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Impact of Entomopathogenic Fungi *Aspergillus flavus* on life history statistics of *Hieroglyphus oryzivorus* (Orthoptera: Acrididae)

S. KUMAR[++], R. SULTANA[++], M. S. WAGAN

Department of Zoology, University of Sindh, Jamshoro

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Abstract: During the present study reproductive activity that include: duration of maturation, into final moult, maturation of adult, copulation timing of individuals, No. of mating and fecundity rate of healthy and unhealthy samples of *Hieroglyphus oryzivorus* effective by infection of *Aspergillus flavus* was studied. It was noted that 6th instar took (8.01±1.02 days) for maturation and infected individual took prolong time for converting into adult. Beside this, infected individual did not copulate for longer time and mating was observed once in the life of infected insects.

Keywords: Maturation, Infection, *Aspergillus flavus*, Life parameter, Mating & Fecundity

1. INTRODUCTION

Grasshopper, through their diversity in types, number, life cycles and habitats expose themselves to a wide range of pathogens. Infact in the agriculture, population of insect pests can be devastated by natural outbreak of pathogens. In 19th century researchers were also utilizing the pathogen for control of different insect in the field addition, there are many recent example of the effectiveness of pathogens when used against insect pests. Many researchers carried work on this subject i-e (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 and Fagade 2004, Bidochka and Khatchatourias, 1992, Paraiso *et al.*, 1992, Riffat *et al.*, 2012, Kumar *et al*., 2013, 2014).

Although; majority of studies have been done to assess the mortality ratio of target pest after treating with various entomopathogenic fungi (Diver, *et al*., 2000, Moore, *et al*., 1992, Inglis, *et al*., 1996 and Blanford, *et al*., 1998) but still now nothing has been published with exception of (Johnson and Pavlikova 1986, Olfert and Erlandson 1991 and Fargues *et al*., 1991) who carried work on the infection on feeding after pathogenic treatment.

But, mostly these scientists carried work under environment constant region that are condition for more infection and could not consider how this behavior and the overall impact of pathogen might change under more realistic, variable condition experiment in the field. The aim of this study, therefore, was to examine the effect of *A. flavus* on the life history statistics of *H. oryzivorus* which is considered a severe pest of paddy field in Sindh during July to October in different rice producing districts i-e Larkana, Khairpur Nathan Shah District Dadu and Badin. (Riffat, 2008). This pest cause heavy damage to all growing stages of rice however, after harvesting of the paddies, they migrate from paddy field to the nearby grassland, research so for conducted to control this pest mainly concentrated with application of pesticides. Therefore, there is a need for integrated pest management (IPM) approach to reduce the use of chemical pesticides with environment friendly methods. The present study is key step to initiate the IPM in Sindh.

2. MATERIAL AND METHODS

2.1 *Sampling:*

For the collection of grasshopper's samples monthly many rice fields located in Dadu, Larkana and Badin districts were visited from time to time in the year 2014. Mostly fields were inspected during May to October. Samples were collected from rice field which were surrounding by different vegetation of grasses, jawar and maize.

2.2 *Collection of infected samples:*

For the collection of infected insects keen observation were made in field and only those insects were collected which having clear symptoms of mycoses viz: (1)insect don't move fast, (2)change its color from original one, (3)cuticle fully covered with fungal mycelia and (4)they were very sluggish and found very easy to pick up. Infected specimens were picked with large forceps. Collected specimens were brought into laboratory and were sorted out into

[++]Corresponding author Email: riffatumer@hotmail.com

different developmental stages and kept in clean cages having diameter of (30.5cms length and 26.5cms width) fresh *Zea mays* leaves were provided to insects as described by Prior *et al*., (1995) and Riffat *et al*., (2013). Food plant change daily and observation has been noted.

2.3 *Isolation of entomopathogenic fungi:*

For the isolation of different entomopathogenic fungi cadavers with mycelia cushions preparations of conidia were obtained by film methods these preparation were kept on the slide and slide was colored with lactophenol cotton blue for clear view after this hyphae and conidia were studied under Stereoscopes Binocular Microscope. Shape and size of conidia were observed under the microscope for identification of fungi species. This method has been adapted from Kumar, (2014).

2.4 Identification *of fungal isolates:*

During the present study identification of fungal isolated was mainly based on their conidia shape and size and other description given by de Hoog, (1972); Domsch, *et al.,* (1980); IMI, (1983); Balazy, (1993) and Humber, (2005) were adopted.

3. RESULT AND DISCUSSION

During the present study a total of 164 samples (Both nymphs and adults) of *H. oryzivorus* were collected from three main rice producing areas i-e Dadu Larkana and Badin Districts of Sindh. Out of 164 individual of *H. orzivorus* 56 samples 34% were contaminated with infection of *Aspergillus flavus* (Table-I). It was observed that majority of sample were badly infected by *A. flavus* its possible reason might be due to high moisture level and increase in temperature. Table-II indicate that sexual reproductive activities of *H. oryzivorus* was also affected by the infection of *Aspergillus*. The healthy range of 6^{th} instar maturation was given 6.00±1.3 days by Riffat and Wagan (2010) on opposing to this, the normal individual treated with *A. flavus* under laboratory condition took more days (8.01±1.02) for maturation. Average normal maturation period for adult was reported (10.93±2.6) by Riffat and Wagan (2010) on contrasting to this, infected individual took prolong time for converting into adult stage. Addition to this total mating timing during entire life of *H. oryzivorus* was noted 139.06±55.3 hrs (Riffat, 2008) but during recent observation we had failed to observe the total mating time among insects because of less survival of insects.

Beside this, healthy individual showed maximum copulation duration that remains together for prolong time however, in case of infected samples they close to each other for lesser time (7.6±3.95 hrs) then, immediately leave each other and never attempt again for copulation. Similarly only single mating was observed in contaminated individual while there was maximum mating i-e (12.17±4.12) observed by Riffat, (2008) in the healthy individual of *H. oryzivorus.*

10 healthy female which were ready for oviposition after 24 hrs were picked from kept stock of grasshoppers, maintained under laboratory condition. They all were treated with prepared medium of *Aspergillus* in order to know the fecundity activity oh unhealthy individual (Table III). This table suggested that oviposition time of unhealthy samples was (21.02±0.21 mints) and female deposing only single and broken egg pods with lesser No. of eggs (17.23±0.2) and size of egg pods (16.30±0.01 mm) it was also reduced compare to its normal size. However, there was no significant difference in the length of eggs. It was very interesting to note that female secrete less quantity of brownish foamy mass instead of yellowish and took just (3.42±0.23 minutes) for foamy mass secretion while in case of normal individual it take (14.53±3.39 mints) for secretion of foamy mass and covered the whole opening. Present observation showed that *A. flavus* had a broad infection range. Current investigation recommends that *A. flavus* not only influence the survival-ship of *H. oryzivorus* but it also infect the other reproductive activities of this pest and hence could be exploited as a microbial control agents of the *H. oryzivorus* in rice producing areas.

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Table. I. Collection of *Hieroglyphus oryzivorus* from various localities of Sindh.

S #	District	Crop	Total No. of samples	Infected No. with %	Uninfected No. with %	Stages
1	Dadu	Rice	38	13/34%	25/65%	Nymph /Adult
2	Dadu	Maize	21	8/38%	13/61%	Nymph /Adult
3	Larkana	Maize	17	6/35%	11/64%	Nymph /Adult
4	Larkana	Rice	45	16/35%	29/64%	Nymph /Adult
5	Badin	Jawar	12	3/25%	9/75%	Nymph /Adult
6	Badin	Rice	31	10/32%	21/67%	Nymph /Adult
T.	---	---	164	56/34%	108/65%	

Table. 2. Reproductive activity of healthy and unhealthy sample of *H. oryzivorus.*

Life History Statistics	Healthy Range (n=15)	Unhealthy Range (n= 10)
6th instar Duration of Maturation	6.00±1.3(days)	8.01±1.02 (days)
Maturation of Adult	10.93±2.6 (days)	13.02±1.00 (days)
Total Mating time during entire life	139.06±55.3hrs	Nil.
Duration of Copulation	33.26±13.9(hrs)	7.6±3.95(hrs)
No. of Mating	12.17±4.12	1.00±0.00

Note: 10 sample (both sex) were analysis for this activity.

Table. 3. Fecundity rate of healthy and unhealthy sample of *H. oryzivorus.*

Life History Statistics	Healthy Range (n= 15)	Unhealthy Range (n= 10)
Oviposition Time	47.26±6.0 mints	21.02±0.21mints
No. of Egg Pods	3.26±0.96	1.00±0.1 (Broken)
No. of Egg	35.65±14.64	17.23±0.2
Size of Egg Pods	34.68±0.84mm	16.30±0.01 mm (Broken)
Size of Egg	4.52±0.07mm	3.10±0.01 (mm)
Weight of Egg Pods	1.30±0.03 gm	0.20±0.01gm
Secretion of foamy mass	14.53±3.39 (mints)	3.42±0.23 (mints)

Note: 10 infected individual were observed for this activity.

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