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SUSCEPTIBILITY OF THREE *HIEROGLYPHUS* SPECIES (HEMIACRIDINAE: ACRIDIDAE: ORTHOPTERA) TO SOME STRAINS OF THE ENTOMOPATHOGENIC FUNGI FROM PAKISTAN

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ABSTRACT

The species of genus *Hieroglyphus* are a voracious and destructive pest of cash crops in Pakistan in order to decline their population three species of *Hieroglyphus* were treated with some strains of the entomopathogenic fungi under laboratory conditions. During the present study three pathogenic fungi species i-e *Metarthizium flavoviride* Gams and Roszypal, *Beauveria bassiana* (Bals.-Criv.) and *Aspergillus* sp. Micheliwere isolated and identified with infection the following incidence rates: (53%), (35%)and (12%) respectively on *Hieroglyphus* species. The proportional cumulative survival of *Hieroglyphus* in the different treatments of fungi is showed that insects treated with the pathogen began to die with full signs of mycosis on day 5th. All treated insects died by day 6th by application of *M. flavoviride* while other replicates of the *B. bassiana* and *Aspergillus* spp. all dying by day 7th. In contrast, control mortality was extremely low with only (6, 3, & 8) deaths of *H. perpolita, H. oryzivouous* and *H. nigrorepletus* respectively and with no signs of mycosis. This study recommended that *M.flavoviride*, *B. bassiana* and *Aspergillus* spp. among all the isolated entomopathogenic fungi are major factors of mortality in *Hieroglyphus* population and it might be used as bio-control agent to suppress the grasshopper's population in field.

Keywors: *Hieroglyphus,* pest, cash crops entomopathogenic fungi, bio-control agent, population.

INTRODUCTION

The species of genus *Hieroglyphus* is a voracious and destructive pest of cash crops in Pakistan and India (Roonwal, 1978; Riffat and Wagan, 2007, 2012). This genus is considered polyphagous and cause damage of millions of rupees annually. Control of these grasshoppers has generally involved "Knock off" chemical pesticides. On pesticides expenses reached in billions of rupees each year. However, because of increasing concern on its effect on non-target organism, human health and persistence in the environment, there is the need for environmental friendly alternative biological control that involves the use of natural enemies and pathogens to control pests among these; entomopathogenic fungi are very important to reduce the grasshoppers population in field (Gerson and Smiley, 1990; Moore *et al*., 1992; Seyoum *et al*., 1994; Shah *et al*., 1998). They are important biological control agents because of their observed capacity to cause spectacular epizootics. The fungi species affecting grasshoppers i-e *Entomopha gagrylli* (Fresenius), Batko attracted attention long ago as a potential bio-control agent and was briefly marketed in South Africa in 1898 as a bio-pesticide (Lomer *et al*., 2001).

Chapman and Page (1979) stated that entomopathogenic fungi are a group of fungi with complete and complex life cycles. They further urged that they could be used as classical biological control agents because of their observed capacity to cause spectacular epizootics. Furthermore, Prior *et al*. (1992) recommended the oil based formulation of entomopathogenic fungus *Metarhizum flavoviride* Gams and Rozsypal for control of locusts and grasshoppers in Africa. Douro-Kpindou *et al*. (1995) used *M. flavoviride* against the variegated grasshopper *Zonocerus variegatus* (L.) is the humid zones of southern Benin and get significant result. In addition to this, Thomas *et al*. (1997) treated rice grasshoppers *Hieroglyphus daganesis* Krauss with the fungi, and Kooyman *et al*. (1997) treated sencgalose grasshoppers *Oedaleus senagalensis* (Krauss) in optional scale application in the Sahelian zone of southern Niger. Steedman (1990) applied *M. flavoviride* against the *Z. variegatus*. Shah *et al*. (1994) recorded natural level of fungal infections in Acrididae and Pyrgomorphidae. Gunnarsson (1988) demonstrated an immune response in insects for (12hrs) after pathogen application and Gotz and Vey (1974) have shown rapid humoral encapsulation of *B. bassiana* hyphae, even within the cuticle, thus it is possible for an insect to be alerted for infection at a very early stage. There is bulk of information available on the use of entomopathogenic fungi against grasshoppers from abroad but unfortunately this area is completely neglected from Pakistan. It was therefore, felt necessary to undertake a laboratory examination, which involves

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evaluating the impact of various fungi species affecting grasshopper population from Pakistan. The basic aim of this study is to note susceptibility of *Hieroglyphus* species i-e *H. perpolita* (Uvarov), *H. oryzivorous* Carl and *H. nigrorepletus* Bolivar against different species of entomopathogenic fungi under controlled condition. The finding of this research will be helpful to determine the suitability of entomopathogenic fungi as agents for the biological control of grasshoppers. Moreover, with the help of these microbial components we can easily save our precious crops as well as huge amount expend on pesticide.

MATERIALS AND METHODS

Collection of samples

Hieroglyphus species were collected from agriculture fields of rice, maize, sugarcane, millets, fodder crops and their surrounding vegetation of grasses using sweep net (8.89cm in diameter and 50.8cm in length) as well as by hand picking. The collection was made during the year 2012 in the months of June to November from various provinces of Pakistan. Collected insects were taken to the laboratory and kept in cages (length 30.5cm, width 26.5cms). Grasshoppers fed maize leaves, and twigs surface, sterilized in 5% sodium hypochlorite solution as described by Prior *et al*. (1995).

Rearing of Insects

Insects were divided into groups of 50 to four replicates per treatment. No. discrimination was made between (age, class or sex) insects were then placed in cages (length 16.5 cm, width 13.5 cms) under laboratory (25^{o}-$23^{/}$N, 68^{o}-$24^{/}$E) conditions where the temperature fluctuated between 28 ± 2^{o}C to 39 ± 2^{o}C and relative humidity was 26 to 61%. A total of 4065 individual of *Hieroglyphus*, comprising a mix of final instars nymphs and immature, then mature adults were collected and maintained in the laboratory for up to 1 week prior to use.

Fungal isolation and sporulation test

Hieroglyphus cadavers removed from the cages, were surfaced sterilized in 5% Sodium hypochlorite and 75% ethanol solution and then rinsed in sterile distilled water. The cadavers were then left to dry for 48hrs as described by Dourou-Kpinduo *et al*. (1995). After drying these cadavers, they were humid incubated in clean desiccators at room temperature as described by Luz and Fargues (1998). Sporulation cadavers were regarded as being positive while non-sporulating cadavers were negative. The sporulating fungi on cadavers were isolated in pure culture on sabouraud dextrose agar (SDA), slopes and formulated in ground nut oil these fresh suspension were placed in both sonicator for 1minute to break up the conidial chains and conidial counts were made with a haemocytometer as described by Poinar and Thomas (1984).

Identification of fungal isolates

Identification of fungal isolates was carried by description given by International Mycological Institute (IMI), Manual of pathogenic fungi and bacteria (1983), the incidence of occurrence of the isolated fungi was recorded (Table 1).

Table 1. Collection of *Hieroglyphus* species from the different districts of the Pakistan during the year 2012.

a. (Sindh)

Districts	Species		
	H.perpolita *(n=591)*	*H.nigrorepletus* *(n=887)*	*H.oryzivorous* *(n=705)*
Karachi	5	13	0
Jamshoro	6	23	12
Thatta	16	67	17
Badin	17	101	0
Tharparkar	43	56	0
Umerkot	18	53	16
Mirpurkhas	23	134	13
Tando Allahyar	37	34	16
Tando M. Khan	41	55	0
Hyderabad	78	67	9
Khairpur	63	34	0
Shaheed Benaziabad	52	43	0
Dadu	66	123	179
Larkana	42	18	303
Jacoabad	41	43	117
Sukkur	43	23	23

Table continued…

Table 1 continued

b. (Punjab)

Districts	Species		
	H.perpolita (*n*=349)	*H.nigrorepletus* *(n=76)*	*H.oryzivorous* *(n=290)*
Bahawalpur	13	0	21
Chakwal	44	9	32
Faisalabad	24	6	18
Jhelum	19	5	11
Jhang	21	0	19
Kasur	13	3	10
Lahore	19	0	8
Mianwali	27	4	21
Multan	33	7	12
Rawalpindi	78	21	108
Rahim Yar Khan	35	13	12
Sahiwal	14	8	10
Sialkot	9	0	8

c. Khyber Pakhtunkhwa

Districts	Species		
	H.perpolita *n=(195)*	*H.nigrorepletus* *(n=91)*	*H.oryzivorous* *(n=617)*
Abbatabad	11	0	43
Battagrum	6	8	31
Charsadda	31	7	57
Dera Ismail Khan	10	4	18
Hairpur	8	3	127
Kohat	9	6	16
Mansehra	10	11	203
Mardan	31	9	11
Nowshehra	28	13	29
Peshawar	44	21	38
Swat	7	9	44

d. Balochistan

Districts	Species		
	H.perpolita *(n=72)*	*H.nigrorepletus* *(n=103)*	*H.oryzivorous* *(n=89)*
Barkhan	18	44	10
Kalat	21	13	12
Khuzdar	11	7	16
Lasbela	22	39	51

Pathogenicity Bioassay

Three fungal isolates used for the pathogenicity bioassay on *Hieroglyphus* the isolates include *Metarhizium flavoviride*, *Beavveria bassiana* and *Aspergillus* spp. The isolates were cultivated at 28^{o}C at photoperiod of 12hrs light and darkness 12h L:D) for 15 days as described by Balogun and Fagade (2004). 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 the ground oil. Then fungal spore's suspension in oil was prepared and the spore concentration determined using the Neuberger Haemocytometer as described by Lomer and Lomer (1996). Before the commencement of the bioassay insects were bred and conditioned to their cages for one week. Then 0.1ml of the spores' suspension was applied

Table 2. Identification of Entomopathogenic fungi isolated from *Hieroglyphus* species.

Isolates	Growth Morphology	Color	Phialidas	Spores	Probable organism
D1	Powdery mycelia	White or pale yellow	----	Clustered globular to flask shaped conidia	*Beauveria bassiana*
D5	Surface is powdery & finally crustose	Dark herbage green	Conidia in chain form	Globose conidia	*Metarthizium flavoviride*
D6	Fast growing & heavily sporing	Dirty green	Typically radiate	Typically globose to subglobose	*Asperillus* species

International Mycological Institute (IMI) Manual of pathogenic fungi and bacteria (1983)

Table 3. Mortality of *Hieroglyphus* population by treating with different pathogenic fungi species during the year 2012.

Treatments	Period days (Mean ±SE)						
	Ist	2nd	3rd	4th	5th	6th	7th
M. flavoviride	0.33±0.33[b]	0.00±0.00[d]	2.00±0.57[a]	7.00±1.15[a]	10.66±2.02[a]	27.00±2.30[a]	3.00±3.00[a]
B. bassiana	0.00±0.00[c]	2.00±1.00[a]	0.66±0.66[c]	4.33±1.45[b]	6.00±0.57[b]	10.33±1.20[c]	26.66±3.84[b]
Aspergillus Sp.	1.33±0.33[a]	1.00±0.57[b]	1.00±0.57[b]	4.33±0.66[b]	5.66±1.20[c]	13.33±2.02[b]	23.33±1.85[c]
Control	0.00±0.00[c]	0.66±0.33[c]	0.00±0.00[d]	2.00±0.57[c]	0.00±0.00[d]	1.00±0.57[d]	2.00±0.00[d]

Note: Mean in the same column followed by the same letters are not significantly different from one another at 5% level of probability.

carefully under the pronotal shield of the grasshoppers using sterile Pasteur pipette (Dourou-Kpindou *et al*., 1995; Thomas *et al*., 1997).

However, for the control experiment blank oil without spores was applied on the pronotal shield of the grasshoppers. In the last infected and uninfected grasshoppers were transferred into separate clean cages. Daily mortality was recorded, cadavers removed from the cages, were surface sterilized humid incubated and the causative fungi isolated in pure culture. This method was adopted for all the species studies viz: *Hieroglyphus perpolita, H. oryzivorous* and *H. nigrorepletus.*

RESULTS

Out of 4065 specimens of *Hieroglyphus* collected from field used for the study 90% of them died in the cages (Table 2) from which 74% of the cadavers recorded positive fungal sporulation results three fungi species were isolated and identified with infection the following incidence rates: *M. flavoviride* (53%), *B. bassiana* (35%) and *Aspergillus* sp (12%) (Fig. 1). The virulence bioassay involves the treatment of *Hieroglyphus* population with spore's suspension of *M. flavoviride*, *B. bassiana* and *Aspergillus* sp. The proportional cumulative survivals of *Hieroglyphus* in the different treatments of fungi are shown in (Table 3, Figs. 2-4). Insects treated with the pathogen began to die with full signs of mycosis on day 5th. All treated insects died by day 6th by application of *M. flavoviride* while other replicates of the *B. bassiana* and *Aspergillus* spp. all dying by day 7th. In contrast, control mortality was extremely low with only (6, 3, 8) deaths of *H. perpolita, H. oryzivorous* and *H. nigrorepletus* were recorded respectively with no signs of mycosis.

The highest lethal time of 6 days recorded for *Hieroglyphus* species by application of *M. flavoviride* suggested that its spores are lethal to *Hieroglyphus* species and could cause significantly high mortality in all treated species. This suggests that *M. flavoviride* might be a severe pathogen of *Hieroglyphus* species. The study provides further evidence that infection by *M. flavoviride*, *B. bassiana* and *Aspergillus* spp. causes a significant reduction in host feeding well before deaths. The average survival times of the treated insects in the present study were shorter than those typically observed in control trials. The high fungal infection incidence recorded on grasshopper's cadavers suggests that the fungi entomopathogens isolated are significantly important pathogens in the population of the *Hieroglyphus* (Fig. 4). This study provides further evidence that infection by *M. flavoviride* causes a significant reduction in host feeding well before death. The average survival times of the treated insects in the present study were shorter than those typically observed control treatment.

DISCUSSION

Orthoptera are attacked by many vertebrates and invertebrates natural enemies (Greathead, 1992) while recent review have emphasized the large number of pathogenic diseases being studied as possible biological control agents (Bidochka and Khatchatourians, 1991;

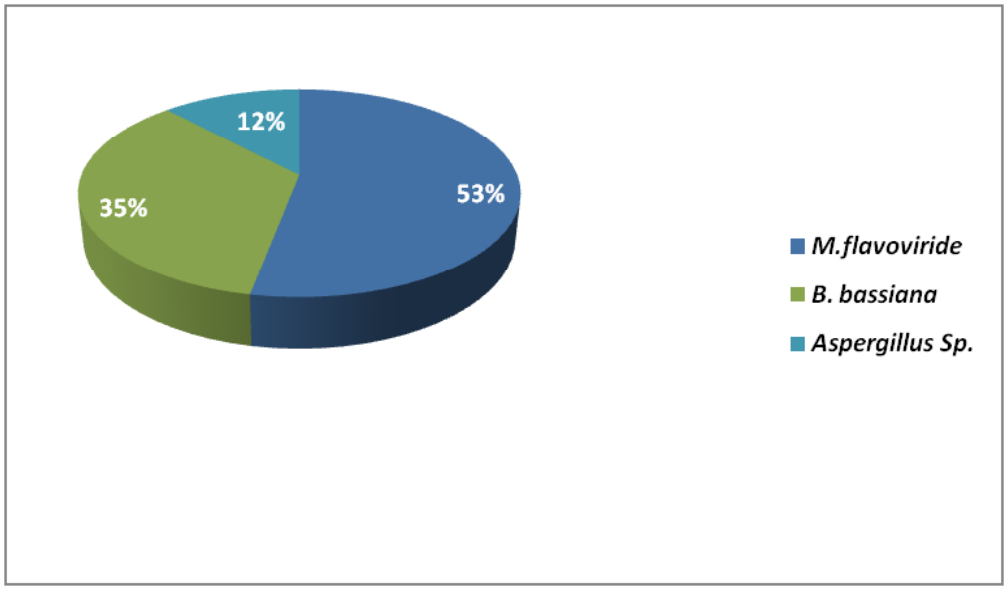

Fig. 1. Incidence of entomopathogenic fungi isolated from *Hieroglyphus* species.

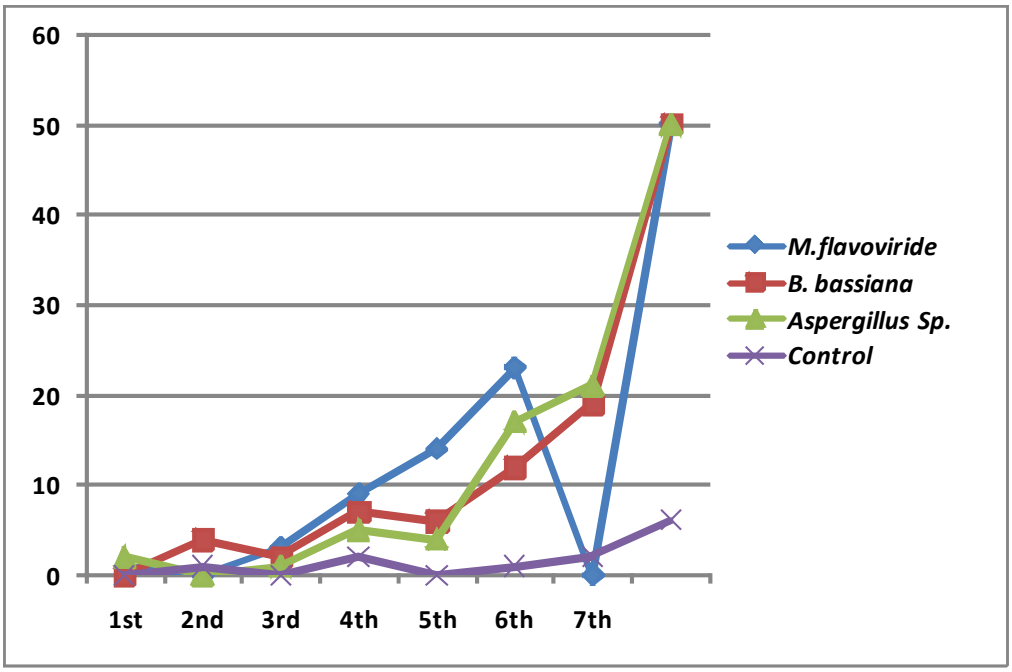

Fig. 2. Mortality of *Hieroglyphus perpolita* by treating with different species of fungi during the year 2012.

Streett and McGuire, 1990). The high fungal infection incidence recorded on *Hieroglyphus* populations suggests that the fungi entomopathogens isolated are important pathogens in the population of the *Hieroglyphus* are correlated with the finding of Hernardez-Crespo and Santiago Alvarez (1997). *B. Bassina* isolated in this study agree with the observation of Paraiso *et al*. (1992). Isolation of *Metarhizium* species from *Zonocerus variegates* (Linnaeus) Cadavers have been reported by Shah *et al*. (1994). Present study agreed on this account.

For the past century, entomopathogenic fungi have been known to come drastic decline among grasshoppers and locust populations. Consequently, most of the research has described fungal epizootics or attempted to utilized fungi as biological control agents. Presently we also did study under controlled condition and pathogen injected on the pronotum sheets of insects. It almost gave similar result as obtained by previous workers. A number of studies have shown both chemicals and pathogens to affect insect feeding rates. Haynes (1988) reviewed the

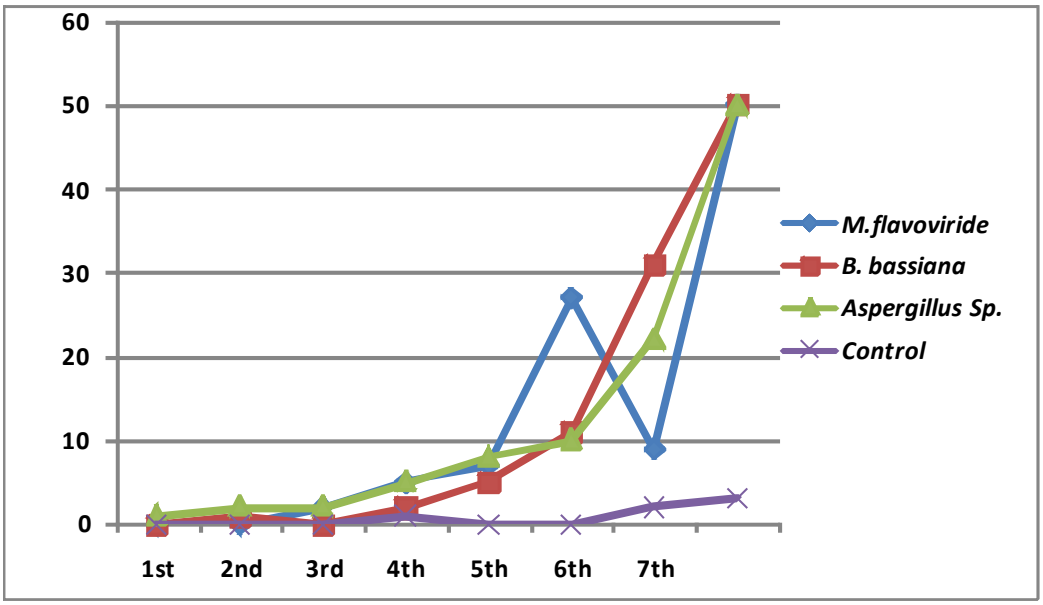

Fig. 3. Mortality of *Hieroglyphus oryzivorus* by treating with different species of fungi during the year 2012.

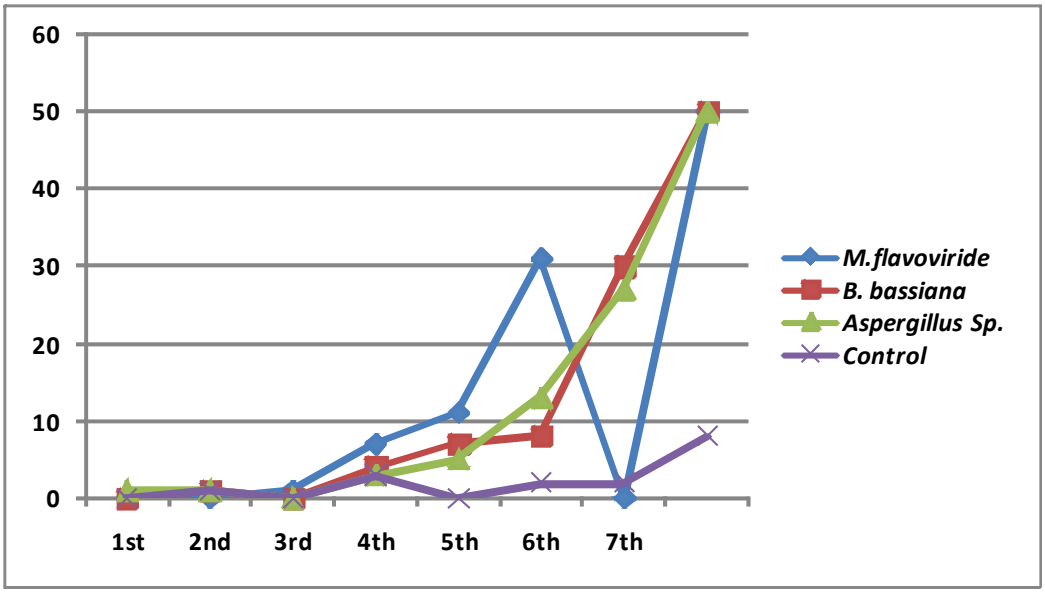

Fig. 4. Mortality of *Hieroglyphus nigrorepletus* by treating with different species of fungi during the year 2012.

sub-lethal effect of neuro-toxic insecticides on insect behavioral and found examples of both increases and reductions in food intake following exposure.

Moore *et al*. (1992) and Seyoum *et al*. (1994) both reported significant reductions in feeding by the Desert locust, *Shistocerca gregaria* (Forskal), following infections with *M. flavoviride*. Similar results have also been obtained from other host pathogen combinations including the North American rangeland grasshopper, *Melanoplus sanguinipes* (F.) infected with *Nosema locustae* Canning (Johnson and Pavlikova, 1986) and the armyworm, *Spodoptera exigua* Hubner, infected with *B. bassiana* (Balsamo) Vuillemine (Hung and Boucias, 1992). Evidence for depletion in nutrients in the host haemolymph has also been shown following fungal invasion (Zacharuk, 1971; Funk *et al*., 1993). All of these reported studies were carried out under very controlled, artificial laboratory conditions using insects inoculated with a high dose of pathogen and, in some cases, the

pathogen injected directly into the haemolymph. The aim of the current study was to improve on this by examining the effects of a range of pathogen doses on feeding rate and incubating insects under more natural conditions using cages maintained in the laboratory where the temperature range was optimum.

This study provides further evidence that infection by *M. flavoviride* causes a significant reduction in host feeding well before death. The average survival times of the treated insects in the present study were shorter than those typically observed in control. Thus, it is likely that even the lowest dose, at least when applied as a single source of inoculum under the pronotum, is higher then would be acquired in the field following spray application. That said, the reduction in feeding, as indicated by faeces production, was significant by the second or third day after inoculation for all doses. Even taking into account body size of the test insects, this is a faster and reduction at lower doses of pathogen than observed in other similar studies carried by (Moore *et al*., 1992; Seyoum *et al*., 1994).

Physical and biochemical events associated with the process of infection have been widely studied for *B. bassiana* (Balsamo) and *M. anisopliae* (Metsch.) Sorokin but much less so for *M. flavoviride*. Seyoum *et al*. (1994) suggested that significant colonization of the insect is necessary before feeding is reduced. Gunnarsson (1988) noted that hyphae of *M. anisopliae* did not reach the haemocoel of infected *S. gregaria* until 48 h post-inoculation. Significant colonization of tissues would not be expected to occur until sometime after this. In contrast, a study by Cheung and Grula (1981) in which *Heliothis zea* Boddie larvae were injected with *B. bassiana* revealed the gut walls were infiltered by long hyphae in just 48h. However, injection of the pathogen directly into the haemolymph by passes the processes and time associated with cuticular penetration. Thus, it is unlikely that sufficient colonization of tissues could have taken place within 48 h, it might give significant results.

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REFERENCES

Balogun, SO. and Fagade, OE. 2004. Entomopathogenic fungi in population of *Zonocerus variegatus* (I) in Ibadan, Southwest, Nigeria. Afri. J. Biotech. 3(8):382-386.

Bidochka, MJ. and Khatchatourians, GG. 1991. Microbial and protozoan pathogens of grasshoppers and locusts as potential bio-control Agents. Bio-control Sci. Technoi. 1:243-249.

Chapman, RF. and Page, WW. 1979. Factors affecting the mortality of the grasshopper *Zonocerus variegates*, in southern Nigeria. J. Amim. Ecol. 48:271-288.

Cheung, PYK. and Grula, EA. 1981. In vito events associated with entomopathology of *Beauveria bassiana* for the corn earworm (*Heliothis zea*). J. Inver. Patho. 39:303-313.

Dourou-Kpindou, OK., Godonou, I., Houssou, A., Lomer, CJ. and Shah, PA. 1995. Control of Zonocerus variegates by ultra-low volume application of an oil-formulation of *Metarhizium flavoride* conidia. Bio-control Sci. Tec. 5:131-139.

Funk, CJ., Ramoska, WA. and Betchel, DB. 1993. Histopathology of Entomophaga grylli pathotype 2 infections in *Melanoplus differentialia.* J. Inver. Patho. 61:196-202.

Gerson, U. and Smiley, RL. 1990. Acarine Biocontrol Agents.Chapman & Hall, London.

Gotz, P. and Vey, A. 1974. Humoral encapsulation in Diptera (Insecta): defense reactions of Chiroonoms lavae against fungi. Parasito. 58:193-205.

Greathead, DJ. 1992. Natural enemies of tropical locusts and grasshoppers. In: their impact and potential as biological control agents. Eds. Lomer, CJ. and Prior, C. Biological control of locust and grasshoppers. Wallingford, Oxon, CAB. International.105-121.

Gunnarsson, SGS. 1988. Infection of *Schistocerca gregaria* by the fungus *Metarhizium anisopliae:* cellular reactions in the integument studied by scanning electron and light microscopy. J. Inver. Patho. 52:9-17.

Haynes, K. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Ann. Rev. Ent. 33:149-168.

Hermandez-Crespo, P. and Santiago-Alvarez, P. 1997. Entomopathogenic fungi associated with natural population of the morroccan locust *Dociostarus maroccanus* (Thumberg) (Orthoptera: Gomphocerinae) and other Acidae in Spain. Biocontrol Sc.Technol. 7:353-363.

Hung, SY. and Boucias, DG. 1992. Influence of *Beauveria bassiana* on the cellular defence response of the beet armyworm, *Spodoptera exigua*. J. Inver. Patho. 60:152-158.

IMI, 1983. The international Mycological institute series of Description of pathogenic fungi and bacteria. In:

Institute of CAB international Egham, Surrey, United Kingdom Mycopathologia 130:43-64

Johnson, DL. and Pavikova, E. 1986. Reducation of consumption by grasshopper (Orthoptera: Acrididae) infected with *Nosema locustae* Canning (Microsporidia: Nosematidae). J. Inver. Patho. 48:232-238.

Kooyman, C., Bateman, RB., Langewald, J., Lomer, CL., Ouambama, Z. and Thomas, MB. 1997. Operational-scale application of entomopthogenic fungi for the control of Sahelian grasshoppers. Proc. R. Soc. London. B.264:541-546.

Lomer, C. and C. Lomer, C. 1996. Lubilosa technical Bulletins. 1-7.

Lomer, CJ., Bateman, RP., Johnos, DL., Langewald, J. and Thomas, M. 2001. Biological control of grasshoppers and locusts. Ann. Rev. Ent. 46:667-702.

Luz, C. and Farques, J. 1998. Factors affecting conidia production of *Beauveriia bassiana* from fungus killed cadavers of rhodiniusprolixusj. J. Inv Pathol. 72:97-103.

Moore, DM., Reed, Le-Patourel, G., Abraham, YJ. and Prior, C. 1992. Reeducation of feeding by the desert locust *Schistocerca gregaria*, after infection with *Metarhizium flovoviride*. J. Inver. Patho. 60:304-307.

Paraiso, A., Lomer, CJ., Godonu, I. and Dourou-Kpindou, OK. 1992. Preliminary studies on the ecology of *Zonocerus variegates* in the Republic of Benin. In: biological control of locusts and grasshoppers. Eds. Lomer, CJ. and Prior, C. CAB international Wallingford, UK. Proceeding of a workshop held at IITA, Cotonous Republic of Benin. 133-141.

Poinar, OG. and Thomas, MG. 1984. Laboratory guide to insect pathogen and parasites plenum press. New York and London.

Prior, CC., Lomer, Herren, H., Paraiso, A., Kooyman, NC. and Smit, JJ. 1992. The HBC/IITA/DFPV collaborative research programme on the biological control of locusts and grasshoppers. In: Biological control of locusts and grasshoppers. Eds. Lomer, C. and Prior, C. CAB international, Wallingford. UK. 8-10.

Prior, C., Carey, MA., Brahamy, J. and Moore Dandbateman, RP. 1995. Development of a bioassay method for the selection of entomopathogenic fungi virulent to the desert locust *schistocerca gregaria* (Forskal). J. Appl. Entomol. 119:567-572.

Riffat, S. and Wagan, MS. 2007. Comparative studies on the Ovipositional behaviour of *Hieroglyphus* species *(*Hemiacridinae: Acrididae: Orthoptera) from Pakistan. Pak. J. Zool. 39(5):321-325.

Riffat, S. and Wagan, MS. 2012. Review of genus *Hieroglyphus* Krauss 1877 (Hemiacridinae: Acrididae: Orthoptera) with description of one new species from Pakistan. Pak. J. Zool. 44: 43-51.

Roonwal, ML. 1978. The phadka grasshopper and its control. India Farming. 27(10):3-6.

Seyoum, E., Moore, D. and Charney, AK. 1994. Reducation in flight activity and food consumption by the *Schistocerca gregaria*, Forskal (Orth: Cyrtachthacrinae), after infection with *Metarhizium flovoviride*. J. Appli. Ent. 118:310-315.

Shah, PA., Godonou, I., Gbonoboni, C. and Lomerc, J. 1994. Natural levels of fungal infection in grasshoppers in northern Benin. Biocontrol Sci.Tech. 4:331-341.

Shah, PA., Godonou, I., Gbongboui, C., Hossou, A. and Lomer, CJ. 1998. Survival and mortality of grasshopper egg pods in semi-arid cereal cropping areas of northern Benin. Bull. Entomol. Res. 88(4):451-459.

Steedman, A. (ed.). 1990. Locust Handbook (3rd edi.). Natural Resources Institute, Chatham, UK.

Streett, DA. and McGuire, MR. 1990. Pathogenic disease of grasshoppers. In: biology of grasshoppers. Eds. Chapman, RF. and Joern, A. John Wiley and Sons, New York. 484-516.

Thomas, MB., Blandford, S. and Lomer, CJ. 1997. Reduction of feeding by the variegated grasshoppers *Zonocerus variegates* following infection by the fungal pathogen *Metarhizium flavoviride*. Bio control Sci.Tech. 7:327-334.

Zacharuk, RY. 1971. Ultrastructural changes in tissues of larval elateridae (Coleoptera) infected with the fungus *Metarhizium anisopliae*. Canad. J. Micro. 17:281-289.

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