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Full Length Research Paper

Entomopathogenic fungi in population of *Zonocerus variegatus* (l) in Ibadan, Southwest, Nigeria

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Field survey of population of *Zonocerus variegatus* revealed a high fungal incidence of 76% when Sporulation tests were carried out on grasshoppers cadaver. Eight fungi with differing incidence rates were isolated. These are *Fusarium* sp. (8%); *Beauveria bassiana* (18%); *Metarhizium* sp. (20%); *Aspergillus flavus* (10%); *Penicillium* sp. (13%) *Aspergillus niger* (14%); *Mucor* sp. (13%) and unidentified fungus (4%). Fungal isolates virulence bioassay response showed that *B. bassiana* had the highest lethal time (LT_{50}) of 2 days. *Metarhizium* sp with LT_{50} of 5 days was selected in lieu of *A. niger* which does not prove to be pathogenic to grasshoppers. The results were discussed in relation to the ecology of fungal pathogens of the variegated grasshopper and their possible role in control of *Z. variegatus* in the agroecosystem of south west, Nigeria.

Key words: Fungal ecology, entomopathogenic fungi, *Zonocerus variegatus* infection incidence.

INTRODUCTION

Zonocerus variegatus (L) is a voracious and destructive insect, native to the humid forest zone of West Africa (Modder, 1984). It belongs to the same order (Order: Orthoptera) as locusts. It is a polyphagous insect that defoliates and destroys the stem bark of food crops at the end of the dry season. 90 and 30 % yield losses due to the infestation of *Z. variegatus* (L) on cowpea and maize, respectively, had been reported in Benin Republic (Langewald et al., 1997). This is of great economic consequence to the African farmer. Hence, *Z. variegatus* had been designated as a major pest of the agroecosystem by the National Agricultural Technical Committee of Nigeria since 1970 (Modder, 1986).

Control of the variegated grasshopper has generally involved 'Knock off' chemical pesticides. However because of the increasing concern on its effect on non-target organisms and persistence in the environment, there is the need for environmentally friendly alternative (Bateman, 1997). Biological control is an environmentally friendly alternative that involve the use of natural enemies and pathogens to control pests (Greathead, 1992). Recently, large number of insect pathogenic microorganisms referred to as entomopathogens have been identified as possible biological control agents for grasshoppers (Bidochka and Khatchatourians, 1992). Among these agents, fungi entomopathogens had been reported to have great potentials for grasshopper's control (Prior and Greathead, 1989).

The aim of this study is to survey the habitat of *Z. variegatus* for entomopathogenic fungi in Ibadan, South

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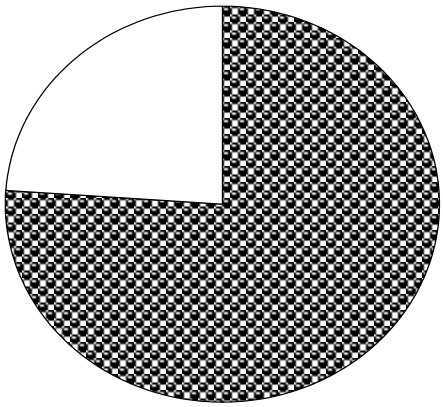

Figure 1. Fungal sporulation test on Zonocerus variegatuscadavers

West, Nigeria and their possible role in the control of this pest.

MATERIALS AND METHODS

Materials

Grasshoppers were collected for two years between September and February, 1999 and 2000 consecutively, using sweep net. They were collected from fallow plots close to the Zoological Garden of the University of Ibadan and the west bank of the Lake in the International Institute of Tropical Agriculture (IITA), Ibadan. Collected insects were taken to the laboratory in clean cages measuring 30 cm x 30 cm x 40 cm high, with wooden floors, wire mesh sides and roof (Dourou-kpindou et al.,1995).

Grasshoppers were fed with cassava stems, leave and twigs surface sterilized in 5 % sodium hypochlorite solution as described by Prior et al, (1995). Five cages containing 50 adults grasshoppers (sexually immature) per cage were used for the study as described by Shah et al. (1994). They were kept in the cages for 2 weeks. Mortality in caged grasshoppers was recorded.

Fungal Isolation and Sporulation test

Grasshopper cadavers removed from the cages were surfaced sterilized in 5 % sodium hypochlorite and 75 % ethanol solution and rinsed in plenty of sterile distilled water. The cadavers were then left to dry for 48 h (Dourou-kpinduo et al., 1995). After drying they were humid incubated in clean dessicators at room temperature as described by Luz and Farques (1998).

Sporulating cadavers were regarded as being positive while non-sporulating cadavers are negative. The sporulating fungi on cadavers were isolated in pure culture on Sabourand Dextrose Agar (SDA) as described by Poinar and Thomas (1984).

Identification of Fungal Isolates

Identification of fungal isolates was done as described by the International Mycological Institute (IMI), Manual of Pathogenic Fungi and Bacteria (1983). The incidence of occurrence of the isolates was recorded.

Pathogenicity Bioassay

Five fungal isolates were used for the pathogenicity bioassay on *Z. variegatus*. The isolates include *Beauveria bassiana*, *Metarhizium* sp., *Penicillium* sp., *Aspergillus niger* and *Mucor* sp. The isolates were cultivated at 28^0C at photoperiod of 12 h light and darkness (12 h L:D) for 15days. After the incubation, sterile spatula was used to harvest the conidia from the fungal culture. The harvested conidia were transferred into sterile McCartney bottles containing groundnut oil.

Then fungal spores suspension in oil was prepared and the spore concentation determined using the Neuberger Heamocytometer as described by Lomer and Lomer (1996). Before the commencement of the biassay, post-fledgling adult grasshopper were bred and conditioned to their cages for one week. Then 0.1 ml of the spores' suspension was applied carefully under the pronotal shield of the grasshoppers using sterile Pasteur pipette (Dourou- Kpindou et al., 1995) and (Thomas et al., 1997). For the control experiment, blank oil without spores was applied to the pronotal shield of the grasshoppers. Infected and uninfected grasshoppers were transferred into separate clean wooden cages. Daily mortality was recorded. Cadavers removed from the cages were surface-sterilized, humid incubated and the causative fungi isolated in pure culture.

RESULTS

Out of 250 field collected *Z. variegatus* used for this study, 90 of them died in the cage. From which 76 % of the cadavers recorded positive fungal sporulation results (Figure 1).

Eight fungi species were isolated and identified (Table 1) with infection the following incidence rates: *Fusarium sp.* (8%); *Beauveria bassiana* (18%); *Metarhizium* sp. (20%); *Aspergillus flavus* (10%); *Penicillium sp.* (13%); *Aspergillus niger* (14%); *Mucor sp.* (13%) and an unidentified fungus (4%) (Figure 2).

The virulence bioassay involves the treatment of *Z. variegatus* with spores' suspension of *B. bassiana, Metarhizium* sp. *A. niger, Penicillium* sp. and *Mucor* sp. (Figure 2). *Fusarium* sp., *A. flavus* and the unidentified

Table 1. Identification of Entomopathogenic fungi isolated from *Z. variegates.*

Isolates	Growth Morphology	Colour	Phialides	Spores	Probable Organism
B3	Sparse to abundant mycelium, wrinkled in old cultures	white or peach with purple tinge	Simple lateral phialides	Oval, ellipsoidal, cylindrical to straight micro and macroconidia	Fusarium sp
D1	Powdery mycelia	white or pale yellow		Clustered globular to flask shaped conidia	*Beauveria bassiana*
D5	Surface is powdery and finally crustose	dark herbage green	Conidia in chain form	Globose conidia	*Metarhizium sp*
D6	Fast growing and heavily sporing	dirty green	Typically radiate	Typically globose to subglobose	*Aspergillus flavus*
E8	Rapid growing, non-spreading and often wrinkled	violet	Penicillate ending on phialides	Mostly globose or ovoid	*Penicillium sp*
F4	Fast growing and heavily sporing	Black to dark brown		Rough echinulated globose conidia	*A. niger*
F6	dense sporangiophores	Ash-coloured	not seen	Not seen	Unknown fungus
F7	dense sporagiosphores	Grey	–	Ovoid zygospores	*Mucor sp*

Z. variegates as described by The International Mycological Institute Manual of Pathogenic Fungi and Bacteria (1983).

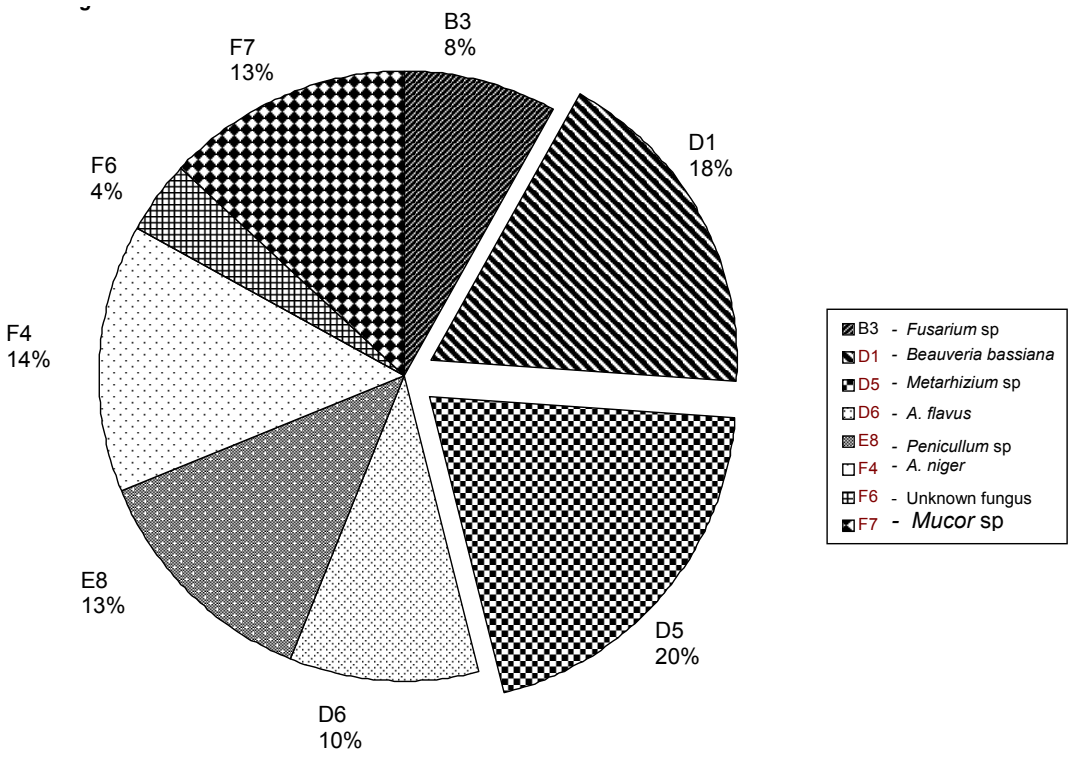

Figure 1. Incidence of Entomopathogenic Fungi Isolated from *Z. variegates.*

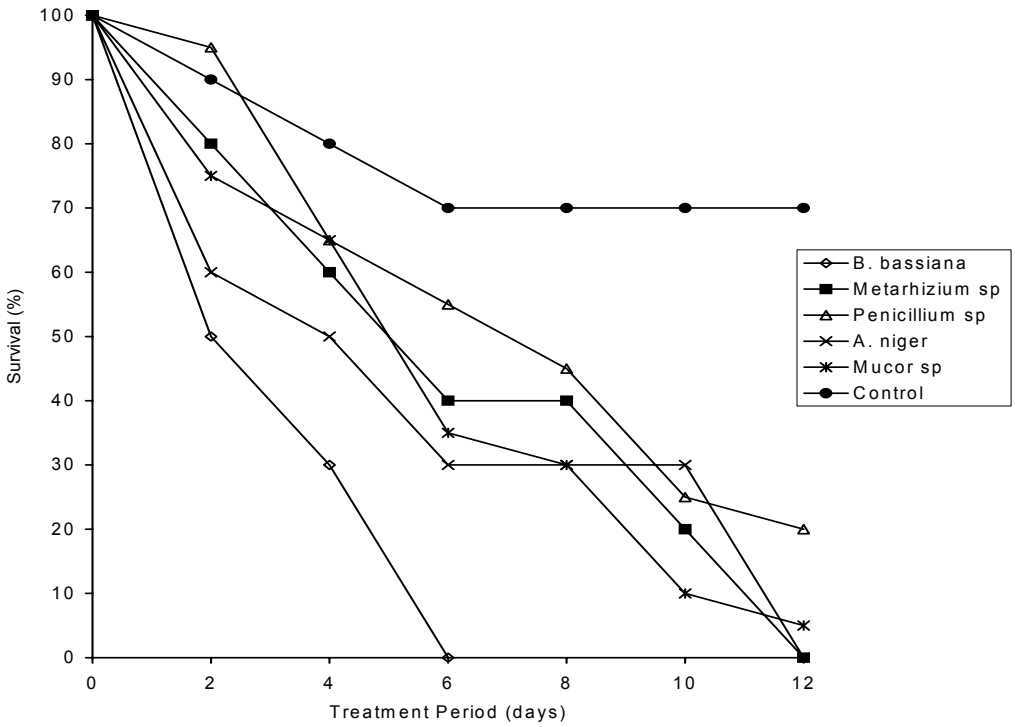

Figure 3. Selected fungal isolates virulence bioassay.

fungus were not chosen for the bioassay because of their low incidence rates. The highest LT_{50} (lethal time) of 2 days for *B. bassiana* and the lowest of 7 days for *Penicillium* sp were recorded. A. *niger* showed the second highest LT_{50} of 4 days, *Metarhizium* sp. achieved 100 % of kill at the end of the bioassay, *Mucor* sp. could only achieve 95 % of kill at the end of the bioassay.

Statistical analysis showed a good relationship between dose concentration of fungal spores and grasshoppers mortality.

DISCUSSION

The high fungal infection incidence recorded on grasshoppers cadavers, suggests that the fungi entomopathogens isolated are important pathogens in the population of the grasshoppers in the study (Hernardez Crespo and Santiago Alvarez, 1997). Also, the result indicates that despite that, there are other microbial pathogens of *Z. variegatus* in the study area (Bidochka and Khachatourians, 1991).

Fusarium sp. and *B. bassiana* isolated in this study agrees with the observation of Paraiso et al, (1991). Isolation of *Metarhizium* sp *Zonocerus variegatus* cadavers have also been reported by Shah *et al.* (1994). *A. flavus* and *A. niger* isolated in this study have previously been observed by Smit (1991) and Hernandez Crespo and Santiago Alvarez (1997) with significantly frequent incidence rates. *Penicillum sp.* isolated in this study had been reported by Poinar and Thomas (1984) from insects populations. *Mucor* sp belongs to the family Mucoraceae and Lomer and Lomer (1996) had reported the isolation of members of this family from grasshoppers. The unidentified fungus (F6) does not show the presence of spores, hence it is not easy to classify and even its use in bioassays way difficult. This suggests that it may not be an important pathogen in *Z. variegatus* population.

The highest lethal time (LT_{50}) of 2 days recorded for *B. bassiana* suggests that its spores are lethal to *Z. variegatus* and could cause significantly high mortality in population of grasshoppers. This agreed with Johnson et al. (1992) who reported a highly significant level of mortality in *B. bassiana* treated grasshoppers in Mali. This further suggests that it has great potentials as a microbial control agent of *Z. variegatus*. The lowest LT_{50} of 7 days and 80 % of kill at the end of the bioassay for *Penicillium sp.* (Figure. 3) suggests that it might not be a strict entomopathogen of *Zonocerus* rather an entomogenous fungus that cannot cause epizootics in grasshopper's population (Shah et al., 1994; Hernandex Crespo and Santiago Alvavez, 1997). The second highest LT_{50} of 4 days recorded for *A. niger* (Figure 2) is quite high but on re-inoculation, it does not prove to be the causative agent (Hernandez-Crespo and Santiago-

Alvarez, 1997). This suggests that *A. niger* might not be a strict pathogen of *Z. variegatus* but an opportunistic organism. *Metarhizium* sp. and *Mucor* sp. recorded the same LT_{50} of 5 days with 100% and 95% of kill, respectively, at the end of the bioassay. The high virulence of *Metarhizium* sp to grasshoppers had been reported (Bateman et al. (1997), Thomas et al. (1997), and this suggests it could be used as an environmentally friendly control agent for grasshoppers.

The isolation of *Mucor* sp. from insects had been reported by Lomer and Lomer (1996). But its use might not be desirable due to the fact that it is a known pathogen of plants (Duggar, 1989).

This study demonstrates that B. *bassiana* and *Metarhizium* sp. among all the isolated entomopathogenic fungi are major factors of mortality in *Z. variegatus* population and hence could be exploited as a microbial control agent of the variegated grasshopper in South West, Nigeria.

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