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CONTROL OF BLACK POD DISEASE OF CACAO THROUGH SPORE-DISPERSAL CONTROL WITH *THAUMATOCOCCUS DANIELLI.*

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ABSTRACT

A field and laboratory experiment were conducted in two (2) cocoa plantations in Osonba village in Oban Clan, Akampka Local Government Area of Cross River State, Nigeria to test the role of *Thaumatococcus danielli;* as undergrowth in the control of vertical and horizontal spread of *Phytophthora palmivora* the causal agent of black pod disease of cocoa. Results from the experiment showed that the disease development was acropetal, progressing from pods near the plantation floor to those at the apex. Incidence and severity were high with increase in rainfall implicating rain splash as the main means of inoculum dispersal. Spore count taken from horizontal traps with a hemacytometer, showed that more spores (2.8×10^4) of the fungus were trapped in plots without *T. danielli* growing as undergrowth while in plots with *T. danielli,* only few spores were trapped (0.7×10^2). Quantity of spores trapped by vertically-pitched traps increased with decrease in distance between traps and cacao tree implying that more spores are dropped under the cacao canopy than outside. Disease incidence (I) was higher in the control (without *T. danielli*) plot (71%) than in the treatment (34%). When disease severity index (S) was worked out and the two experiments compared, that in the control was higher (5.6) than in treatment (1.7). Results of this research show that rainsplash is the major means of inoculum dispersal and, incidence and severity were directly proportional to amount of rainfall. However, (I) and (S) reduced where *T. danielli* grew as undergrowth. This implies that there was reduction in rainsplash impaction by the glabrous and wide ovate leaves of *T. danielli* hence, the possibility of growing this plant in a mixed cropping system with *T. cacao* with intent to control the cacao black pod disease as well as conserving this very important sweetener (*T. danielli*).

Keywords: Cross River State, *Phytophthora palmivora*, *Thaumatococcus danielli*, rain splash impaction, cacao black pod.

1. INTRODUCTION

Cacao (*Theobroma cacao* L) is a member of the family Sterculiaceae. Members are distributed in the tropical region of the world. The seeds from cacao pods when extracted and dried are used for the production o chocolate which is a very popular beverage (Thurston, 1998). However, according to Mossu (1992), the ability to extract fat from chocolate and the invention of milk chocolate have increased the use and demand for cacao. Today, it is popular as a beverage, as candy and in many deserts.

According to FAO (1996), though the largest consumption of cacao beverages is in the developed countries, the major producers are the tropical countries. The plant is raised in nurseries and transferred to permanent sites where they are planted in plantations.

Most cacao plantations are sited in deforested areas. This production environment provides very conducive condition for the multiplication of pathogens especially those of fungal origin thus predisposing the plant to different fungal diseases.

According to Gregory (1974), Luz (1989) and Danquah (1995), diseases are serious limiting factors in cacao production and according to results of their works, the most serious and widely distributed disease of cacao worldwide, is probably the black pod rot caused by some species of *Phytophthora*. The most virulent species in this genus which has been isolated from diseased pods in nearly all cacao growing countries is *P. palmivora*.

This fungus which belongs to the family Pythiaceae infects pods at different ages but the greatest losses are recorded at two (2) months prior to ripening. Extensive epidemiological studies of black pod disease have shown that splashing rain appears to be the most important

means of the disease spread (Nambiar and Sarma, 1982, Maddison and Griffin, 1972).

Recently in Nigeria, according to Government Report, there has been a down-turn in cacao bean production from 330,000 metric tones in 2006/2007 season, to 212, 000 metric tones in 2008/2009 season (The Nation Newspaper, 9th March, 2010). One of the reasons for this fall in production among others is the high cost of chemicals which makes purchase of agrochemicals for control of pests and diseases very difficult. It is based on this that 14 Governors from cacao producing States met in Ondo State, Nigeria to deliberate on ways of reverting the trend (The Nation Newspaper, 9th March, 2010) through alternative and environmental-friendly means.

Thaumatococcus danielli (Benn.) Benth. Commonly called Miraculous fruit, belongs to the family Maranthaceae. In Nigeria, the plant is highly valued as a sweetener. It is a natural source of Thaumatin; an intensely sweet protein which is located on the aril of the seed. The plant has large papery leaves with glabrous adaxial surface which measures up to 46cm long. The plant is popular locally because the leaves are used to wrap food locally, the sturdy petioles are used as building materials and for mat production while the seeds contain Thaumatin and have a number of traditional medicinal uses (Adeyemi *et al*., 2014; Shalom *et al*., 2014).

Because of its various uses locally and internationally, a stakeholders' forum was held in Calabar at the instance of the Federal Government of Nigeria with the theme "Unveiling the sweet prospects of Thaumatin: a natural sweetener from *Thaumatococcus danielli*". This research is therefore aimed at controlling Black-pod disease of Cacao through a mixed cultivation system thereby conserving the very important sweetener: *Thaumatococcus danielli*.

2. MATERIALS AND METHODS:

2.1 Study site:

Two (2) cacao plantations with history of cacao black pod disease incidence were chosen as sites for the study. The estates are located in Osonba village, Oban clan in Akamkpa Local Government Area of Cross River State, Nigeria. Mapping-out of plots was done in January, 2010 before the rains. The plantations were separated in space by 3.5 kilometres. One of the plantations had herbs of not more tan 0.36m as undergrowth (treatment) while the second plot had *Thaumatococcus danielli* (control) .with varying heights as undergrowth.

2.2 Plant material:

The variety, Forastero amazona is the most commonly cultivated cacao variety in Cross River State, Nigeria and it was the variety used for this study. Plants were planted at standard spacing of 4m x 4m as recommended by Mossu (1992).

2.3 Isolation of fungal pathogen from host plants and identification.

Test plant pods with characteristic black-pod symptoms were harvested from the field. These pods were washed under a flowing tap, surface sterilized with ethyl alcohol and finally rinsed with several changes of sterile distilled water. The diseased portions were cut out at the interface between the healthy and the diseased tissues.

These portions were placed on a gelled potato dextrose agar (PDA) in Petri dishes, incubated at 28±1°C until growth was observed. Through series of sub-culturing, pure cultures were obtained and maintained on agar slant in a refrigerator (Udo *et al*, 2008). Identification was done with the help of a microscope.

2.4 Pathogenicity test:

Ascertaining the causative agent was through Koch's postulate and done according to Heist *et al* (2001). With a sterile hypodermic syringe equipped with a 20 gauge needle, approximately 5×10^5 spores were sprayed on a wet healthy cacao pod by spraying to run-off level. The pods were covered with transparent polyethylene bags and allowed to stay under observation for symptoms development. Spores measurement was done with a hemacytometer. The control experiment was carried out with sterile distilled water without spores.

All the experimental set-ups were observed for symptom development. The experiments were replicated five (5) times each.

2.5 Experimental design and field samples:

In every study plot of 1acre each, 5 plots of 20m x 20m were mapped out as sub-plots. The different spore traps were randomly pitched. Cacao pods showing symptoms of black pod disease (brown spots at early stage of infection and dark grey pods at late stage of attack) were sampled and recorded as being infected. From each sampled tree, last pod infected and closest to the plantation floor and the one towards the apex but closest to the pod without disease symptoms were recorded as samples for pathogenicity test (Newbook, 1982).

2.6 Spore dispersal, collection and disease progress:

A uni-directional spore traps protocol as described by Ahimera *et. al.* (2004) was adopted. In April of the study year, some 9cm diameter funnels connected by stoppers to 250ml conical flasks were attached to 2.54cm diameter galvanized pipes.

Disease progress was analyzed using traps of different dimensions. For horizontal disease progress, the spore traps were designed to have conical flasks with funnels at varying heights of 20cm, 30cm, 40cm, 50cm and 100cm above the plantation floor litter where the pathogen is known to overwinter. All the traps were located 1m away from cacao stand (Ramachandran *et al* 1990).

For vertical disease progress, 2 traps were pitched at equidistant positions of 1m, 2m 3m and 4m away from the cacao stem. The experiment was set up in a site with *T. danielli* growing as the undergrowth and site where undergrowth was made up of normal plantation weeds such as *Hygrophilia* sp. with height not above 30cm. Traps were located 1m away from cacao stand (Ramachandran *et al* 1990). Water samples collected in the conical flasks was decanted after a day, 2 or 3 days of exposure depending on the amount of rainfall. The number of the fungal spores was quantified in 2ml of agitated water (from every flask) with the use of a haemocytometer (Neubauer-improved 0630010 model) under a binocular microscope.

Experiments were conducted within the months of May and June when incidence normally occurs.

2.7 Incidence of disease:

Disease incidence (I) was recorded as the proportion of diseased stands to total number of plants in the study plot. The percentage incidence was calculated using the formula below;

$$\% \text{ incidence} = \frac{\text{No. of stands infected}}{\text{Total stands sampled}} \times \frac{100}{1}$$

2.8 Disease severity:

Disease severity (S) was recorded as the sum of the proportion of infected leaves (within a point scale) per stand divided by the total number of stands sampled including those with zero incidence rates (Groth *et. al.*, 1999). The disease score was measured on a 1-to-3 point scale (1 = no disease, 2 = few spots on pod and 3 = pods severely affected with grey patches) rating scale. The formula below was used:

$$\frac{(X_1 x1) + (X_2 x2) + (X_3 x3)}{(X_1 + X_2 + X_3)}$$

Where X is the stand severity multiplied by the different rating class scales (1, 2. .....n).

2.9 Analysis of data:

Number of spores in the different water samples was compared for the various months within the study period. A regression analysis was performed to determine relationship between the quantity of *P. palmivora* conidia and the amount of rain water collected over the study period.

3. RESULTS AND DICUSSION

3.1 Disease incidence and severity:

Incidence measurement was calculated based on the ratio of infected stands to the total plants population sampled in a particular plot. Fig. 1 shows the percentage incidence of the black pod disease. It was recorded that the control (without *T. danielli*) had 5.0% incidence 1n the month of May, 28% for June and 71% in June. Incidence in the treatment was the reverse scoring 0.3%, 12% and 34% for the months of April, May and June respectively.

The highest disease severity index was obtained in June for the control (Fig. 2). In this month,

the infected spots coalesced to produce large dead pods on the tree. For this month, *the* treated plots recorded a severity index of 5.6, followed by 3.3 in May and 0.7 in April. For the treated samples, the same trend was followed as in disease incidence. The reason for the high incidence of the disease in June may be because of the heavy amount of rainfall (224mm).

This amount of rainfall must have triggered the germination of spores of the pathogen, mycelial growth and subsequent production of spores for infection to take place. This was also suggested by Pusey (1997) for fir blight. It therefore implies that the amount of rains can be used to predict the incidence of this disease. Severity index followed the same trend and the same reason can be given for the high severity index in the control experiment. However, more spores were trapped at a vertical distance of 1m but beyond this, there was no significant trend in the amount of spores collected.

3.2 Disease progress:

The result in Table 1 also gives an indication that more spores were trapped at a height of 30 cm in the control than at other heights (20, 40, 50 and) and in the treated plots. The implication here may be that the distance between the cacao canopy and the plantation floor was such that the raindrop impaction could lift more spores above 20cm with impact reducing beyond 30cm. This agrees with the works of Ogawa and English, (1991) on the spread of disease propagules in temperate trees. There was a general reduction in disease incidence on cacao trees growing where *T. danielli* thrived as undergrowth. The reason for this is simple. *T. danielli* has a large glabrous leaf with an interlocking growth habit thus forming a cover over the plantation floor. This cover therefore prevents the direct contact of raindrops with the plantation floor there reducing the potential of drop impaction to disperse the spores. This is suggested by Ramachandran *et al* (1990) for the control of Phytophthora leaf infection in black pepper in Areca-black pepper mixed cropping system.

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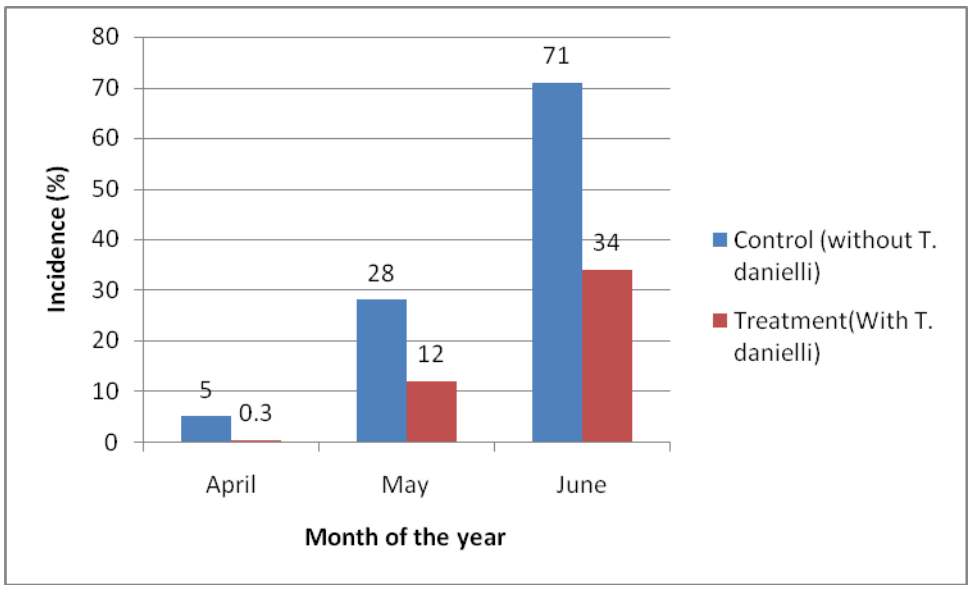

Fig. 1: Percentage incidence of black pod disease in study area

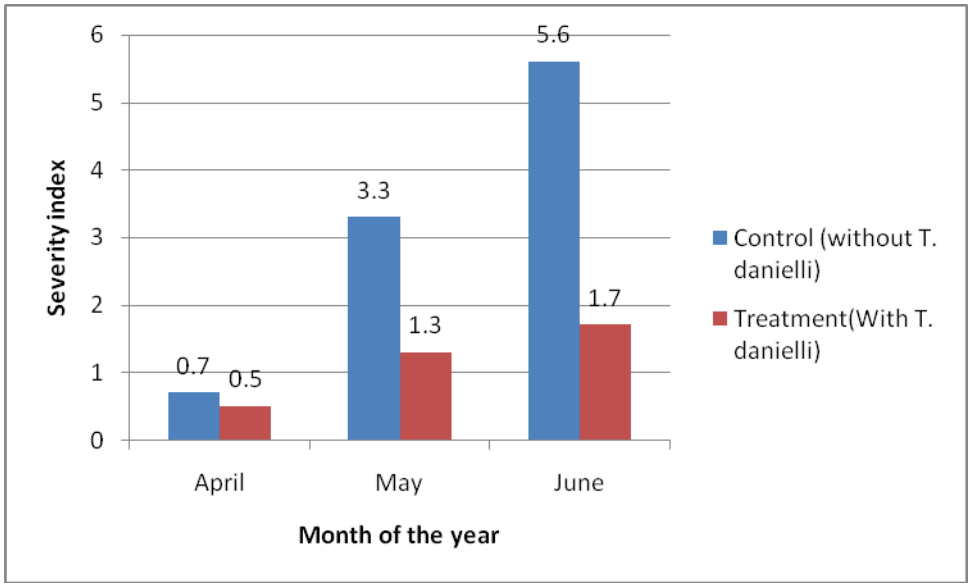

Fig. 2: Severity index of black pod disease in study area

Table 1: Horizontal progress of black pod disease within the study period.

Month	Plot with *T. danielli* as undergrowth					
	Rainfall measurement (mm)	Trap horizontal distance/quantity of spore trapped.				
		20	30	40	50	60
April	118	$12x10^1$	$2.7x10^1$	$1.9x10^1$	$0.2\ x10^2$	$0.4\ x10^1$
May	213	$0.6x10^1$	$0.5\ x10^2$	$0.8\ x10^2$	$1.3\ x10^1$	$0.6\ x10^1$
June	224	$1.0\ x10^1$	$0.7\ x10^2$	$9.1\ x10^1$	$0.5\ x10^2$	$1.1\ x10^1$

Table 2: Vertical progress of black pod disease within the study period.

Month	Plot with *T. danielli* as undergrowth				
	Rainfall measurement (mm)	Trap horizontal distance/quantity of spore trapped.			
		1	2	3	4
April	118	$6.2\ x10^2$	$4.3\ x10^2$	$9.5\ x10^2$	$2.0\ x10^1$
May	213	$8.0\ x10^2$	$7.1\ x10^2$	$4.6\ x10^2$	$\mathbf{3.1}\ x10^1$
June	224	$2.7\ x10^2$	$5.8\ x10^2$	$2.6\ x10^2$	$2.4\ x10^1$
	Plot without *T. danielli* as undergrowth				
April	118	$4.1\ x10^4$	$5.1\ x10^2$	$8.0\ x10^2$	$3.1\ x10^2$
May	213	$6.8\ x10^3$	$3.8\ x10^3$	$5.2\ x10^2$	$4.4\ x10^2$
June	224	$6.0\ x10^3$	$3.6\ x10^3$	$6.6\ x10^2$	$3.2\ x10^2$

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