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Root and Stem Damage Caused by Termite-fungi Interaction on Rice

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Abstract: Termites are serious biotic threat to rainfed upland rice ecology. The objective of this study was to evaluate the root and stem damage caused by the effect of termite-fungi (*Botryodiplodia theobromae* and *Trichoderma* sp.) interaction on three upland rice varieties (OS 6, LAC 23 and NERICA 1) planted on ultisol soil under screenhouse condition. It was laid on Randomized Complete Block Design (RCBD) with three replications. The results showed that rice variety OS 6 was found to be most susceptible to termite infestation and the termite- fungi interaction as the damage caused on root was high. The root weight was low indicating the level of susceptibility of rice varieties (OS 6>NERICA 1>LAC 23) to termite infestation and damage. NERICA 1 treated with termite, termite+*Botryodiplodia* and *Botryodiplodia* only had significant low root weight, OS 6 treated with *Trichoderma* only, termite+*Botryodiplodia* and Termites+*Trichoderma* had significant root weight reduction. The stem girth taken was significantly low in some treatments with termite and fungi. LAC 23 treated with termite+ *Botryodiplodia* had low stem girth even at three weeks after treatment. NERICA 1 treated with termites had significant low stem girth while OS 6 treated with termite+*Trichoderma* had low stem girth. The nutrient uptake by the roots of rice plant treated with the two fungi was significantly low. The studies harness the economic importance of *Botryodiplodia theobromae* and *Trichoderma* spp., which penetrate into rice plant via termite attack.

Key words: Upland ecology, rice, termites, fungi, damage, interaction

INTRODUCTION

Rice (*Oryza* spp.,) is one of the most important food crops in the world. It is consumed by nearly three billion people almost on daily basis (Nwilene *et al.*, 2011). There is increasing demand for rice on daily basis because of expanding human population. In Africa, there is a wide gap between the production and consumption due to a number of factors. Apart from the inconsistent whether condition during planting, inadequate agronomic practices, diseases and low soil fertility, insects have a considerable impact on rice production causing yield losses of about 30% in upland rice and up to 100% in lowland rice (Umeh *et al.*, 2001; Togola *et al.*, 2010; Togola *et al.*, 2012). They are regularly present in rice fields damaging rice plants and compromising farmers' efforts. Upland rice is mostly at risk of soil-borne organisms including termites and fungi. Termites are most significant soil pests of rice in Africa and are mainly found in the upland rice ecosystems. Of the approximately 2,500 termite species in the world, about 300 are recorded as pests (Logan *et al.*, 1990). In Nigeria, 120 species have been identified, but only 20 spp., damage crops and buildings .Although most termite species feed on dead plant materials, few attack living plants root in the soil. The most damaging termite in Nigeria is *Macrotermes* spp. Damage is caused by the adults (workers) that consume the roots and fill the stem with soil. Root is an integral part of plant where water conduction, food transport and osmotic regulations in plant take place. The damage on the root reduces the translocation of water and nutrients to other parts of the plant leading to the dryness and death of the attacked plant. Yield losses of about 50 to 100% have been attributed to termites in farmers' fields (Togola *et al.*, 2010). Termite damage on rice roots can also predispose the roots to secondary infection or invasion by pathogens including fungi. Any insect infestation and infection of rot causing pathogen can lead to significant yield reduction. Root and crown rot is a significant disease on rice and caused most yield losses in various part of the rice grown areas. The prevalence and economic importance of rot has been linked to culturing susceptible varieties and environmental conditions particularly

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moisture (Saremi and Okhowat 2004). The most common symptoms of foot rot disease in some cases may be abnormal elongation of the plant stem). However, the disease causes poor seedlings (Groth *et al*., 2004). It is also called foot rot in some regions and occurs widely and sporadically in other areas of rice production. In addition the disease has been reported from the rice tracts of south Asia, European countries and America (Desjardins *et al*., 2000). The classic and most conspicuous symptom of the disease is the hypertrophy effect or abnormal elongation of plant. Several studies have focused on the effect of termite or fungal pathogen on root but little attention was paid to the effect of the association between termites and fungal pathogens. Therefore, this study evaluates the detrimental effect of the fungi-termite association on rice root and plant stem.

MATERIALS AND METHODS

This study was conducted at International Institute of Tropical Agriculture (IITA), Africa Rice Ibadan, Nigeria station where three upland rice varieties (NERICA1, OS 6 and LAC 23) were used for evaluation. The experiment was laid out on a Randomized Complete Block Design (RCBD) with three replicates (21 pots per variety and total of 63 pots for the three varieties). For each rice variety, seven pots were filled with soil -six pots with sterilized soil and one pot with naturally infested (unsterilized) soil. The soil sterilization was done with the sterilizing machine-Terra force manufactured by Horticultural Engineers maid stone, New York, USA. The soil was heated up to temperature of 80.96°C for $1^1/_2$ h. The sterile soil was the collected and filled into pots when sufficiently cool. About five seeds were planted per pot. A week after germination, rice stand was thinned down to three stands per pot. About ten living termites (*Microtermes* sp.) were introduced into the soil four weeks after planting. The termites were gently covered with soil. The fungal isolates (*Botryodiplodia theobromae* and the *Trichoderma* sp.) used were collected from laboratory stock culture. Each fungus was prepared with 100 mL of sterile distilled water using conidial inoculation. About 2 mL of each inoculum was injected with needle and syringe into the fibrous root of the plant. Out of the three stands in a pot, two of the stands were treated with the inoculum while one stand served as the destructive samples at one month after the introduction of inoculum while the other two plants were kept until maturity. The stem girth was taken on weekly basis with the use of venial caliper while the root weight was taken with the use of beam balance. The soil sample was collected before planting and after the experiment for analysis of major nutrients in the laboratory. The difference between the soil nutrients before planting and after the experiment represents the major soil nutrients assimilated by the plant root. The treatments were as follows: BT = *B. theobromae*, TS = *Trichoderma* sp., TM = Termite alone, TMB = Termite+*Botryodiplodia theobromae*, TMT = Termite+*Trichoderma* sp., NIS = Naturally Termite Infested Soil and SS = Sterile Soil. Data collected was subjected to analysis of variance (ANOVA) using the SAS 9.1, (SAS, 1999). Analysis of variance was carried out using General Linear Model (GLM) and the significant difference was further determined by Fisher's Least Significant Difference (LSD) to separate means at a significance level of $p<0.05$.

RESULTS

The results revealed the significant variations of the treatments among rice varieties with various levels of susceptibility to termite attack. Among the three varieties used for the study, LC 23 had the highest stem girth, followed by NERICA 1 and OS 6, respectively. There were various degrees of reaction of rice varieties to each treatment. However, each of the varieties planted on naturally infested soil have low stem girth. There was significant reduction in the stem girth of LAC 23 treated with Termite+*Trichoderma* sp., combine (TMT) between 2nd week and 6th week in comparison with the control. *Trichoderma* sp., (TS) did not have significant effect on the stem girth of LAC 23. The plant in naturally infested soil had very low stem girth almost throughout the experiment. For LAC 23 at the end, the plant treated with Termites+*B. theobromae* (TMB) had the least stem girth while plant treated with TS has the highest stem girth (Table 1). At third week, NERICA 1 treated with Termite alone (TM) had significantly small girth followed by plant with Naturally Infested Soil (NIS) and then plant treated with Termite+*Trichoderma* sp (TMT) while others did not give significant effect at 3rd week. The stem girth of NERICA 1 treated with Termite+*B. theobromae* (TMB) was higher than the control although the difference was not significant. At seventh week, the stem girth of the plant in naturally infested soil was the lowest followed by the plant treated with TMT. At the end of the experiment, plant treated with TMB had the least stem girth followed by the plant treated with TMT and then the plant in naturally infested soil. The plant in naturally infested soil had least stem girth almost throughout the experiment (Table 2). The stem girth of OS 6 was smaller than that of LAC 23 but relatively the same with NERICA 1. The stem girth of OS 6 treated with TS was significantly small as well as the OS 6 treated with TMB and the OS 6 treated with TMT with 0.393, 0.393 and 0.367 cm stem girth

Table 1: Plant girth of LAC 23 weeks after treatment

	Weeks after treatment (cm)									
Treatment	1	2	3	4	5	6	7	8	9	10
BT	0.320^{abc}	0.360^{ab}	0.403^{ab}	0.473^{ab}	0.54^{ab}	0.580^{a}	0.527^{ab}	0.550^{a}	0.530^{ab}	0.530^{ab}
TS	0.373^{ab}	0.400^{ab}	0.460^{a}	0.523^{a}	0.583^{a}	0.587^{a}	0.613^{a}	0.633^{a}	0.633^{a}	0.643^{a}
TM	0.320^{abc}	0.323^{b}	0.410^{a}	0.430^{ab}	0.46^{abc}	0.480^{ab}	0.513^{ab}	0.463^{a}	0.523^{ab}	0.523^{ab}
TMB	0.240^{bc}	0.213^{c}	0.253^{c}	0.350^{b}	0.33^{c}	0.360^{b}	0.440^{b}	0.463^{a}	0.450^{b}	0.450^{b}
TMT	0.340^{ab}	0.363^{ab}	0.443^{a}	0.457^{ab}	0.563^{a}	0.607^{a}	0.617^{a}	0.563^{a}	0.617^{a}	0.617^{a}
NIS	0.183^{c}	0.207^{c}	0.300^{bc}	0.353^{b}	0.377^{bc}	0.507^{a}	0.517^{ab}	0.530^{a}	0.533^{ab}	0.537^{ab}
SS	0.387^{a}	0.413^{a}	0.463^{a}	0.470^{ab}	0.473^{abc}	0.507^{a}	0.540^{ab}	0.540^{a}	0.567^{ab}	0.568^{ab}

Column means followed by the same letters are not significantly different at $p \leq 0.05$, TS: *Trichoderma* sp., BT: *Botryodiplodia theobromae*, TM: Termites, TMB: Termites+*B. theobromae*, TMT: Termites+*Trichoderma* sp., NIS: Naturally infested soil, SS: Sterile soil

Table 2: Plant girth of NERICA 1 weeks after treatment

	Weeks after treatment (cm)									
Treatment	1	2	3	4	5	6	7	8	9	10
BT	0.367^{a}	0.353^{a}	0.360^{a}	0.363^{ab}	0.413^{ab}	0.427^{a}	0.430^{a}	0.430^{ab}	0.430^{a}	0.430^{a}
TS	0.353^{a}	0.353^{a}	0.357^{bcd}	0.367^{aB}	0.400^{abc}	0.420^{a}	0.430^{a}	0.43^{ab}	0.433^{a}	0.433^{a}
TM	0.320^{ab}	0.317^{ab}	0.333^{d}	0.370^{aB}	0.378^{abc}	0.387^{ab}	0.40^{ab}	0.393^{bc}	0.393^{ab}	0.393^{ab}
TMB	0.320^{ab}	0.337^{ab}	0.417^{ab}	0.333^{b}	0.377^{bc}	0.370^{ab}	0.373^{abc}	0.373^{cd}	0.367^{bc}	0.367^{ab}
TMT	0.303^{b}	0.300^{ab}	0.350^{cd}	0.367^{ab}	0.473^{a}	0.370^{ab}	0.347^{bc}	0.370^{cd}	0.370^{bc}	0.370^{bc}
NIS	0.240^{c}	0.280^{c}	0.337^{d}	0.330^{b}	0.327^{c}	0.323^{b}	0.323^{c}	0.330^{d}	0.333^{c}	0.333^{c}
SS	0.367^{a}	0.373^{a}	0.403^{abC}	0.350^{ab}	0.400^{abc}	0.373^{ab}	0.410^{a}	0.450^{a}	0.450^{a}	0.450^{a}

Column means followed by the same letters are not significantly different at $p \leq 0.05$, TS: *Trichoderma* sp., BT: *Botryodiplodia theobromae*, TM: Termites, TMB: Termites+*B. theobromae*, TMT: Termites+*Trichoderma* sp., NIS: Naturally infested soil, SS: Sterile soil

Table 3: Plant girth of OS 6 weeks after treatment

	Weeks after treatment (cm)									
Treatment	1	2	3	4	5	6	7	8	9	10
BT	0.313^{ab}	0.373a	0.400^{ab}	0.440^{a}	0.460^{a}	0.480^{a}	0.493^{a}	0.537^{a}	0.547^{a}	0.547^{a}
TS	0.300^{ab}	0.302^{abc}	0.380^{a}	0.383^{ab}	0.377^{a}	0.387^{a}	0.393^{a}	0.393^{a}	0.407^{ab}	0.393^{b}
TM	0.320^{ab}	0.320^{abc}	0.417^{a}	0.367^{ab}	0.430^{a}	0.450^{a}	0.483^{a}	0.483^{a}	0.517^{ab}	0.517^{a}
TMB	0.343^{a}	0.330^{ab}	0.343^{ab}	0.340^{ab}	0.357^{a}	0.303^{a}	0.377^{a}	0.380^{a}	0.390^{ab}	0.393^{b}
TMT	0.247^{bc}	0.287^{bc}	0.310^{ab}	0.257^{b}	0.340^{a}	0.347^{a}	0.387^{a}	0.367^{a}	0.367^{b}	0.367^{b}
NIS	0.183^{c}	0.243^{c}	0.283^{b}	0.33^{ab}	0.397^{a}	0.450^{a}	0.477^{a}	0.490^{a}	0.483^{ab}	0.400^{a}
SS	0.357^{a}	0.363^{ab}	0.373^{ab}	0.380^{ab}	0.43^{a}	0.437^{a}	0.453^{a}	0.487^{a}	0.487^{ab}	0.487^{a}

Column means followed by the same letters are not significantly different at $p \leq 0.05$, TS: *Trichoderma* sp., BT: *Botryodiplodia theobromae*, TM: Termites, TMB: Termites+*B. theobromae*, TMT: Termites+*Trichoderma* sp., NIS: Naturally infested soil, SS: Sterile soil

respectively (Table 3). There were variations in the level of damage caused to the roots of each variety after treatments. The root weight followed relatively the same trend with that of the severity of damage that was scored. In LAC 23, there was no significant difference in the root weight of the rice plants treated singly with each fungus. There was no significant reduction in the weight of plants treated with TM only. The plants treated with each of the fungi and termite combine had root weight reduction. There was slight root damaged on LAC 23 treated with BT and TS and mild root damage caused on LAC 23 treated with TM, TMB, TMT and NIS. As for NERICA 1, there was a slight difference in the trend of the reaction of the treatments on the root weight. The treatment of each of BT and TS caused significant low root weight of 8.0 g and 13.0 g, respectively. NERICA 1 treated with TM only had significantly low root weight of 11.0 g. Naturally infested soil manifested the usual trend of significantly low root weight (Table 4). The root damage caused by the fungi-termite interaction on NERICA 1 was mild with most treatments while NERICA 1 treated with TMB had moderate root damage. NERICA 1 treated with BT, TS, TM, TMB and TMT had 15, 10.2, 12.2, 9.2 and 14.5g root weight, respectively lower than the untreated control. The root weight of OS 6 was generally low. The root weight of OS 6 treated with TS only and TMB was significantly low with 1.0 and 3.1 g, respectively in relative to control (Table 4). OS 6 was the most susceptible variety

Table 4: Root weight of the 3 rice varieties on different treatment

Treatment	LAC 23 (g)	NERICA 1 (g)	OS 6 (g)
BT	25.0^{ab}	8.2^{c}	16.0^{ab}
TS	25.1^{ab}	13.0^{bc}	1.0^{c}
TM	31.0^{ab}	11.0^{c}	19.0^{a}
TMB	15.0^{ab}	11.1^{c}	3.1^{c}
TMT	19.0^{ab}	14.0^{abc}	8.0^{bc}
NIS	19.0^{ab}	8.7^{c}	14.0^{ab}
SS	35.0^{a}	23.2^{a}	16.0^{ab}

Column means followed by the same letters are not significantly different at $p \leq 0.05$, TS: *Trichoderma* sp., BT: *Botryodiplodia theobromae*; TM: Termites, TMB: Termites+*B. theobromae*, TMT: Termites+*Trichoderma* sp., NIS: Naturally infested soil, SS: Sterile soil

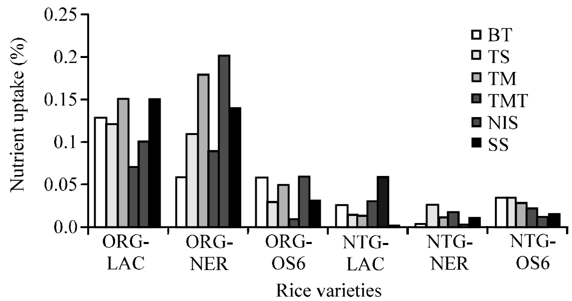

Fig. 1: Nutrient Uptake by the root from the Ultisol Soil, BT: *Botryodiplodia theobromae*, TS: *Trichoderma* sp., TM: Termites, TMT: Termites+*Trichoderma* sp., NIS: Naturally Infested soil, SS: Sterile soil, LAC: LAC 23, NER: NERICA 1, OS6: OS 6, ORG: Organic compound, NTG: Nitrogen

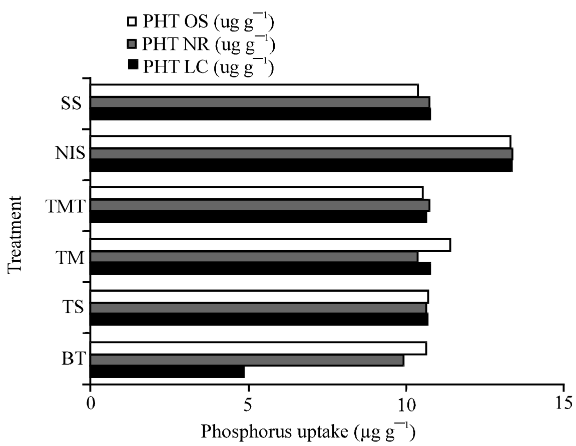

Fig. 2: Level of Phosphorus u ptake by the root, BT: *Botryodiplodia theobromae*, TS: *Trichoderma* sp., TM: Termites, TMT: Termites+*Trichoderma* sp., NIS: Naturally Infested soil, SS: Sterile soil, LC: LAC 23, NR: NERICA 1, OS: OS 6; PHT: Phosphate

among the three rice varieties used for the study to fungi and termite infestation. There was severe damage or rot of more than 60% damage on OS 6 treated with TS, TMB and TMT while there were moderate damage on OS 6 treated with BT, TM and mild damage on OS 6 treated with NIS (Table 5). The assimilation of organic compound by the three rice varieties varied and it was affected by various treatments. There was varietal effect on the assimilation of the nutrients with LAC 23 taking the lead followed by NERICA 1 and OS 6, respectively. The assimilation of nutrients especially the organic compound by OS 6 was very low among the three varieties. The assimilation of organic compound by OS 6 treated with TS and TMT was very low (Fig. 1). There was little variation in the trend of assimilation of Nitrogen and phosphorus by the treatments at varietal level. The amount of Organic compound assimilated by root of LAC 23 treated with TMT was significantly low. The amount of Nitrogen assimilated by the NERICA 1 treated with BT and NIS was significantly low. The amount of phosphorous assimilated by LAC 23 and NERICA 1 treated with BT was significantly low (Fig. 2).

Table 5: Rate of damage and rot caused by termites and fungal pathogens on the three rice varieties

Treatment	LAC 23	NERICA 1	OS 6
BT	+	++	+++
TS	+	++	++++
TM	++	++	+++
TMB	++	+++	++++
TMT	++	++	++++
NIS	++	++	++
SS	-	-	-

-: No damage, little or no reduction from the control, +: Slight damage; about 20% reduction, ++: Mild damage, about 40% reduction, +++: Moderate damage, about 50% reduction, ++++: Severe damage/ rot; above 60% reduction, TS: *Trichoderma* sp., BT: *Botryodiplodia theobromae*, TM: Termites, TMB: Termites+*B.theobromae*, TMT: Termites+*Trichoderma* sp., NIS: Naturally infested soil, SS: Sterile soil

DISCUSSION

The result of this study revealed the effect of termite infestation and fungi infection on the three rice varieties among which OS 6 was the most susceptible to termite infestation and fungi infection while LAC 23 was the most tolerant. NERICA 1 also showed some level of tolerance to termite attack. The tolerance of NERICA variety could have been a trait from *O. glaberrima* parent CG 14 which is tolerant to termite infestation and other biotic constraints as reported by Jones *et al*. (1997). Apart from the treatment effect, the study showed low stem girth of the rice planted on the naturally infested soil. The naturally infested soil still has a lot of organisms that affected the plant vigour of rice plants. Indeed, soils contain diverse communities of microscopic organisms and macro-organisms that are able to damage plants. A detrimental interaction between soil organism and plant is often highly specific. Highly specialized interactions between soil organisms and plants can kill seedlings and even adult trees. The study showed that termite is a serious insect pest of rice that paves way for fungi to gain entry into the plant. This is in conformity with the discovery of Wood and Cowie (1988) who considered termites to be the most significant soil pests of crops in

Africa. Harris (1968), IITA (1971) and Malaka (1973) have reported that *Macrotermes, Microtermes* and *Trinervitermes* feed on upland rice in Nigeria.

The effect of treatment on the stem girth was varietal-specific. There was significant decrease in the stem girth of LAC 23 treated with each of TMT and TMB. This confirms the work of (Agrios, 1988), when he described the phenomenon that when a pathogen interferes with the upward movement of inorganic substances, diseased conditions will result in the part of the plant denied of these materials. The results showed the low effect of termite and fungi on reduction of stem girth on LAC 23 and NERICA 1. This supports the explanation of (Togola *et al*., 2012) that five NERICA varieties are tolerant to termites infestation which is an element of the performance of NERICA against field insects in general (Bidaux, 1978; Arraudeau, 1992; Nwilene *et al*., 2008). He stated that NERICA 1 had very low termite infestation during the early vegetative stages (tillering and heading) but was heavily infested at maturity. This variety is aromatic and the aroma produced especially during the vegetative stages would repel termites during this period. But at maturity this variety was heavily infested probably because of the dryness of the stems and the diminution of the aroma. Despite the level of tolerance of LAC 23, the stem girth of LAC 23 treated with Termites+*B. theobromae* (TMB) had the least stem girth at the end. This showed the effect of *Botryodiplodia theobromae* on the rice varieties. It then implies that when termite gains entry into the plant root, it paves way for fungi like *Botryodiplodia theobromae* into the root. *Botryodiplodia theobromae* was reported to be pathogenic on cereal, mango fruits and cocoa bean (Fagbohun *et al*., 2011). It caused *Botryodiplodia* rot in maize (Kader and Arpaia, 2013). It has been reported to cause black rot of cereals in Nigeria, India, Pakistan and Thailand. *B. theobromae* was responsible for *Botryodiplodia* stalk rot in maize which was reportedly found in Africa, Asia and America . It produced stalk rot with a black discoloration in moist, hot environments. The diseased plant dries prematurely. The rotten portion shows some shredding of the pith and a dark gray to black discoloration of the vascular bundles. Abundant grayish mycelia are conspicuous in the rotten areas. The entry of termite into the plant root did not only pave way for *Botryodiplodia theobromae* in this study, it did for *Trichoderma* sp., also. Although there have been many reports on the use of *Trichoderma* sp., as a biological control agent against fungal disease in rice and many other crops (Gomathinayagam *et al*., 2010; Harman, 2006), it was shown as rot causing fungus in this study which is contrary to the popular view. Notwithstanding, there were reports of *Trichoderma* sp., as pathogenic agent to some crops. Harman *et al*. (2004) and Bailey and Lumsden (1998) reported *Trichoderma* spp., as pathogenic fungus to plants causing diseases of crops such as apples, maize and alfalfa and some of its strains can also produce highly phytotoxic metabolites. Also, Farr *et al*. (1995) reported *Trichoderma* sp., as a causative organism for green mold rot of corn and wheat. It was also reported to be causing green mold of mushroom (Samuels *et al*., 2002).

There were variations in the assimilation of nutrients by the plant root. The assimilation of nutrients especially the organic compound by OS 6 was very low among the three varieties. The assimilation of organic compound was adversely affected by TS and TMT. This suggests the utilization of the nutrients by these organisms for metabolic activities thereby competing for the available nutrients in the soil. And since TM was not adversely affected, it therefore depicts that *Trichoderma* sp., was responsible for the depletion of organic matter. It therefore suggests that it can be used to control some organic component in the soil which may be detrimental to the host plant. Conversely, the relative abundance of *Trichoderma* sp., in the soil can be detrimental to soil bio-ecosystem. Soil microorganisms transform organic matter into plant nutrients that are assimilated by plants (Bot and Benit, 2005). Carbon is an important constituent of organic matter and indeed of every organism either macro or micro. World soils are important reservoirs of active Carbon and play a major role in the global carbon cycle. The importance of soil organic matter to plant cannot be over emphasized. Micro-organisms, earthworms and insects help break down crop residues and manures by ingesting them and mixing them with the minerals in the soil and in the process recycling energy and plant nutrients (Bot and Benit, 2005). The amount of nitrogen assimilated by NERICA 1 treated with BT and IS was significantly low while the amount of phosphorous assimilated by the root of LAC 23 and NERICA 1 treated with BT was significantly low. It therefore depicts that *Botryodiplodia theobromae* affects nutrient uptake in some rice varieties. Low root weight, stem girth and nutrient uptake in some of the rice varieties planted on naturally infested soil suggests that there are other organisms responsible for the negative effect. There are myriad of organisms or factors that could militate against the growth of plants in the soil.

CONCLUSION

The damage caused by fungi on the rice plant was made possible by the infestation of termite which pre-disposed the root for fungi attack. The study showed the detrimental effects of damage caused by *Trichoderma* sp. and *Botryodiplodia theobromae* on the root and stem of rice plant and adverse effect caused by these fungi on the nutrient uptake by the plant.

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