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Research Article

Oviposition Deterrence of Fruit fly in Treated Mangoes with Ant cues and Fungus *B. bassiana.*

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Abstract

Bacterocera zonata **is a very serious pest of fruit crops especially mangoes over the globe. The present study was conducted to observe oviposition deterrence in *B. zonata* under lab conditions. Mangoes (Desi, Chaunsa and Anwar Ratool) were treated with ant cues and entomopathogenic *Beauveria bassiana*. Oviposition deterrence was recorded when nonspecific and variety specific (Chaunsa, Anwar Ratol) mangoes were treated with ant cues and *B. bassiana* at different intervals (exposure to 4 days). It showed that the mean number of oviposition punctures tend to increase with time. Minimum fruits puncture was found at day 1 in *B. bassiana* treated mangoes, while at 4th day, maximum number of punctures were observed in untreated mangoes. *B. bassiana* treated mangoes showed minimum number of punctures than ant cues and untreated mangoes were observed with highest number of punctures.**

Keywords: ***B. zonata*, Ants cues, *Beauveria bassiana*, Oviposition deterrence**

Introduction

Fruit flies (Diptera: Tephritidae) use both visual and olfactory signals that function at various spatial scales to identify suitable host plants and fruits (Clarke 2016; Jacob *et al.*, 2017; Masry *et al.*, 2019). Behaviour of fruit flies especially dealing with oviposition and sexual behaviour, is of greater importance (Díaz-Fleischer & Aluja, 2000; Peña *et al.*, 2002; Vayssières *et al.*, 2009; Minekawa et al., 2018; Jose *et al.*, 2019). Information flows within food webs influence the behaviour, ecology and population dynamics of animals (Vos *et al.*, 2006; Goldenberg *et al.*, 2018; McInturf *et al.*, 2019). The extrinsic information that plays a crucial role in the survival of animals includes cues on the availability of food and mates, oviposition sites, abiotic factors as well as the presence of competitors and natural enemies (Dicke & Grostal, 2001; Brodeur *et al.*, 2017; Billeter and Wolfner 2018; Røstelien 2019). Fruit fly population dynamics and fitness are influenced greatly by sexual, feeding and oviposition behaviours, which mainly take place in tree canopies, it is to be expected that arboreal ant species will directly and indirectly influence these behaviours (Leskey and Nielsen 2018; Bekker *et al.*, 2018).

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Studies on substances that deter oviposition, albeit of great potential economic significance, are confined to botanical repellents, petroleum spray oils and host-marking pheromones on previously occupied hosts (Prokopy & Roitberg, 2001; Cook *et al.*, 2007; Ekesi *et al.*, 2016; Himanen *et al.*, 2017; Figueroa Candia 2018). Pupation of economically important fruit flies takes place in the soil, after final-instar larvae have left the infested fruit. Ant predation can be considerable but inadequate to regulate fruit fly populations (Aluja *et al*,. 2005; Moore and Duncan 2017; Muhmed 2018; Greenberg 2019) reported on the effect of predation by ants on fruit flies but, likewise, they limit their observations to the ground level (De la Mora *et al.*, 2015; Ennis and Philpott 2019).

Many prey species have evolved chemical senses such as olfaction and taste for detecting their predators. Cues of predator presence may be emitted by the predators either directly [e.g., chemicals (kairomones) (Pereira *et al.*, 2016; Weiss 2018), or indirectly [e.g., chemicals from disturbed/injured (alarm pheromones) or dead conspecific arthropods (Kersch-Becker *et al.*, 2017; Hiltpold and Shriver 2018). Fruit flies have a strong adverse economic impact on commercial horticulture in tropical regions (Clarke, 2005; Vayssières *et al.*, 2016; Mathew *et al.*, 2018; Sultana *et al.*, 2019).

Studies on the detection and potential of chemical cues which can deter the oviposition in important insect pest is the need of time. Understanding the role of such vibrabt chemical could greatly help to fight fruitfly beacuase of which, the imports of Pakistan are at the stake of ban by international agenices. The present studies represent that fungus and ant cues negatively affect the ovipositional behavior *B. zonata.* There was comparatively less ovipostion in fungus treated mangoes as compared to ant cues treated mangoes.

Material and Methods

Collection and Rearing of *B.* zonata

Adults of *B. zonata* (male ♂ and female ♀) were collected by installing pheromone traps (Methyl eugenol) in the Mango orchards in District, Multan. The various life stages of *B. zonata* larvae were collected from damaged and rotten fruits found under the trees. The fruits were bought to the insect rearing lab of MNS- University of Agriculture, Multan and culture of *B. zonata* was established in plastic cages (30×60×60 cm). Infested fruits were provided to adults for feeding, and egg-laying. The larvae emerged after about three days after hatching of eggs and fed on an artificial diet prescribed by Vayssières *et al*. (2015). The larvae were maintained for 12 weeks on artificial diet till pupation. The rearing conditions were maintained at 25±2°C and 60-75% R.H.

Table 3.2.1 Artificial diet provided to larvae of *B.zonata* is as follows:

Sr.No.	Ingredients	Quantity
1	Banana	2 fingers
2	Honey	2 tea spoons
3	Egg	1
4	Vitamin B	1 tea spoon

Isolation and identification of Fungi

Gallaria bait method was used to isolate the fungus from soil samples (Zimmermann 1986). Distilled water was used to moistened the samples before autoclaving. Glass tubes were filled with soil leaving 1 cm space empty foe aeration purpose. The larvae of Wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) were collected from old bee hives and shifted to lab conditions of 26 °C for mass culture. When larvae reached to 3rd instar, silk webbing in soil was avoided by exposing to 56 °C water for 15s (Woodring and Kaya, 1988). The larvae were added to each vial for 12 days at 25 °C. During incubation the vials were shaked after every 12 hours. After 12 days of incubation the dead larvae were removed and then washed with ethanol and double distilled water. These larvae were sterilized and then shifted to PDA (potato dextrose agar) media plates and sealed with parafilm. Until the emergence of fungus, the media plates were incubated at room temperature (25°C). When the fungal growth appeared, it was identified under microscope with the help of taxonomic keys (Barnett & Hunter 1999; Domsch *et al*.

2007). Identified fungus was further purified and cultured again to use it as bio-control agent.

Effect on ovipositor of fruit fly by ant cues

Ant colony was located at research farm of MNS- University of Agriculture, Multan and ants were collected by digging the soil with hand shovel. Collected ants were brought to Ecology lab of MNS- University of Agriculture, Multan to observe their effect on fruit flies oviposition.

A medium sized (12×24″) plastic tub was used for trial. The top layer of 5 cm of tub was covered with the Vaseline to prevent the ants coming out of tub. Tub was filled with soil containing ants colony along with 15 un-riped (greenish) mangoes of size (2.5 pounds). Then tub was tightly covered with polythene sheet to minimize all chances of ants escape. The tub was placed for 24 hours in the ecology lab of MNS-University of Agriculture, Multan at 25 °C and 60-75% R.H.

After 24 hours, the mangoes were removed from tub and used to observe the oviposition of fruit flies. Six plastic jars (6×4″) were prepared, whose 2 sides were replaced with nylon mesh for the aeration. Five treated mangoes were placed in each jar and five females of B. zonata females. Similar batch of untreated mangoes were placed with *B. zonata* females. The experiment was laid in three replicate at conditions of 25 °C and 60-75% R.H. After 24, 48, 72 and 96 hours, the number of punctures on mangoes were counted and analyzed under microscope.

Effect of fungus on oviposition of fruit fly

The powder of isolated fungus (*Beauveria bassiana*) was taken in the Ecology lab of MNS- University of Agriculture, Multan. The powder was then weighed on the weighing balance. The

1g fungus powder and 1500 ml of distilled water was taken from distillation unit of central lab of MNS-University of Agriculture, Multan.

The distilled water was then poured in a beaker of 2000 ml and weighed 1g fungus (*Beauveria bassiana*) was then added to 1500 ml of distilled water in a beaker this then stirred with a stirrer to make it a homogenized mixture. A medium sized (12×24″) plastic tub was used for trial. Tub was filled with fungus solution along with 15 un-riped (Greenish) mangoes of size (2.5 pounds). Tub was placed for three hours in the ecology lab of MNS-University of Agriculture, Multa at 25 °C and 60-75 % R.H.

After three hours the mangoes were removed from tub and placed in the lab for drying purpose. To observe the oviposition of fruit flies six plastic jars (6×4″) were prepared, whose 2 sides were replaced with nylon mesh for aeration. Five treated mangoes were placed in three jars and five *B. zonata* females were placed in each jar tightly covered. Similar batch of untreated mangoes were placed with *B. zonata* females. The experiment laid in three replicates at conditions of 25 °C and 60-75 % R.H. After 24, 48, 72 and 96 hours, the number of punctures on mangoes were counted and analyzed under microscope.

Statistical analysis

The mortality rate of fruit fly in the bioassay of pathogenicity was recorded from the number of dead insects. The percent mortality was corrected by using Abbott's (1925) formula.

Means were spread at 5% level of significance using Tukey's Kramer test. Means significance was tested using analysis of variance technique in Minitab v13.2 (Minitab, 2002)

Results

Oviposition detterance in *B. zonata* with ant cues and *B. bassiana*

Oviposition deterrence in *B. zonata* was recorded when mangoes of Chaunsa variety were treated with ant cues and *B. bassiana* at different intervals. Significant differences were observed in oviposition puncture on mangoes ($F_{2,8}$=18.3; P=0.0028). Mean No. of oviposition puntures tend to increase with the days after exposure increased as minimum were recorded at day-1 in *B. bassiana* treated mangoes (12%) after 4 days of exposure, maximum oviposition punctures were recorded in untreated mangoes.

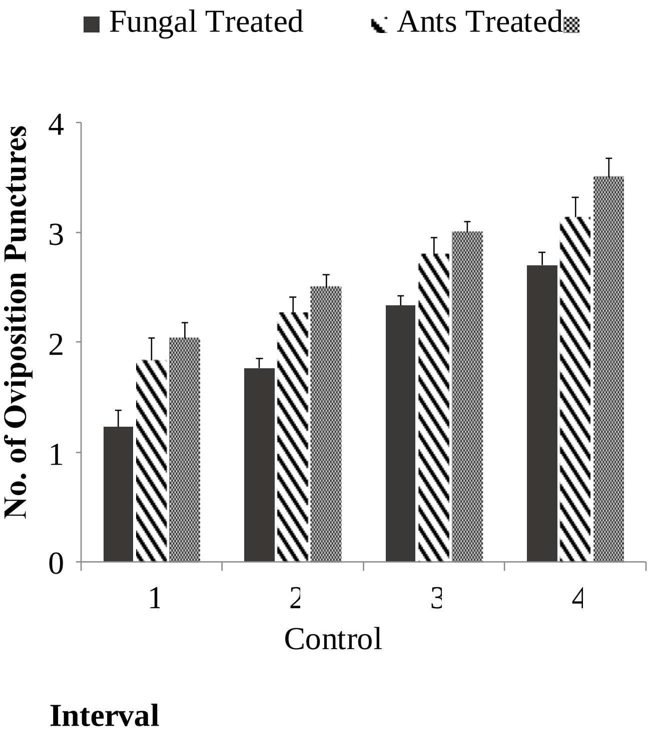

Fig. Oviposition puncture (Mean±S.E) of *B. zonata* on mango variety Chaunsa when treated with ant cues and *B. bassiana* on different days after exposure under lab conditions.

Comparison of oviposition between fungal treated, ants treated and untreated mangoes

Oviposition deterrence in *B. zonata* was recorded when mangoes of Chaunsa variety were treated with ant cues and *B. bassiana* at different intervals. Significant differences were observed in oviposition puncture on mangoes ($F_{2,35}$=29.96; P=0.0000). Minimum oviposition punctures were observed in fungal treated followed by ants treated and then maximum punctures were recorded in untreated mangoes.

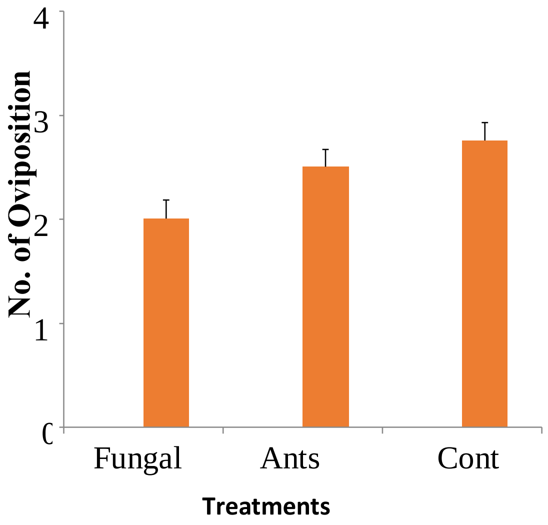

Fig.3.3.2 Comparison of oviposition puncture (Mean±S.E) of *B. zonata* on mango variety Chaunsa when treated with ant cues and *B. bassiana* under lab conditions.

Anwar Ratol mangoes were treated with ants and fungus to check the oviposition detterance

Oviposition deterrence in *B. zonata* was recorded when mangoes of Anwar Ratol variety were treated with ant cues and *B. bassiana* at different intervals. Significant differences were observed in oviposition puncture on mangoes ($F_{2,8}$=70.6; P=0.0001) Mean No. of oviposition puntures tend to increase with the days after exposure increased as minimum were recorded at day-1 in *B. bassiana* treated mangoes (10%) after 4 days of exposure, maximum oviposition punctures were recorded in untreated mangoes.

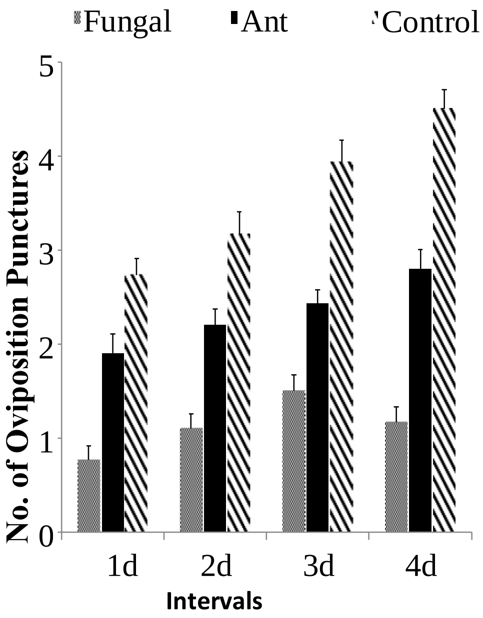

Fig. Oviposition puncture (Mean±S.E) of *B. zonata* on mango variety Anwar Ratol when treated with ant cues and *B. bassiana* on different days after exposure under lab conditions.

Cummulative comparsison of oviposition between fungal treated, ants treated and untreated mangoes

Oviposition deterrence in *B. zonata* was recorded when mangoes of Anwar Ratol variety were treated with ant cues and *B. bassiana* at different intervals. Significant differences were observed in oviposition puncture on mangoes ($F_{2,35}$=295.96; P=0.0000). Minimum oviposition punctures were observed in fungal treated followed by ants treated and then maximum punctures were recorded in untreated mangoes.

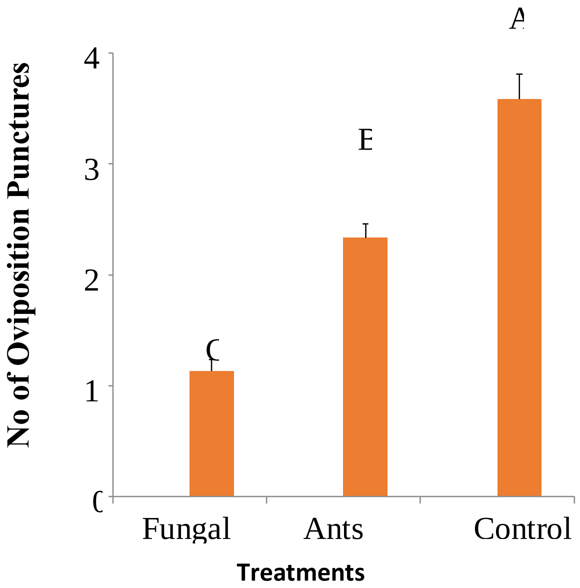

Fig. Cummulative comparison of oviposition puncture (Mean±S.E) of *B. zonata* on mango variety Anwar Ratol when treated with ant cues and *B. bassiana* under lab conditions.

Mangoes (Desi) were treated with ants and fungus to check the oviposition detterance

Oviposition deterrence in *B. zonata* was recorded when mangoes which were of desi variety treated with ant cues and *B. bassiana* at different intervals. Significant differences were observed in oviposition puncture on mangoes ($F_{2,8}$=17.4; P=0.0032). Mean No. of oviposition puntures tend to increase with the days after exposure increased as minimum were recorded at day-1 in *B. bassiana* treated mangoes (8%) after 4 days of exposure, maximum oviposition punctures were recorded in untreated mangoes.

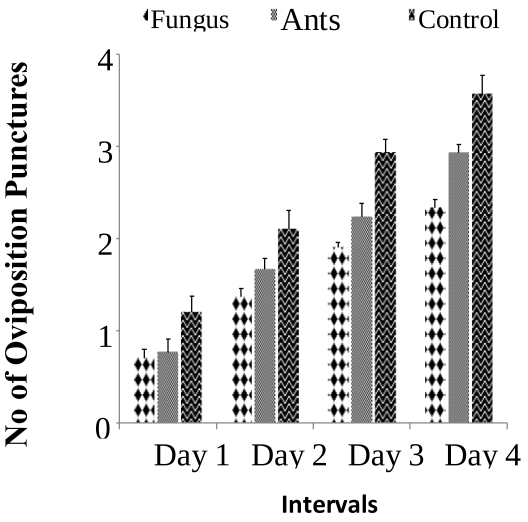

Fig. Oviposition puncture (Mean±S.E) of *B. zonata* on mango (Desi) when treated with ant cues and *B. bassiana* on different days after exposure under lab conditions.

Cummulative comparsison of oviposition between fungal treated, ants treated and untreated mangoes

Oviposition deterrence in B. zonata was recorded when Desi mangoes were treated with ant cues and *B. bassiana* at different intervals. Significant differences were observed in oviposition puncture on mangoes ($F_{2,35}$=43.83; P=0.0000). Minimum oviposition punctures were observed in fungal treated followed by ants treated and then maximum punctures were recorded in untreated mangoes.

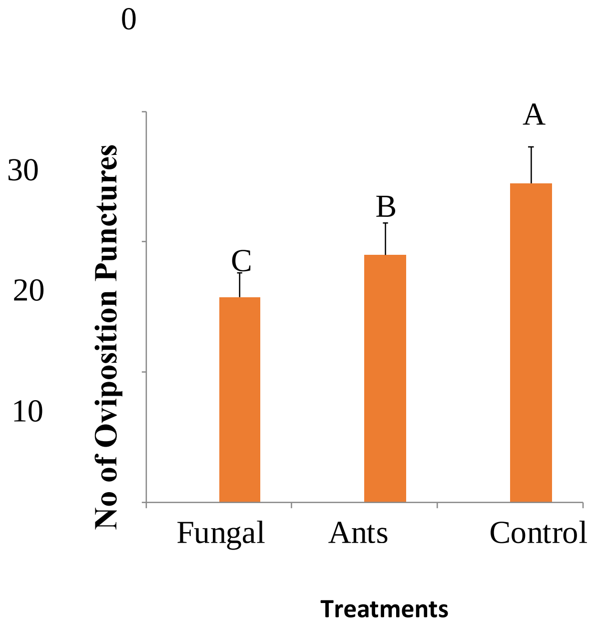

Fig. Cummulative comparison of oviposition puncture (Mean±S.E) of *B. zonata* on mango (Desi) when treated with ant cues and *B. bassiana* under lab conditions.

Discussion

The receptivity of fruit repellents and deterrents influences the fruit flies' perceptual process of ovipositional acceptance (Jang & Light, 1981; Scarpati *et al.*, 1993). Different varieties of mangoes were treated with enotomopathogenic fungus, *B. bassiana* and ants, which negatively affect the ovipositional behaviour of *B. zonata* fruit flies. The oviposition preference was decreased with the increase in concentration. Minimum oviposition was observed in fungal treated mangoes followed by ants treated mangoes. There was less oviposition in anwar ratool variety followed by Desi variety in all treatments as compared to chaunsa variety.

Significant differences were observed in oviposition puncture on mangoes. Fruit flies uses relatively unspecific visual and odour stimuli to identify oviposition sites (Daniel, 2010). The less oviposition in mangoes treated with entomopathogenic fungi could be due to the repellent effect of the EPF to fruit fly (Salvatore *et al.*, 2009). In another study, Salvatore *et al.*, (2009) reported that fruit fly prefers untreated mangoes for oviposition as compared to mangoes treated with some biopesticide like EPF.

There were less oviposition on mangoes on which higher concentration fungi were applied. The EPF firmly stick with the skin of the fruit and produce some volatile chemicals that results in the repellency of fruit fly for oviposition. The higher the concentrations, the higher will be the deterrent chemicals produced. The production of to such metabolites that are repellent to insects, is the indirect mode of action of fungi (Daisy *et al.*, 2002).

In the current study, there was also less ovipostion in mangoes treated with ant cues of *Monomorium* sp. Our results are similar to Mele *et al.* (2009) who observed quite less oviposition in mangoes treated with ant cues of arboreal ant *Oecophylla longinoda* (Latreille) by two fruit fly species, *Ceratitis cosyra* (Walker) and *Bactrocera invadens.* However, still the nature of these chemical cues is not known because ants deploy a range of pheromones from exocrine glands (Hölldobler & Wilson, 1990; Billen & Morgan, 1998).

However, the study by Offenberg *et al.*, (2004) is same as that of our results that the cues are chemical or visual. Ants mark their entire territory and trails with visible cues produced in the rectal sac (anal spots) and with invisible trail pheromones produced in the rectal gland (Dejean & Beugnon, 1991). These anal spots are not randomly distributed; they lead from the nest to a food source (Dejean & Beugnon, 1991). Although Offenberg *et al.*, (2004) suggest that herbivores are repelled by ant pheromones, it is still unclear whether the visible or invisible cues (or a combination) are the principal cause.

Conclusion

The present study represent that fungus and ant cues negatively affect the ovipositional behavior *B. zonata*. There was comparatively less ovipostion in fungus treated mangoes as compared to ant cues treated mangoes.

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