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Settling, Oviposition and Reproduction Response of Peach Fruit Fly, *Bactrocera zonata* (Saunders) to the Plant Extracts of Native Species of Kalat Division in Balochistan

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NS and ZH designed the idea. NS and ZS carried out the lab trials. MI supervised the study, analyzed the data. NS and MI wrote the manuscript. K and ZH provided help in preparation of the manuscript by critical analysis.

Key words
Fruit fly, Botanical extract, Settling, Insect repellent, Oviposition deterrent

ABSTRACT

Fruit flies are notorious insect pests causing considerable loss to agriculture produce. This effort was meant to evaluate the response of different life stages of peach fruit fly to plant extracts having insecticidal properties. Extract of three native plant species (*Azadirachta indica, Zataria multiflora*, *Achillea santolina*) and their various concentration (2, 1, and 0.5%) were used for the purpose. Firstly, the artificial diet of fruit flies was subjected to these treatments, while on the other hand chikoo fruits which were used for flies to settle on, were dipped in same concentrations of these plant extracts, and dried under shade and exposed to peach fruit flies for feeding for 15 days. All the three plants exhibited insect repelling potential but these had no significant difference from each other, while *A. indica* showing nonsignificant but most promising results. Similarly, there was no significant combine effect of plants extracts and its various concentrations. However, various doses of plant extracts showed significant difference in reducing the number of oviposition, pupae developed, flies settled and post settling reproduction. There was inverse response of peach fruit flies to increasing concentration of plant extracts. Minimum significant number of fruit flies (0.58) settled after 18 h, reaching to its peak (1.73) after 42 h showing a decreasing trend subsequently. Hence, all the plants exhibited insecticidal potential and resulted in reduction of fruit fly population in laboratory condition. Thus, these plant extracts could be considered as potent biological insecticides for peach fruit fly owing to field trails.

INTRODUCTION

The demand for fruits has increased in many folds especially in the form of canned or fresh fruits due to increasing population. However, disease and insect pest problems affect both quality and quantity of fruits. Among insect pest fruit flies of family Tephritidae (Diptera) are most harmful insect pests of fruits and vegetables. About 4000 species of fruit flies were reported out of which 700 species found throughout the world (Fletcher, 1987). Approximately, 250 species of *Bactrocera* fruit flies are widely found in most tropical and temperate areas throughout the world. Nearly, 11 fruit fly species including *B. cucurbita*, *B. zonata* and *B. dorsalis* are examined to cause losses of fruit and vegetable production in Pakistan.

Peach fruit fly is one of the most harmful species of Tephritidae. It is a serious pest of peach and custard apple in neighboring country (Butani, 1976; Grewal and Malhi, 1987), as well as guava and mango in Pakistan (Syed *et al*., 1970). It is a polyphagous species attacking some 40 species of fruit and vegetables (White and Elson-Harris, 1992).

The presence of male adults of *B. zonata* in the orchards of mango and guava was about 74.66% and

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46.62%, respectively in Pakistan (Khan *et al*., 2005). In semi-desert and coastal areas of Balochistan and Punjab the fruit fly *B. zonata* has been found as a serious threat for peach orchards, although these pests were rarely found in hilly areas of Pakistan (Marwat *et al*., 1992; Sarwar, 2006). There is urgent need to adopt eco-friendly technologies such as Integrated Pest Management to enhance productivity of fruits and vegetables.

Number of control methods used to reduce damage of the fruit flies to vegetation. For this purpose, many cultural, biological, chemical practices including insecticidal and pesticide sprays have been used. The pesticide and insecticide sprays are able to kill the larvae of fruit flies and other insects to some extent but it affects the quality and taste of the fruits. Additionally, chemical sprays also affect environment and surrounding vegetation. Due to lack of awareness, local farmers use many insecticide sprays without knowing the effect of those sprays, which further causes toxic effects to environment and fruit quality.

A wide range of natural botanical extracts against plant diseases (Matrose *et al*., 2020; Shamsullah *et al*., 2020) and insect pests (Schmutterer and Singh, 2002) have become popular to achieve their environment friendly management. These plant extracts have been used against peach fruit fly (Siddiqi *et al*., 2011; Ilyas *et al*., 2017) as well as other fruit flies (Riaz *et al*., 2018; Ugwu *et al*., 2021).

Among natural insect repellents, neem has a great commercial potential. It has an advantage over synthetic insecticides in a way that it has no or less toxic effect on man, animals and useful insects (Schumutterrer, 1985). As many as 540 insect species including all key agriculture insect pests have already been reported to be vulnerable and exhibit various behavioral and physiological effects of neem (Schmutterer and Singh, 2002). Neem plant extract affects the insect potency, long life and productivity (Arora and Dhaliwal, 1994). The secondary metabolites present in neem extract prevent the growth and productivity to insects (Prakash and Rao, 1997). Different experiments proved that the neem extracts are the best sprays to check the insects and pests. However, little is known about the efficacy of similar insect repelling properties of *Zataria multiflora* (Karim and Yousefi, 2013) and *Achillea santolina* (Yonus *et al*., 2016).

The upper and lower highlands of Kalat division in Balochistan have a diverse resource of endemic plants with ethnomedicinal properties including *Z. multiflora* and *A. santolina* (Tareen *et al*., 2010; Bibi *et al*., 2016), which can also be explored for their potential to coup with agricultural insect pests and diseases. Therefore, present research work was carried out to determine the effect of neem along with two other plant extracts, with similar properties, on settling and reproduction response of *B. zonata* peach fruit fly.

MATERIALS AND METHODS

Laboratory studies

This study was undertaken in the Department of Entomology, Faculty of Agriculture, Lasbela University of Agriculture, Water and Marine Sciences, Lasbela, Pakistan. The experiment, to study the potential of different plant extracts at different doses, included two factors; different plant extracts (with 3 levels) and concentrations (with 4 levels including control) and each treatment factor was replicated four times.

Water extract of 'Neem' seed (*Azadirachta indica* A. Juss.) Meliaceae, stem and leaves extract of 'Izghand' (*Zataria multiflora* Boiss.) Lamiaceae, and extract from leaves and flower of 'Boh-e-Madran' (*Achillea santolina* L.) Asteraceae, were used in this study. These plant materials were brought from Ayurvedic shop and ground to fine powder in a grinding machine. From each plant sample, 30 g of powder was added in 500 ml of water and shaken after 24 h to mix evenly. These samples were used at 0.5, 1 and 2% concentrations in laboratory experiments. The culture of *B. zonata* was maintained under controlled laboratory conditions at 28 ± 2°C and 60 ± 5% R.H.

Effect of plant extract on the oviposition of peach fruit fly

The water extracts of the plants were used to determine its effect on oviposition of fruit fly. Extracts were mixed with chikoo juice at 0.5%, 1 and 2 concentrations. This treated juice was applied inside plastic glasses having pin-holes all around the wall of glass. The glasses applied with treated juice were exposed to 15 days old 20 fruit fly adults (mixed population) for 24 h for egg laying in the pin-holes. Untreated chikoo juice was used as control. The eggs laid in different treatment were collected separately with a camel hairbrush in water filled petri dishes. The number of eggs laid in different treatments were compared for determining the effect of extracts.

Effect of plant extracts on the progeny of peach fruit fly

Water extract of *A. indica*, *Z. multiflora*, and *A. santolina* were mixed with the diet of fruit fly at 0.5, 1 and 2 concentrations. The flies were separately offered untreated chikoo fruit for oviposition for three days. Five pairs of fruit flies were fed with the treated and control (untreated) diet at each concentration for 15 days. The fruits were labelled and then kept in glass jars having one-inch layer of sand at the bottom. After 15 days, number of pupae were counted and emerged adults were also counted and compared.

Table I. Mean number of fruit flies at its different life stages fed on fruits treated with various concentrations of plant extracts.

Concentration (%)	Plant extracts			Total mean
	A. indica	*Z. multiflora*	*A. santolina*	
(a) Number of eggs laid				
Control	28.75 a	32.50 a	31.00 a	30.75 A
0.5	19.50 bcd	21.25 bc	22.50 b	21.08 B
1	13.75 ef	16.50 cde	15.75 de	15.33 C
2	6.25 g	9.50 fg	8.25 g	8.00 D
Total mean	17.06 B	19.94 A	19.38 B	
(b) Number of pupae developed from eggs				
Control	48.75 a	50.00 a	52.50 a	50.42 A
0.5	30.00 bcd	37.50 b	32.50 bc	33.33 B
1	25.50 cde	30.00 bcd	30.00 bcd	28.50 BC
2	18.25 e	22.50 de	20.50 de	20.42 C
Total mean	30.63 A	35.00 A	33.88 A	
(c) Number of adults emerged from pupae				
Control	42.25 a	43.25 a	47.50 a	44.33 A
0.5	25.50 bc	32.50 b	27.50 bc	28.50 B
1	21.25 cde	24.75 cd	24.50 cd	23.50 B
2	12.25 f	17.75 def	15.75 ef	15.25 C
Total mean	25.31 A	29.56 A	28.21 A	
(d) Number of fruit flies settled on fruits				
Total mean	0.92 A	1.00 A	0.96 A	
0.5	0.81 abcd	0.94 abcd	0.86 abcd	0.87 B
1	0.69 bcd	0.75 bcd	0.75 bcd	0.73 BC
2	0.56 d	0.61 d	0.64 cd	0.60 C
Control	1.61 ab	1.72 a	1.58 abc	1.64 A
(e) Number of pupae developed after settling				
Control	56.50 ab	47.00 bcd	58.75 a	54.08 A
0.5	33.75 ef	42.00 cde	49.50 abc	41.75 B
1	29.50 fg	34.75 ef	36.25 def	33.50 BC
2	22.00 g	26.25 fg	29.50 fg	25.92 C
Total mean	35.44 B	37.50 B	43.50 A	
(f) Number of adults emerged after settling				
Control	49.25 ab	40.75 bcd	53.75 a	47.92 A
0.5	27.75 ef	37.50 cde	44.00 abc	36.42 B
1	23.00 fg	29.50 def	31.25 def	27.92 BC
2	16.25 g	21.50 fg	25.00 fg	20.92 C
Total mean	29.06 C	32.31 B	38.50 A	

Means sharing similar letters are not significantly different by DMRT at $P \leq 0.05$.

Settling and post settling reproduction response of peach fruit fly on chikoo fruit treated with different plant extracts

For the purpose, individual fruits were treated by dipping them in various concentrations of the plant extracts and were air dried in the laboratory. Untreated fruits were used as control. These treated fruits were placed in rearing cages. About 20 flies were introduced in the cages and their settling response was recorded after each 6 h for three days. Development of pupae and emergence of adults were also recorded from the fruits exposed to treatments.

Statistical analysis

The collected data was statistically analyzed using MSTAT-C software and means were compared by Duncan's Multiple Range Tests (DMRT) at $P \leq 0.05$.

RESULTS

Effect on the oviposition

The results showed that exposure of fruit flies to the water extracts of tested plants, reduced the number of eggs laid. It indicates that these plant extracts acted as oviposition deterrents against fruit fly. The interaction effect of plant extracts with its doses was non-significant. However, various doses of these extracts had significant difference on reducing oviposition of peach fruit fly *B. zonata* (Table 1a). Minimum significant (average 8 number of eggs) oviposition was at 2% concentration. Comparatively, the treatment of three plant extracts did not exhibit any significant variance on the number of eggs laid.

Effect on the progeny of peach fruit fly

Table 1b shows the number of pupae developed from the eggs laid by female peach fruit flies fed on diet treated with different concentrations of plants extracts. An interaction of plants extracts and their concentration, applied to artificial diet of peach fruit flies, did not show any significant effect. However, lowest number of pupae 18.25 recovered at 2% concentration of *A. indica* extract which were lower than 1, 0.5% and control of same plant extract but pupae recovered from controls were significantly higher than those recovered at all other concentrations of plant extracts. Similarly, 22.50 in *Z. multiflora* and 20.50 in *A. santolina* extract at 2% concentration were lower than those of all concentrations and their controls. The comparison of plants extract shows that there was no significant difference in mean number of pupae recovered from all three extracts. However, the different concentrations of plants extract showed significant difference that the significantly lowest number of 20.42 pupae were recovered at highest concentration.

Table 1c shows the number of adults emerged from the developed pupae. The plant extracts alone and its interaction with its various concentrations had no significant difference on the number of adults emerged from the developed pupae. In contrast, the concentration factor had significant effect on the number of adults emerge from developed pupae. Minimum significant adults 15.25 emerged at 2% followed by 23.50 and 28.50 at 1 and 0.5%, respectively.

Effect on settling response of peach fruit fly

Table II shows the number of fruit flies settled on chikoo fruits treated with various concentrations of plant extracts after given age intervals. Chikoo fruits were dipped in different concentration of plants extract, dried under shade and exposed to fruit fly in order to determine the settling response of fruit fly. Overall, there was no significant difference in interaction effect of plant extracts, its doses and age intervals. However, the lowest settling observed was 0.25 flies settled fruit^{-1} after 18 h at 0.5, 1 and 2% concentrations of *A. indica* and *Z. multiflora* extracts and while at same time interval in control treatments the settled flies were 1.5 and 2.0 fruit^{-1}, respectively which were higher than those treated. This lowest value was also observed after 36 and 54 h at 2% concentration of *A. Indica* and after 36 h at 1% concentration of *Z. multiflora*. Similarly, lowest settling response was observed 0.25 flies settled fruit^{-1} after 18 and 36 h at 2% concentration in *A. santolina* extract. The highest settling response on treated chikoo fruits was observed after 42 h at 0.5% in *Z. multiflora* (2.25 flies) which was higher than 2 and 1% concentrations. Moreover, the values of control were higher than values of each concentration showing 3.0 flies after 24 h in the control of *Z. multiflora* (Table II).

Unlike interaction of all three factors, settling response was significantly different at different concentrations. Minimum settling was observed at 2% and maximum settling was observed at control treatment (Table II). However, the interaction of different concentration of plant extract with various time intervals was non-significant by DMRT at $P \leq 0.05$ (Table IIIb).

In accordance with concentration factor, time interval also showed a significant effect by DMRT at $P \leq 0.05$ on settling response of peach fruit fly in this experiment (Table II). Significantly lowest number of flies 0.58 and 0.60 settled on fruits after 18 and 36 h, respectively. Whereas, highest significant number of flies 1.73 settled after 42 h. However, the interaction of various time intervals with different plant extract had no significant effect on settling response of peach fruit flies (Table IIIa).

Table II. Mean number of flies showing hourly settling response to various concentrations of different plant extracts.

H	*Azadirachta indica*				*Zataria multiflora*				*Achillea santolina*				Total means
	0.5%	1%	2%	0%	0.5%	1%	2%	0%	0.5%	1%	2%	0%	
6	0.75 fgh	0.75 fgh	0.75 fgh	1.00 efgh	0.75 fgh	0.50 gh	0.75 fgh	1.50 cdef	0.50 gh	0.75 gfh	0.50 gh	1.75 bcde	0.85 D
12	0.50 gh	0.50 gh	0.50 gh	1.50 cdef	0.75 fgh	0.75 fgh	0.50 gh	1.50 cdef	0.75 fgh	0.50 gh	0.75 fgh	2.00 bcd	0.88 D
18	0.25 h	0.25 h	0.25 h	1.50 cdef	0.25 h	0.25 h	0.25 h	2.00 bcd	0.50 gh	0.50 gh	0.25 h	0.75 fgh	0.58 E
24	0.75 fgh	0.50 gh	0.75 fgh	1.25 defg	0.75 fgh	0.75 fgh	0.75 fgh	3.00 a	1.00 efgh	0.75 fgh	0.75 fgh	1.75 bcde	1.06 C
30	0.75 fgh	0.75 fgh	0.50 gh	1.75 bcde	1.00 efgh	1.00 efgh	0.50 gh	1.00 efgh	0.75 fgh	0.75 fgh	0.50 gh	1.50 cdef	0.90 D
36	0.50 gh	0.50 gh	0.25 h	1.75 bcde	0.50 gh	0.25 h	0.50 gh	0.75 fgh	0.50 gh	0.50 gh	0.25 h	1.00 efgh	0.60 E
42	1.75 bcde	1.25 defg	1.25 defg	2.00 bcd	2.25 abc	1.75 bcde	0.75 fgh	2.25 abc	1.75 bcde	1.75 bcde	1.50 cdef	2.50 ab	1.73 A
48	1.00 efgh	1.00 efgh	0.50 gh	2.00 bcd	1.50 cdef	1.00 efgh	1.00 efgh	2.00 bcd	1.25 defg	0.75 fgh	0.75 fgh	2.25 abc	1.25 B
54	1.00 efgh	0.75 fgh	0.25 h	1.75 bcde	0.75 fgh	0.50 gh	0.50 gh	1.50 cdef	0.75 fgh	0.50 gh	0.50 gh	0.75 fgh	0.79 D
Total Means	0.81 ABCD	0.69 BCD	0.56 D	1.61 AB	0.94 ABCD	0.75 BCD	0.61 D	1.72 A	0.86 ABCD	0.75 BCD	0.64 CD	1.58 ABC	

Means sharing similar letters are not significantly different by DMRT at $P \leq 0.05$. H, hour.

Table III. Mean number of fruit flies settled on fruit treated with plant extracts (a) and by different concentrations (b) after various time intervals.

	Time (h)								
	6	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**54**
(a) Plant extracts									
A. indica	0.82 bcd	0.75 cd	0.56 d	0.81 bcd	0.94 abcd	0.75 cd	1.56 abc	1.13 abcd	0.94 abcd
Z. multiflora	0.88 bcd	0.88 bcd	0.69 cd	1.31 abcd	0.88 bcd	0.50 d	1.75 ab	1.38 abcd	0.81 bcd
A. santolina	0.87 bcd	1.0 abcd	0.50 d	1.06 abcd	0.88 bcd	0.56 d	1.87 a	1.25 abcd	0.63 cd
(b) Concentration (%)									
Control	1.42 a	1.67 a	1.42 a	2.00 a	1.41 a	1.17 a	2.25 a	2.08 a	1.33 a
0.5	0.67 a	0.67 a	0.33 a	0.83 a	0.83 a	0.50 a	1.92 a	1.25 a	0.83 a
1	0.67 a	0.58 a	0.33 a	0.67 a	0.83 a	0.42 a	1.58 a	0.92 a	0.58 a
2	0.67 a	0.58 a	0.25 a	0.75 a	0.50 a	0.33 a	1.17 a	0.75 a	0.42 a

Means sharing similar letters are not significantly different by DMRT at $P \leq 0.05$.

Accordingly, the interaction of different plant extracts with its various concentrations had no significant difference on settling response of fruit fly (Table 1d). Nevertheless, 2% concentration of *A. indica* and *Z. multiflora* showed lower number of fruit flies settled than at 0.5, 1% and control. The comparison of the effects of plant extracts on mean settling response showed no significant difference, however different doses of plant extracts had a significant difference on settling response of fruit fly, showing inverse trend to the increasing concentration of extracts. The lowest number of 0.60 fruit fly fruit^{-1} settled at 2% concentration fallowed by 0.73 at 1% and 0.87 at 0.5% dose as compared to 1.64 in control, which were significantly highest.

Table 1e shows the number of pupae developed after settling on the fruits treated with different concentrations of plant extracts. As the fruit flies lay eggs beneath the skin of fruit and counting of eggs is not possible, therefore, the oviposition response of the test insect would either be noted by counting the larvae in the infested fruit by dissecting them or by counting the number of pupae recovered. The results indicate that both different plant extracts and its various doses alone had significant effect on the number of pupae developed on the fruits treated but their interaction had no significant difference. In case of *A. indica* seed extract, the lowest number of 22.00 pupae were recovered from 2% and most pupae 56.50 were recovered in control. Similar trend was seen while treated with other two plant extracts. However, plants extract results show that significantly lowest number of pupae 35.44 recovered from *A. indica* seed extract treated fruits followed by 37.50 from *Z. multiflora* and 43.50 from *A. santolina* extract. Likewise, at various doses lowest significant number of pupae 25.92 were recovered from the fruits treated with 2% concentration which was significantly lower than those 54.08 recovered at control.

Table 1f shows the number of adults emerged from pupae developed after settling on the fruits treated with different concentrations of plant extracts. The results indicate that both factors plant extracts and its various doses had significant effect on the number of adults emerged from collected pupae on the fruits treated but the interaction of these two factors had no significant difference (Table 1f). The comparison of plants extract shows that lowest significant number of 29.06 adults emerged in *A. indica* seed extract which was significantly lower than 32.31 in *Z. multiflora* and 38.50 in *A. santolina*. Whereas, the results of different concentrations of plants extracts shows that in 2% concentration significantly lowest number of adults 20.92 emerged as compared to 27.92 at 1%, 36.42 in 0.5% and dose and 47.92 at control.

DISCUSSION

Neem contains an array of chemicals having different complex mode of action on insects. Azadirachtin, a chemical compound from the neem seeds, is the main component responsible for the toxic effects in insects. Azadirachtin from neem effects insects in a variety of different ways: as an antifeedant, insect growth regulator and sterilant (Mordue and Nisbet, 2000).

Results of our study are in accordance with a study which implies neem seed kernel extract, for oriental fruit fly, as an ovipositional deterrent (Chen *et al*., 1996). Similarly, neem seed kernel extract had effect on the fecundity and post-embryonic development of fruit flies *B. cucurbitae* and *B. dorsalis* (Singh, 2003). This idea was further supported the ingestion of neem can significantly reduce the longevity and fertility of melon fly

and the oriental fruit fly (Khan *et al*., 2007). Accordingly, significant repellency of the Mexican fruit fly was observed in terms of oviposition at 3 and 5% aqueous neem extract and 4.5% neem oil treatment (Valencia-Botin *et al*., 2004). Lower number of *B. zonata* adults settled on fruits treated with petroleum ether extract of sweet flag followed by neem acetone extract and sweet flag extract. The number of pupae and adults obtained from fruits decreased with increase in the extract dosage (Naheed *et al*., 2004).

Effect of crud extracts of *A. santolina* determined for feeding toxicity against adult of *Oryzaephilus surinamensis*. The results of the study showed that Hexane extract of *A. santolina* was effective reaching survival rate of adult to 66.2% at concentration of 10% (Yonus *et al*., 2016). Antifeedant activity of *Z. maltiflora* oil was reported to be more effective on feeding deterrence index than *Thymus daenensis* (Karim and Yousefi, 2013).

Looking at the settling response our findings agreed with the similar study, where settling of melon fruit fly was adversely affected by neem seed water extract and neem oil (Khattak *et al*., 2009). The study further reported that significantly lower number of pupae was recovered from the fruit treated with 2% concentration of neem oil and 3% of neem seed water extract as compared to control.

CONCLUSION

All the plant extracts exhibited insecticidal properties and resulted in reduction of fruit fly population in laboratory condition and decreasing trend of fruit fly population continued with increasing concentration. *A. indica* showed most promising results followed by *A. santolina*. These plants studied in this research work, contain an array of chemicals having different complex mode of action on insects, hence further investigation is needed to evolve strategies for integrated pest management techniques. Furthermore, the increasing dosage of these plant extracts had increasing degree of fruit fly repellence. Hence, more research is needed to standardize the dosage. Moreover, these findings are based on laboratory trials and may differ in field trials due to the various biotic and abiotic conditions in different agro-ecological zones.

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Ethical statement

Essential ethical guidelines and approval of the Department of Entomology, LUAWMS were adopted for this study.

Statement of conflict of interest

The authors have declared no conflict of interest.

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