Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 3, No. 12, p. 223-229, 2013
http://www.innspub.net

RESEARCH PAPER **OPEN ACCESS**

Pathogenic application of *Aspergillus* species for the control of agricultural important grasshoppers

Santosh Kumar*, Riffat Sultana, M. Saeed Wagan

Department of Zoology, University of Sindh, Jamshoro, Pakistan

Article published on December 23, 2013

Key words: Grasshopper, survivalship, *Aspergillus* spp., control, infection.

Abstract

During the present study a total of 08 species of grasshopper came in collection amongst these survivalships of three grasshopper i-e Oxya velox (Fabricius, 1787), Poekilocerus pictus (Fabricius, 1775) and Hieroglyphus nigrorepletus Bolivar, 1912 were note following the infection of three Aspergillus species i-e Aspergillus flavus, A.fumigutus and A.niger under laboratory condition. The average survival times of the treated grasshopper in the present study were significantly shorter than those typically observed in control trails. The high fungal infection incidence recorded on grasshopper cadavers suggested that fungi entomopathogen isolated are significantly important pathogen in the reduction of grasshopper's population.

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

Introduction

Grasshopper and locust are responsible for significant loss to the agricultural industry in grassland biomes of the world (Lomer *et al*., 2001). Over 97 species of important grasshopper exist in Sindh and of these 19 are considered to be minor and major pest (Wagan, 1990, Riffat and Wagan., 2012).Microbial agents considered so far for the control of Acrididae include all major types i-e fungi, bacteria, virus, nematodes and protozoan amongst all these, Entomopathogenic fungi are of interest as classical biological control agents because of their observed capacity to cause spectacular epizootics. (Lomer *et al*., 2001 & Riffat *et al*., 2013).

Investigations have been conducted in many countries of the world to document the parasites, predators and pathogens including fungi, (Aldrovandi, 1923, Christie, 1929, 1936, Greathead, 1963, 1992, Nickel, 1972, Poinar, 1975, Roonwal, 1976, Henry *et al*., 1985, Prior and Greathead, 1989, Shah *et al*., 1998, Balfour-Browne, 1960, Hermandez-Crespo and Santigo-Alvarez, 1997, Shah *et al.,* 1994, Balogun & Fagade 2004, Bidochka and Khatchatourians, 1992, and Paraiso *et al.,* 1992). But still now; this practice is currently under consideration as a potential alternative to chemical insecticides for grasshopper control in Pakistan. It was therefore felt necessary and an attempt has been made to apply entomopathogens fungi against important grasshopper from district Badin. Because, Research in this project has contributed data required for the registration of biological control agents in Pakistan generally and Badin particularly. The basic aim of the present study is to contribute the knowledge about the pathogeneic application of *Aspergillus* species for the control of agricultural important grasshoppers.

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. (Fig. 1) 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* 2012.

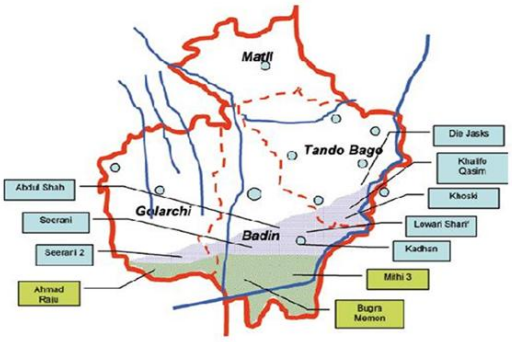

Fig. 1. Map of Badin showing surveyed areas.

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 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.,* 2012.

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-II).

Table 1. Identification of Entomopathogenic Fungi.

Growth Morphology	Color	Phialides	Spores	Probable organisms
Fast growing and heavily sporing	Dirty Green	Typical 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.

Pathogenicity bioassay

Different fungi species were isolated and then isolates was cultivated at 28°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 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.,* 2012.

Result

During the present study a total of 3701 specimens comparing on mix population were collected from various agricultural field. The collected material was sort out into 08 species i-e *Truxalis examia examia, Oxya velox, Poekilocerus pictus, Oxya hyla hyla, Hieroglyphus nigrorepletus, Hieroglyphus perpolita, Acrida exaltata,* and *Aiolopus thalasinus* among them grasshopper only 03 species i-e *Poekilocerus pictus, Hieroglyphus nigrorepletus* and *Oxya velox*. Amongst these grasshopper only 03 host species i-e *Poekilocerus pictus, Hieroglyphus nigrorepletus* and *Oxya velox* with over all collection ratio of 2563 were treated with 03 pathogen fungi i-e *Aspergillus flavus, A.fumigutus* and *A.niger*.(Table:I)

Table 2. Showing the collection of grasshoppers from different localities of district Badin during the year 2012-2013.

Species	Badin Proper	Tando Bago	Khoski	Kadhan	Abdul Shah
Truxalis examia examia	50	37	22	10	13
Oxya velox	157	68	193	203	143
Poekilocerus pictus	103	201	204	151	217
Oxya hyla hyla	63	103	207	72	133
Hieroglyphus nigrorepletus	103	217	302	158	143
Hieroglyphus perpolita	37	22	19	31	14
Acrida exaltata	05	17	03	19	07
Aiolopus thalasinus	57	63	54	37	43

Note: Total no. of specimen was collected 3701.

At the present it was observed that out of 2563 collected specimen of treated species from field used for the study 90% of them died in the cages. It has been observed that grasshoppers treated with the pathogen began to die with full signs of mycosis on day 4^{th} and 5^{th}. All treated insects died by day 6^{th} following the application of *A.flavus* while other replicates of the *A.fumigutus* and *A.niger* all dying by day 7^{th}. Opposing to this significantly low mortality ratio was obtained for control treatments without anus sign of myosine (Table-III). The highest lethal time of 6 days recorded for treated grasshopper after the infection of *Aspergillus flavus* recommend that its spore are severely lethal to grasshopper and could cause high mortality in all treated species of insects. This suggests that *A.flavus* might be proving good pathogen agent against grasshopper. This study also indicated that infection by *A.flavus , A.fumigutus* and *A.niger* cause a significant reduction in host feeding well before deaths. It might be one of the cause they can net survive for longer period of time. It has been observed that average survival times of the treated insect in the present study were shorter than those typically observed in control traits (Table 3)

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

Treatment	Period days (Mean ± S.E)						
	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	6^{th}	7^{th}
A flavus	0.35 ± 0.32^{b}	0.00 ± 0.00^{d}	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.fumigutus	0.00 ± 0.00^{c}	2.5 ± 0.10^{a}	0.61 ± 0.32^{c}	3.8 ± 1.32^{b}	5.8 ± 0.43^{b}	9.8 ± 1.20^{c}	27.0 ± 3.9^{b}
A.niger	1.42 ± 0.31^{a}	1.00 ± 0.58^{b}	1.00 ± 0.43^{b}	4.5 ± 0.53^{b}	4.9 ± 1.20^{c}	11.42 ± 1.30^{b}	22.8 ± 1.90^{c}
Control	0.00 ± 0.00^{c}	0.75 ± 0.31^{c}	0.00 ± 0.00^{d}	1.9 ± 0.46^{c}	0.00 ± 0.00^{d}	1.00 ± 0.57^{d}	1.8 ± 0.00^{d}

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

Discussion

For the past few centuries, entomopathogenic fungi have been registered as best biological tool for controlling the grasshopper and locust population in many countries of world including Pakistan (Bidochka and Khatchatourians, 1992, Streett and McGuire, 1990, Shah *et al.,* (1994), Riffat *et al.,* 2012). The high fungal infection incidence recorded on grasshopper population suggested that *Aspergillus* isolated are important pathogen in the population of the grasshopper on these observation agreed with the finding of Hernardez Crespo and Santiago Alvarez, (1997) and Riffat *et al.,* (2012).

Most of the researchers strongly recommend the utilization of fungal epizooties as biological control agents against grasshopper (Paraiso *et al.,* (1992), Moore *et al.,* (1992) Funk *et al.,* 1993, Hung and Boucias, 1992). Presently we also did experiment under laboratory condition and pathogen in the form of oil formation has been injected on the pronotum sheets of insects. This treatment gave similar result as obtained by Haynes, (1988), Johnson and Parlikova 1986, Hung and Boucias, 1992 and Zacharuk, 1971). Earlier Riffat *et al.,* 2012 reported that M.flavoviride cause a significant reduction in host feeding well before death. Presently we obtain similar result for 03 treated species. During the present study survival times of the treated insect was found shorter than those typically observed in control. It might be due to insertion of pathogen directly on the pronotum sheet of insect and insect soon with infection compare with the field following the spray application.

Acknowledgments

We are highly thankful to Prof. Dr. Tahir Rajput Ex. Dean Faculty of Natural Science for providing all necessary data regarding, the fungi identification and Mr. Aqeel Bhutto for access to his laboratory for rearing, culturing and isolation of fungi media.

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