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Research Article

Repellency of aqueous and acetone based plant extracts against melon fruit fly, *Bactrocera cucurbitae* under lab conditions

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ABSTRACT

Melon fruit fly (*Bactrocera cucurbiate*), a cosmopolitan insect pest, attacks on many fruits and vegetables. Study was conducted to evaluate the comparative repellency of three plant extracts viz; *Azadirachta indica*, *Datura stramonium* and *Parthenium hysterophorus* against melon fruit fly under laboratory conditions. Each plant extract was prepared in two types of solvents i.e. water and acetone. Three concentrations (i.e. 2%, 4% and 6%) of each plant extract (i.e. aqueous and acetonic) were applied on bitter gourd fruit, with one positive (i.e. malathion 1ml/L) and one negative control (i.e. water/acetone). Studies revealed that in aqueous solutions, *Datura stramonium* provided the maximum (6.00±0.17%) fruit weight loss and 68.86±2.28% repellency corresponding to highest concentration. While in case of acetonic extracts, best results were given by the *Parthenium hysterophrous* (4.47%±0.75 fruit weight loss and 79.3%±0.90 repellency) when applied @ 6% concentration. *Azadirachta indica* was least effective in both types of extract. Overall, acetone based plant extracts were more efficient as compared to the aqueous one exception to that was *D. stramonium* which was more effective in aqueous based extract. Furthermore, raising the concentration improved the effectiveness of both types of extracts.

Keywords: Plant extracts; fruit fly; repellency; *Bactrocera cucurbitae*.

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INTRODUCTION

Cucurbitaceae is an important plant family comprising of vital horticultural crops i.e. water melon, musk melon, cucumber, pumpkins, gourds, squash etc. Majority of these are imperative vegetable crops, mostly are harvested and consumed in summer season. Among the aforementioned horticultural crops cucurbits are excellent food stuff in nature having almost all essential constituents which are required by human body for healthy life (Irshad et al., 2010). About 30 insect pests attack on cucurbits in Indo-Pak region, like white flies, thrips, midges, fruit flies and red pumpkin beetle. But the penultimate pest is regarded as very destructive. Melon fruit fly, *Bactrocera cucurbitae* is a notorious pest of cucurbits particularly in bitter gourd (Sharma and Sohal, 2013). Female fly punctures the fruit and insert its ovipositor for egg laying.

Depending upon the environmental conditions and localities this pest has potential to destroy 40-100% crops (Kibira et al., 2015). Control of the fruit flies is a challenging task as the maggots are internal feeder and consume the fruits internally (Heve et al., 2017). Fruit flies are considered cosmopolitan pest and can damage other crops if their host plants and fruits are not available. Different methods are used to control fruit flies but synthetic chemicals are mostly used against this pest in spite of their hazardous effects on human beings (Wirtu, 2024). Beneficial insects and pollinators are present in agricultural fields in a notable amount by playing an important role in natural pest control and pollination. The use of this type of synthetic insecticides also disturb their population in nature and ultimately crop production is affected (Ndakidemi et al., 2016). Some are highly carcinogenic particularly in children and some are responsible for headache, vomiting, blood pressure abnormalities and dizziness like abnormalities. Due to high residual effects of most of the insecticides they also deteriorate food stuff (Ccanccapa et al., 2016). To diminish the impacts of these toxic substances on non-targeted organisms, there is a keen desire to develop some safe and promising materials against pests (Debashri and Tamal, 2012). Plants have some natural chemicals in their different parts which can be used against insects (Regnault-Roger, 1997). These botanicals have repellent effects and killing effects only against selective organisms. Plant extracts have amazing properties to repel the harmful insets from the crops. The have ability to easily penetrate in the cuticle of insect which enhance their bioavailability (Moretti et al., 2013). This penetrating property of plant extracts can reduce the stay duration of any insect pest on host plant due to their repellent effects. As the insect sensilla are specialized for detecting and sensing the unfavorable aroma, so when they are sit on plant extracts treated surface they feel uncomforting and left the treated substances (Moretti et al., 2013).

Plant extracts of *Azadirachta indica* (Neem) leaves, seeds of *Datura stramonium* (Datura), fruits of *Zingiber officinale* (Ginger) and *Allium sativum* (Garlic) have potential to repel the fruit fly. Due to the repellent effect of these extracts the female fruit flies did not showed a good number of egg deposition (Hafiz et al., 2020). Usually beneficial insects are not disturbed by these bio-pesticides (Gupta and Dikshit, 2010). A lot of plant extracts are known now a day which can be used as repellent against insect pests without side effects and they are also economical for farmers (Isman, 1997). Botanicals may replace of these chemical insecticides but a lot of work is required to be done on this aspect as currently the botanicals based chemicals comprise only 1% of the pesticides market (Debashri and Tamal, 2012). Aqueous and acetone extracts of *Azadirachta indica*, *Datura stramonium* and *Parthenium hysterophorus* have been used against different insect pests according to previous literature. But their efficacy against the melon fruit fly was needed to explore more as only small number of literature is available.

MATERIALS AND METHODS

Research was conducted under laboratory conditions and the methodology is given below in detail.

Rearing Fruit Flies

Infested cucurbits were collected from the vegetable market of Islamabad (33.7294° N, 73.0931° E), Pakistan. These fruits were placed in the transparent plastic cage (45×40×40 cm). Autoclaved sand of 5cm thick layer was placed at the bottom of the cages for the pupation of molted maggots from the fruits. As the last stages of the internal feeder maggots of fruit flies come out from the fruits for pupation. Cages were observed on daily basis. After pupation in the sand, this was sieved out to separate the pupae in a petri dish. This petri plate was placed in another clean cage. On emergence of adults from the petri dish the adults of the *Bactrocera cucurbitae* were separated (identified on the basis of morphological characteristics). Then females of *B. cucurbiate* were also separated from males (identifying on the basis of their ovipositor) and were fed on 10% honey solution. After 2 weeks, male flies were released in female's cage for mating. After 24 hours' females were again separated for experimental use. All of this procedure was conducted under controlled lab condition (25±5 °C, 65±5% RH and photoperiod of 14: 10 (L: D) hours)

Plant Extracts Preparation

Aqueous extract

Leaves of *Azadirachta indica*, seed of *Datura stramonium* and upper 3-inch portion of *Parthenium hysterophorus* bearing flowers were taken, washed out with tap water, dried at 40 °C for 48 h. Then these materials were ground in electric blender machine at 25000 rpm. After sieving the dried plant material, 60 grams from each plant material was placed in 1L distilled water and placed in hermetically sealed glass containers to attain 6% aqueous solution, following the procedure by (*Poderoso* et al., 2016). The other two concentrations 2% and 4% were prepared by adding more distilled water.

Acetone extraction

After obtaining the fine powder by following the above-mentioned procedure, from each plant material plant extracts were obtained by using Soxhlet's apparatus at 55-60 ºC at Animal nutrition laboratory of Faculty of Veterinary and Animal Sciences, PMAS Arid Agriculture University Rawalpindi. In Soxhlet's apparatus, a proportion of 50g (botanical powder) and 250 ml (acetone) was used for three times to get the required amount of each plant extract. To get the stock solution, excessive amount of acetone was evaporated from the solution by using electric rotary evaporator. Required concentrations were made by using this extract as stock solution.

Repellency test of plant extracts

Three concentrations (i.e. 2%, 4% and 6%) from aqueous and acetone-based plant extracts were prepared and were used as treatments with positive control (malathion 1ml/L of water) and negative control (only distilled water in case of aqueous based) and (only acetone in case of acetone based extract). For each treatment bitter gourd fruits were dipped in each concentration for 1 minute and then placed for dry. After drying the treated bitter gourd were placed in plastic cages (same sized as used for rearing). Each treatment was replicated three times. Ten numbers of female fruit flies (mated 24 hours before release) were released in each cage for 24 hours. Then they were released from cages and the cages were placed for eight days in controlled conditions (i.e. 25±5 ºC, 65±5% RH and 14:10 L: D). Mixture of sterile fine sand and dust was also placed at the bottom of rearing cage for pupation. After eight days' bitter gourd fruits were weigh and soil was sieved out for counting emerged pupae. Fruit weight loss (%) calculated by applying the formula described by Usman et al., (2013) and repellency (%) was calculated by using formula described by Murugesan et al. (2021).

$$\text{Fruit weight loss (\%)} = \frac{(Wb - Wa)}{300} \times 100$$

Where: Wb = weight of fruits at time of placing in cage

Wa = weight of fruits after eight days

$$\text{Repellency (\%)} = \frac{(Pc - Pt)}{(Pc + Pt)} \times 100$$

Where: Pc = No of pupae emerged in negative controlled

Pt = No. of pupae emerged in treated

Statistical Analysis

Analysis of variance regarding number of emerged pupae, fruit weight loss and repellency was carried out using Statistix 8.1 package (Analytical Software, 2005). Means were compared by LSD test at 5% probability level.

RESULTS AND DISCUSSION

Weight Loss

Results related to fruit weight loss are given in (Table 1). Experiment was repeated two times and given data is the pooled data of repeated experiments. All plant extracts have effects against melon fruit fly that's the reason in extracts treated bitter gourd fruits, weight loss was low which shows their repellent effects. In case of aqueous extract maximum weight loss was (18.72±0.96%) in 2% concentration of *A. indica* treated fruits. So it can be assumed that the maggots of the melon fly can efficiently feed on bitter gourd in presences of *A. indica* spray due to low repellent effect as compared to the *P. hysterophorus* and *D. stramonium*. Minimum weight loss in aqueous based extract was shown by *D. stramonium* (6±0.17%) at highest concentration. While in case of *P. hysterophorus* and *A. Indica* at same concentration weight loss was (9.11±0.38% and 13.28±0.51%) respectively. It shows that the aqueous extract of *D. stramonium* repel the fruit flies more efficiently as compared to *P. hysterophorus* and *A. Indica* and also disturb their feeding efficiency. The results also indicate that by increasing concentration the efficacy of extracts was also increased. In case of acetone based extract maximum weight loss (18.94±0.35) was also noted in *A. indica* treated bitter gourd fruits at lowest concentration. Minimum weight loss (4.47±0.75) in acetonic extract was noted in *P. hysterophorus*. Table (1) also indicates that overall acetone based plant extracts were more efficient than the aqueous extracts except *D. stramonium*, in which aqueous based extract was more efficient than acetone based extract. Results regarding the fruit weight loss were also compared to the positive and negative control.

Repellency

Results related to repellency of different plant extracts is given in table 2.

Results of the three plant extracts (i.e. *P. hysterophorus, A. indica* and *D. stramonium*) are given in table (1). Among these three extracts, repellency of each extract with three concentrations (i.e. 2%, 4% and 6%) and two types of

Table1: Percent fruit weight loss of bitter gourd treated with the aqueous and acetone extracts of *P. hysterophorus*, *A. indica* and *D. stramonium*

	Concentrations	*P. hysterophorus*	*A. indica*	*D. stramonium*
Aqueous Extract	2%	15.22±0.19b	18.72±0.96b	14.86±0.71c
	4%	12.83±0.60c	15.278±0.25c	10.96±0.88d
	6%	9.11±0.38d	13.28±0.51cd	6.00±0.17ef
	Control (p)	3.76±0.11e	3.99±0.15f	4.18±0.19f
	Control (n)	26.30±0.69ea	25.96±0.68a	26.33±1.74a
Acetone Extract	2%	15.11±0.42b	18.94±0.35b	19.67±0.44b
	4%	7.97±0.52d	12.73±1.19d	11.56±0.49d
	6%	4.47±0.75e	9.72±1e	7.72±1.25e
	Control (p)	3.58±0.25e	3.99±0.15f	3.87±0.15f
	Control (n)	25.78±0.38a	27.16±0.63a	26.33±1.74a
	LSD	1.43	2.1	2.7

Means followed by the same letter in each column for each main effect are not significantly different according to LSD at P= 0.05

Table 2: Percent repellency of the aqueous and acetone extracts of *P. hysterophorus*, *A. indica* and *D. stramonium* against melon fruit fly on bitter gourd.

	Concentrations	*P. hysterophorus*	*A. indica*	*D. stramonium*
Aqueous Extract	2%	51.62±0.66e	45.13±0.60e	53.67±1.42d
	4%	57.53±2.58d	52.85±0.66d	62.44±2.42c
	6%	67.52±0.80c	60.02±0.32c	68.86±2.28b
	Control (p)	84.83±2.59a	84.09±4.01a	83.29±2.03a
Acetone Extract	2%	55.44±1.24de	45.52±0.67e	46.55±1.74e
	4%	70.41±2.59c	59.00±0.87c	61.48±0.79c
	6%	79.28±0.90b	66.69±2.31b	69.66±1.51b
	Control (p)	84.89±1.75a	85.23±1.05a	84.51±3.58a
	LSD	5.52	5.38	5.88

Means followed by the same letter in each column for each main effect are not significantly different according to LSD at P= 0.05.

extraction method (i.e. aqueous and acetonic) was tested against melon fruit fly (*Bactrocera cucurbitae*). Malathion was used as positive control to compare the results of extracts. Results show that all plant extracts have repellency against fruit fly. Repellency was maximum at highest concentration in both, aqueous and acetone extracts. Our findings are in line with Roonjho et al. (2013) who also checked the repellency of different plant extracts against cotton mealybug and observed the maximum repellency at highest concentration.

In aqueous based extract maximum repellency was given by *D. stramonium* (68.86±2.28b) at 6% concentration. At the same concentration repellency was (67.521±0.80 and 60.02±0.32) given by *P. hysterophorus* and *A. indica* respectively. Minimum repellency in aqueous extract (45.13±0.60) was examined in *A. indica* at 2% concentration. While at the same concentration, *P. hysterophorus* and *D. stramonium* repellency was (51.62±0.66 and 53.67±1.42) respectively. So the best results in aqueous extract were given by *D. stramonium*. The findings of Ali et al. (2018) also revealed that that aqueous extract of datura has mortality effect against the *Trogoderma granarium*, but its efficacy was less than the aqueous extract of neem. The reason of less efficacy of datura as compared to the neem extract might be due to different insect pests.

On other hand in acetone-based extracts, maximum repellency (79.28±0.90%) was given by *P. hysterophorus* at 6% concentration. Repellency at 4% (70.408±2.59) by *P. hysterophorus* was also more than other two extracts (*A. indica & D. stramonium*). Minimum repellency in acetonic extract (45.52±0.67) was given by *A. indica* at 2% concentration. *D. stramonium* was the only extract which showed high repellency in aqueous extract instead of acetonic extract at 6%, while others were more repellent in acetonic extraction at highest concentration. The efficacy of each plant extract can be changed when prepared in different type of solvent as it was also noticed by Rima et al., (2021) that acetone extracts which are rich in green pigments present the best insecticidal activities against *Drosophila melanogaster* fruit

fly. On the other hand, regarding the repellent activity, the aqueous extract of spinach displayed higher effectiveness. At the lowest concentration, repellency of both aqueous and acetonic extracts was almost same in case of *A. indica*, anyhow it was increased in acetonic extract as compared to the aqueous extract. So acetonic extract gave more repellency as compared to the aqueous extract in case of *P. hysterophorus* and *A. indica*. Overall maximum repellency was also given by acetonic extract (79.28±0.90).

Plant extracts are not usually harmful for non-targeted organisms as they have been consumed by mammals as feed and some plants which are being used by human beings in their cooking can also be used for extraction against insect pests (Scott et al., 2004). Sultana et al. (2013) checked the efficacy of neem, basil, mahua and other plant extracts against melon fruit fly. They concluded that neem extract does not give results as were given by *M. indica and C. fistula*. In our research work neem extract was less effective as compared to the *P. hysterophorus.* So his results support our results. The efficiency of any plant extract may depend upon the method of extraction, way of application, concentration and environmental conditions. Neem, Parthenium, Eucalyptus and Methomyl extracts were also used by Ali et al. (2011). They concluded that these all extracts have repellent effects against melon fly, *Bactrocera cucurbitae*. So when these extracts are applied on fruits and vegetables they repel harmful insects due to their odor. So the spoilage of food can be checked in such a way. These results were also supportive to our outcomes as we also found these extracts (Neem and Parthinium) effective against melon fruit fly. They repel the fruit flies from bitter gourd as compared to the control treatment.

According to the current studies, in case of aqueous extracts the highest repellency was observed when *D. stramonium* extract with highest concentration was sprayed. These findings are in line with hafiz et al. (2020) who also sprayed the plant extracts at different concentrations to observe their repellency against melon fruit fly and concluded that *D. stramonium* gave the most promosing results. *D. stramonium* is an effective plant, which has repellent effects against many insect pests as the larvacidal effects of this plant extract were reported by Pascual-Villalobos and Robledo (1998). Moreover, Datura Spp effectiveness against acari families also have been reported (Guirado et al. 2001; Thevan et al. 2005). In case of acetone extracts, *P. hysterophorus* extract was the most reliable in repelling the fruit flies. *P. hysterophorus* is also considered a toxic and aggressive weed which has repellent and pharmacological properties. Due to its toxic effects it can be used against various insect pests. In mosquito management program this weed was used and gave good results according to the studies of the Kumar et al. (2011). Singh (2003) elaborated the efficacy of the aqueous extraction of neem against *B. cucurbitae* and *B. dorsalis*. Flies were fed on neem seed kernel aqueous extract as a water source and then fecundity and post embryonic development was checked at three different time intervals. Fecundity power was reduced up to 72% in *B. cucurbiate* and 75% in *B. dorsalis* as compared to control in which fecundity was 99%. Eggs hatching was reduced up to 56% and 86%, respectively in both species while this extract did not affect the pupation. At the end of experiment recommendation of aqueous extract of neem use as cheap, safe, renewable and best alternative to synthetic pesticides was given. So it is clear that the use of plant extracts against harmful insects is a safe method and no extra charges are required to purchase the material.

Fauna of pollinator and natural enemies of harmful insect exist in the field crops and perform their duty to controlling insect pest in efficient way. But use chemical insecticides in field crops disturb their population. Usually they are more susceptible to theses insecticides as compared to pests. The synthetic pesticides have lethal and sub-lethal effect on the natural enemies. So the use of bio-pesticide is considered benign in this situation against these beneficial insect and at the same time they give promising results against pest insects (Amoabeng et al., 2019). A lot of research work is required on plant species to use them against insects. Egg deterrent power, repellency, infertility, toxicity and other aspects of the plants extracts are dependent on the properties of their source plants. Different part of plants has different insecticidal properties as studied by Ahmad et al. (2019). They used different parts of Z. *officinale, A. sativum* C. *citratus*, *Eucalyptus. Nicotiana tabacum* and *A. indica* (Meliaceae) against *T. castaneum* in stored grains. After drying these plants, extracts were prepared and tested regarding the mortality. *Allium sativum* (Alliaceae), *Zingiber officinale* (Zingiberaceae) demonstrated more efficiency among all the extracts. Biology of the insect is disturbed by applying plant extracts as Silva et al. (2013) checked the efficacy of *Azadirachta indica* extracts against fruit fly. Extracts were applied directly on the adults to check the longevity and pre-oviposition. The extract affected the longevity of adults fly significantly but the pre-oviposition was not affected.

CONCLUSION

Insecticides are very harmful for our environment due to their residual effects and targeting beneficial living organisms. So plant extracts can be used as an alternative of these insecticides. *Parthinium hysterophorus* is a common weed in

Pakistan. Aqueous and acetone based extract of this weed can be used against melon fruit fly, *Bactrocera cucurbiate* as an alternative to recommended insecticide (malathion). Use of this plant extract might be an economically and environmental friendly as compared to the insecticide.

ABBREVIATIONS

RH: relative humidity; *A. indica*: *Azadirachta indica*; *D. stramonium: Datura stramonium; p. hysterophorus: Parthenium hysterophorus;* C (0): Negative Control; C(p): Positive control; L: D: Light to dark ratio; h: hours.

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AVAILABILITY OF DATA AND MATERIALS

The data used and analyzed during this project are available from the corresponding author on reasonable request.

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