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Full Length Research Paper

Effects of macro-parasitic mite *Eutrombidium trigonum* (Hermann) on the life history characteristics of *Hieroglyphus* species from Sindh, Pakistan

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***Trombidiidae* species are reported as ecto-parasites in their larval stages for the variety of acrididae, which are pests of many economic crops in the world including Pakistan. The current parasitism ratio of red mite, *Eutrombidium trigonum*, was studied on three host species of *Hieroglyphus,* which are presently destroying valued crops. It was observed that proportional survival was found to be lower in mites-parasitized specimens. Females of these three species had reduced initial and total reproduction as compared to un-parasitic species. Egg production significantly reduced to 47.23±510.23, 98.67±11.43 and 51.56±12.40 with mite's parasitism during its entire life in these three studied species. As far as the survival of individuals is concerned, it is also significantly affected by mites' parasitism. At present, survival of *Hieroglyphus* species was reported at 20.32±3.43, 42.39±8.46 and 37.25±7.93 days for male and 16.30±2.61, 35.73±10.26 and 25.63±8.40 days for females of *Hieroglyphus perpolita Hieroglyphus oryzivorous* and *Hieroglyphus nigrorepletus* respectively. Infestation was reported at 18.25% on *H. oryzivorus* followed by 12.86 and 12.31% on *H. nigrorepletus* and *H. perpolita* respectively. Similarly, the infestation of adults was reported to be significantly high at 15.60% for *H. oryzivorus*, followed by 8.89% on *H. perpolita* while it was reported to be significantly low at 6.53% for *H. nigrorepletus*. Overall, parasitism percentage was reported at 43.42% for hopper stages and 31.02% for adult individual. This study suggests that these mites could be the major controlling factors in the dynamics of natural population of *Hieroglyphus* species.**

Key words: *Eutrombidi*um *trigonum,* ectoparsite, *Hieroglyphus,* pest*,* major controlling factors.

INTRODUCTION

Trombidiidae species (red mite) are reported as ectoparsite in their larval stages for the variety of acrididae, which are pests of many economic crops in the world including Pakistan. Many of the more interesting effects of mites on grasshoppers are sub-lethal or indirect in nature, and include change behavior and host fecundity. Mite species are known to parasitize grasshopper, the most common are the red mite *(Euthrombidium locustarum)*, *Euthrombidium trigonum*, and *Empusa (Entomophthora) grylli* found on the wings of grasshoppers (Peswani, 1961; Rizvi and Aziz, 1966, Huggans and Blickenstaff, 1966; Roonwal, 1976). They are characterized by a complex life cycle having heteromorphic larvae and predatory post-larval instar, and both stages are considered very injurious to host. Review of literature showed that a mite parasitizes a large number of economically important insect pests. That is why this group is regarded as potential agents of biological control (Welbourn, 1983; Gerson and Smilay, 1990). Amongst the mites species, *Eutrombidium* is considered the most frequent parasite of grasshoppers (Huggans and Blickenstaff, 1966; Southcott, 1993) and is

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therefore regarded as being a promising manager for the biological control of acridid pests (Welbourn, 1983).

There has been a flood of relevant information on grasshopper pathogen since the last comprehensive review on the biology of grasshopper given by Uvarov (1966, 1977). Researches, for the most part, have emphasized on the use of pathogen for controlling grasshopper population because indiscriminate use of chemical pesticides not only increases the rate of resistance evolution, but also poses a risk to human health and environment (Shahid and Riazuddin, 1999). Accordingly, review on grasshopper pathogens have had an application prospectively and dealt mainly with suppression of grasshopper population (Henry, 1977; Streett, 1986).

Rizvi and Aziz (1966) observed that mites (*Eutrombidium*) infect thorax under the wing and elytron pods of older hoppers and adults. During August and September, about 5% of the hoppers and 2 to 11% of the adults were found to be infested by this mite without causing any serious injury infestation. Mites provide an excellent example of the potential opportunity for pest managers to exploit macro parasite in grasshopper control but actually, the general lack of understanding about the ecology of parasites prevents the pest managers from using them successfully. In this manuscript, we try to explain the parasitism nature of *E. trigonum* species and how it influences the host and put the significant effect on the survival, fecundity and other activities of host species. The finding of this research will be helpful to determine the suitability of this closely related species as agents for the biological control of grasshoppers.

MATERIALS AND METHODS

Collection of host species

The stock of *Hieroglyphus* nymphs and adults were collected from the agricultural fields of rice, maize, sugarcane, millets, fodder crops and their surrounding vegetation of grasses with the help of traditional insect hand-net (8.89 cm in diameter and 50.8 cm in length), as well as by hand picking. The collection was made during the year 2011 in the months of June to November from various districts of Sindh province in the field. The collected material was transferred into polythene bags and later transferred to the laboratory.

Killing and preservation of host species and mites

The following method has been adapted from Vickery and Kevan (1983). Collected material brought into the laboratory was killed by means of potassium cyanide in standard entomological killing bottles. The host species were not left too long (10 to 15 min) in cyanide because the colour, particularly that of the green specimens, changed. Then the *Hieroglypgus* species were thoroughly examined under the stereoscopic dissecting microscope and some of the mites were scraped off with a needle (attached on head, thorax, wings and legs region) and were put in preservative medium (containing the 70% alcohol and few drops of glycerin); this medium is more preferable for longer preservation of samples. For further study, mites were then thoroughly washed in tap water and examined in glycerol on a cavity slide (without a cover glass), using a stereoscope dissecting binocular microscope. Glycerol was preferred to 70% ethyl alcohol as a mountant for two principal reasons. Firstly, this medium cleared the structures to a suitable degree, and secondly, it did not evaporate significantly either on a slide under a strong microscope lamp, or in the microvials to which the specimens were later transferred for storage. The microvials were pinned through their rubber stoppers beneath the host species from which the mites had originally been collected. Total numbers of mites were counted and infestation on the head, thorax, leg and wings pads of host species was observed with the help of microscope.

Rearing of host species

To study the fecundity and survival of host species, fifty specimens from each species collected from various fields were reared in crowded conditions in cages (Length1 6.5 Width 13.5cms) as well as in separate glass jars (ordinary jam bottles) under laboratory (25° to 23' N, 68° to 24' E) condition where the temp erature fluctuated between 28±2 and 39±2℃ with relative hum idity of 26 to 61%. These temperature and relative humidity regimes are similar to field conditions. Each cage was provided with cup containing sieved garden sand for oviposition. Fresh drops of water were added daily to keep the sand moist. Green shoots of fresh maize leaves were clipped and placed in 50 ml conical flask filled with water. Experimental cages and jars were thoroughly cleaned and placed in the sunlight for 2 to 3 h after 10 to 12 days. The paper sheet placed on the bottom of the cage was changed daily.

Collection of egg-pods and eggs

All eggs' cups were checked daily in the morning. Egg-pods were collected and opened carefully by following the method of Pradhan and Peshwani (1961). Egg-pod and the number of eggs in each egg-pod were recorded.

Identification

The scheme of identification for mites species followed here is that of Robaux (1971) and Southcott (1993).

Statistical analysis

Data obtained from experimental groups were subjected to one-way analysis of variance (ANOVA) (SPSS 10.0 Soft-ware) with repeated measures, and significant means were determined using Duncan's New Multiple Range test (DNMRT), and Latter Significantly Different Range Test (LSD). These tests were used to compare the means of the various treatments.

RESULTS

During the present study, significant infestation was also reported on some parts of femur. Actually, the thickness of the skin is probably the key factor because the mite feeds by thrusting its smaller hook, commonly called chelicerae (fangs), into the surface layers of the skin; where the skin is unusually thick, they find it difficult to thrust their hook, and may be brushed off by the

Table 1. Collection of *Hieroglyphus* species from the different districts of the Sindh during the year 2011.

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 2. Infection caused by microbial agents (*Euthrombidium* spp.) on the different developmental stages of *Hieroglyphus* species.

Species	Developmental stages					
	Hoppers (n=1632)			Adults (n=551)		
	Un-effective	Effective	Infection (%)	Un-effective	Effective	Infection (%)
H. perpolita	237	201	12.31	104	49	8.89
H. oryzivorous	365	298	18.25	138	86	15.60
H. nigrorepletus	321	210	12.86	138	36	6.53
Total	923	709	43.42	380	171	31.02

movements of the host. Infestation occurs during the months of July to September when humidity percentage was reported to be significantly high in the field.

Table 1 shows the collection of host species of *Hieroglyphus* species in various districts of Sindh. It stated that *Hieroglyphus perpolita* was significantly reported to be the highest from Hyderabad, Dadu and Khairpur, while *Hieroglyphus nigrorepletus* was recorded in greater number in Badin, Mirpurkhas and Dadu as opposed to *Hieroglyphus oryzivorous* which was significantly reported to be the highest from Dadu, Larkana and Jacoabad districts while in the other districts, its ratio was significantly low.

Table 2 suggested that hopper infestation was reported at 18.25% on *H. oryzivorus* followed by that reported on *H. nigrorepletus* and *H. perpolita* as 12.86 and 12.31% respectively. Similarly, the infestation of adults was significantly reported to be the highest (15.60%) on *H. oryzivorus* followed by that (8.89%) reported on *H. perpolita*, while that found on *H. nigrorepletus* was significantly reported to be the lowest (6.53%). Overall, parasitism percentage was reported as 43.42% for hopper stages and 31.02% for adult individuals. This finding indicates that hoppers are more vulnerable to infestation as compared to adults. During the present study, it has been observed that ectoparasitic larval and adult mites attach to grasshoppers by piercing either the integument or a wing vein, often during moulting. The mites feed on hemolymph and remain attached for 3 to 14 (mean 7.33±3.60) days on the host body. Infection of parasites can lead to the death of the grasshopper, sterility or a reduced amount of energy within the insect causing illness and skin irritation. *E. trigonum* survive on the exterior of the grasshopper and can cause stress and discomfort for host species.

At present, it was observed that proportional survival was found to be lower in mites-parasitized specimens. Females of these three species had reduced initial and total reproduction when compared to un-parasitic species. Egg production significantly reduced to 47.23±510.23, 98.67±11.43 and 51.56±12.40 in *H. perpolita H. oryzivorous* and *H. nigrorepletus* respectively with mite's parasitism during its entire life cycle, while the fecundity ration of un-parasitic host species was

Table 3. Comparison of the fecundity of *Hieroglyphus* species infested by *Eutrombidium trigonum* during year 2011.

Species	Parasitism			Non-Parasitism		
	Pods per female	Eggs per pod	Total eggs during life	Pods per female	Eggs per pod	Total eggs during life
H.perpolita	1.23±0.31	19.32±2.10	47.23±510.23	2.48±0.38 [a]	23.21±5.36 [b]	57.02±17.93 [a]
H.oryzivorus	1.88±0.41	28.66±5.34	98.67±11.43	2.94±0.51 [b]	37.82±7.32 [c]	114.16±39.05 [c]
H.nigrorepletus	2.1±0.23	17.23±3.21	51.56±12.40	3.10±0.43 [b]	20.96±4.51 [a]	60.16±18.22 [b]
$F_{.(0.05)}$	$_{(1.73)}$ 4.36 ^{ns}	$_{(21.73)}$ 39.27*	$_{(65.82)}$ 16.06 ^{ns}	$_{(2.83)}$ 06.11*	$_{(27.33)}$ 48.00*	$_{(77.11)}$ 35.26 ^{ns}

Mean in the same column followed by the same letter are not significantly different from one another at 50% level of probability (DNMRT); * = P≤ 0.05; ns = not significant at P≥ 0.05.

Table 4. Comparison of the survival time of adult *Hieroglyphus* species infested by *Eutrombidium trigonum* during year 2011.

Species	Parasitism		Non-Parasitism	
	(Mean ±Sd) ♂	(Mean ±Sd) ♀	(Mean ±Sd) ♂	(Mean ±Sd) ♀
H. perpolita	20.32±3.43	16.30±2.61	25.81±3.79	19.92±2.13
H. oryzivorus	42.39±8.46	35.73±10.26	50.83±8.40	41.82±10.89
H. nigrorepletus	37.25±7.93	25.63±8.40	44.62±7.60	35.10±7.85

significantly high (Table 3). As far as survival of individuals is concerned, it is also significantly affective by mites' parasitism. At present, survival of *Hieroglyphus* species was reported to be 20.32±3.43, 42.39±8.46 and 37.25±7.93 days for males, and 16.30±2.61, 35.73±10.26 and 25.63±8.40 days for females of *H. perpolita*, *H. oryzivorous*, and *H. nigrorepletus* respectively contrasting to that which was significantly high in non-parasitic mites (Table 4).

During the present study, it was observed that *Eutrombidium* in its larvae 6-legged condition attached itself to the wing bases of grasshopper species, and often appears attached in great numbers to adults while the nymphs and adults of this mite are free living and feed voraciously on the eggs of grasshoppers. They are thought to be of considerable aid in biological control of agriculture pests. It was also observed that the larvae displayed a preference for the weaker scherotized parts of the host's bodies and more particularly, the pliable connection between cuticular plates. Infestation of red mites was reported significantly as the highest on the hoppers stages of *Hieoglyphus* species.

DISCUSSION

Mites often have large effects on the life-history pattern of their host species. There is a need to examine life-patterns of parasitized organisms under field condition and laboratory condition. During the present study, the laboratory experiment carried out to examine the effect of an ectoparasitic grasshopper mite showed that it could significantly affect the survival and reproduction rates of *Hieroglyphus* species. Red mite's *E. trigonum* significantly infest the host species (beings), generally by way of the head, pronotum and particularly the thorax under the wing-and elytron-pads of older hoppers and adults.

Earlier, Peswani (1961), and Rizvi and Aziz (1966) reported that *Eutrombidium* spp. causes infestation on the thorax and wigs -pads of *H. nigrorepletus,* but did not report any infestation of the legs and head region. Similarly, Mason (1973) and Roonwal (1978) recorded mild infestation of mites on the same species. Roonwal (1978) collected two mites both belonging to Trombidiidae with 5% and 2 to 11% on the hoppers and adults stages respectively. In addition, Peswani (1961) mentioned mite larvae on adults, while Mason (1973) regarded this as *Trombidium* spp. At present, we reported significantly high percentage of *E. trigonum* on the three species of *Hieroglyphus* (Table 5). Records on the preferred attachment sites reveal that *Eutrombidium* larvae prefer the weaker sclerotized parts of their hosts, particularly the wings or the soft cuticle of the body. Severin (1944) reported from North American 3 to 30 grasshopper eggs being sucked out by 9 single *Eutrombidum* species for the entire duration of its life. Further, Huggans and Blickenstaff (1966) recorded that *Eutrombidium* species feed exclusively on the eggs of Saltotoria.

Khl (1994) while studying the Australian *Eutrombidum* species reported 248 specimen belonging to various subfamilies of Morabinae, Pyrgomorphinae, Oxyinae, and Catantopinae (including Cyrtacanthacridini), and Acridinae were significantly affected by mites. The dominant sites of attachment are the insertion of leg and

Table 5. Infestation of *Eutrombidium trigonum* on *Hieroglyphus* population.

Host species	Infestation (%)		References
	Hoppers	Adults	
H. nigrorepletus	--	6-11	Peswani (1961)
	--	4-7	Rizvi and Aziz (1966)
	--	3-8	Mason (1973)
	5	2-11	Roonwal (1978)
H. perpolita	12.31	8.89	Present finding
H. oryzivorous	18.25	15.60	Present finding
H. nigrorepletus	12.86	6.53	Present finding

the prosternum between them, accounting for 80% of cases, with the first, more recurrent. He further added that only one mite attacked most of the host individuals, but more than 20 were occasionally recorded. Mostly, the mites are absent in cold conditions. During the present study, infestations of mites was recorded high in months of July to September, and this might be due to the high humidity percentage in the field. During this study, distributions of *Hieroglyphus* species vary in the different districts of Sindh which might be due to the geographical conditions of the region.

Eutrombidium larvae mostly refer to Acrididae as hosts (Anderson and May, 1982; Welbourn, 1983; Southcott, 1993). This study is in agreement with this account. The results of this study showed that host range of *Eutrombidium* is obviously restricted to Acrididae. Earlier, Huggans and Blickenstaff (1996) reported mild infestation on Gryllidae among thousands of parasitized Acrididae.

At present, it was observed that proportional survival was found to be lower in mites-parasitized specimens. Females of these three species had reduced initial and total reproduction as compared to un-parasitic species. Egg production significantly reduced to 47.23±510.23, 98.67±11.43 and 51.56±12.40 with mite's parasitism species while Riffat and Wagan (2007) collected significantly high numbers of eggs as 57.02±17.93, 114.16±39.05 and 60.16±18.22 for *H. perpolita*, *H. oryzivorous* and *H. nigrorepletus* respectively. As far as survival time of individuals is concerned, mites parasitism is also significantly effective for the present survival of *Hieroglyphus* species and was reported as 20.32±3.43, 42.39±8.46 and 37.25±7.93 days for male and 16.30±2.61, 35.73±10.26 and 25.63±8.40 days for females of *H. perpolita*, *H. oryzivorous* and *H. nigrorepletus* respectively, while for non-parasitic species, Riffat (2008) recorded 25.81±3.79, 50.83±8.40 and 44.62±7.60 days for male and 19.92±2.13, 41.82±10.89 and 35.10±7.85 days for female of *H. perpolita H. oryzivorous* and *H. nigrorepletus* respectively. These findings showed that mite's parasitism has significant effect on the reduction of the fecundity and longevity of host species.

Conclusion

In view of the aforementioned facts, it could be suggested that ectoparasitism by larval and adult mites in controlling grasshopper numbers depend upon the abundance of mites because the effect of ectoparasitism will be of little importance if there are not enough mites relative to grasshopper numbers. So it is recommended that large numbers of mites are essential to control the grasshopper's population.

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