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Effect of Entomopathogenic Fungi on the Food Consumption of Acrididae Species

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***Abstract*—**This study was conducted to evaluate the effect of *Aspergillus* species on acridid populations which are major agricultural pests of rice, sugarcane, wheat, maize and fodder crops in Pakistan. Three and replicates i.e. *Aspergillus flavus, A. fumigatus* and *A. niger,* excluding the control, were held under laboratory conditions. It was observed that consumption faecal production of acridids was significantly reduced after the pathogenic application of *Aspergillus*. In the control replicate, the mortality ratio for stage (N_4-N_6) was maximum on day 2nd i.e. [$F_{10.7} = 18.33$, $P < 0.05$] followed by [$F_{4.20} = 07.85$, $P < 0.05$] and [$F_{3.77} = 06.11$, $P < 0.05$] on 4th and 3rd day, respectively. Similarly, it was a minimum i.e. [$F_{0.48} = 84.65$, $P < 0.05$] on the 1st day. It was also noted that faecal production of Acridid nymphs was not significantly affected when treated with conidial concentration in H_2O formulation; however, it was significantly reduced after the contamination with conidial concentration in oil. The high morality of acridids after contamination of *Aspergillus* supports their use as bio-control agent for reducing pest population. The present study recommends that exploration and screening must be conducted to provide additional pathogens for evaluation as potential biological control against grasshoppers and locusts.

***Keywords*—**Acridid, agriculture, *Aspergillus,* formulation, Grasshoppers.

I. Introduction

ENTOMOPATHOGENIC fungi are regarded as bio-pesticides and expected to have a significant and increasing role for the control of locust and grasshopper populations in the world, including Pakistan [1]. These microbial agents are commonly famous as myco-insecticides that have great potential to kill locust and grasshopper species. Beside this, it is also beneficial to control flies, beetles and aphids in field [2]. Pathogenic fungi quickly penetrate into the host's external surface and it was observed that after utilization of pathogenic fungi large number of grasshoppers and locusts were killed, within short time. This finding suggests that this microbial agent is very useful against many pest species. Microbial agents that include: bacteria, virus, nematodes, protozoan and pathogenic fungi are good bio-control agents. Reference [3] stated that pathogenic fungi are very important and interesting bio-control agent due to its observed capacity that lead to formation of epizootics.

Earlier, many researchers conducted studies on this [4], [6], [8]-[10], [14]. About 35 genera, comprising 400 species/sub-species of pathogenic fungi have been identified. Previously, [15] reported that about 1800 entomopathogenic fungi have very close association with the insect population in the field.

After application of different insecticides and pesticides, a large number of scale insect (Hemiptera) are killed in the field, but on the other hand, it can cause health and environmental issues, and it is this problem that has led to renewed interest in the development of eco-friendly microbial agents that are now incorporated into an integrated pest management (IPM) strategy. Utilization of entomopathogenic fungi for practical pest control is very limited due to the lack of scientific literature on the epidemiology of infection, in particular the host parasitic system. Therefore, efforts are being made in the investigation to isolate, identify and characterize mycoflora associated with natural mortality of various pest species of grasshopper and to study the prospect of bio-control from this area. The utilization of different chemicals has a very harsh impact on the environment and the frequent use of chemicals enhances the resistance power in an insect's body. For example, in 1987, an outbreak of locusts was not able to be controlled by pesticides.

II. Experimental Procedure

A. Insects Sampling

Stocks of grasshoppers both mature and immature were collected from various districts of Sindh province, Pakistan. The specimens were captured with a sweep net having a 25×25 cm diameter, 82 cm in length (without the diameter). Some specimens were also captured by hand picking, sweeping, trapping, night trap, aerial netting and in black light pan traps when-ever found. Collected insects were taken to the laboratory where two cages of different measurement i.e. (42 cm in length, 30 cm in width) and (35 cm in length, 32.5 cm in width) were maintained. All collected individuals were equally divided and put into cages. Fresh leaves of *Zea mays* L. serve to rearing the insects. The leaves and twigs were previously sterilized in 5% solution of Sodium hypochlorite (NaOCl). This methodology has been adopted from [1], [5], [7], [11]-[13], [16]. For identification of the samples, the scheme given by [17] was followed.

B. Collection of Infected Samples

For capturing of insects contaminated with pathogenic fungi, careful observation has been made in the field and only those insects were collected which show clear symptoms of mycoses such as (i) insect does not move fast, (ii) de-coloration not original, (iii) fungal mycelia fully spread on the cuticle, and (iv) insects look sluggish/inactive and very easy to capture. Infected specimens were easy to capture with large forceps and after collection were transferred into glass jars and

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brought to laboratory for further analysis. All were sorted out into different host species and kept in clean cages. Fresh *Zea mays* L. leaves were provided for the insects. Food plant change daily and food consumption, through analysis of faecal material and the mortality of the insect after every 24hrs were noted.

C. Incubation in Laboratory

Different species of Acrididae were divided into group of about 50 individuals for each treatment. However, there was no differentiation in age, sex and developmental stage. All collected specimens were placed into wooden cages under laboratory conditions, where the temperature range between 28±2 °C to 41±2 °C and the relative humidity (RH) was 26.5% to 60.5%. The population of grasshoppers comprised of all developmental stages which were collected from the field and maintained in the laboratory at the Entomology and Bio-Control Research Lab. (EBCRL), Department of Zoology, University of Sindh, Jamshoro (25°-23/N, 68°-24/E).

D.Fungal Isolation and Sporulation Test

The sporulating fungi were separated into pure culture on Sabouraud Dextrose Agar (SDA), after which, it was formulated into oil (coconut); after preparing the oil formulation this fresh suspension was kept in a sonicator for 60 sec to break the conidial chain. After breaking, conidial was counted with the help of a haemocytometer, this method has been adopted from [18], [19].

E. Identification of Fungal Isolates

Various species of *Aspergillus* have been identified on the basis of conidia shape and size. Beside this, for detail and authentic identification, element concentration has been determined under scanning electron microscope (SEM). For reorganization of fungi terminology given by [20]-[24] was followed.

F. Pathogenicity of Aspergillus Isolates

Aspergillus isolates were incubated at 37°C, photoperiod was 12L: 12D for 24-hours. [19], [25]. Sterile spatula after incubation was used to harvest the conidia from the fungal culture. This harvested conidia were shifted into small McCartney bottles (fully sterilize and contained coconut oil) with fungal spores suspension prepared in oil and spore concentration measured with Neuberger Haemocytometer [26].

G.Formulation of Aspergillus Conidia

Two different formulations were selected in order to determine which formulation is more effective. Before starting the experiment, different parts of *Zea mays* L. (consisting of leaves and stem) were broken and shaken under tap water several times. After this, about 2.5 gm of maize was placed in small jars and 26 gm in cages. Ten insects were reared in 4-liter plastic jars, while 50 specimens were kept in different cages.

1. Formulation for small jars:

5×10^{6} *(Conidial concentration) + 20ml (Coconut oil) = Oil formulation*

2. Formulation for colony:

5×10^{30} *(Conidial concentration) + 100ml (Distal water) = Water formulation*

The conidial oil distilled water formulation was sprayed on the insects using a hard held sprayer. Each insect was directly and individually sprayed with 3.5 ml of the appropriate concentration. After 15 to 20 minutes, the treated insects were transferred to the jars as well as in cages. The control groups received only a water formulation, without conidia. The insect in each replicate were fed on *Zea mays* L. (30 gm every 48 hrs).

H.Bio-Pesticides Application

Before the commencement of the bioassay test, the insects were reared in cages for one week. After that, 0.1 ml of conidial oil suspension was carefully applied beneath the pronotum shield of the insect with the help of a sterile Pasteur pipette. Beside this, in control replicate blank oil with spores was applied on the pronotum shield of the grasshoppers that were reared in jars individually, while in the second replicate, the conidial (mix in distilled water) formulation were sprayed on the insects (reared in captivity) using a hard held sprayer. Each insect was directly and individually sprayed with 3.5 ml of the appropriate concentration. After 15 to 20 minutes the treated insects were transferred to the cages. The control groups received the same water formulation without conidia. The insects in each replicate were fed on *Zea mays* L. (30 gm after every 48 hrs).

Food consumption of the insets was noted every 48 hrs. Insects contaminated with *Aspergillus* and the healthy control grasshoppers were shifted into separate cages and placed in the laboratory, where they were monitored and deaths were noted daily.

III. Result

Food consumption and faecal production by the insects treated with different formulations of the *Aspergillus* species were analyzed under laboratory conditions. The three species replicates i.e. *A. flavus, A. fumigatus* and *A. niger,* while the fourth replicate was the control. As can be seen from Tables I-II, a greater reduction in faecal production was noticed after the treatment with the oil formulation. A reduction in the feeding of the infected insects stages (N_1-N_3) was started after treatment of the 1st to 2nd days. Significant reduction in faecal production in stages N_1 to N_3 was observed for almost all specimens and with the exception of a few, most had died early and within four days. Besides this, faecal production in (N_4-N_6) developmental stages was significantly reduced on 2nd day, i.e. [$F_{0.18} = 32.29$, $P < 0.05$] and it was [$F_{0.03} = 68.94$, $P < 0.05$] on 1st day; however, there was no significant difference in the faecal production of the insects deposited i.e. [$F_{0.20} = 35.78$, $P < 0.05$] on the 3rd and 4th day, respectively.

In a comparison of the oil formulations, the rate of faecal production of acridid (nymphs) treated with conidial

concentration in H_2O and maintain in cages, indicates that the maximum faecal production was obtain on the 2nd day [$F_{0.24}$ = 42.76, $P < 0.05$], followed by [$F_{0.23}$ = 41.02, $P < 0.05$] on 5th and 6th day, as shown in Table IV. However, least amount of faecal material was obtained on the 1st day [$F_{0.08}$ = 14.84, $P < 0.05$]. Table V shows the faecal production of acridid (adults) when treated with conidial concentration maximum in H_2O. It was seem that the greater ratio of faecal material was obtained on 8th day [$F_{0.22}$ = 39.27, $P < 0.05$] and it was non-significant on the 2nd to 7th day, while it was significantly low [$F_{0.10}$ = 18.33, $P < 0.05$] on the 1st day. Beside this, faecal production of adult acridid, cultured in small jars when treated with oil formulation, the maximum value [$F_{0.21}$ = 37.52, $P < 0.05$] was noted on day 6th day, while a significant least value [$F_{0.09}$ = 16.58, $P < 0.05$] was observed on the 1st day, while the observations for day 2nd to 5th and 7th were non-significant, as shown in Table III.

The cumulative percentage of faecal production in the treated insects differed significantly compared to the control, and the entomopathogenic fungi was seen to have a significant impact on the food consumption and feeding behavior of the insects. It was noted that insect pathogen, unlike chemical insecticides, do not have a quick response on pest feeding; however, a gradual reduction in feeding was recorded after the 2nd day. The reduction in feeding can be attributed to the pathogenic effect that may also alter body fat accumulative, and therefore, the insects become thin and sluggish during the treatment process.

TABLE I
FAECAL PRODUCTION OF IMMATURE (NYMPHS STAGES 1TO 3 ACRIDID CULTURE IN SMALL JARS UNDER LABORATORY CONDITIONS (AFTER TREATMENT OF *ASPERGILLUS* OIL FORMULATION)

Treatments	Days of Observation (Mean±Se)						
	1st	2nd	3rd	4th	5th	6th	7th
A. flavus	0.020 ± 3.242^{b}	0.031 ± 4.374^{b}	0.033 ± 2.827^{c}	0.028 ± 2.733^{c}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
A. fumigatus	0.019 ± 2.598^{c}	0.029 ± 4.995^{c}	0.031 ± 3.550^{b}	0.030 ± 3.099^{b}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
A. niger	0.017 ± 2.766^{c}	0.030 ± 3.181^{b}	0.035 ± 2.820^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Control	0.692 ± 0.033^{a}	0.641 ± 0.040^{a}	0.794 ± 0.040^{a}	0.715 ± 0.026^{a}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
$F_{(0.05)}$	$_{(0.18)}$ 32.29	$_{(0.18)}$ 32.29	$_{(0.22)}$ 39.27	$_{(0.19)}$ 34.03	-------	-------	-------

TABLE II
FAECAL PRODUCTION OF IMMATURE (NYMPHS STAGES 4 TO 6 ACRIDID CULTURE IN SMALL JARS UNDER LABORATORY CONDITIONS (AFTER TREATMENT OF *ASPERGILLUS* OIL FORMULATION)

Treatments	Days of Observation (Mean±Se)						
	1st	2nd	3rd	4th	5th	6th	7th
A. flavus	0.022 ± 5.042^{C}	0.026 ± 3.643^{B}	0.032 ± 2.874^{B}	0.031 ± 3.181^{C}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}
A. fumigatus	0.032 ± 4.395^{D}	0.025 ± 3.562^{C}	0.030 ± 2.947^{C}	0.032 ± 2.582^{C}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}
A. niger	0.026 ± 5.740^{B}	0.024 ± 2.283^{C}	0.031 ± 3.137^{B}	0.033 ± 2.769^{B}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}
Control	0.077 ± 7.781^{A}	0.657 ± 0.047^{A}	0.745 ± 0.044^{A}	0.707 ± 0.030^{A}	0.032 ± 3.501^{A}	0.714 ± 0.031^{A}	0.778 ± 0.040^{A}
$F_{(0.05)}$	$_{(0.03)}$ 68.94	$_{(0.18)}$ 32.29	$_{(0.20)}$ 35.78	$_{(0.20)}$ 35.78	-------	-------	-------

TABLE III
FAECAL PRODUCTION OF ADULT ACRIDID CULTURE IN SMALL JARS UNDER LABORATORY CONDITIONS (AFTER TREATMENT OF *ASPERGILLUS* OIL FORMULATION)

Treatments	Days of Observation (Mean±Se)						
	1st	2nd	3rd	4th	5th	6th	7th
A. flavus	0.061 ± 3.115^{b}	0.032 ± 6.535^{b}	0.030 ± 2.427^{c}	0.031 ± 2.773^{c}	0.033 ± 3.273^{b}	0.027 ± 4.633^{d}	0.030 ± 5.049^{d}
A. fumigatus	0.060 ± 5.498^{b}	0.031 ± 2.759^{c}	0.033 ± 2.680^{b}	0.036 ± 3.772^{b}	0.031 ± 2.424^{c}	0.031 ± 3.315^{c}	0.033 ± 2.840^{b}
A. niger	0.039 ± 0.011^{c}	0.055 ± 4.565^{d}	0.031 ± 3.173^{c}	0.032 ± 3.247^{c}	0.035 ± 2.827^{b}	0.033 ± 2.769^{b}	0.034 ± 3.116^{c}
Control	0.227 ± 0.113^{a}	0.642 ± 0.038^{a}	0.700 ± 0.031^{a}	0.722 ± 0.033^{a}	0.715 ± 0.041^{a}	0.711 ± 0.032^{a}	0.732 ± 0.033^{a}
$F_{(0.05)}$	$_{(0.09)}$ 16.58	$_{(0.19)}$ 34.03	$_{(0.19)}$ 34.03	$_{(0.20)}$ 35.78	$_{(0.20)}$ 35.78	$_{(0.21)}$ 37.52	$_{(0.20)}$ 35.78

TABLE IV
FAECAL PRODUCTION OF ACRIDID (NYMPHS) POPULATION TREATED WITH CONIDIAL CONCENTRATION IN H_2O CULTURED MAINTAINED IN THE LARGE CAGE

Treatments	Days of Observation (Mean±Se)									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
A. flavus	0.075 ± 3.106^{b}	0.068 ± 3.419^{d}	0.063 ± 5.158^{c}	0.068 ± 2.605^{b}	0.073 ± 2.314^{b}	0.070 ± 2.608^{d}	0.073 ± 2.356^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
A. fumigatus	0.062 ± 3.496^{c}	0.074 ± 4.571^{c}	0.070 ± 2.656^{b}	0.059 ± 5.328^{c}	0.067 ± 3.102^{d}	0.072 ± 2.149^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
A. niger	0.073 ± 3.279^{b}	0.083 ± 3.077^{b}	0.072 ± 2.150^{b}	0.067 ± 2.582^{b}	0.070 ± 3.229^{c}	0.074 ± 2.959^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Control	0.125 ± 5.217^{a}	0.736 ± 0.036^{a}	0.709 ± 0.031^{a}	0.704 ± 0.026^{a}	0.713 ± 0.024^{a}	0.731 ± 0.024^{a}	0.739 ± 0.022^{a}	0.731 ± 0.019^{a}	0.714 ± 0.026^{a}	0.721 ± 0.023^{a}
$F_{(0.05)}$	$_{(0.08)}$ 14.84	$_{(0.24)}$ 42.76	$_{(0.22)}$ 39.27	$_{(0.22)}$ 39.27	$_{(0.23)}$ 41.02	$_{(0.23)}$ 41.02	-------	-------	-------	-------

TABLE V
FAECAL PRODUCTION OF ACRIDID (ADULTS) POPULATION TREATED WITH CONIDIAL CONCENTRATION IN H_2O CULTURED MAINTAINED IN THE LARGE CAGE

Treatments	Days of Observation (Mean±Se)									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
A. flavus	0.082±3.177[d]	0.067±2.769[c]	0.063±5.537[c]	0.069±2.477[d]	0.068±3.101[d]	0.071±2.415[c]	0.072±2.499[c]	0.074±2.695[b]	0.075±2.624[b]	0.072±3.492[b]
A. fumigatus	0.085±5.740[c]	0.057±6.099[d]	0.060±4.790[d]	0.071±2.385[c]	0.072±2.354[c]	0.070±2.357[d]	0.075±2.413[b]	0.073±2.356[b]	0.00±0.00[c]	0.00±0.00[c]
A. niger	0.089±4.600[b]	0.085±3.969[b]	0.064±4.061[b]	0.073±1.800[b]	0.076±2.793[b]	0.072±2.417[b]	0.074±2.207[b]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]
Control	0.163±3.667[a]	0.753±0.071[a]	0.733±0.026[a]	0.741±0.029[a]	0.732±0.023[a]	0.766±0.031[a]	0.739±0.025[a]	0.734±0.027[a]	0.695±0.026[a]	0.768±0.023[a]
F (0.05)	(0.10) 18.33	(0.24) 42.76	(0.23) 41.02	(0.23) 41.02	(0.23) 41.02	(0.24) 42.76	(0.24) 42.76	(0.22) 39.27	-------	-------

IV. DISCUSSION

It was observed that virtually all insects were found to be susceptible to fungal disease. It was also noted that thermoregulatory behavior of acridid species was observed in the laboratory following a spray application of oil- and water-based formulation of *Aspergillus* and (unsprayed) individuals. All treated grasshoppers kept in (jars and cages) were carefully monitored for three days. During the present study, it was noticed that infected insects altered their thermoregulatory behavior and showed a *behavioral fever* response to the pathogen, the body temperature increased as a way of literally toasting the fungal invader. Further, these behavioral responses may result in enhanced spore diffusion and fungal fitness. Earlier, Faria and Wraight [27] compiled a comprehensive list of fungal species from Asia, Africa, Europe, America, Australia/Oceania etc. and stated that different bio-pesticides are useful to control pest populations in the field; however, no findings were available on the utilization of this bio-pesticide from Pakistan.

After the pathogenic application, it was also noted that cuticular antimicrobial lipids, protein, metabolites shedding of the cuticle during development and behavior environmental adaptation that includes: fever, burrowing and growing, were affected. It was also noted that after the application of the oil and water based formulation of *Aspergillus,* the acridid species showed interesting behavioral changes prior to death. Beside this, it was noticed that the insect become thin and sluggish, and showed a reduction in feeding due to fungi infection which may affect body fat accumulation at sexual maturity. From the present study, it was observed that if total elimination of an insect pest is not needed, pathogenic fungi provides excellent results. It is suggested that entomopathogenic fungi (EPFs) play a vital role in the implementation of IPM techniques in the field and can offer sustainable pest control when combined with other techniques.

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S. Kumar was born in Sukkur Sindh, Pakistan. I have completed my earlier study from the same Division throughout my carrier I always got topmost position in class. I have passed B.S (Zoology) in 2009 from Shah Abdul Latif University, Khairpur. After this, I was registered for earning M.S leading to Ph.D degree on the title: "Analysis of the pathogenic applications of *Aspergillus* Species against Acridid grasshoppers of agriculture importance in Sindh" in University of Sindh Jamshoro. At present I have submitted thesis now waiting for Ph.D viva voce Examination. In meanwhile, I also published 18 research papers in National and International journals of repute having 18.54 impact factor (Google scholar Citation indices: 06 h-indexes: 01). Beside this, 02 research papers are accepted in pre review journal and 03 are in process of review in different journal. I also attended several National and International scientific conferences, seminars and workshops and presented 09 Research Papers in field of biological sciences. Further, I also display my work in poster format in different conferences. So far as the research guidance is concerned. I am actively involved with my supervisor in her different project such as,

- Identification of species
- Changing in climatic condition due to influence of insecticides and pesticides on crops.
- Utilization of fungi against pest and its impact on environment.
- Biology of different species under laboratory condition.