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Field and *In-vitro* Studies on *Corynespora* Leaf Fall Disease of *Hevea brasiliensis* in Nigeria

E. O. Akpaja[1], D. Omorogbe[2] and N. A. Nwogu[3*]

[1]Department of Plant Biology and Biotechnology, University of Benin, P.M.B.1154, Benin City, Edo State, Nigeria.
[2]Rubber Research Institute of Nigeria, Iyanomo, Edo State, Nigeria.
[3]Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author EOA designed the study and wrote the first draft of the manuscript. Authors DO and NAN managed the sample study and analyses of the study. Author NAN managed the literature searches and wrote the final manuscript. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aims: This study evaluated the current incidence and severity of *Corynespora* leaf fall disease (CLFD) in the fields among cultivated rubber clones grown in Nigeria and the growth performance of selected isolates of the pathogen on Potato Dextrose Agar (PDA).
Study Design: The field survey was made in randomized complete block design consisting of three replicates.
Place and Duration of Study: The field study was carried out in the bud wood nursery of Rubber Research Institute of Nigeria (RRIN), Iyanomo, Edo State, Nigeria between April 2013 and August 2013.
Methodology: Ten (10) leaves per replicate were picked at random and assessed for incidence of CLFD based on severity. Disease-scoring rating chart was used in assessment of infection and calculation of Disease Index. The pathogenicity of all the different types of symptoms observed in CLFD was evaluated based on radial growth and biomass of the *Corynespora cassiicola*. Data collected were subjected to analysis of variance (ANOVA) using SPSS software (version 16.0).

**Corresponding author: E-mail: nwogunkem@yahoo.com;*

Mean separation was done using Duncan's Multiple Range Test. All tests were carried out at 5% probability level.

Results: The incidence of CLF disease was found to be between 80 to 100% in all the rubber clones screened. When the isolates were grown on PDA, there were significant differences (P = .05) in the growth of the different isolates. The least mycelial diameter (2.08±0.25 cm) was observed in NIG 910 while the highest (6.60±0.13 cm) was recorded in NIG 804. NIG 800 showed the least mycelial biomass of 0.078±0.05 g while the highest mycelial biomass was recorded in NIG 900 (0.35±0.27 g) and NIG 910 (0.35±0.26 g).

Conclusion: In this study, it was observed that *C. cassiicola* infected all the rubber clones investigated with varying aggressiveness. Newly bred rubber clones should be developed and screened for resistance against *C. cassiicola* prior to recommendation to growers. Further studies to determine the exact role of cassiicolin in *C. cassiicola* pathogenicity would prove very useful in *Hevea* breeding programme.

Keywords: Hevea brasiliensis; rubber clones; phytopathogenic; CLF disease.

1. INTRODUCTION

Hevea brasiliensis Muell. Arg. is an economic plant principally valued for its latex or Natural Rubber (NR). Generally, the industrial sector depends on NR production especially considering the diminishing reserves of petroleum with increasing environmental hazards [1]. NR tree grows well in rain forest regions of lowland tropics with temperature ranges of between 21 – 35ºC and as well distributed rainfall of 2000 mm or more on a well-drained soil [2]. The rubber tree is affected by plethora of economically important pathological problems which many of them are of fungal origin [3]. In Nigeria, the most important rubber diseases of rubber seedlings and budded plants in the nursery are leaf disease [4,1], while in some countries like United States of America, the South American Leaf Blight (SALB) is the most devastating leaf disease especially in mature plantations [5].

Corynespora casiicola (Berk. and Curt) Wei. is the causative agent of *Corynespora* Leaf Fall Disease (CLFD) in young and matured rubber trees [6]. Cassiicolin (cas), a glycosylated cysteine-rich small secreted protein (SSP) has been identified as a potential CLF disease effector in rubber tree [7]. Harinidi et al. [8] reported that susceptible clones affected by *C. cassiicola* could suffer continuously in such a long period that crown becomes leafless for the whole year. Folial infection by this pathogen could cause dieback, stunted increment, while on mature trees it could obviously reduce latex production [9].

This study evaluated the current incidence and severity of CLFD in the fields among cultivated rubber clones grown in Nigeria and the growth performance of selected isolates of the pathogen on Potato Dextrose Agar (PDA).

2. MATERIALS AND METHODS

2.1 Experimental Location for Field Survey

The field study was carried out in the bud wood nursery of Rubber Research Institute of Nigeria (RRIN), Iyanomo, Edo State, Nigeria. Twenty four (24) clones of *Hevea brasiliensis* were evaluated for pathogenicity test to *Corynespora* leaf fall disease (CLFD). The clones included thirteen (13) indigenous and eleven (11) exotic clones obtained from Malaysia, Sri-Lanka and Indonesia (Table 1).

2.2 Experimental Design

The field survey was made in randomized complete block design consisting of three replicates. Ten (10) leaves per replicate were picked at random and assessed for incidence of CLFD based on severity as described by [10]. Disease-scoring rating chart of IRRDB [11] and method of [12] was used in assessment of infection and calculation of Disease Index.

Disease Index (DI) =

$$\frac{(0\text{xa}) + (1\text{xb}) + (2\text{xc}) + (3\text{xd}) + (4\text{xe})}{\text{a} + \text{b} + \text{c} + \text{d} + \text{e}} \times \frac{100}{\text{X}}$$

Where;

0, 1, 2, 3, 4, 5 = Infection categories.

No Infection spot = 0
Less than 10% of leaves infected (Very light) up to 5 spot = 1
Light – 5 to 10 spots and 10-25% leaves fall = 2
Moderate > 10 spots and 26 to 50% leaf fall = 3
Severe – Large lesions and 51 to 75% leaf fall = 4
Very Severe – Large lesions and > 75% leaf fall = 5
a, b, c, d, e, f, = No of leaves/ plant that falls into the infection categories.
X = Maximum number of infectious categories.

2.3 *In vitro* Evaluation

The pathogenicity of all the different types of symptoms observed in CLFD was evaluated based on radial growth and biomass of the *Corynespora cassiicola*. Isolates of *C. cassiicola* were identified based on colony and conidial morphology.

2.4 Mycelial Growth Measurement

The leaf samples were collected with clean polythene bag. The disease lesions were cut to size and sterilized with 0.1% mercury chloride. The sterilized samples were inoculated aseptically and incubated at 27ºC. After 48 hours colonies of the inoculum were observed and pure culture of it was made and re-inoculated for radial dimension. The procedure of [13] on radial extension was employed. The inoculated dishes were incubated at 27ºC and readings were taken at 12 hourly intervals from the second day after inoculation by measuring the diameter. The experiment was carried out in 4 replicates.

2.5 Biomass Determination

After three weeks of incubation, the petri-dishes were autoclaved at 121ºC for 15 minutes according to the method of [14]. The mycelia mat in each petri dish was carefully scrapped on to Whatman No. 1. Filter paper and mycelia biomass was determined as described by [15].

2.6 Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using SPSS software (version 16.0). Mean separation was done using Duncan's Multiple Range Test. All test were carried out at 5% probability level.

3. RESULTS AND DISCUSSION

The severity of *Corynespora* leaf fall disease in studied rubber clones is shown in Table 1. The severity was highest in AV 163 with disease index of 69.3. This was followed by NIG 803 having value of 57.7.

Table 2 shows the symptoms of *Corynespora cassiicola* found in 24 rubber clones examined with percentage incidence ranging from 80 to 100%. NIG 801, 803, 804 and 901 were among the clones with lower percentage incidence, while RRIM 612, 701, 707, PB 217 and G11 clones had highest rate of CLFD incidence.

There was significant difference (*P = .05*) in the performance of the different isolates at 95% level of probability measured by mycelia diameter of the pathogen on PDA as shown in Table 3. The least mycelia diameter (2.08±0.25 cm) was observed in symptoms type D (NIG 910) while the highest mycelia diameter of (6.60±0.13 cm) was found observed in NIG 804 with symptom type D.

As shown in Table 4; there was little variation in biomass among the selected isolates. NIG 800 showed the least mycelia biomass of 0.078±0.05 g while the highest mycelia biomass was recorded in NIG 900 (0.35±0.27 g) and NIG 910 (0.35±0.26 g).

This study showed that the pathogen *Corynespora cassiicola* (Berk. and Curt) infected the twenty four rubber clones examined (Table 1). This is contrary to the observation of [16] that *C. cassiicola* infect selected clones of *Hevea brasiliensis*. The ability of C*. cassiicola* to infect all the clones in this study may be attributed to the fact that though *C. cassisola* is known to vary in its pathogenicity, and it makes rubber clones to become more susceptible with time according to the findings of [17].

The severity of CLF disease was highest in AV 163 followed by NIG 803 (Table 1). The difference in severity among the different clones may not be attributed to qualitative variation in the sequence or structure of the toxin produced by various isolates considering the findings of [7].

Table 1. Severity of *Corynespora cassiicola* leaf fall disease in selected rubber clones

*Symptoms	Rubber clones																						
	NIG 800	NIG 801	NIG 802	NIG 803	NIG 804	NIG 900	NIG 901	NIG 903	NIG 906	NIG 907	NIG 908	NIG 909	NIG 910	HAR 1	RRIM 612	RRIM 701	RRIM 707	PB 217	PB 28/59	PB 5/51	GT 1	RRIC 45	PR 107	AV 163
A	38.8	56.7	3.3	57.7	16.7	0.8	0.8	-	-	7.5	-	-	62	-	5.0	-	0.8	-	4.2	0.8	0.8	-	-	69.2
B	-	-	-	5.0	-	1.7	4.2	-	-	31.7	2.5	12.5	-	12.5	56.7	-	-	3.3	16.7	11.7	52.8	21.7	14.1	5.0
C	2.5	-	-	8.0	41.7	-	15.8	-	11.7	-	4.2	-	4.2	-	-	-	0.8	-	-	14.2	-	-	11.2	-
D	-	-	-	-	6.7	35.0	-	36.7	-	-	-	5.8	-	-	-	45.8	39.2	24.2	-	14.3	-	5.0	5.0	-
E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.1
F	-	-	-	-	-	-	2.5	-	-	-	-	-	-	-	-	13.3	6.7	-	-	-	-	5.8	-	-
G	3.7	-	-	4.2	5.8	5.8	1.7	2.5	-	5.0	-	7.6	3.3	3.3	-	-	-	-	-	3.3	-	-	-	-
H	4.2	1.7	13.3	3.3	-	-	-	4.2	0.8	-	0.8	6.7	-	-	-	7.5	-	0.8	-	3.3	-	-	-	-
I	2.5	10.0	-	-	-	0.8	-	-	-	2.5	-	5.8	7.5	-	-	6.7	5.8	2.5	-	0.8	2.5	1.7	-	-
J	-	-	-	-	2.5	-	-	-	2.5	-	-	-	1.7	-	-	-	4.2	-	-	-	-	-	-	-
K	-	-	6.7	-	-	-	-	-	-	-	0.8	1.7	-	-	-	-	-	-	-	1.7	-	1.7	0.9	0.8
L	-	-	-	-	-	-	-	-	0.8	1.7	-	-	-	-	-	-	-	1.7	-	-	-	1.7	-	-

**The disease manifest in various symptoms*

Table 2. Incidence of *Corynespora cassiicola* in selected rubber clones

S/no	Clones	Source	Incidence (%)
1	NIG 801	Nigeria	80.0
2	NIG 803	Nigeria	83.3
3	NIG 901	Nigeria	83.3
4	NIG 804	Nigeria	86.7
5	NIG 800	Nigeria	90.0
6	NIG 906	Nigeria	90.0
7	NIG 907	Nigeria	90.0
8	NIG 909	Nigeria	90.0
9	NIG 910	Nigeria	90.0
10	HAR 1	Malaysia	90.0
11	PB 5/51	Malaysia	90.0
12	NIG 900	Nigeria	96.7
13	NIG 903	Nigeria	96.7
14	NIG 908	Nigeria	96.7
15	RRIC 45	Sri- Lanka	96.7
16	NIG 802	Nigeria	96.7
17	RRIM 612	Malaysia	100.0
18	RRIM 701	Malaysia	100.0
19	RRIM 707	Malaysia	100.0
20	PB 217	Malaysia	100.0
21	PB 28/59	Malaysia	100.0
22	GT 1	Malaysia	100.0
23	PR 107	Indonesia	100.0
24	AV 163	Indonesia	100.0

Table 3. *In-vitro* mycelial growth of selected isolates of *Corynespora cassiicola* five days after inoculation

Isolates	Type of symptom	Mycelial diameter (cm)[x]
NIG 800	A[Y]	2.83±0.99[c]
NIG 801	C	3.03±0.70[c]
NIG 803	G	3.43±0.14[c]
NIG 804	D	6.60±0.13[d]
NIG 900	H	2.25±0.35[b]
NIG 910	A	2.08±0.25[a]
HAR 1	I	2.28±0.38[b]

X = Means of four (4) replicates ± standard error
Y = Alphabet correlate with the description of isolate's symptom

Table 4. Mycelial biomass of selected isolates of *Corynespora cassiicola* grown *In-vitro*

Isolates	Type of symptom	Mycelial biomass (g)[x]
NIG 800	A[Y]	0.08±0.05[a]
NIG 801	C	0.27±0.11[b]
NIG 803	G	0.18±0.02[a]
NIG 804	D	0.33±0.02[b]
NIG 900	H	0.35±0.27[c]
NIG 910	A	0.35±0.26[c]
HAR 1	I	0.13±0.02[a]

X = Means of four (4) replicates ± standard error
Y = Alphabet correlate with the description of isolate's symptom

There was variation in mycelial diameter and biomass of selected isolates of *C. cassiicola* among the different clones studied (Tables 3 and 4). The mycelial diameter and biomass was used as a crude measure of isolates aggressiveness. Artificial infection tests using these isolates will be conducted in our subsequent experiment to evaluate their aggressiveness. Aggressiveness of isolates may be due to transcriptional regulation of the cassiicolin gene. Transcriptional regulation of cassiicolin gene has been shown to contribute to differences in aggressiveness between isolates in the same cultivar, but not to difference in pathogenicity/ virulence of one isolate in different cultivars [7]. However, no expression experiments have been conducted during present study.

4. CONCLUSION

In this study, it was observed that *C. cassiicola* infected all the rubber clones investigated with varying aggressiveness. It should be remembered that there are various physiological races of *C. cassiicola* infecting the rubber tree. Therefore, newly bred rubber clones should be periodically screened for resistance against this fungus before recommendation to growers. Further studies to determine the factors responsible for the role of cassiicolin in organism virulence would prove very useful in *Hevea bresiliensis* breeding programme.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Omorusi VI, Ikerodah-Omo EE, Mokwunye MUB. Evaluation of effect of antagonistic fungi and arbuscular mycorrhizal fungi (AMF) on incidences of some disease *Hevea brasiliensis* (Muell. Arg). Nature and Science. 2011:9(12):151–154.
2. Chee KH. Present status of rubber diseases and their control. Review of plant Pathology. 1990:69(7):430–443.
3. Igeleke CL. Disease of Rubber (*Hevea brasiliensis*) and their control. 10th Annual Conference of Horticultural Society of Nigeria; 1998.
4. Begh ER. Hevea plantation establishment. Proceeding of training workshop on the Hevea Plantation establishment held at RRIN Iyanomo; 1995.
5. Ogbebor ON. The status of three common leaf disease of Para rubber in Nigeria. Journal of Animal and Plant Sciences. 2010:6(1):567–570.
6. Evueh AG, Okhuoya JA, Osemwegie OO, Idress HA, Ogbebor ON. Evaluation of phylloplane fungi as biocontrol agent of *Corynespora* leaf fall disease of Rubber (*Hevea brasiliensis* Muell. Arg). World Journal of Fungal and Plant Biology. 2011:2(1):0 –05.
7. Déon M, Bourré Y, Gimenez S, Berger A, Bieysse D, Lamotte F, Poncet J, Roussel V, et al. Characterization of a cassiicolin – encoding gene from *Corynespora cassiicola*, pathogen of rubber tree (*Hevea brasiliensis*). Plant Science. 2012;185–186:227–237.
8. Harinidi SS, Wisma S. Chemical control of *Corynespora* Leaf - fall. Proceedings of the Workshop on *Corynespora* Leaf fall disease of *Hevea* Rubber, Indonesian Rubber Research Institute, Medan, Indonesia; 1996.
9. Ogbebor NO, Adekunle AT. Inhibition of conidial germination and mycelial growth of *Corynespora cassiicola* (Berk and Curt) of rubber (*Hevea brasiliensis* Muell. Arg.) using extracts of some plants. African Journal of Biotechnology. 2005:4(9):996–1000.
10. Breton F, Sanier C, d' Auzac J. Role of Casiicolin, a host selective toxin in pathogenicity of *Corynospora cassiicola* causal agent of a leaf fall disease of *Hevea*. Journal of Rubber Research. 2000:3:115–128.
11. International Rubber Research and Development Board (IRRDB). International Rubber Research and Development Board (IRRDB) Workshop on *Corynespora* leaf fall of Rubber; 2000.
12. Parry D. Plant Pathology in Agriculture. Cambridge University Press. U. K; 1990.
13. Okwulehie IC, Okwujiako IA, Igbojionu V. Studies on nutritional requirement and growth of *Pleurotus pulmoarius* (Fries) Quelet, an exotic mushroom. Nigerian Journal of Botany. 2006:19(2):308-316.
14. Schisler LC, Volkoff O. The effect of safflower oil on mycelial growth of Boletaceae in submerged liquid cultures. Mycologia. 1977:69:118-125.
15. Nwanze PI, Khan AU, Ameh JB, Umoh VJ. The effect of media, oil type and rate on

the mycelial wet and dry weights of *Lentinus squarrosulus* (Mont). Sing and *Psathyrellaaroumbonata* Pegler in submerged liquid culture. African Journal of Biotechnology. 2005:4(3):326-331.

16. Jaysuriya KE, Deacon JE, Fernando S. Weakening effect of 2-furaldehyde on *Rigidoporus lignosus.* Journal of Rubber Research Institute of Sri-Lanka. 1996;77: 54–65.
17. Yanxiang Qi, YixianXie, Xin Zhang, Jingi Pu, Huiqiang Zhang, Silaing Huang, He Zhang. Molecular and pathogenic variation identified among isolates of *Corynespora cassiicola*. Molecular Biotechnology. 2009: 41(2):145–151.

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