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Research Paper

Pathogenicity of entomopathogenic *Entomophthora grylli* on grasshoppers *Diabolocatantops axillaris* and *Zonocerus variegatus* causing leaf damage of shea tree seedlings

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Abstract. The grasshoppers *Diabolocatantops axillaris* and *Zonocerus variegatus* were found feeding on the leaves of shea tree seedlings which caused damage that appears as rough patches on the surface. The objectives of this study were to provide healthy shea tree seedlings and control grasshoppers causing leaf damage using *Entomophthora grylli* as bioagent. Out of a total of 18 deaths termite mounds sand and forest soil samples that were examined, only 7 were found hosting *E. grylli* from Ihievbe Owan West with 26.5%, Ivbiaro Ebiaro Esako West with 9.2%, Eko-Igohebhe Esan Central with 8.7% and Umegbe Oredo with 2.8% respectively, all in Edo State. For the insect bait method, Orthoptera, *D. axillaris* and *Z. variegatus*, picked *E. grylli* from soil forest. Pathogenicity test revealed that injected *E. grylli* on grasshoppers with approximately 1×10^3 conidia consisting of mature resting spores (germinated and ungerminated) caused sluggish movement on grasshoppers on the eighth day. Grasshopper was found death as it clings to the aerial top of the container cover lid. Death of *D. axillaris* was observed on the eleventh day with mycelia growth on the abdomen. The results suggest that *E. grylli* could be an effective bioagent for the control of grasshoppers.

Keywords: Pathogenicity, *Entomophthora grylli,* grasshopper, shea, leaf.

INTRODUCTION

Shea tree *Vitelleria paradoxa* is one of the most abundant indigenous tree species in the Sudanian zone that forms the backbone of livelihoods for most of its 5000 km range (Boffa, 1999; Ezema and Ogujiofor 1992). Shea butter is sometimes used as a base for medicinal ointments. Some of the isolated chemical constituents are reported to have anti-inflammatory activity (Akihisa *et al.*, 2010). Almost every part of the tree has its use, e.g. the fruit is eaten and the leaves are used as fodder, and serve as an ingredient for making alkaline and paint (Lovett and Haq, 2000).

Grasshoppers (Orthoptera: Acididae) are serious pests both in agriculture and pasture in many countries of the world. In Africa, grasshoppers are widespread and their ecology and importance have been studied by many researchers (Mestre, 1988; Chapman and Joern, 1990). Most grasshoppers are polyphagous, eating vegetation from multiple plant sources (Davidowitz, 2015), but some are omnivorous and also eat animal tissue and animal faeces. In general their preference is for grasses, including many cereals grown as crops (O'Neil *et al.*, 1997). Grasshoppers are plant-eaters, sometimes becoming serious pests to cereals, vegetables and pasture, especially when they swarm in millions as locusts and destroy crops over wide areas. They protect themselves from predators by camouflage; and when detected, many species attempt to startle the predator with a brilliantly-coloured wing-flash while jumping and (if

Figure 1. Grasshoppers *Diabolocantops axillaris* feeding on the leaves of shea tree seedlings.

adult) launching themselves into the air, usually flying for only a short distance. Other species such as the rainbow grasshopper have warning coloration which deters predators. Grasshoppers are affected by parasites and various diseases, and many predatory creatures feed on both nymphs and adults. The eggs are the subject of attack by parasitoids and predators (Capinera, 2008; Cott, 1940; O'Toole, 2002).

Grasshoppers are affected by diseases caused by bacteria, viruses, fungi and protozoa. The bacteria *Serratia marcescens* and *Pseudomonas aeruginosa* have both been indicated in causing disease in grasshoppers, also entomopathogenic fungus *Beauveria bassiana*. This widespread fungus has been used to control various pest insects around the world, but although it infects grasshoppers, basking in the sun as a result of raising the insect's temperature above a threshold tolerated by the fungus, and the infection is not lethal. The fungal pathogen *Entomophthora grylli* is able to influence the behaviour of its grasshopper host, causing it to climb to the top of a plant and cling to the stem as it dies. This ensures wide dispersal of the fungal spores liberated from the corpse (Valovage and Nelson, 1990).

Grasshoppers eat large quantities of foliage both as adults and during their development, and can be serious pests of arid land and prairies. Pasture, grain, forage, vegetable and other crops can be affected. Insecticides can be used, but adult grasshoppers are difficult to kill, and as they move into fields from adjoining plantations, crops may soon become re-infested. The Shea tree seedlings face high level of leaf damage caused by Grasshoppers *Catantops Stramineus*. This reduction in the number of leaves may be a serious problem in the photosynthetic ability of the plant leading to stunted growth. The use of *E. grylli* ensures wide dispersal of the fungal spores liberated from the dead grasshoppers to healthy adult grasshoppers.

MATERIALS AND METHODS

Sampling location

A survey for soil samples was conducted in Edo state. GARMIN trex 10 - Geographical Positioning System (GPS) of Coordinates was used to collect samples from forest soil and dead termite mounds.

Insect bait method and pathogenicity test

We used Galleria insect bating method with perforated plastic containers 4 cm × 7 cm (Figure 1). The grasshoppers used belong to family Orthoptera, genus Acrididae of *Diabolocatantops axillaris* and *Zonocerus variegatus.* Topical method was used.

During fungus conidia production, healthy adult grasshoppers, *D. axillaris* and *Z. variegatus* were infected with injected doses of *E. grylli* for pathogenicity test. Each cadaver was coarsely chopped and immersed in 0.15 M sodium chloride. After stirring, the chopped tissue was poured through two layers of cheesecloth and centrifuged. The supernatant was drawn down to 0.7 ml and the remaining pellet of fungus was re-suspended in eppendof tubes of sterile distilled water and injected onto the grasshoppers using a sterile syringe needle. Each was injected with approximately 1×10^3 conidia consisting

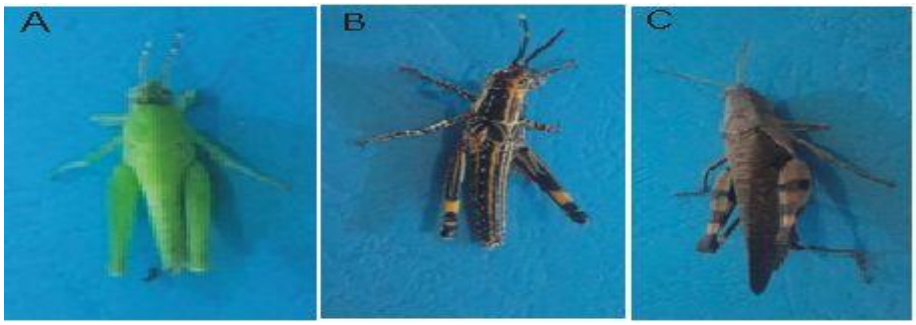

Figure 2. Different species of grasshoppers found feeding on the leaves of shea tree seedlings. A) Larva of *Diabolocatantops axillaris* (Thunberg, 1815); B) Larva of *Zonocerus variegatus* (Linnaeus, 1758); C) Adult of *Diabolocatantops axillaris* (Thunberg, 1815).

Figure 3. Samples from dead termite mounds soil and forest soil.

of mature resting spores (germinated and ungerminated). The dose per grasshopper was determined with Neubauer hemacytometer.

Infected grasshoppers were kept in perforated plastic containers 4 cm × 7 cm. They were maintained at between 26 and 28°C until death (14 days post injection) on a diet of shea tree seedling leaves. As soon as death was observed, grasshoppers were removed from containers and the fungus was immediately re-extracted again.

Monitoring

Insect cultures were monitored, stained with lactophenol cotton blue and examined at ×10 and ×40 magnifications using light microscope after 14 days incubation. Light photographs of conidia were taken using Moticam 2300 camera connected to the microscope and computer. Light photographs of Grasshoppers were also taken from lower to total magnification using Samsung Galaxy Note 10.1 2014 Edition.

Identification of entomopathogen

The entomopathogen was identified using commonwealth mycological institute (CMI 1979) description of pathogenic fungi and bacteria (Nos. 601-610).

Statistical analysis

Data were analyzed as % abundance and student test analysis (t-test).

RESULTS AND DISCUSSION

The authors surveyed Shea tree nursery of the Nigerian Institute for Oil Palm Research (NIFOR), Bida substation that were disrupted by Grasshoppers activities (Figure 1). Different species of grasshoppers (*Diabolocatantops axillaris* and *Zonocerus variegates*) found feeding on the leaves were collected from the seedlings leaflets and surrounding weeds (Figure 2). The damage grasshoppers cause on leaves appears as rough patches on the surface.

Soil samples were collected randomly from selected dead termite mounds sand and forest soil (Figure 3). Coordinates of samples from forest soil and dead termite mounds in Edo State were recorded (Table 1). The grasshoppers first, were noticed with sluggish movement on the eighth day. Death of *D. axillaris* was observed on

Table 1. Coordinates of samples from dead termite mounds soil and forest soil in Edo State.

No. of samples	Characteristics	Coordinates		Elevation (m)	Locations
		N˚	E˚		
1	Termite Mound Sample (M S) 1	06˚13.183”	005˚33.840	38	Umegbe Comm. Oredo L.G.A
2	Termite M S 2	06˚13.149”	005˚33.858	37	Umegbe Comm. Oredo L.G.A
3	Termite M/S 3	07˚02.516”	006˚09.405	243	Umegbe Comm. Oredo L.G.A
4	Soil Sample 1	06’13.145”	006’13.145”	41	Umegbe Comm. Oredo L.G.A
5	Termite M/S 4	07’01.955	006’08.298	209 *	Ivbiaro Vill Esako West L.G.A
6	Termite M/S 5	07’01.955	006’08.298	209 *	Ivbiaro Vill Esako West L.G.A
7	Soil Sample 2	06˚56.151	006˚16.248	70 *	Egeuno Vill Esako West L.G.A
8	Soil Sample 3	06˚56.151	006˚16.248	70 *	Ivbiaro Vill Esako West L.G.A
9	Termite M/S 6	07’01.925	006’08.317	203 *	Ihievbe Vill Owan West L.G.A
10	Soil Sample 4	07’01.925	006’08.317	203 *	Ihievbe Vill Owan West L.G.A
11	Soil Sample 5	07’01.925	006’08.317	203 *	Ihievbe Vill Owan West L.G.A
12	Soil Sample 6	06˚44.322	006˚14558”	386	Eko-Igohebhe Vill Esan Central L.G.A
13	Soil Sample 7	06˚30.350	006˚15.021	309	Igueben Town Igueben L.G.A
14	Soil Sample 8	06˚30.251	006˚12.420	247	Ogwa Vill Esan West L.G.A
15	Termite M/S 7	06˚30.251	006˚12.385	254 *	Ogwa Vill Esan West L.G.A
16	Soil Sample 9	06˚30.251	006˚12.385	254 *	Ogwa Vill Esan West L.G.A
17	Termite M/S 8	06˚30.699	006˚12.000	257 *	Ogwa Vill Esan West L.G.A
18	Termite M/S 9	06˚30.699	006˚12.000	257 *	Ogwa Vill Esan WestL.G.A

Same coordinates: * * * * *

Table 2. Abundance of *Entomophthora grylli* from dead termite mounds soil and forest soil in Edo State.

Sample no.	Locations	Mean % of *Entomophaga grylli*	
		Termite mound sample (MS)	Forest soil (FS)
1	Umegbe Comm. Oredo L.G.A (MS)	0.0	Nil
2	Umegbe Comm. Oredo L.G.A (MS)	0.0	Nil
3	Umegbe Comm. Oredo L.G.A (MS)	0.0	Nil
4	Umegbe Comm. Oredo L.G.A (FS)	Nil	2.8^{e}
5	Ivbiaro Vill Esako West L.G.A (MS)	Nil	0.00
6	Ivbiaro Vill Esako West L.G.A (MS)	9.2^{c}	0.00
7	Egeuno Vill Esako West L.G.A (FS)	0.0	0.0
8	Ivbiaro Vill Esako West L.G.A (FS)	0.0	0.0
9	Ihievbe Vill Owan West L.G.A (MS)	26.5^{a}	0.0
10	Ihievbe Vill Owan West L.G.A (FS)	0.0	0.0
11	Ihievbe Vill Owan West L.G.A (FS)	0.0	0.0
12	Eko-Igohebhe Vill Esan Central L.G.A (FS)	0.0	8.7^{c}
13	Eko-Igohebhe Vill Esan Central L.G.A (FS)	0.0	0.0
14	Ogwa Vill Esan West L.G.A (FS)	0.0	18.4^{b}
15	Ogwa Vill Esan West L.G.A (MS)		0.00
16	Ogwa Vill Esan West L.G.A (FS)	0.0	0.0
17	Ogwa Vill Esan West L.G.A (MS)	6.3^{d}	0.00
18	Ogwa Vill Esan West L.G.A (MS)	3.0^{e}	0.00

Mean with the same letters indicate non-significant differences while different letters in the same column indicate significant differences in regard to abundance at p = 0.05 using student test (t-test), to test for standard error of mean (SEM).

the eleventh day with mycelia growth on the abdomen. This study shows that entomopathogen *E. grylli* was pathogenic to grasshoppers *D. axillaris* and *Z. variegatus*. This agrees with work of Eziashi *et al*. (2016a, b) on the use of entomopathogenic fungi *Metarhizium anisopliae* and *Beauvaria bassiana* against

Figure 4. Isolation of *Entomophthora grylli* from the soil using insect bathing method.

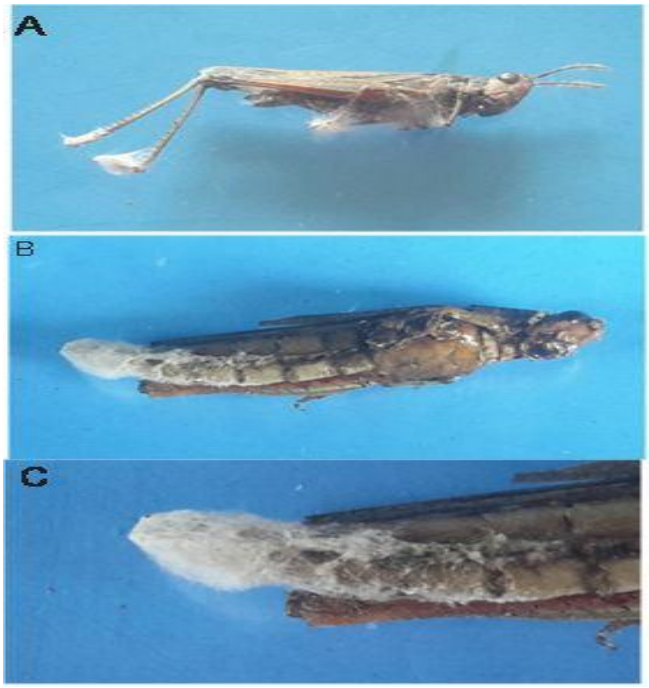

Figure 5. Infected grasshoppers *Catantops stramineus.* A. Picked *Entomophthora grylli* from the soil (at ×2.0 magnification); B. Pathogenicity Test of *Entomophthora grylli* on healthy *Catantops stramineus* (at ×3.0 magnfication); C. Pathogenicity Test of *Entomophaga grylli* on healthy *Catantops stramineus* (at ×4.0 magnification).

termites, and Michael *et al.* (1995) using *E. grylli* species against grasshoppers. Out of a total of 18 death termite mound sand and forest soil samples examined, only 7 were found hosting *E. grylli* from Ihievbe Owan West with 26.5%, Ivbiaro Ebiaro Esako West with 9.2%, Eko-Igohebhe Esan Central with 8.7% and Umegbe Oredo with 2.8% respectively, all in Edo state (Table 2). For the insect bait method (Figure 4), Orthoptera, *D. axillaris* and *Z. variegatus* picked *E. grylli* from soil forest (Figure 5A). Grasshopper was found dead as it clings to the aerial top of the container cover lid. This agrees with similar study by Valovage and Nelson (1990), who reported that *E. grylli* was able to influence the behavior of the grasshopper.

Pathogenicity test revealed that the fungus was pathogenic and was the same with the initial inoculum (Figure 5B, C). Death was observed by motionlessness

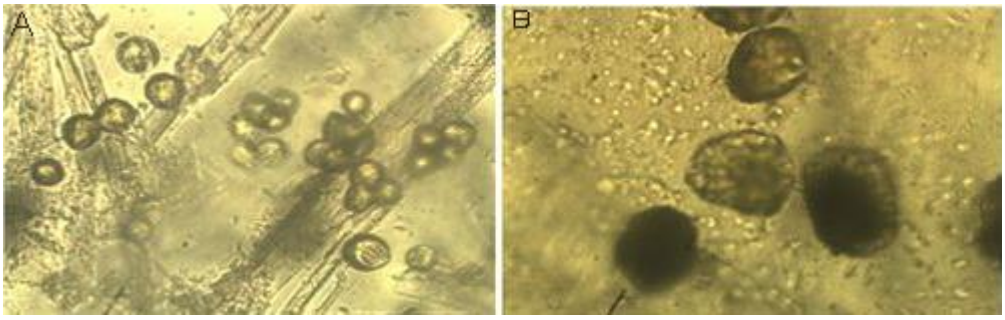

Figure 6. Microscopic view of *Entomophthora grylli.* A, at ×10 magnification and B, at ×40 magnification.

of the grasshoppers and the emergence of mycelia growth on the abdomen. The insect culture was examined using light microscope with conidia obovate to pyriform with a broad papillae base and an evenly rounded apex 25-45 × 20-27 m, single walled. The hyphal body grow rapidly, giving rise to conidiophores. Resting spores azygospores. The fungus has so far not been able to grow in culture media (Figure 6A, B).

CONCLUSION

The results suggest that *E. grylli* could be an effective bioagent for the control of grasshoppers. Further study is necessary to determine the effectiveness of *E. grylli* under field condition and to examine its impact on non target insects.

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