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A new Dipteran parasitoid, *Phasia varicolor* (Diptera: Tachinidae) found in the field attacking the red cotton bug, *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae)

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Abstract

Dysdercus koenigii is a serious pest of cotton and other plants of economic importance. It was collected from the field and was observed that they were infested with a new tachinid parasitoid. This parasitoid was preserved and identified as *Phasia varicolor*. *Dysdercus koenigii* were observed for change in their fecundity and fertility due to parasitoid infestation. Their unhealthy nymphs were subjected to survival analysis and antioxidant (ascorbic acid) treatment. Based on the parameters studied, it was concluded that *Phasia varicolor*, a tachinid parasitoid might prove to be an effective biocontrol agent vice insecticides for the *Dysdercus koenigii*.

Keywords: Biological control, *Dysdercus koenigii*, fecundity, tachinid parasitoid

1. Introduction

Dysdercus koenigii is one of the serious pests of cotton plant. This bug also causes damage to other economically important plants. Nymph and adult feeds on seeds in the developing cotton bolls leaving a stain on the lint of cotton [15]. That is why it is known as the red stainer also. Over the years, *D. koenigii* has gained polyphagous status, thriving on okra, hollyhock [10] and plants of Bombacaceae family [11]. The bug has a high fecundity power in the field and efficient egg development [20]. This pest is being controlled largely by using indiscriminate chemical insecticides leading to chemical hazards in the environment [1]. In order to get rid of these hazards, scientists are looking toward the application of biological control methods of pests, which include predators, parasites and parasitoid. Needless to mention, the biological control using dipteran parasitoid is an effective promising means to reduce insect pest population.

Order Diptera consists of more than 100 families and at least 20 of them act as parasitoids. Out of these, the tachinids are the most important, widespread and abundant parasitoids in all the terrestrial ecosystems. Over 90% of the tachinid species attack the phytophagous insects and about 60% of them are parasitoids of ditrysian Lepidoptera [16, 17, 3], which comprise the most diverse group of plant-feeding organisms.

Most tachinids are endoparasites. Eggs are laid down inside the host body wherein developing larvae feed. This fly typically infests only the adult insects. Adult female tachinid flies retain a variety of methods to shelter their young ones; some lay eggs on leaves, others insert eggs or maggots directly into the host and still others attach eggs or maggots to the outside of the host. Parasitoid's eggs may also be consumed by the host or inserted by the female parasitoid, hatch into maggots inside the victim's body. In some cases, such eggs are affixed to the skin of host, which hatch and then maggots bore into the haemocoel of the host, where in they complete their development devouring the host tissues.

Our lab has been working on *Dysdercus koenigii* for the last 40 yrs. Of course, these bugs are initially collected from the field and then cultured in the lab [20]. Very recently the field collected bugs revealed that they are being infested with a parasitoid. Hence we were curious to work further and found that a Dipteran parasitoid namely *Phasia varicolor* has emerged out as a new biological control agent, which could replace indiscriminate use of the hazardous chemicals for the control of *Dysdercus koenigii*.

The present work reports the incidence of tachinid flies, *Phasia varicolor* parasitizing *Dysdercus koenigii* during the winter season in India.

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2. Materials and Methods

2.1 Collection and rearing: Adults and nymphs of *Dysdercus koenigii* were collected randomly once in a week from early November to March in 2014-2015 from the agricultural fields in BHU and its abounding regions. All the bugs were sorted and reared in the glass jars under laboratory conditions. They were provided with water pre-soaked cotton seeds, which were replaced with new ones every day [13]. The bugs were inspected daily for the ejection of parasitoid larvae. Tachinid larvae emerging from the bugs were put in plastic cups with a sheet of moistened filter paper and kept in BOD incubator (NSW, New Delhi) at 25 ± 2 °C in a regime of 16L-8D photoperiod and relative humidity of 70-80% until adult flies emerged. After the adult emergence, the flies were preserved and processed for identification. Images of whole insects were taken using a stereomicroscope (Leica, EZ4 D) having a digital camera (Leica EC3, resolution: 3.1 mega pixel).

2.2 Identification of parasitoid and study of life cycle: Dipteran parasitoid was collected and identified according to Sun and Marshall [18]. Its life cycle was studied in the laboratory.

2.3 Fecundity and fertility: Infested mated females were kept in a separate jar. 50 females of both groups comprising infested and healthy ones were observed for egg laying. For fertility test, eggs of infested and healthy adults were reared in BOD incubator until adult bugs emerged from the said eggs.

2.4 Survival analysis: Survival study was done on the two groups of bug. Nymphs of both groups were observed daily for mortality and life table of insect from healthy and infested eggs was prepared. Data were analysed by Kaplan Meier survival analysis using Graph pad 6 software.

2.5 Antioxidant Treatment: Ascorbic acid is commonly used as antioxidant. Its deficiency is associated with the abnormalities during ecdysis process. To see the effect of antioxidant on the development, nymphs from the eggs of infested female were treated with three different concentration of ascorbic acid i.e., 10mM, 20mM and 30mM, diluted in water and studied till death.

2.6 Statistical analysis: Statistical analyses were performed using Graph pad 6. Student's *t* tests (significance at $p < 0.0001$) were used to compare the difference in fecundity and fertility between control and experimental insect. Kaplan-Meier survival analysis was used to calculate median survival time (in days). Comparisons of survival curves were carried out by using log-rank test. After survival analysis, hazard ratio was calculated by using Mantel Haneszel test.

3. Results

To check the effect of parasitism due to parasitoid on *Dysdercus koenigii*, two experiments of survival analyses were performed over a period of 26 days. In one of the analyses, two groups of insects were taken; group 1 denoting the nymphs emerged from eggs of the uninfested *D. koenigii* and group 2 denoting the nymphs emerged from the adult bug eggs which were infested by the parasitoid. Results of the analyses are shown in Fig 1(A) and median survival time for group 1 and 2 are 14.5 and 9 days respectively. A significant reduction in survival time of Group 2 insect was observed ($p < 0.0001$) as compared to that of group 1 insects with chi-

square value, 20.30 at degree of freedom 1 (log rank, Mantel cox test). Hazard ratio for group 1/2 was 0.1736 0.1736.

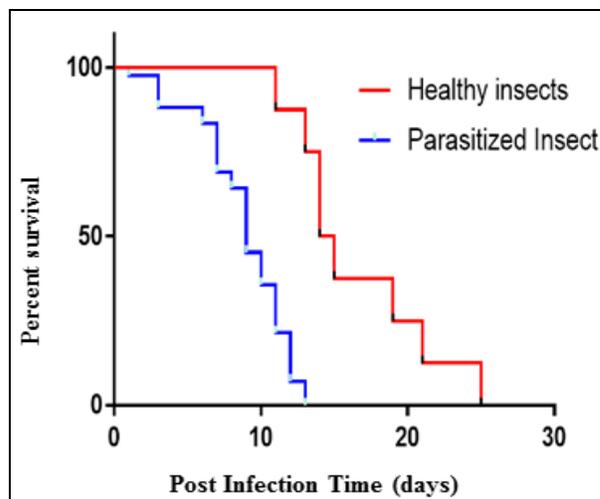


Fig 1A: Kaplan-Meier Survival probability plot of healthy and parasitized group of host *Dysdercus koenigii*.

In the second survival analysis experiment, treatment of ascorbic acid was given to the nymphs emerged from the eggs of infested *D. koenigii* in order to check its effect on survival and moulting. Survivability of the nymphs treated with 20 mM concentration of ascorbic acid was significantly increased as compared to 10mM and 30mM ascorbic acid treated nymphs and the control (untreated infested nymphs) (Fig. 1B). Log rank (Mantel-cox) test gives significant difference with the chi-square value, 59.69 at degree of freedom 3. Median survival time of nymphs of control, 10mM, 20mM and 30mM ascorbic acid treated group is 9,7,18.5 and 10.5 days respectively.

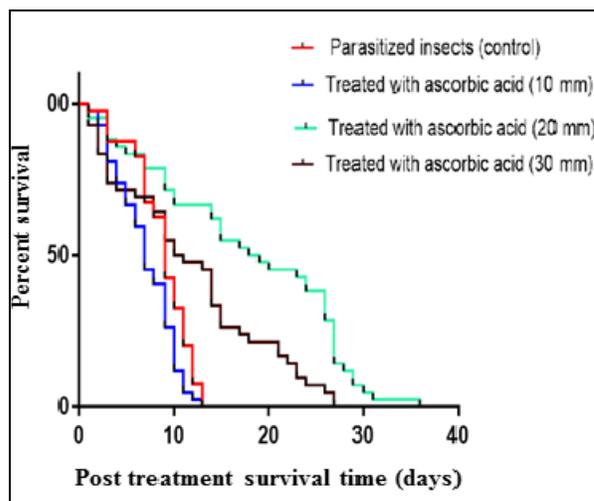


Fig 1B: Kaplan-Meier survival probability plot of the parasitized insects after the treatment of different concentrations of antioxidant, ascorbic acid.

Average number of eggs in case of healthy females was 70.4 ± 0.588 per female. However, this number decreased to 36.56 ± 0.421 eggs per female in case of the infested female (Fig. 2). The fertility rate of healthy and infested adult bugs were 88.84 ± 0.5277 and 7.90 ± 0.099 respectively. Maximum fertility rate was 96.7% and 9.6% in the healthy and infested nymph respectively as per analysis of box plot (Fig 3).

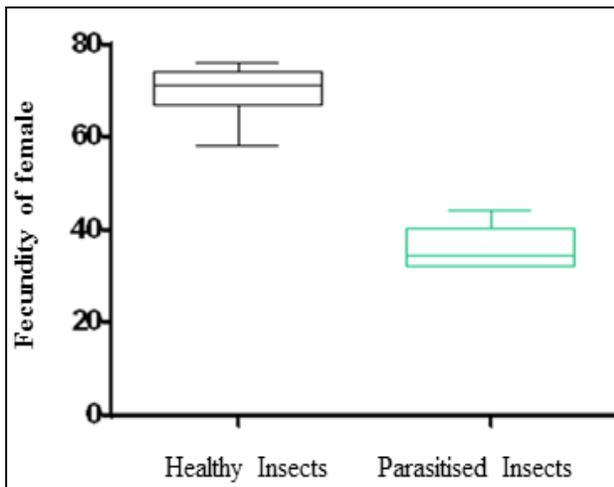


Fig 2: Fecundity rate of female *Dysdercus koenigii*.

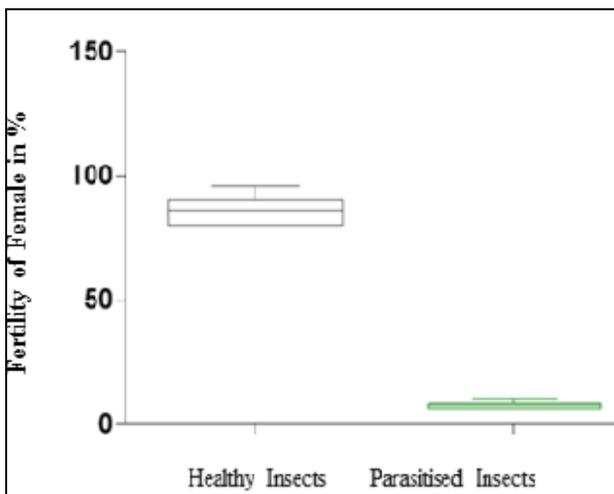


Fig 3: Fertility rate of host, *Dysdercus koenigii*.

4. Discussion

The Tachinidae consists of large diverse family of bristly flies with a postscutellum. *Phasia* species of Tachinidae family belongs to subfamily Phasiinae. The parasitism by this tachinid species was observed from November to the end of March for the three consecutive years. The population of the parasitoid varies widely in a year. It was observed that the tachinid fly infested only the female *Dysdercus koenigii*. The reason for this could be that the female bug provides more food and nutrition to the parasitoid's larvae as compared to male bugs. Adult parasitoids are free-living. Female tachinid flies insert eggs or maggots directly into the haemocoel of *D. koenigii*. Some other female tachinids attach their eggs to the surface of the host. Eggs inserted by the tachinid mother hatch into maggots inside the haemocoel of host. The maggots complete their development therein, consuming the host tissues as they grow. These larvae develop by feeding first on haemolymph and non-vital tissues and organs and then devour vital parts, with few exceptions killing their hosts before completing their larval development [7, 21, 12, 19, 5]. In the present case, the larvae appeared to affect oocyte development of the host bug probably due to loss of haemolymph, which is the storehouse of biomolecules which are sequestered into developing oocytes.

It has been observed that the life cycle of *Phasia varicolor* completes in two phases. Adults complete its life cycle outside the host and larvae develop inside the female *D.*

koenigii. Female parasitoid inserts many eggs into a host, but surprisingly, only single larva survives. Larva develops within their host in ~2 week time, then exit to pupate outside the host body (fig 4). Adult parasitoid emerges in 2 week time (males emerge before females) and mates within 48 hrs. Adults live further for 10-31 days.

Parasitism of female *D. koenigii* renders abnormality of its reproductive system, causing degeneration of the developing oocytes. However, other metabolic changes of the host bug are not otherwise much affected due to parasitism. With the result, some host often survives for a short duration even after the larvae of the Tachinid have come out of the host body.

It appears that the selection of a host by the parasitoid is crucial i.e. size of the host is important. The female bug, being bigger in size than the males, is parasitized by the parasitoid. It is but natural that the female bug would have more food resources for the parasitoid development [6, 8]. Because of the relationship between the size of a parasitoid and that of the host wherein it developed, female bug is more vulnerable to the parasite infection. Further the body size plays an important role in determining the fitness of female in comparison to male in terms of different parameters like longevity and fecundity. Still further, females are selected as the host to determine the sex of the offspring of the parasitoid that will benefit the most from the host size [14, 6, 2]. It is well established that the reproductive capacity of individuals can be used to estimate the rate of their increase at the population level. Ordinarily the two parameters used to measure the reproductive capacity of insects are fecundity and fertility.

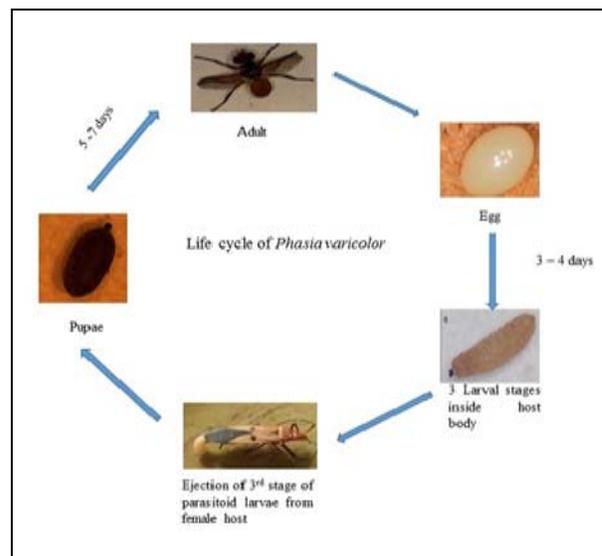


Fig 4: Life cycle of *Phasia Varicolor*

Fecundity refers to the animal's reproductive output (i.e. number of eggs laid) whereas the fertility refers only to the number of viable progeny coming out from eggs [9]. Our data clearly demonstrate that the fecundity of the infested bug significantly decreased ($p < 0.0001$) in comparison to the healthy bug (Fig 2). This is due to parasitism of female *D. koenigii* (host), which results in degeneration of the developing oocytes in the female bugs. Rate of conversion of nymph into adult in healthy insects was significantly high (96%) in comparison to the infested host insects (Fig 3). Thus, fertility appears to be the true parameter because it gives an exact estimation of the number of individuals entering in the next generation [9]. However, we observe that the fertility of the host is being down regulated by the parasitoid.

Initially survival analysis was done on the two groups of healthy nymph and infested nymph. Median survival was significantly high in the healthy nymphs as they moult into adults, while almost nymphs from the infested host died before transforming into adult.

It is believed that ascorbic acid plays an important role in the normal development and moulting of bug [4]. Therefore, infested nymphs were treated with ascorbic acid in their diet along with water. It is observed that treatment with ascorbic acid resulted in the extension of the developmental period. But this extension did not induce moulting of infested nymphs into imago. Thus based on our findings, it could be said that *Phasia varicolor* could be used as a new biological control agent (parasitoid) vice insecticides in controlling the *Dysdercus koenigii* population, as it hampers the moulting of infested nymph into adult bug. However, further investigations in this regard are much to be designed required.

5. Conflict of interest

The authors declare that they have no conflict of interest.

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