtained shows a better behavior of non-inoculated seedlings in the majority of hybrid genotypes

except two (T1N and T3F) where inoculated seedlings behave better than controls (Figure 6). Overall, the leaf width of the inoculated seedlings (3.05 ± 0.02 cm) was 2.78% lower than that of the control seedlings (3.13 ± 0.04 cm). In the T1N and T3F hybrid genotypes, the leaves of the inoculated seedlings were wider than those of the non-inoculated seedlings. The distribution of the variable around the mean shows a low dispersion according to the standard deviations (0.4 to 0.9).

Leaf area

The leaf area has significant differences with several subsets (Figure 7). The results show a good performance of the T6N and T3N hybrid genotypes within the control seedlings and of the T1N and T6N hybrid genotypes within the non-inoculated seedlings and which are also non-tolerant. However, in general, the leaf area of the control seedlings was 3.68% higher than those inoculated. Inoculated seedlings whose foliar surfaces are superior to controls in T1N and T3F are also observed. The standard deviations for the leaf area are fairly high (10.3 to 16.4) indicating a wide dispersion of values around the means.

Number of leaves.

There is a slight difference (1.40%) between the numbers of leaves of non-inoculated plants which is higher than that of inoculated plants, although many seedlings exhibit contradictory tendencies (Figure 8). The standard deviations (1.3 to 1.7) also show a strong dispersion of values around the means.

Variance analysis of morphological parameters

The leaf area was strongly correlated with the width ($r = 0.94$, $P < 0.01$) and the length ($r = 0.91$; $P < 0.01$) of the leaves, indicating that it was effectively obtained from these parameters (Table 2). On the other hand, the number of leaves was not correlated with any other morphological parameter. The leaf area also has a strong correlation ($r =$

0.89, $P < 0.01$) with the height of the seedlings and slightly with the stem diameter.

Analysis of the variance of the *Fusarium* wilt effect on morphological traits

The results of the analysis of variance (Table 3) show significant differences for all the observed parameters. These results make it possible to detect the difference in susceptibility of the genotypes to *Fusarium oxysporum* f. sp. *elaeidis*.

Incidence of *Fusarium*

The degree of infection is assessed by the incidence of the *Fusarium* on bulb and leaves (Figure 9). The incidence is determined by the relative number of seedlings that show symptoms of the disease. The variation in the incidence of the disease was observed both on the bulbs and on the leaves (Figure 9). It is observed that the incidence shows the higher infection rate on the bulbs than on the leaves (Figure 9). The correlation between disease incidence and seedling morphology was not significant ($P > 0.05$).

Severity of *Fusarium*

The degree of infection is also assessed by the severity of *Fusarium*. The severity is determined by the rate of spread of symptoms on the leaves and bulb of the plant (Figure 10). The results obtained showed that the

severity of the disease is higher in the leaves than in the bulbs (Figure 10), which means that once the seedling is attacked, the symptoms of the disease develop much more on the leaves than on the bulb. The correlation between disease severity and seedlings morphology was not significant ($P > 0.05$).

Index of *Fusarium*

The *Fusarium* wilt index is the essential parameter in determining the tolerance or not of a hybrid genotype to *Fusarium oxysporum*. Table 4 presents the distribution of the index of *Fusarium* between seed hybrid genotypes. It can be noted that among the 5 seed hybrid genotypes that have a *Fusarium* count less than 100, the first three (T3F, T1N and T1F) hybrid genotypes have also always been morphologically more efficient. Among these genotypes with a low index of *Fusarium*, three (T3F, T1F, and T2F) are hybrid genotypes that confirm their status as highly tolerant while T1N and T7N hybrid genotypes are potential tolerant. There was a positive and significant correlation ($r = 0.67$, $P < 0.05$) between the *Fusarium* index and the severity disease, between *Fusarium* index and incidence of disease ($r = 0.85$, $P < 0.01$). However, the correlation between *Fusarium* index and seedling morphology was negative and not significant ($r = - 0.17$, $P > 0.05$).

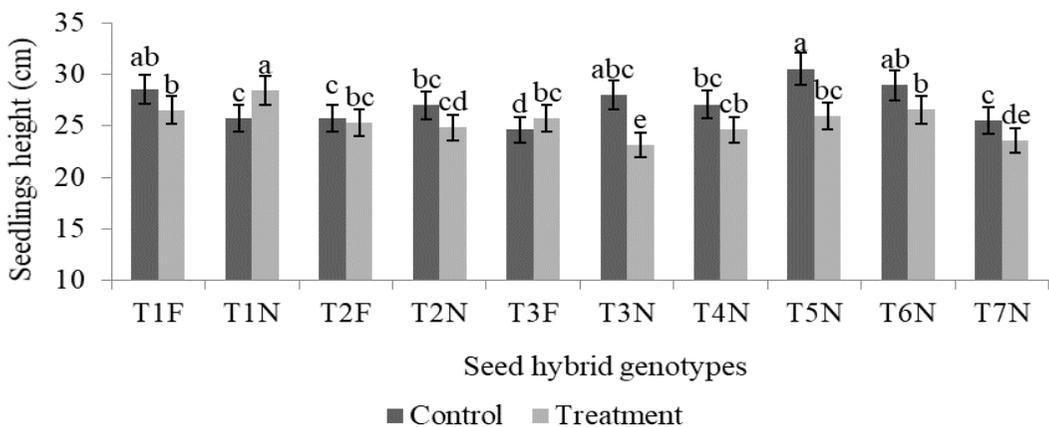


Figure 3: Comparison of the heights of the inoculated seedlings and the non-inoculated seedlings (control). Hybrid genotypes from the same group with the same letters are not significantly different

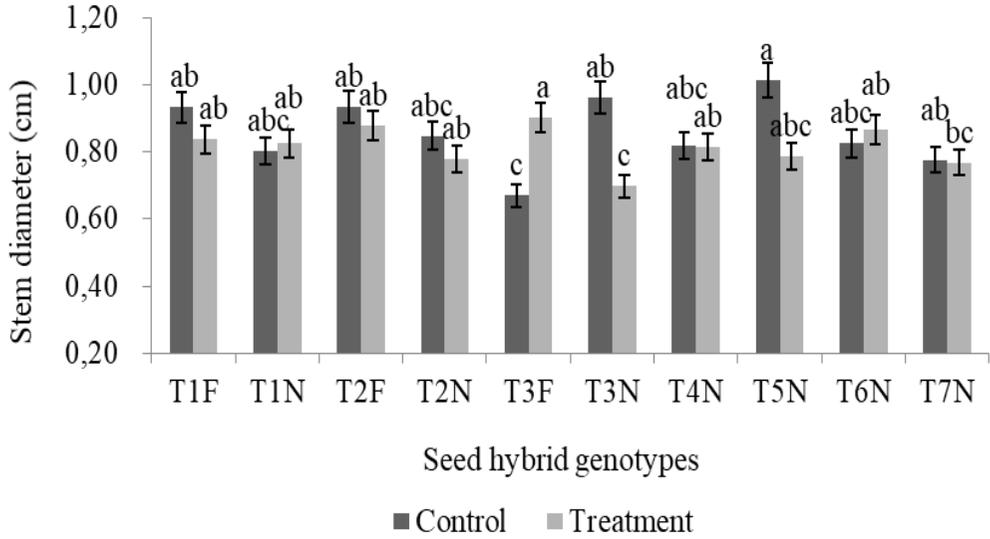


Figure 4: Comparison of stem diameter of inoculated seedlings and non-inoculated seedlings (control). Hybrid genotypes from the same group with the same letters are not significantly different

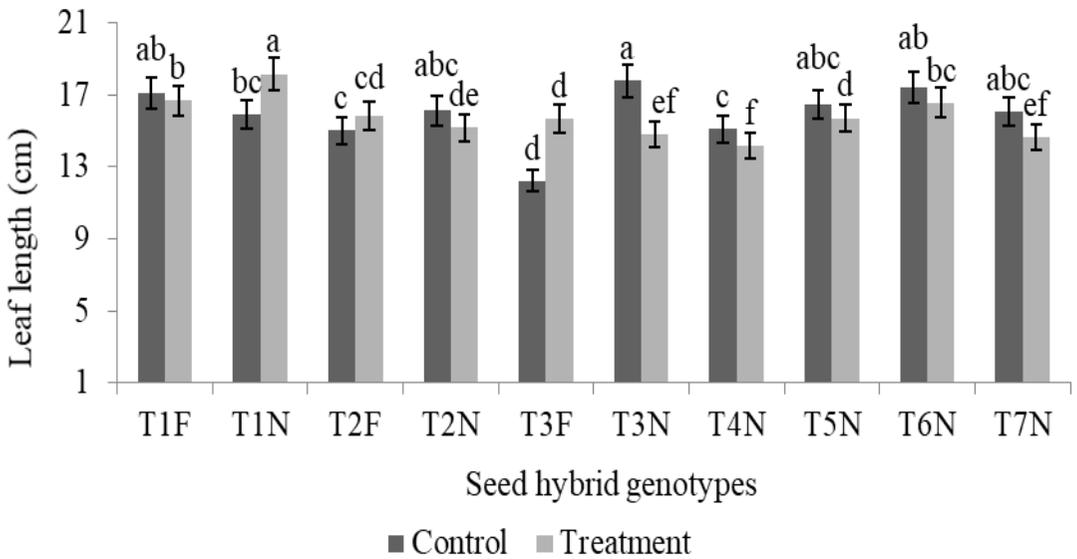


Figure 5: Comparison of leaf length of inoculated seedlings and non-inoculated seedlings (control). Hybrid genotypes from the same group with the same letters are not significantly different.

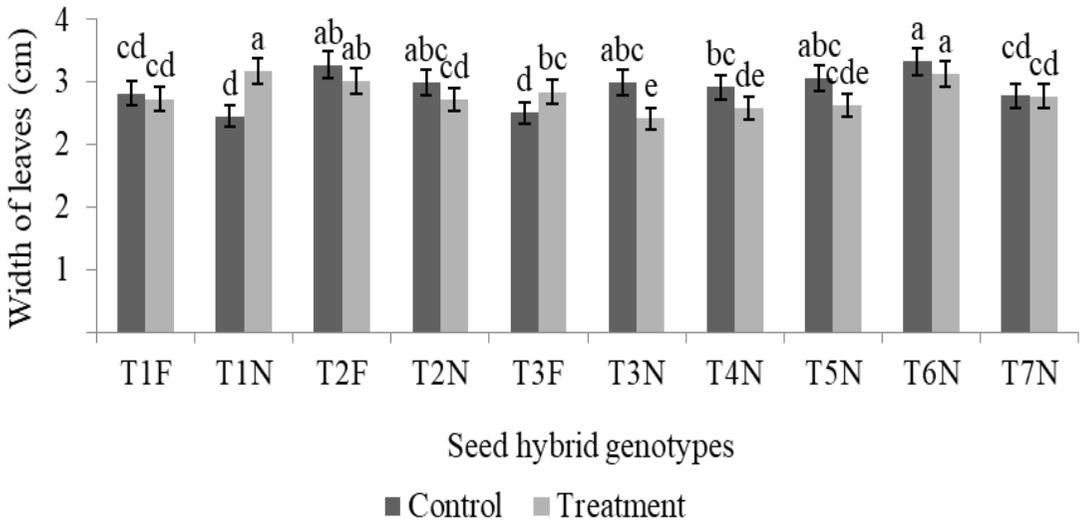


Figure 6: Comparison of leaf width of inoculated seedlings and non-inoculated seedlings (control). Hybrid genotypes from the same group with the same letters are not significantly different.

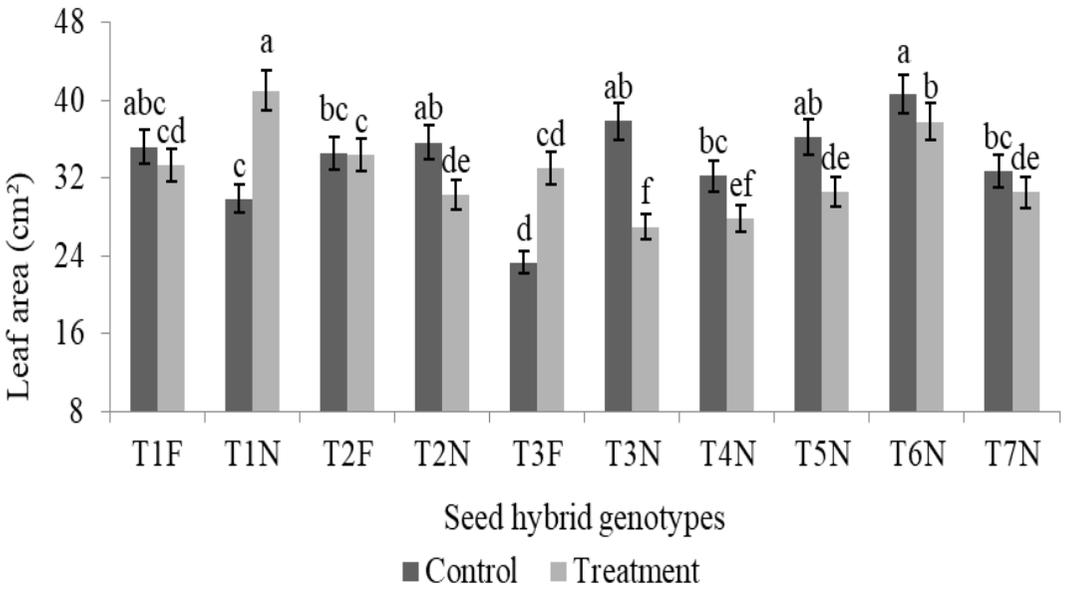


Figure 7: Comparison of the leaf area of the inoculated seedlings and the non - inoculated seedlings (control). Hybrid genotypes from the same group with the same letters are not significantly different.

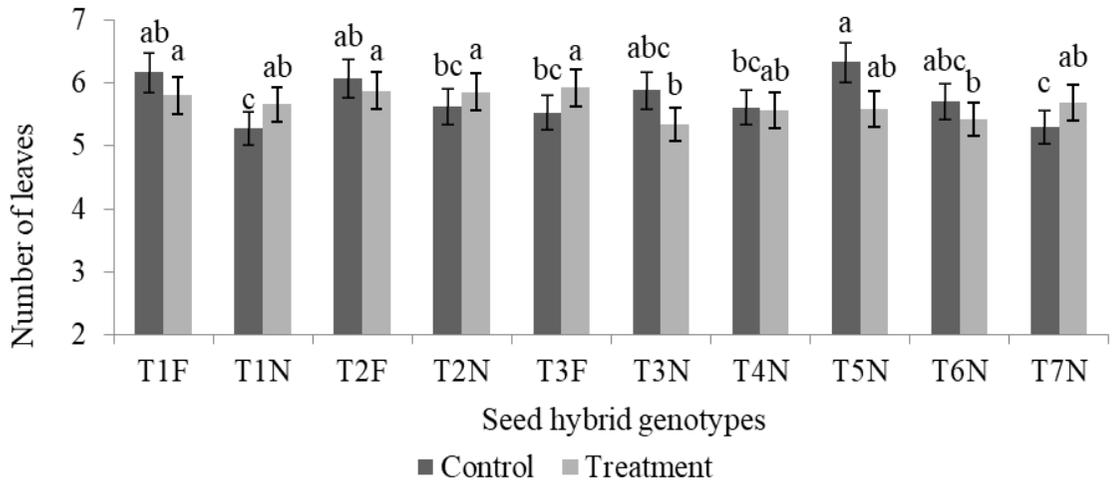


Figure 8: Comparison of the number of leaves of inoculated seedlings and of non-inoculated seedlings (control). Hybrid genotypes from the same group with the same letters are not significantly different.

Table 2: Correlations between different growth parameters.

Parameters	Leaf area	No. of leaves	Leaf width	Leaf length	Stem diameter	Seedling height
Leaf area	1					
No. of leaves	0,16 ^{ns}	1				
Width of leaf	0,94 ^{**}	0,21 ^{ns}	1			
Length of leaf	0,91 ^{**}	0,02 ^{ns}	0,72 [*]	1		
Stem diameter	0,64 [*]	0,60 ^{ns}	0,69 [*]	0,42 ^{ns}	1	
Seedling height	0,89 ^{**}	0,15 ^{ns}	0,73 [*]	0,89 ^{**}	0,61 ^{ns}	1

** Correlation is significant at P < 0.01, * Correlation is significant at P < 0.05, ^{ns} Correlation is non-significant.

Table 3: Analysis of the variance of the *Fusarium* wilt effect on morphological traits of oil palm seedlings in pre-nursery.

Parameters		Sum of squares	Degree of freedom	Mean squares	F	p
Seedling height	between groups	3773.19	9	419.24	10.48	0.00**
Stem diameter	between groups	5.62	9	0.62	2.53	0.01*
Leaf length	between groups	2103.94	9	233.77	18.34	0.00**
Leaf width	between groups	60.14	9	6.68	11.35	0.00**
No. of leaves	between groups	49.34	9	5.48	2.52	0.01*
Leaf area	between groups	30575.88	9	3397.32	17.83	0.00**

*,**Significant at P<0.05 and P<0.01, respectively, ns non-significant.

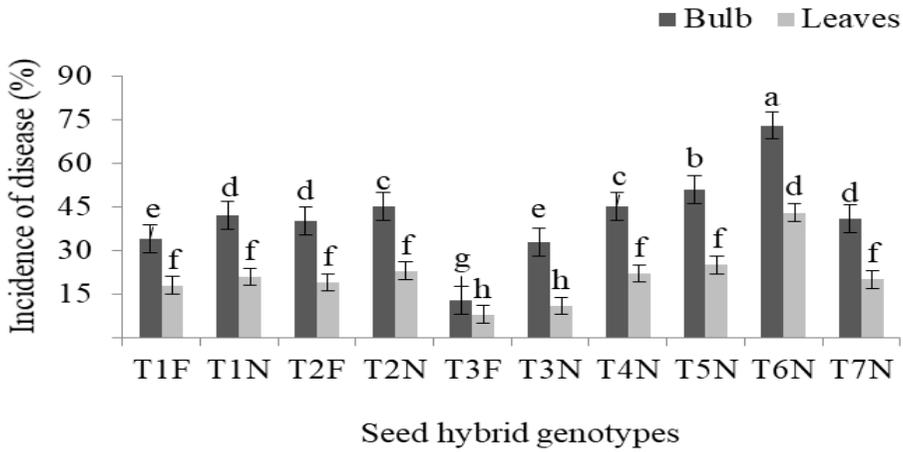


Figure 9: Incidence of *Fusarium* wilt observed in the bulb and leaves. Hybrid genotypes from the same group with the same letters are not significantly different.

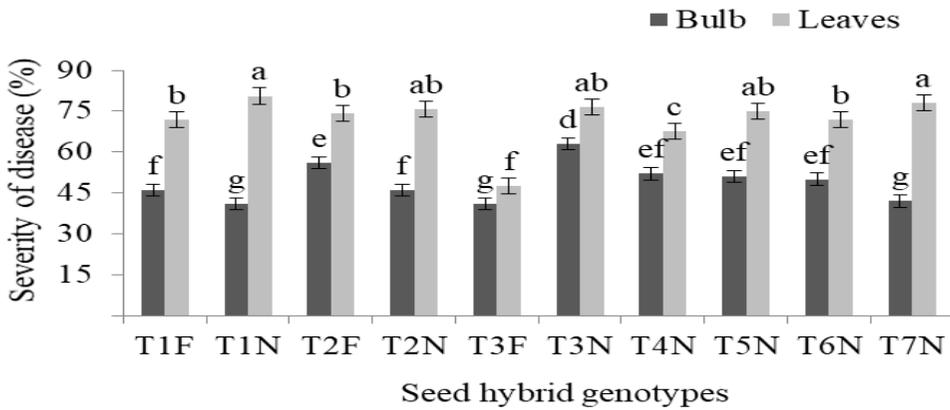


Figure 10: Severity of the *Fusarium* wilt observed in the bulb and leaves of inoculated seedlings. Hybrid genotypes from the same group with the same letters are not significantly different.

Table 4: Tolerance index of *Fusarium* of different hybrid genotypes.

Classification	Genotypes	Index de wilt
1	T3F	22
2	T1N	49,5
3	T1F	66
4	T7N	77
5	T2F	93,5
6	T4N	104,4
7	T3N	109,9
8	T5N	120,9
9	T2N	131,9
10	T6N	164,9

DISCUSSION

The morphological difference observed between inoculated and non-inoculated seedlings shows the effects of *Fusarium* wilt which led to the retardation of growth of the different parameters of the inoculated seedlings. This corroborates the results obtained in several studies (Flood, 2006; Vouli Bi et al., 2016; Claudine et al., 2019; Kenneth et al., 2019). The description of the effects of *Fusarium* wilt on adult oil palms shows that all morphological parameters, namely palm height and diameter, leaf length and width, leaf area are similar to the results of our study and correspond to the typical symptoms of *Fusarium* wilt (Gaetán and Madia, 2007; Ntsomboh-Ntsefong, 2012). However, the morphological parameters are affected differently, the height of the seedlings being the most affected. This makes it possible to understand why this same phenomenon is most remarkable on adult palm trees presenting typical symptoms. It is shown that *Fusarium* affects the growth of certain plants such as rice (*Oriza sativa*) by reducing the number and length of roots (Hinde et al., 2009). In a study on the pathogenicity involving inoculation with different *Fusarium* species, it was also found that *Fusarium* species strongly affect germination and root growth of pine seedlings. The same authors found that pine seeds were more sensitive to infection where the germination and root growth inhibition were more important (Lazreg et al., 2014). It has also been reported that *Fusarium* species affect germination, root growth, shoot growth and the index vigour in *Oriza sativa* (Lazreg et al., 2013). Our leaf length and foliar surface results were also similar to those obtained on nursery-damaged (wilted by *Fusarium*) and water stressed seedlings (Flood, 2006). The correlation between the different morphological parameters (height and diameter of palm trees, leaf length and width, leaf area) shows that they all undergo growth retardation due to the effects of *Fusarium* wilt. *In vitro* and *in vivo* experiments with *Fusarium solani* were also reported to show severe inhibitory effect on morphology (growth and development) of pea

cultivars (Smykalová and Griga, 2011). Growth parameters of pea (*Pisum sativum* L.) were also influenced negatively after infection with *Fusarium oxysporum* f. sp. *Pisi* (Tariq et al., 2015). Morphological parameters such as those measured in this study are important indicators for potential uptake of water and nutrients (Himmelbauer et al., 2004; Yergeau et al., 2006; Da Silva et al., 2016). However, the morphological parameters observed on the seed of hybrid genotypes tested in this study are not correlated with the internal symptoms observed on the same hybrid genotypes by Ntsomboh-Ntsefong et al. (2015b). These observations are similar to those of Buchanan (1999) who have shown that the visible symptoms of *Fusarium* wilt are much uncorrelated to internal symptoms. It is therefore inappropriate to speculate on the tolerance of progenies on the basis of the effects of *Fusarium* wilt on morphological parameters. The overlapping classification of the inoculated seedling hybrid genotypes is necessarily linked to the difference in sensitivity of both hybrid genotypes and morphological parameters to *Fusarium*.

The incidence of *Fusarium* wilt that we observed is similar to that of Yergeau et al. (2006) in a study in which we took into account the different types of symptoms, expressed or not, healthy-looking palms on which observations were made on their stipe to detect any presence of brown fibers. The number of seedlings infected at the level of the bulb being higher than that observed at the leaf level is explained by the fact that the disease appears first on the bulb and a few days later on the leaves, hence the need to observe both internal and external symptoms of *Fusarium* wilt for characterization is necessary (Ntsomboh-Ntsefong et al., 2015a). The progenies considered as non-tolerant and which proved to be as efficient as the proven tolerant progenies, could be justified by the insufficiency of root contamination by the pathogen because of the low roots and consequently have no openings to allow penetration of the pathogen (Hamel et al., 2005). The tests initially carried out for the characterization of these progenies were not

under the same environmental conditions (Ntsomboh-Ntsefong et al., 2015a) which constitute an important factor in the behavior of plants (Goh, 2000). Validation of the tolerance of progeny to *Fusarium* wilt is conditioned by confirmation in plantation of results obtained in pre-nurseries (Allou et al., 2003; Lazreg et al., 2014). The present work showed that the T1N and T7N which have a *Fusarium* index lower than 100 but considered as non-tolerant, would have been invalidated at the level of the plantation; in this case the results obtained can be in agreement with those of Chang et al. (2014) who found a strong stability of the pathogenicity. These results therefore indicate that seedling resistance assessment tests are of general significance. The correlation between incidence, severity, and *Fusarium* index indicates that they are closely related, so *Fusarium* tolerance could be inferred from incidence or severity. The environmental conditions were favorable to the expression of the pathogen which continued to evolve in an environment from which it is derived. It is therefore estimated that the variations in the different parameters observed are mainly due to the nature of the progenies.

Conclusion

This study showed that all growth parameters were strongly correlated, with plant height being the most affected parameter with 5.95% less of inoculated seedlings compared to control seedlings. On the other hand, leaf length was the least delayed parameter. There were also two hybrid genotypes (T1N and T3F) among the ten that showed no morphological symptoms of *Fusarium* wilt, whereas the T3N and T5N genotypes initially known to be susceptible to *Fusarium* wilt were the most affected (19.05% and 13.33% respectively) of the inoculated seedlings compared to the control seedlings. T1N initially known to be susceptible to *Fusarium* wilt manifested signs of tolerance confirming the observations made in this study on internal symptoms. Moreover, T3F, T2F and T1F hybrid genotypes recorded an IF<100 and have also been morphologically

more efficient thus confirming their *Fusarium* tolerance status. The correlation between incidence, severity, and *Fusarium* index indicates that they are closely related, so *Fusarium* tolerance could be inferred from incidence or severity. These results may serve as a pre-diagnostic index of *Fusarium* wilt in oil palm.

COMPETING INTERESTS

There is no competing interests for this article.

AUTHORS' CONTRIBUTIONS

GM: methodology, statistics, interpretation, draft of manuscript, proof reading. G N-N: conceptualization, methodology, experimentation, interpretation, draft of original manuscript, proof reading and editing. GFNE: conceptualization, interpretation, writing and reviewing. ANN: draft and editing of manuscript. VDT: interpretation, writing and reviewing.

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REFERENCES

- Allou K, Ahoussou N, Ake S, Diabate S, De Franqueville H. 2003. Behavior of oil palm clones in the field in high density zones of F.O.E in Ivory Coast. *Agronomie Africaine*, **15**: 29-38.
- Buchanan AG. 1999. Molecular genetic analysis of *Fusarium oxysporum* wilt resistance in oil palm. Ph.D. Thesis, Bath University, Bath.
- Chang T-H, Lin Y-H, Chen K-S, Huang J-W, Hsiao S-C, Chang P-F. 2014. Cell wall reinforcement in watermelon shoot base related to its resistance to *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *Niveum*. *Journal of Agricultural Science*, 1-10. DOI: 10.1017/S0021859614000057
- Claudine KS, Koussinou L, Adandonon A, Nodichao L. 2019. Distribution and incidence of *Fusarium* wilt in oil palm in Benin. *Journal of Applied Biosciences*, **135**: 13831-13839. DOI: <https://dx.doi.org/10.4314/jab.v135i1.9>
- Cochard B, Philippe A, Tristan DG. 2005. Oil palm genetic improvement and sustainable development. *OCL.*, **12**: 141-147. DOI: <https://doi.org/10.1051/ocl.2005.0141>
- Cooper RM. 2011. *Fusarium* wilt of oil palm: A continuing threat to South East Asian plantations. *The Planter-Kuala Lumpur*, **87**(1023): 409-418.
- Da Silva MP, Tylka GL, Munkvold GP. 2016. Seed treatment effects on maize seedlings co-infected with *Fusarium* spp. and *Pratylenchus penetrans*. *Plant Disease*, **100**: 431-437. DOI: <https://dx.doi.org/10.1094/PDIS-03-15-0364-RE>
- De Franqueville H, Diabate S. 1995. La fusariose du palmier à huile en Afrique de l'Ouest. *Plantation Recherche Développement*, **24**: 5-13.
- Edel V, Steinberg C, Gautheron N, Recorbet G, Alabouvette C. 2001. Genetic diversity of *Fusarium oxysporum* populations isolated from different soils in France. *FEMS Microbiology Ecology*, **36**(1): 61-71. DOI: <https://doi.org/10.1111/j.1574-6941.2001.tb00826.x>
- Flood J. 2006. A Review of *Fusarium* Wilt of Oil Palm Caused by *Fusarium oxysporum* f. sp. *elaeidis*. *Phytopathology*, **96**(6): 660-662. DOI: 10.1094/PHYTO-96-0660
- Gaetan SA, Madia M. 2007. Occurrence of *Fusarium* wilt caused by *Fusarium oxysporum* on common sage in Argentina. *Plant Disease*, **90**(6): 833. <https://doi.org/10.1094/PD-90-0833A>
- Goh KJ. 2000. Climatic requirements of the oil palm for high yields. In *Managing oil palm for high yields: agronomic principles*, Goh KJ. (ed.). Malaysian Society Soil Science and Param Agriculture Surveys: Kuala Lumpur; 1-17.
- Hamel C, Vujanovic V, Nakano-Hylander A, Jeannotte R, St-Arnaud M. 2005. Factors associated with *Fusarium* crown and root rot of *asparagus* outbreaks in Quebec. *Phytopathology*, **95**: 867-873. DOI: 10.1094 / PHYTO-95-0867
- Himmelbauer ML, Loiskandl W, Kastanek F. 2004. Estimating length, average diameter and surface area of fine roots using two different image analyses systems. *Plant and Soil*, **260**: 111-120. DOI: 10.1023/B: PLSO.0000030171.28821.55
- Hinde Boudoudou H, Hassikou R, Ouazzani Touhami A, Badoc A, Douira A. 2009. First manifestations of *Fusarium* wilt on germination and rice seedlings. *Bulletin de la Société de Pharmacie de Bordeaux*, **148**: 45-54. DOI: 10.1094 / PDIS-94-9-1170A.
- Jourdan C, Michaux-Ferrière N, Perbal G. 2000. Root System Architecture and Gravitropism in the Oil Palm. *Annals of Botany*, **85**: 861-868. DOI: 10.1006/anbo.2000.1148
- Kenneth GP, Lindel MC, Wayne TO, David WT. 2019. The epidemiology of *Fusarium* wilt of banana. *Frontiers in Plant Science*, 10: 1395. DOI: <https://doi.org/10.3389/fpls.2019.01395>
- Lazreg F, Belabid L, Sanchez J, Gallego E, Garrido-Cardenas JA, Elhaitoum A.

2013. First report of *Fusarium acuminatum* causing damping off disease on Aleppo pine in Algeria. *Plant Disease*, **4**: 557. DOI : doi/abs/10.1094/PDIS-06-12-0608-PDN
- Lazreg F, Belabid L, Sanchez J, Gallego E, Bayaa B. 2014. Pathogenicity of *Fusarium* spp. associated with diseases of Aleppo-pine seedlings in Algerian forest nurseries. *Journal of Forest Science*, **60**(3): 115-120.
- Ntsomboh-Ntsefong G, Ngando-Ebongue GF, Koona P, Bell JM, Youmbi E, Ngalle HB, Bilong EG, Madi G, Anaba B. 2012. "Control approaches against vascular wilt disease of *Elaeis guineensis* Jacq. caused by *Fusarium oxysporum* f. sp. *elaeidis*". *Journal of Biology and Life Science*, **3**(1): 160-173.
- Ntsomboh-Ntsefong G, Madi G, Nyaka NA, Nsimi MA, Epoh GT, Namuene KS, Fontem LA, Ngando Ebongue GF. 2015a. Vascular wilt disease tolerance status of some oil palm (*Elaeis guineensis* Jacq.) progenies with local strains of *Fusarium oxysporum* f. sp. *elaeidis* in Cameroon. *Int. J. Curr. Res. Biosci. Plant Biol.*, **2**: 111-122.
- Ntsomboh-Ntsefong G, Epoh-Ngunea T, Madi G, Nsimi-Mva A, Ngando Ebongue GF, Kounga Tagne S, Mpondo Mpondo E, Dibong D. 2015b. "Isolation and in vitro characterization of *Fusarium oxysporum* f. sp. *elaeidis*, causal agent of oil palm (*Elaeis guineensis* Jacq.) vascular wilt. *Research in Plant Sciences*, **3**(1): 18-26. DOI:10.12691/plant-3-1-4
- Oil world. 2014. Major vegetable oils: world supply and distribution. Oil world.
- Oritsejafor JJ. 1986. Weed hosts of *Fusarium oxysporum* f.sp. *elaeidis*. *Oléagineux*, **41**: 1-7.
- Paul CP. 1995. *Fusarium* wilt of oil palm. Studies on resistance and pathogenicity. Ph.D. Thesis, University of Bath, Bath.
- Renard JL, Gascon JP, Bachy A. 1972. Research on vascular wilt disease of the oil palm. *Oléagineux*, **27**: 581-591.
- Smykalová I, Griga M. 2011. Comparison of the effects of *Fusarium solani* filtrates *in vitro* and *in vivo* on the morphological characteristics and peroxidase activity in pea cultivars with different susceptibility. *Journal of Plant Pathology*, **93**: 19-30.
- Tailliez B, Ballo KC. 1992. A method of measuring the leaf area of the oil palm. *Oléagineux*, **47**: 537-545.
- Tariq RMS, Sahi ST, Ahmad T, Hannan A. 2015. Changes in mineral profile and morphological traits of pea (*Pisum sativum* L.) as influenced by *Fusarium oxysporum* f. sp. *Pisi* under natural conditions. *Journal of Plant Pathology and Microbiology*, **5**: 244.
- Tengoua FF, Bakoumé C. 2008. Pathogenicity of Cameroon strains of *Fusarium oxysporum* f. sp. *elaeidis* – the causal agent of oil palm vascular wilt. *The Planter*, **84**: 233-237.
- Tucker CC, Chakraborty S. 1997. Quantitative assessment of lesion characteristics and disease severity using digital image processing. *Journal of phytopathology*, **145**: 273-278.
- Voui bi BNB, N'guessan KA, Kassik FJM, Tape bi FA, Kamanzi K. 2016. Insectes ravageurs et champignons parasites associés au dépérissement des peuplements de *Tectona grandis* (teck) régénérés à Téné, zone semi-décidue de Côte d'Ivoire. *Int. J. Biol. Chem. Sci.*, **10**(1): 87-105. DOI : http://ajol.info/index.php/ijbcs
- Yergeau E, Sommerville DW, Maheux E, Vujanovic V, Hame C, Whalen JK, St-Arnaud M. 2006. Relationships between *Fusarium* population structure, soil nutrient status and disease incidence in field-grown *asparagus*. *FEMS Microbiology Ecology*, **58**: 394-403. DOI: 10.1111/j.1574-6941.2006.00161.x.