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Reduction in faeces production and food consumption by three rice grasshoppers after infection with *Aspergillus* species from Badin, Sindh

Santosh Kumar*, Riffat Sultana, Muhammad Saeed Wagan, Waheed Ali Panhwar, Faheem Haider Solangi

Department of Zoology, University of Sindh, Jamshoro, Pakistan

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Abstract

During the present study three rice pest species i-e *Oxya velox* (Fabricius, 1787), *Oxya hyla hyla* (Serville, 1831) and *Hieroglyphus nigrorepletus* (Bolivar, 1912) were treated with three *Aspergillus* species under laboratory condition. It was observed that infestation of *Aspergillus* cause significant reduction in the feeding and over all faeces production per insect i-e ($F_{(0.75)}$ = 13.09, $F_{(0.81)}$ = 14.84, $F_{(0.79)}$ =13.09 & $F_{(1.89)}$=04.36 P<0.05) for *Oxya velox,* ($F_{(0.57)}$ = 0.96, $F_{(0.72)}$= 13.09 , $F_{(0.84)}$= 14.84 & $F_{(1.71)}$= 0.436 P<0.05) for *O.hyla hyla* and ($F_{(0.98)}$ = 16.58 , $F_{(1.08)}$= 0.262, $F_{(0.77)}$= 13.09 & $F_{(1.41)}$= 0.262 P<0.05) for *H.nigrorepletus* when treated with three pathogenic fungi and control respectively. Individual analyses for each date revealed that, generally, mean faeces production per insect decreased with dose, with controls significantly higher than the treated populations.

* **Corresponding Author:** Santosh Kumar ✉ santoshsonu@hotmail.com.

Introduction

Rice (oryza sativa) is the stable food crop of majority of the population of Pakistan. On an average the area under cultivation of the crop is about 22.8 million acres (Janjua, 1957). Grasshoppers are important pest of crops including paddy throughout the world causing considerable damage to rice nurseries as well as grown up plants (Irshad *et al*., 1977). During the present study three grasshoppers i-e *Oxya velox* (Fabricius, 1787), *Oxya hyla hyla* (Serville, 1831) and *Hieroglyphus nigrorepletus* (Bolivar, 1912) was enlisted as severe pest of Paddy field in Badin during August to October. During the month of November to December after harvesting of the paddies, they migrate from paddy fields to the nearby grass land. Research so far conducted on rice grasshopper has mainly concentrated on the control of the noxious pest with pesticides. Therefore, there is a need for integrated pest management (IPM) approach to reduce the use of chemical pesticides with environment friendly methods. IPM is very beneficial to farmers by this they can get help from a range of different pest control methods. Amongst these methods biological control is considered most effective and sustainable method for controlling the grasshopper by utilization of various microbial agents such as virus, bacteria, protozoa and fungi etc.

Earlier, many workers carried work on the utilization of fungi against grasshopper and locust and get significant result (Aldrovandi 1923, Christie 1929, 1936, Greathead 1963, 1992, Nickel 1972, Poinar 1975, Roonwal 1976, Henry *et al*., 1985, Prior & Greathead 1989, Shah *et al*., 1998, Balfour-Browne, 1960, Hermandez-Crespo & Santigo-Alvarez, 1997, Shah *et al.,* 1994, Balogun & Fagade 2004, Bidochka & Khatchatourias, 1992, Paraiso *et al.,* 1992 and Riffat *et al.,* 2013). As far as research on the application of pathogenic fungi is concerned there is no detail information is available except Kumar *et al*., 2013 from here that is why this attempt has been carried out.

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. Hopefully this research will be guideline for future researchers who intend to study the commercialization of fungi on large scales for controlling the grasshopper and locust in Pakistan.

Materials and methods

Collection of samples

The stock of grasshopper were collected from agriculture fields of rice, maize, sugarcane, millets, fodder crops and their surrounding vegetation of grasses using sweep net (8.89 cms in diameter and 50.8cms in length) as well as by hand picking. Collected insects took to the laboratory then were kept in clean cages having length 30.5cms and width 26.5cms. Insects fed on maize leaves, leaves and twigs surface sterilized in 5% sodium hypochlorite solution as described by Prior *et al.,* (1995) and Riffat *et al* (2013).

Incubation in laboratory

Grasshopper were divided into groups of 50 to form replicates per treatment there was no discrimination between age and sex then all insect placed in cages (length 16.5cms, width 13.5cms) under laboratory condition where temperature range between 28±2°C to 39±2°C and humidity was 26% to 61%. Population of grasshoppers comprising on all developmental stages which were collected from field maintained in the laboratory (25°-23/N, 68°-24/E) for up to 1week prior to use.

Fungal isolation and sporulation test

Insects cadavers were removed from the cages than surfaced sterilized in 5% Sodium hypochlorite and 75% ethanol solution and then will rinsed in sterile distilled water. The cadavers were then left to dry for 48hrs (Dourou-Kpinduo *et al.,* 1995). After drying these cadavers, they were humid incubated in clean dessicators at room temperature as described by Luz and Fargues, (1998). 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 was placed in both sonicator for 1 minute to break up the conidial chains and conidial counts were made with a haemocytometer as described by Poinar and Thomas, (1984) and Riffat *et al.,* (2013) and Kumar *et al.*, (2013).

Identification of fungal isolates

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

Pathogenicity Bioassay

Different fungi species were isolated and then isolates was 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) and Kumar *et al.*, (2013). 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 spores' 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 was 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). However, for the control experiment blank oil without spores was applied to the pronotal shield of the grasshoppers. In the last infected and uninfected grasshoppers was transferred into separate clean cages. Daily mortality was record and dead insects were removed from the cages Riffat *et al.,* (2013).

Statistical analysis

Data was analyzed with the help of statistical software SPSS version 10.0. Obtained data from experimental groups was subjected to one-way analysis of variance (ANOVA), with repeated measures and significant mean were determined using Least Significant Difference (LSD) Test. These tests were used to compare the means of the various treatments.

Results

The results of the faecal assessments are shown in (Table II). One way analysis of variance (ANOVA) revealed a significant effect of treatment and time on weight of faeces produced per insect i-e ($F._{(0.75)}$ = 13.09, $F._{(0.81)}$ = 14.84, $F._{(0.79)}$ =13.09 & $F._{(1.89)}$=04.36 P<0.05) for *Oxya velox,* ($F._{(0.57)}$ = 0.96, $F._{(0.72)}$= 13.09 , $F._{(0.84)}$= 14.84 & $F._{(1.71)}$= 0.436 P<0.05) for *O.hyla hyla* and ($F._{(0.98)}$ = 16.58 , $F._{(1.08)}$= 0.262, $F._{(0.77)}$= 13.09 & $F._{(1.41)}$= 0.262 P<0.05) for *H.nigrorepletus* when these insects treated with *Aspergillus flavus* (Friedrich link, 1809) , *A.fumigutus* (Fresenius, 1863) and *A.niger* (Van Tieghem, 1867) and control respectively. Individual analyses for each date revealed that, generally, faeces production per insect decreased with dose, with controls significantly higher than the treated populations.

Table 1. Identification of Entomopathogenic Fungi.

Growth Morphology	Color	Phialides	Spores	Probable Organisms
Fast growing and heavily sporing	Dirty Green	Typically radiate. (Splitting to several poorly defined column)	Typically globose to subglobose	*Aspergillus flavus*
Fast growing and heavily sporing	Black to dark brown	Globose, Tangled . (Splitting into columns)	Rough echinulated globose conidia	*Aspergillus niger*
Fast growing and moderately sporing	Grey-Green	Chain basipetally	Conidia (air borne spores)	*Aspergillus fumigates*

Note: International Mycological Institute (IMI) manual of pathogenic fungi and bacteria.

Analysis of the average total weight of faeces produced per treatment per day revealed a similar but more marked pattern to the above. Examining the data in this way indicate the total treatment effect by combining both changed in faeces production per insect and changes in the number of individuals, this show that over all control (i.e. effective reduction in feeding as indicated by total faecal production can be far greater than indicated by mortality alone).

Table 2. Faeces production of important grasshoppers after the treatments of different pathogenic fungi during the year 2013.

a) Oxya velox (Fabricius, 1787).

Days	Treatments			
	Aspergillus flavus	*A.fumigatus*	*A.niger*	Control
1st	2.00gm	1.86 gm	2.12 gm	1.68 gm
2nd	1.32gm	1.52 gm	1.35 gm	2.52 gm
3rd	0.98gm	1.00 gm	0.97 gm	2.32 gm
4th	0.32gm	0.80 gm	0.62 gm	1.92 gm
5th	0.41gm	0.31 gm	0.21 gm	1.62 gm
6th	0.20gm	0.10 gm	0.24 gm	1.78 gm
7th	0.02gm	0.10 gm	0.06 gm	1.45 gm
F.(0.05)	(0.75) 13.09	(0.81) 14.84	(0.79) 13.09	(1.89) 0.436

(b) Oxya hyla hyla (Serville, 1831).

Days	Treatments			
	Aspergillus flavus	*A.fumigatus*	*A.niger*	Control
1st	1.32gm	2.01gm	2.02gm	2.62gm
2nd	0.98gm	1.32 gm	1.00 gm	1.82 gm
3rd	0.82gm	0.12 gm	2.01 gm	2.02 gm
4th	0.43gm	0.62 gm	0.33 gm	1.62 gm
5th	0.33gm	0.73 gm	0.21 gm	1.42 gm
6th	0.10gm	0.22 gm	0.10 gm	1.32 gm
7th	0.02gm	0.04 gm	0.23 gm	1.20 gm
F.(0.05)	(0.57) 0.96	(0.72) 13.09	(0.84) 14.84	(1.71) 0.436

(c) Hieroglyphus nigrorepletus (Bolivar, 1912).

Days	Treatments			
	Aspergillus flavus	*A.fumigatus*	*A.niger*	Control
1st	2.32 gm	2.01 gm	1.52 gm	2.64 gm
2nd	1.52 gm	2.03 gm	1.18 gm	1.32 gm
3rd	1.56 gm	1.03 gm	0.92 gm	2.01 gm
4th	0.82 gm	0.87 gm	0.56 gm	1.05 gm
5th	0.32 gm	0.52 gm	0.42 gm	1.23 gm
6th	0.10 gm	0.32 gm	0.23 gm	0.94 gm
7th	0.24 gm	0.82 gm	0.62 gm	0.73 gm
F.(0.05)	(0.98) 16.58	(1.08) 0.262	(0.77) 13.09	(1.41) 0.262

The study provides further evidence that infection by *Aspergillus flavus, A.fumigutus* and *A.niger* 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 were significantly important pathogens in the population of the grasshopper.

During the present study it was also observed that

treated grasshopper undergoes an interesting behavioral modification after infection with *Aspergillus* and just Prior to death. Beside this in the same cases insects start unusually movement without feeding and suddenly stop the same while in some cases it was also observed that their body muscles begin to swollen and reddish pitch appeared on the entire abdomen surface. Due to these pathological changes insect became sluggish day by day in last insect climb on stalk of grass kept in cages, clasp their legs around the stem and die in this position.

Table 3. Mortality of grasshopper's population treating with different pathogenic fungi during the year 2012-2013.

Treatments	Period days (Mean ± SE)						
	1st	2nd	3rd	4th	5th	6th	7th
A. flavus	0.35±0.32b	0.00±0.00d	1.5±0.47 a	6.9±1.41 a	11.0±2.10 a	26.8±1.30 a	3.4±2.80 a
A.fumigatus	0.00±0.00c	2.5±1.00 a	0.61±0.32c	3.8±1.32b	5.8±0.43b	9.8±1.20c	27.0±3.9b
A.niger	1.42±0.31 a	1.00±0.58b	1.00±0.43b	4.5±0.53b	4.9±1.02c	11.42±1.30b	22.8±1.90c
Control	0.00±0.00c	0.75±0.31c	0.00±0.00d	1.9±0.46c	0.00±0.00d	1.00±0.57d	1.8±0.00d

Note: The letter indicate a significant difference (P<0.05) according to LSD test.

Discussion

Nearly, one million species of insects has been described in world (May, 2000) which comprise approximately 67 % of the world's described fauna and flora. Amongst insect due to diversify number of grasshoppers they destroy several billion dollars worth of valued crops worldwide (Bidochka and Khatchatourians, 1992). But indiscriminate use of insecticides against these pest can also promote resistance among the target or another pest population so in order to resolve this hardles specific biocides should be developed commercially to target particular pest and minimize it effects on non- target organisms. For this purpose present study has been initiated. Earlier the large number of pathogenic disease has been studied as possible biological control agents (Bidochka and Khatchatourians, 1992; Streett and McGuire, 1990, Riffat *et al.*, 2013) from many countries of the world including Pakistan. In addition to this Cheema *et al.*, (1973), Irshad (2003) carried work on the biology of *Aspergillus flavus* and *A.fumigatus* and observed that these fungi infect several insect species in Pakistan presently we are agreed on this account. 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) suggest that significant colonization of the insect is necessary before feeding is reduced.

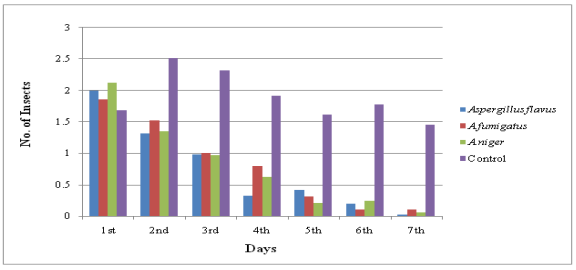

Fig. 1. Faeces production of *Oxya velox* after the treatments of different pathogenic fungi during the year 2013.

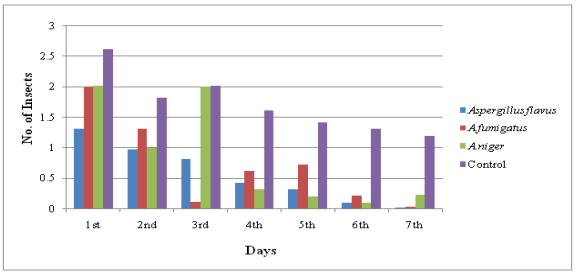

Fig. 2. Faeces production of *Oxya hyla hyla* after the treatments of different pathogenic fungi during the year 2013.

During the present study it was noted that all the host species treated with different pathogenic fungi under laboratory condition gave significant results after infection insect stop the feeding and there was

significant reduction in their faeces production. So the existing study suggested that these microbial agents must be utilized at the commercial level these entomopathogenic fungi considered safe towards the non-targets organisms present in the field. Further, they also offer a safer alternative for use in IPM than chemical insecticides previously same observation was also reported by (Goeltel and Hajek 2000 and Pell *et al*., 2001). Finally, Present study suggests that utilization of entomopathogenic fungi against pest species of grasshopper and locust is a under explored research area in Pakistan that is why it is really important to improve our knowledge and keep doing research in this field.

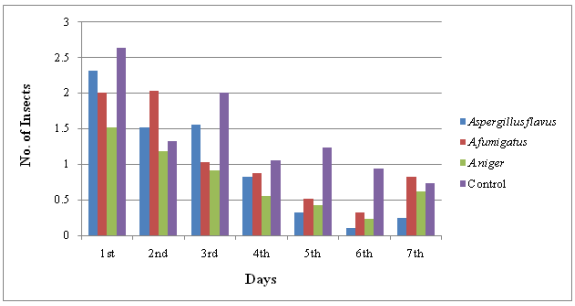

Fig. 3. Faeces production of *Hieroglyphus nigrorepletus* after the treatments of different pathogenic fungi during the year 2013.

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