



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com



Original article

Insecticidal, biological and biochemical response of *Musca domestica* (Diptera: Muscidae) to some indigenous weed plant extracts



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ARTICLE INFO

Article history:

Received 25 April 2019

Revised 22 May 2019

Accepted 28 May 2019

Available online 29 May 2019

Keywords:

Mortality, repellency, genotoxicity

Enzyme inhibition

FTIR

Musca domestica

ABSTRACT

In the current study; insecticidal, growth regulation, oviposition deterrence and repellency of petroleum ether extracts of *Azadirachta indica*, *Penganum harmala*, *Datura stramonium*, *Tribulus terrestris* and *Chenopodium murale* against 2nd instar larvae of housefly was investigated. Five different concentrations (5%, 10%, 15%, 20% and 25%) were used through larval feeding and the mortality data was recorded after 24, 48 and 72 hrs. Highest mortality was induced by *P. harmala* (63.87%) followed by *D. stramonium* (62.78%), *A. indica* (53.84%), *T. terrestris* (41.86%) and *C. murale* (4.09%) after 72 h at 25% concentration, respectively. Increased mortality was observed with increased time duration and concentration. Longest larval duration (9.33 ± 0.33 days) and pupal duration (7.33 ± 0.33 days) was recorded in larvae treated with 25% concentration of *P. harmala* which also caused a decrease in the activity of AChE, ACP, AKP, α -Carboxyl, and β -Carboxyl enzymes. However, at 25% concentration, *C. murale* showed highest oviposition deterrence activity (81.88%) followed by *D. stramonium* (79.58%). In comet assay test, at highest concentration (25%) the mean comet tail lengths represented by *Penganum harmala*, *Datura stramonium* and *Azadirachta indica* (Reference plant) were 10.20 ± 0.49 , 9.20 ± 0.37 and 7.80 ± 0.49 μm while percent DNA damage was 10.56 ± 0.77 , 10.67 ± 1.62 and $8.11 \pm 0.85\%$ respectively compared to controls cells. Phytochemical analysis indicated the presence of flavonoids, steroids, saponins, cardiac glycosides, tannins, alkaloids, terpenoids and anthraquinones. Fourier Transform Infrared spectroscopy (FTIR) analysis revealed the presence of phenolic flavonoids, saponins, tannins as major functional groups. Further studies are needed to explore and thus, to incorporate weed plant extracts for the management of house flies.

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1. Introduction

House fly (*Musca domestica* Linn.) is an important ubiquitous insect pest of both homes and farms as well. It acts as vector of more than hundred diseases in humans and animals caused by

protozoans, bacteria, viruses, pathogens. These pathogens are transported by means of feces, mouthparts, mouth saliva, gut, and through surface of their body. It, usually disseminates harmful pathogens when get in touch with human and/or animals (Malik et al., 2007).

For the management of house fly population, chemical insecticides are commonly used. However, resistance has been developed against nearly all types of insecticides used for its control and now become a global issue. Additionally, these flies are well-known for their capability to develop metabolic and behavioral adaptations to abstain and for the detoxification of synthetic insecticides. Resistance of flies to DDT was reported after some years of its utilization. New synthetic insecticides are being launched consistently from years, and the result was the development of resistance of

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Peer review under responsibility of King Saud University.



flies to carbamate, organophosphate, and pyrethroid classes of insecticides. Such type of challenging circumstances demands such management strategies which restrict the development of insect in order to sustain adult populations via controlling larval populations. The huge costs of chemical insecticides and concerned environmental harmful impacts encouraged scientists to search less toxic, ecofriendly and cheaper biopesticides as alternative way for the pest management in future (Sultana et al., 2016).

Plant-derived products have emerged as a leading source of alternatives for pest control. Plant extracts have bioactive compounds (polyphenols, alkaloids, terpenoids, essential oils, steroids, lignins, fatty acids, and sugars) which are basically secondary metabolites of plants and show broad spectrum of actions against variety of insect pests. Therefore, these products are considered as good substitutes to chemical insecticides for insect pest control and have little effect on human health, environment and predators, parasites and pollinator insects due to minimum residual activity (Regnault-Roger et al., 2012). Sometimes, plants that are being utilized as pest control agents are insect static (growth regulators) more than being insecticides as they are associated with the inhibition of insect development (Pavela, 2008). Most of the plants act as feeding inhibitors in various aspects: feeding repellents, or feeding suppressors and others are involved in the inhibition of growth, production of eggs and development.

P. harmala L. is a herbaceous plant growing in semi-arid lands has multiple potentials and used in curing of various human ailments, such as asthma, lumbago, gastrointestinal problems, jaundice, abortifacient agent and as a stimulant. The most commonly found phytochemical constituents in *P. harmala* include flavonoids, alkaloids, and anthraquinones (Bukhari et al., 2008).

D. stramonium is recognized as Jimson weed or Devil's trumpet and efficacy of this plant has been recognized as useful remedy in traditional medicine (Lee, 2007). *D. stramonium* is also commonly used in numerous human diseases including wounds, ulcers, inflammation, gout, rheumatism, sciatica, swellings, bruises, bronchitis asthma, fever, and toothache etc (Gaire and Subedi, 2013).

Recently, weed plants have been reported against various insect pests. The compounds present in weed plants exhibit toxicological effects on insects (Riaz et al., 2018). Therefore, the present research work was designed to assess the insecticidal activity of *P. harmala*, *D. stramonium*, *A. indica*, *T. terrestris* and *C. murale* against 2nd instar larvae of *M. domestica*.

2. Materials and methods

2.1. Rearing of *Musca domestica* L.

The study was conducted in the Entomology Lab, Department of Zoology, Government College University Faisalabad. Susceptible lab strain was used for bioassays at 28 ± 2 °C temperature and 65–70% relative humidity. Cages of 40 cm × 40 cm × 40 cm size were used for the rearing of house flies. The cages were enclosed with mesh screens and have cloth sleeves at front and back sides. The front sleeve used for the introduction of milk solution, sugar solution and oviposition trays. A cotton pad soaked in the sugar solution (3%) was housed in the cage to provide sugar and water. An adult diet which is comprised of glucose (50%) and powder of MacConkey agar (50%) was prepared. Sugar solution and food was provided daily. Fresh milk soaked cotton wool was furnished to newly emerged flies for three days to augment production of eggs and later they were given milk sugar solution in the same way mentioned above. For rearing of larvae, a mixture of sterilized wheat bran (38 g), milk powder (2 g) and 60 ml water was employed as described by Pavela (2008). The temperature was maintained at 27 ± 2 °C with relative humidity (RH) of $70 \pm 5\%$.

2.2. Collection and extraction of weed plants

All weeds were collected from different rural areas of district Faisalabad and then identified from Department of Botany, Government College University Faisalabad. To remove dust weed plants were then thoroughly cleaned using running tap water and then dried under shade for fifteen days. Crushed dried plants were again oven dried at 60 °C for 20 min. Grinded the oven dried crushed weeds in electrical grinder by using the mesh sieve#2 to obtain the plants in powder form. For extraction, 100 g of sample was mixed with 300 ml of petroleum ether and then the mixture was subjected to Rotary Shaker for 24 h at 220 rpm. Then filtration was made with the help of Whatman filter paper. The filtrate were kept in sterilized grey color air tight screwed glass bottles and stored in refrigerator at 4 °C for use. Following concentrations viz. 5, 10, 15, 20 and 25% were prepared using petroleum ether as solvent from the stock solution of each weed.

2.3. Mortality bioassay

Different concentrations (5%, 10%, 15%, 20% and 25%) of botanical extracts prepared in petroleum ether were used for 5 g wheat bran while the control group was only treated with petroleum ether. Then treated food material was air dried for 15–30 min to evaporate the petroleum ether. Twenty 2nd instar larvae were exposed to each concentration of extract in 250 ml glass beakers labeled for different concentrations. The beakers were covered with muslin cloth. Each experiment was replicated three times along with the control and completely randomized design was followed. Those larvae were also considered dead that showed no feedback to sharp pin probing. Larval mortality was recorded after 24 h, 48 h and 72 h of time duration (Sultana et al., 2016).

2.4. Growth regulatory impact of weed plant extracts on *M. domestica*

To check the growth regulatory impact, treated the wheat bran with different botanical extracts concentration (5, 10, 15, 20, and 25%) while the control was treated with petroleum ether after the evaporation of petroleum ether (15–30 min.). Twenty 2nd instar larvae were released to complete their life cycle in each concentration of extract in 150 ml glass beakers labeled for different concentrations. The beakers were wrapped with muslin cloth with the help of rubber band. Each experiment was carried out with three replication with the control and completely randomized design was monitored (Sultana et al., 2016; Regnault-Roger et al., 2012). Data was collected for survival, pupation and adult emergence from treated larval media after 6–11 days.

2.5. Enzyme assay

2.5.1. Preparation of whole body homogenate

For enzymatic estimation, the larvae of *M. domestica* were washed thoroughly with distilled water and the extra water adhering to larvae was cleared by using blotting paper. Then, larvae were subjected to homogenization utilizing ice-cold sodium phosphate buffer (pH 7.0, 20 mM) with the help of Teflon hand homogenizer. Then, centrifugation was done at 8000g and 4 °C for 20 min and resultant supernatant was used for the estimation of Esterases or Phosphatases. Solutions and glassware used for homogenization were kept at 4 °C before use, and homogenates were also placed on ice until utilized for enzyme assays (Huang et al., 2008)

2.5.2. Quantitative estimation of Esterases and Phosphatases

2.5.2.1. Estimation of acetylcholinesterase activity. In the 50 µl of enzyme solution, 50 µl of acetylcholine chloride (2.6 mM) as a substrate and 1 ml of SBP (sodium phosphate buffer, pH 7.0, 20 mM)

were added. It was placed in incubator at 25 °C for 5 mins. Then 400 µl of 0.3% Fast blue B salt was mixed to stop the reaction. Blank and sample were run through spectrophotometer. Optical density (OD) was checked at 405 nm (Younes et al., 2011).

2.5.2.2. Estimation of carboxylesterase activity. The activity of α -carboxylesterase and β -carboxylesterase was measured as devised by Van Asperen (1962). In 50 µl enzyme solution (homogenates), SBP (1 ml, pH 7.0, 20 mM,) and 50 µl of each β -naphthyl acetate and α -naphthyl acetate (substrate) were added separately to determine the activities of α -carboxylesterase and β -carboxylesterase respectively. The solutions were incubated at 30 °C for 20mins. After incubation, 400 µl of 0.3% fast blue B which was prepared freshly in 3.3% SDS was mixed in each reaction mixture to stop the reaction and after 15 min at 20 °C the color was developed. Blank and sample were run on spectrophotometer. Optical density (OD) was recorded at 430 and 590 nm for α -carboxylesterase and β -carboxylesterase, respectively ((Younes et al., 2011).

2.5.2.3. Estimation of acid- and alkaline phosphatase activity. Acid phosphatase (ACP) and alkaline phosphatase (AKP) level was assessed in larval homogenates following the protocol as already described by Asakura (1978). ACP activity was measured by adding 50 µl larval homogenate with 50 µl sodium phosphate buffer (50 mM, pH 7.0) and 100 µl of 20 mM p-nitrophenyl phosphate (substrate). To assess AKP activity, 50 µl larval homogenate was added to 50 µl Tris HCl buffer (50 mM, pH 9.0) and 100 µl of 20 mM p-nitrophenyl phosphate (substrate). After that both solution of ACP and AKP were incubated in water bath at 37 °C for 15 min, the enzymatic reaction was stop by mixing 0.5 N NaOH solution. The absorbance (OD) of the resulting clear supernatants of sample and blank was recorded at 440 nm (Younes et al., 2011).

The percentage inhibition of the enzyme activity by the test extracts was calculated as follows:

$$\% \text{ Enzyme inhibition} = \frac{\text{OD of Control larvae} - \text{OD of treated larvae}}{\text{OD of Control larvae}} \times 100$$

2.5.3. Oviposition attraction/deterrence

Five pairs of newly emerged (1–2 days old) adult house flies (males and females) were enclosed in the cage. Soaked cotton swab with 1% plant extract plus milk was provided to the flies. The experiments were performed in three replicates. To control group, swab of cotton immersed in milk and carrier solvent was provided only. After the duration of 24 h, eggs were counted and oviposition percentage was evaluated to estimate deterrent activity using protocol of Arivoli and Tennyson (2013) with some minor amendments.

2.6. Repellency bioassay

2.6.1. Double choice area-preference test

The bioassay for the evaluation of repellent efficacy of the plant extracts was evaluated against larvae of *M. domestica*. It was tested by applying area preference procedure as documented by Mohan and Fields (2002) using 9 cm diameter Whitman No. 1 filter paper. Three different test concentrations of plant extracts in petroleum ether were prepared (5%, 15% and 25%). Half part of the filter paper was treated with respective concentration while control part was poured with only petroleum ether. The treated half discs of filter papers were air dried for 15 min. Then treated and untreated halves were reattached with thin cello tape and complete discs of filter papers were adjusted in Petri dishes (9 cm diameter). Ten 2nd instar larvae of house fly were released in the center of filter paper (both halves) in each petridish and covered.

The experiment was carried out in control condition as dark, 27 ± 1 °C and 50–70% relative humidity. Each experiment was replicated thrice for each plant extract and observations were taken after 15, 24 and 48 h. A little amount of feed was provided on each halve to prevent mortality stress due to hunger.

Percentage repellency (PR) data was calculated by applying this formula (Abbott, 1925).

$$\text{PR} = \frac{\text{NC} - \text{NT}}{\text{NC} + \text{NT}} \times 100$$

where,

NC = no. of larvae on control part

NT = no. of larvae on treated part

2.6.2. Comet assay protocol

Comet assay was performed to assess DNA damage following the protocol of Singh et al. (1988). The images were captured and examined at 400× magnification with Komet 5.5 Image Analysis System (Kinetic Imaging, UK) fitted with Olympus BX50 fluorescence microscope equipped with 590 nm barrier filter and 480–550 nm wide band excitation filter. Randomly selected 100 cells per treatment were analyzed. The percentage of DNA in tail (% DNA tail) was used to measure the DNA damage. Percent DNA tail was computed using Komet software version 5.5.

Total comet score was calculated using formula ranging from 0 to 400 arbitrary units.

$$\text{Total DNA Damage Score} = (0 \times n_0 + 1 \times n_1 + 2 \times n_2 + 3 \times n_3 + 4 \times n_4)$$

where,

0–4 is number of comet classes

n0–n4 is the number of comet score in each class

2.6.3. Phytochemical screening of the weed extracts

Qualitative phytochemical analysis of weed extracts (petroleum ether) was carried out by employing standard protocols as prescribed by Harborne (1973), Trease and Evans (1989) and Sofowara (1993).

2.6.4. Test for alkaloids

One ml of 1% HCl was mixed with 3 ml of plant extract in a test tube and then treated with few drops of Meyer's reagent (Potassium mercuric iodine solution). Formation of white yellowish turbidity or precipitate indicated the presence of alkaloids (Siddiqui and Ali, 1997).

2.6.5. Test for terpenoids (Salkowski Test)

Five ml of plant extract was taken in test tube, 2 ml of chloroform (CHCl₃) and 3 ml of Conc. H₂SO₄ was carefully added to form a layer. Reddish brown color at interface indicated the presence of terpenoids (Harborne, 1973).

2.6.6. Test for saponins (Foam test)

To perform test for saponins, 0.5 ml of plant extract was shaken vigorously with 2 ml of distilled water to obtain a stable persistent froth. Retention of foamy lather for 10 min. indicated the presence of Saponins (Sofowara, 1993; Siddiqui and Ali, 1997).

2.6.7. Test for flavonoids

Few drops of sodium hydroxide (NaOH) solution were added to the extract in a test tube. Yellow color becoming colorless when dilute acid added which indicated the presence of flavonoids (Khandelwal, 2004).

2.6.8. Test for tannins

(a) 0.5 ml of crude extract, 1 ml of distilled water in test tube and 1–2 drops of ferric chloride solution were added into it. Appearance of blue and greenish black coloration was an indication of gallic tannins and catecholic tannins respectively.

(b) The test solution was mixed with 1 ml of 1% gelatin solution and 1 ml of 10% NaCl. White precipitate of gelatin indicated the presence of tannins (Trease and Evans, 1989; Sofowara, 1993).

2.6.9. Test for cardiac glycosides (Keller-Killami Test)

Five ml of plant extract, 2 ml of glacial acetic acid and few drop of ferric chloride solution and 2 ml of conc. H₂SO₄ was added along the side of test tube. Formation of a brown ring at the interface showed the presence of glycosides (Trease and Evans, 1989).

2.6.10. Test for phenols

Ferric Chloride Test: 3–4 drops of ferric chloride solution added to crude extract in test tube and shake well. Formation of bluish black color indicated the presence of phenol

2.6.11. Test for quinones

Added 2 ml of test substance (plant extract) conc. H₂SO₄ and shaken well for 5 min, appearance of red color indicated the presence of Quinone.

2.6.12. Test for steroids (Liebermann Test)

For this test was added 10 ml of chloroform in the test solution and then filtered. In a test tube containing 2 ml filtrate, 2 ml of acetic anhydride and few drops of conc. H₂SO₄ were added. Formation of blue green ring specified the presence of steroids (Khandelwal, 2004).

2.6.13. Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR spectrophotometer apparatus was used to detect the characteristic functional groups of the active constituent present in the plant extract based on their vibrational frequencies between the bonds of atoms. The plant extracts prepared in petroleum ether were frozen at –80 °C followed by lyophilization. The IR spectrum of lyophilized extract was obtained by using the FTIR spectrophotometer (Alpha, Bruker, California, USA). The sample was scanned to measure the FTIR spectra in the frequency ranges of 400–4000 cm⁻¹.

2.7. Data analysis

The mortality data of *Musca domestica* obtained from bioassay tests was subjected to Abbot's formula (Abbott, 1925) to obtain corrected mortality. The data of corrected mortality, growth regulatory impact and enzyme assay was analyzed through ANOVA. For oviposition deterrence, methods of Arivoli and Tennyson (2013) were followed with some amendments. Data obtained was subjected to statistical analysis.

$$\text{Oviposition deterring activity \%} = \frac{NC - NT}{NC + NT} \times 100$$

where NC = total number of eggs laid in control group, NT = number of eggs laid in treated group and E = number of eggs laid in treated.

The scoring of comet assay was done by arbitrary units and computer image analysis (Casp^R software). Results statistically analyzed by one factor ANOVA. Mean comet scores of all groups was also analyzed by LSD to isolate significant differences.

3. Results

3.1. Mortality of larvae of *M. domestica* (L.) exposed to weed plant extracts

This experiment was conducted to evaluate insecticidal bioactivities of *Peganum harmala*, *Datura stramonium*, *Tribulus terrestris* and *Chenopodium murale*. *A. indica* (Neem plant) was used as a reference plant against 2nd instar larvae of *Musca domestica* Linn. All the tested weed plant extracts were found substantially fatal at all exposure times and concentrations. The mortality was increased with increase in concentration. Longest exposure time of 72 h showed highest mortality followed by 48 and 24 h, respectively. At the highest concentration (25%), *P. harmala* showed highest mean mortality (63.87%) of all the exposure periods followed by *Datura stramonium*, (62.78%), *A. indica* (53.84%), *Tribulus terrestris* (41.86%) and *C. murale* (4.09%), respectively. At 5% concentration, highest mean mortality was shown by *P. harmala* (24.24%) followed by *D. stramonium*, (14.72%), *A. indica* (8.03%), *T. terrestris* (3.00%) and *C. murale* (0.00%), respectively. All the plant extracts were found active against *Musca domestica* and their order of toxicity observed was 25% > 20% > 15% > 10% > 5%. The order of toxicity at highest concentration (25%) was as follows: *Peganum harmala* > *D. stramonium* > *A. indica* > *T. terrestris* > *C. murale*. Results suggested that the insecticidal activity of the weed plants against *M. domestica* was time and dose dependent (Table 1).

3.2. Growth regulatory impact of weed plant extracts on *M. domestica*

The growth regulatory impact of weed plant extracts was observed for all the tested weed plants and *Azadirachta indica* (Neem). At 25% concentration, the longest larval duration (9.33 ± 0.33 days) was observed in larvae treated with *P. harmala* and *D. stramonium* followed by *A. indica* (8.67 ± 0.33 days), *C. murale* (8.33 ± 0.33 days) and the shortest larval duration (8.00 ± 0.58 days) was recorded against *T. terrestris* compared to the duration of control (5.33 ± 0.33 days). While in case of pupal duration, a longest pupal duration (7.33 ± 0.33 days) was recorded in both *P. harmala* and *A. indica* as compared to *D. stramonium* (7.00 ± 0.58 days), *T. terrestris* and *C. murale* (6.33 ± 0.33 days). However, at 25% concentration, the longest development period (17.00 ± 0.58 days) was shown by *P. harmala* followed by *D. stramonium* (16.67 ± 0.33 days), *A. indica* (15.67 ± 0.33 days), *T. terrestris* (14.67 ± 0.33 days) and *C. murale* (14.33 ± 0.33 days) At 5% concentration, no significant difference with *P. harmala*, *D. stramonium* and *A. indica* whereas *T. terrestris* and *C. murale* represented no significant difference to each other in development time (12.00 ± 0.58 days) (Table 2).

3.3. Oviposition deterrence activity of plant extracts against *M. domestica*

The oviposition deterrence assay was performed at 5, 15 and 25% concentrations of weed extracts which showed that percent deterrence was increased as the concentration of plant extracts was increased. At 25% concentration, *C. murale*, showed highest oviposition deterrence activity (81.88%) followed by *D. stramonium* (79.58%), *A. indica* (74.10%) *P. harmala*, (68.14%) and *T. terrestris* (48.96%). The oviposition deterrence activity of all selected plant extracts was found significant (Table 3).

3.4. Repellency induced by weed plant extracts against *M. domestica*

The mean percent repellency induced by plant extracts against 2nd instar larvae of *M. domestica* is shown in Table 4. It is evident

Table 1
Mean mortality of 2nd instar larvae of *M. domestica* (L) at various concentrations of weed plant extracts.

Plants	Conc.	Treatment			Mean
		T1	T2	T3	
<i>A. indica</i> (F = 51.07; df = 4; P < 0.05)	5%	0.00 ± 0.00f	6.67 ± 3.33ef	17.42 ± 3.37def	8.03 ± 2.88D
	10%	3.33 ± 1.67f	18.33 ± 4.41def	27.54 ± 4.46de	16.40 ± 3.99CD
	15%	6.67 ± 1.67ef	18.33 ± 7.26def	51.13 ± 4.46bc	25.38 ± 7.11BC
	20%	8.33 ± 1.67ef	33.33 ± 4.41 cd	61.24 ± 4.46b	34.30 ± 7.87B
	25%	18.33 ± 1.67def	56.67 ± 7.26b	86.52 ± 6.08a	53.84 ± 10.2A
	Mean	7.33 ± 1.75C	26.67 ± 5.06B	48.77 ± 6.80A	
<i>P. harmalia</i> F = 22.4; df = 4; P < 0.05)	5%	6.67 ± 1.67	21.67 ± 1.67	44.39 ± 2.92	24.24 ± 5.59 ^c
	10%	10.00 ± 5.00	25.00 ± 7.64	52.81 ± 6.08	29.27 ± 7.02C
	15%	20.00 ± 5.00	43.33 ± 1.67	67.98 ± 9.38	43.77 ± 7.59B
	20%	26.67 ± 4.41	46.67 ± 7.26	76.41 ± 7.35	49.91 ± 7.92B
	25%	33.33 ± 4.41	63.33 ± 9.28	94.94 ± 5.06	63.87 ± 9.49A
	Mean	19.33 ± 3.12C	40.00 ± 4.73B	67.31 ± 5.36A	
<i>D. stramonium</i> (F = 55.53 + df = 4; P < 0.05)	5%	15.00 ± 0.00 fg	5.00 ± 0.00 g	24.17 ± 0.00efg	14.72 ± 2.77C
	10%	5.00 ± 2.89 g	15.00 ± 2.89 fg	41.02 ± 3.37cde	20.34 ± 5.58C
	15%	11.67 ± 1.67 fg	30.00 ± 5.00def	61.24 ± 9.38bc	34.30 ± 7.87B
	20%	23.33 ± 6.01efg	50.00 ± 7.64 cd	81.46 ± 4.46ab	51.60 ± 8.95A
	25%	30.00 ± 7.64def	58.33 ± 4.41bc	100.00 ± 0.08a	62.78 ± 10.5A
	Mean	17.00 ± 2.92C	31.67 ± 5.68B	61.58 ± 7.50A	
<i>T. terrestris</i> (F = 46.97; df = 4; P < 0.05)	5%	0.00 ± 0.00e	0.00 ± 0.00e	9.00 ± 2.92de	3.00 ± 1.72C
	10%	0.00 ± 0.00e	8.33 ± 3.33de	17.43 ± 4.46cde	8.59 ± 2.99C
	15%	11.67 ± 4.41de	20.80 ± 3.37 cd	35.96 ± 4.46bc	22.81 ± 4.10B
	20%	18.33 ± 3.33cde	25.85 ± 6.08bcd	44.39 ± 5.05b	29.52 ± 4.60B
	25%	25.00 ± 5.77bcd	32.59 ± 4.46bc	67.98 ± 4.46a	41.86 ± 7.07A
	Mean	11.00 ± 2.98C	17.51 ± 3.50B	34.95 ± 5.79A	
<i>C. murale</i> (F = 9.90; df = 4; P < 0.05)	5%	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00C
	10%	0.00 ± 0.00	0.00 ± 0.00	2.63 ± 1.31	0.88 ± 0.58BC
	15%	0.00 ± 0.00	0.00 ± 0.00	2.63 ± 1.31	0.88 ± 0.58BC
	20%	3.33 ± 1.67	3.33 ± 1.67	2.63 ± 1.31	3.10 ± 0.79AB
	25%	3.33 ± 1.67	5.00 ± 0.00	3.94 ± 0.00	4.09 ± 0.54A
	Mean	1.33 ± 0.59A	1.67 ± 0.63A	2.36 ± 0.52A	

Table 2
Growth regulatory impact of weed plant extracts on *M. domestica* (L.).

Plant name	Duration (days)	Concentration			Control	Significance level P=<0.05;df = 3) F value
		5%	15%	25%		
<i>A. indica</i>	Larval days	7.33 ± 0.33B	7.67 ± 0.33AB	8.67 ± 0.33A	5.33 ± 0.33C	17.58
	Pupal days	5.67 ± 0.33B	6.00 ± 0.00B	7.33 ± 0.33A	4.67 ± 0.33C	14.56
	Dev. time (days)	12.67 ± 0.67B	13.00 ± 0.58B	15.67 ± 0.33A	10.00 ± 0.00C	24.17
<i>P. harmala</i>	Larval days	7.33 ± 0.33B	7.67 ± 0.33B	9.33 ± 0.33A	5.33 ± 0.33C	24.25
	Pupal days	6.33 ± 0.33A	6.67 ± 0.33A	7.33 ± 0.33A	4.67 ± 0.33B	11.58
	Dev. time (days)	13.67 ± 0.33B	14.33 ± 0.33B	17.00 ± 0.58A	10.00 ± 0.00C	59.93
<i>D. stramonium</i>	Larval days	7.67 ± 0.33B	8.33 ± 0.33AB	9.33 ± 0.33A	5.33 ± 0.33D	26
	Pupal days	5.33 ± 0.33BC	6.33 ± 0.33AB	7.00 ± 0.58A	4.67 ± 0.33C	6.44
	Dev. time (days)	13.00 ± 0.58B	14.00 ± 0.00B	16.67 ± 0.33A	10.00 ± 0.00C	68.25
<i>T. terrestris</i>	Larval days	7.00 ± 0.58A	7.67 ± 0.33A	8.00 ± 0.58A	5.33 ± 0.33B	6.33
	Pupal days	5.33 ± 0.33AB	5.67 ± 0.33AB	6.33 ± 0.33A	4.67 ± 0.33B	4.33
	Dev. time (days)	12.00 ± 0.58B	12.67 ± 0.33B	14.67 ± 0.33A	10.00 ± 0.00C	26.67
<i>C. murale</i>	Larval days	6.33 ± 0.33B	6.33 ± 0.33B	8.33 ± 0.33A	5.33 ± 0.33B	14.25
	Pupal days	5.33 ± 0.33AB	5.33 ± 0.33AB	6.33 ± 0.33A	4.67 ± 0.33B	4.25
	Dev. time (days)	12.00 ± 0.58B	12.33 ± 0.67B	14.33 ± 0.33A	10.00 ± 0.00C	14.17

that percent repellency of first two exposure periods of 1st h and 12 h was considerably different. The repellency potential of weed extracts had direct relation with concentration while indirectly related to exposure time. The highest repellent effect was observed after 1st hour at 25% concentration among all tested plants. The highest mean repellency at 25% concentration was shown by *P. harmala* (76.67%) followed by *D. stramonium* (73.16%), *A. indica* (58.55%), *T. terrestris* (48.89%) and *C. murale* (25.82%), respectively. The mean repellency observed at all the concentrations was lowest at 48 h than 1st, 12th and 24 h. Overall, results showed that repellency was highest during 1 h and decreased after 12, 24 and 48 h (1st h > 12 h > 24 h > 48 h).

3.5. Enzyme assay

The effect of the tested weed plants on the enzymatic activity (AChE, AcP, AkP, α -Carboxyl, and β -Carboxyl) in 2nd instar larvae of *M. domestica* was observed at 5, 15 and 25% of concentrations. Maximum decrease was observed at 25% concentration of extracts. *D. stramonium* induced maximum decrease (59.07%) in acetylcholine esterase followed by *P. harmala* (48.10%). Maximum decrease in ACP was induced by *P. harmala* (53.95%), than *D. stramonium* (48.64%) followed by *A. indica* (41.61%), *T. terrestris* (24.20 ± 5.02) and *C. murale* (17.66%), respectively. However, *D. stramonium*, induced maximum percent inhibition activity of AKP

Table 3The percentage of Oviposition deterrence activity of weed plant extracts against *M. domestica* (L.).

Plant name	Concentration of extract		
	5%	15%	25%
<i>Azadirachta indica</i> (F = 182.31; d.f. = 3; P < 0.05)	54.59 ± 3.47C	57.96 ± 2.51C	74.10 ± 3.42B
<i>Datura stramonium</i> (F = 170.55; d.f. = 3; P < 0.05)	59.33 ± 2.62C	65.26 ± 1.58C	79.58 ± 4.23B
<i>Penganum harmala</i> (F = 205.55; d.f. = 3; P < 0.05)	40.25 ± 3.03C	46.50 ± 3.23C	68.14 ± 4.05B
<i>Tribulus terrestris</i> (F = 502.64; d.f. = 3; P < 0.05)	40.60 ± 1.86C	45.45 ± 1.40BC	48.96 ± 0.86B
<i>Chenopodium murale</i> (F = 230.64; d.f. = 3; P < 0.05)	74.99 ± 1.72B	80.09 ± 1.22B	81.88 ± 1.56B
Control	153.67 ± 4.18A	153.67 ± 4.18A	153.67 ± 4.18A

Table 4Repellency data regarding different concentration and exposure of time against 2nd instar larvae of *M. domestica* (L.).

Weed plants	Conc.	Time				Mean
		1 h	12 h	24 h	48 h	
<i>Azadirachta indica</i> (F = 68.31 ; d.f. = 4; P < 0.05)	5%	20.00 ± 7.70	15.56 ± 4.44	6.67 ± 0.00	6.67 ± 0.00	12.22 ± 2.57E
	10%	37.78 ± 4.45	28.89 ± 4.44	15.56 ± 4.44	15.56 ± 4.44	24.44 ± 3.42D
	15%	55.67 ± 0.00	46.56 ± 4.44	24.44 ± 4.44	20.00 ± 7.70	36.67 ± 4.96C
	20%	64.56 ± 4.44	55.44 ± 4.44	33.89 ± 4.44	28.33 ± 1.93	45.56 ± 4.79B
	25%	68.44 ± 4.44	64.89 ± 4.44	58.63 ± 1.01	42.22 ± 4.45	58.55 ± 3.47A
	Mean	49.29 ± 4.48A	42.26 ± 5.82A	27.83 ± 4.89B	22.55 ± 3.80B	
<i>Penganum harmala</i> (F = 50.70; d.f. = 4; P < 0.05)	5%	20.00 ± 7.70	15.56 ± 4.44	12.22 ± 4.45	11.11 ± 4.44	14.22 ± 3.05D
	10%	37.78 ± 4.45	28.89 ± 4.44	24.00 ± 7.70	20.44 ± 4.44	27.78 ± 3.05C
	15%	64.44 ± 16.0	60.00 ± 15.4	37.78 ± 8.89	37.78 ± 4.45	50.00 ± 6.38B
	20%	91.11 ± 4.44	77.78 ± 4.45	51.11 ± 8.89	46.67 ± 7.70	66.67 ± 6.25A
	25%	95.56 ± 4.44	86.67 ± 7.70	68.89 ± 4.44	55.56 ± 4.44	76.67 ± 5.22A
	Mean	61.78 ± 8.52A	53.78 ± 8.01A	38.80 ± 6.79B	34.31 ± 4.67B	
<i>D. stramonium</i> (F = 110.40 d.f. = 4; P < 0.05)	5%	37.78 ± 2.26f	33.33 ± 0.00f	12.22 ± 4.45 h	6.67 ± 0.00gh	22.50 ± 4.86E
	10%	55.56 ± 3.13 cd	42.22 ± 4.45ef	15.56 ± 2.94 g	15.56 ± 2.94 g	32.22 ± 5.42D
	15%	68.89 ± 4.44b	64.44 ± 4.44bc	33.33 ± 1.93f	33.33 ± 0.00f	50.00 ± 5.24C
	20%	91.11 ± 4.44a	68.89 ± 4.44b	44.78 ± 4.45f	37.22 ± 2.45def	60.50 ± 6.60B
	25%	100.00 ± 0.00a	73.33 ± 7.70b	68.21 ± 8.21b	51.11 ± 4.84de	73.16 ± 5.90A
	Mean	70.67 ± 6.21A	56.44 ± 4.59B	34.82 ± 6.26C	28.77 ± 4.61C	
<i>Tribulus terrestris</i> (F = 38.90; d.f. = 4; P < 0.05)	5%	15.56 ± 4.44efg	11.11 ± 4.44 fg	6.67 ± 0.00 g	6.67 ± 0.00 g	10.00 ± 1.74D
	10%	24.44 ± 4.44def	15.56 ± 4.44efg	11.11 ± 4.44 fg	11.11 ± 4.44 fg	15.56 ± 2.51D
	15%	37.78 ± 4.45 cd	24.44 ± 4.44def	20.00 ± 0.00efg	15.56 ± 4.44efg	24.44 ± 3.00C
	20%	60.00 ± 7.70b	28.89 ± 4.44cde	24.44 ± 4.44def	24.44 ± 4.44def	34.44 ± 5.05B
	25%	82.22 ± 4.45a	42.22 ± 4.45c	42.89 ± 4.44cde	28.22 ± 11.76c	48.89 ± 6.73A
	Mean	44.00 ± 6.79A	24.44 ± 3.36B	21.02 ± 2.56B	17.20 ± 4.11B	
<i>Chenopodium murale</i> (F = 16.68; d.f. = 4; P < 0.05)	5%	11.11 ± 4.44	11.11 ± 4.44	6.67 ± 4.45	2.22 ± 0.00	7.78 ± 1.98C
	10%	24.44 ± 4.44	15.56 ± 4.44	11.11 ± 4.44	11.11 ± 4.44	15.56 ± 2.51B
	15%	28.89 ± 4.44	20.00 ± 7.70	15.06 ± 3.86	13.56 ± 4.44	19.38 ± 2.91AB
	20%	33.33 ± 0.00	24.44 ± 4.44	20.00 ± 2.50	15.00 ± 0.00	23.19 ± 2.30A
	25%	37.78 ± 4.45	28.89 ± 4.44	20.62 ± 1.74	16.00 ± 0.00	25.82 ± 3.64A
	Mean	27.11 ± 2.87A	20.00 ± 2.60B	14.69 ± 1.81C	11.57 ± 1.75C	

followed by *A. indica*, *P. harmala*, *T. terrestris* and *C. murale*, respectively. The decreasing order was observed as 46.72% < 44.06% < 35.35% < 20.71% < 12.88%, respectively. In addition, maximum decrease in the activity of α -Carboxyl, and β -Carboxyl was also observed with *D. stramonium* followed by *P. harmala* (Table 5).

3.6. Comet assay

House fly larvae were exposed to extracts of *P. harmala*, *D. stramonium* and *A. indica* to evaluate the genotoxic effects in individual cells using comet assay. Three different concentrations (5%, 15%, 25%) were used to analyze the migration of DNA fragments by agarose gel-electrophoreses (Table 6).

Tail length (TL): The highest mean value of tail length was induced by *P. harmala* (7.60 μ m) at 5% concentration followed by *D. stramonium* (6.20 μ m) and *A. indica* (4.60 μ m). The highest mean value of tail length (10.20 μ m) was observed at 25% concentration by *P. harmala* followed by *D. stramonium* (9.20 μ m) and *A. indica* (7.80 μ m), respectively.

Tail DNA%: At 5% concentration, *P. harmala* was found most active (6.32%) amongst all other tested weed extracts compared to *D. stramonium* (5.48%) and *A. indica* (5.45%) Tail DNA. However,

at 25%, *D. stramonium*, *P. harmala* and *A. indica* forced to move DNA as percentage ratios of 10.67%, 10.56% and 8.11%, respectively. The tail DNA values show that both *D. stramonium*, and *P. harmala* have almost similar results.

Tail moment: The mean T.M values compared to control (0.10) at 5% concentration were observed in *P. harmala* (0.47) followed by *D. stramonium* (0.33), and *A. indica* (0.25), respectively. While at 25% concentration, highest mean TM value was found in *P. harmala* (1.07) followed by *D. stramonium* (0.90) while the lowest mean value was observed in *A. indica* (0.62) (Table 6).

The overall genotoxic trend of TL, Tail DNA% and TM of tested weeds at three concentrations were as followed: *P. harmala* > *D. stramonium* > *A. indica*, 25% > 15% > 5% respectively. It was found that with the increase of concentration the value of TM also increases.

3.7. Phytochemical analysis of weed plant extracts

The qualitative tests for phytochemical components of petroleum ether extract of five weed plants viz: *Azadirachta indica* (Reference plant), *Penganum harmala*, *Datura stramonium*, *Tribulus terrestris* and *Chenopodium murale* revealed positive result for the

Table 5
Effect of different concentrations of plant extracts on the percent inhibition of enzymatic activity in *M. domestica* (L.).

Plants	Enzyme	Concentration			Mean
		5%	15%	25%	
<i>A. indica</i> (F = 9.70; d.f. = 4; P < 0.05)	ACh E	13.93 ± 1.26f	27.52 ± 2.42def	58.51 ± 3.12ab	33.32 ± 6.70B
	ACP	20.74 ± 2.59ef	34.08 ± 2.06cde	70.00 ± 1.28a	41.61 ± 7.43A
	AKP	16.10 ± 1.33f	46.97 ± 1.89bc	69.13 ± 1.68a	44.06 ± 7.73A
	α-Carboxy.	26.74 ± 3.31ef	40.63 ± 3.01 cd	57.64 ± 5.12ab	41.67 ± 4.88A
	β-Carboxy.	23.92 ± 3.49ef	34.51 ± 2.83cde	45.49 ± 1.04bc	34.64 ± 3.39B
	Mean	20.28 ± 1.60C	36.74 ± 1.99B	60.15 ± 2.63A	
<i>P.harmala</i> (F = 28.21; df = 4; P < 0.05)	ACh E	35.15 ± 1.45ef	46.35 ± 2.58cde	62.80 ± 1.43b	48.10 ± 4.13B
	ACP	29.63 ± 2.96fgh	52.96 ± 3.23bcd	79.26 ± 0.37a	53.95 ± 7.28A
	AKP	17.23 ± 1.68 h	30.11 ± 3.70 fg	58.71 ± 1.55bc	35.35 ± 6.26D
	α-Carboxy.	21.18 ± 2.43gh	36.46 ± 3.01ef	59.38 ± 1.80b	39.01 ± 5.68CD
	β-Carboxy.	30.59 ± 4.08 fg	40.78 ± 0.78def	57.65 ± 1.36bc	43.01 ± 4.14BC
	Mean	26.76 ± 2.03C	41.33 ± 2.36B	63.56 ± 2.21A	
<i>D. stramonium</i> (F = 10.33, df = 4; P < 0.05)	ACh E	48.74 ± 1.04bcd	61.13 ± 1.67abc	67.34 ± 1.67ab	59.07 ± 2.83A
	ACP	24.07 ± 1.62f	48.15 ± 4.07 cd	73.70 ± 2.67a	48.64 ± 7.32B
	AKP	25.38 ± 0.76f	37.50 ± 8.68def	77.27 ± 3.28a	46.72 ± 8.28B
	α-Carboxy.	25.69 ± 4.00f	45.83 ± 1.80cde	59.72 ± 3.52abc	43.75 ± 5.20B
	β-Carboxy.	27.84 ± 3.06ef	45.10 ± 3.42cde	52.94 ± 4.75bcd	41.96 ± 4.17B
	Mean	30.35 ± 2.64C	47.54 ± 2.71B	66.20 ± 2.70A	
<i>T. terrestris</i> (F = 10.17, df = 4, P < 0.05)	ACh E	16.31 ± 0.83cde	21.55 ± 1.26bcd	30.62 ± 2.19ab	22.83 ± 2.23A
	ACP	8.52 ± 1.96de	22.96 ± 4.55bc	41.11 ± 3.40a	24.20 ± 5.02A
	AKP	9.47 ± 3.12cde	22.16 ± 1.43bcd	30.49 ± 1.37ab	20.71 ± 3.24A
	α-Carboxy.	8.68 ± 2.11de	15.28 ± 1.25cde	18.06 ± 3.82b-e	14.01 ± 1.91B
	β-Carboxy.	5.10 ± 1.96e	14.90 ± 3.74cde	21.96 ± 3.06bcd	13.99 ± 2.87B
	Mean	9.62 ± 1.27C	19.37 ± 1.42B	28.45 ± 2.40A	
<i>C. murale</i> (F = 6.39; d.f. = 4; P < 0.05)	AChE	5.82 ± 2.27ef	12.73 ± 1.49c-f	29.43 ± 4.38ab	15.99 ± 3.81AB
	ACP	3.33 ± 1.70f	15.19 ± 3.16cde	34.45 ± 3.39a	17.66 ± 4.75A
	AKP	5.49 ± 1.55ef	12.12 ± 1.36c-f	21.02 ± 1.18bc	12.88 ± 2.35ABC
	α-Carboxy.	5.56 ± 0.92ef	11.46 ± 2.08c-f	18.06 ± 0.69 cd	11.69 ± 1.93BC
	β-Carboxy.	3.14 ± 0.39f	9.02 ± 1.41def	18.04 ± 1.96 cd	10.07 ± 2.28C
	Mean	4.67 ± 0.65C	12.10 ± 0.93B	24.20 ± 2.04A	

Table 6
DNA damage and comet parameters of *M. domestica* (L.) larvae at different concentrations of weed plant extracts.

Weed name	Comet parameter	Control	5%	15%	25%
		Mean ± S.E			
<i>P. hermala</i>	L. Tail (μm) (F = 23.96 + d.f. = 3; P < 0.05)	3.60 ± 0.24c	7.60 ± 0.93b	8.60 ± 0.40ab	10.20 ± 0.49a
	Tail DNA % (F = 41.43; d.f. = 3; P < 0.05)	1.14 ± 0.56c	6.32 ± 0.65b	9.05 ± 0.56a	10.56 ± 0.77a
	T.M (μm) (F = 34.42; .f. = 3; P < 0.05)	0.10 ± 0.05c	0.47 ± 0.04c	0.78 ± 0.08b	1.07 ± 0.10a
<i>D. stromium</i>	L. Tail (μm) (F = 22.55 + f. = 3; P < 0.05)	3.60 ± 0.24c	6.20 ± 0.73b	7.60 ± 0.51b	9.20 ± 0.37a
	Tail DNA% (F = 21.74; d.f. = 3; P < 0.05)	1.14 ± 0.56c	5.48 ± 0.34b	8.71 ± 0.37a	10.67 ± 1.62a
	T.M (μm) (F = 11.29; .f. = 3; P < 0.05)	0.10 ± 0.05c	0.33 ± 0.04b	0.65 ± 0.04a	0.90 ± 0.19a
<i>A. indica</i>	L. Tail (μm) (F = 8.7 + d.f. = 3; P < 0.05)	3.60 ± 0.24c	4.60 ± 0.68bc	5.80 ± 0.86b	7.80 ± 0.49a
	Tail DNA (F = 22.29; d.f. = 3; P < 0.05)	1.14 ± 0.56c	5.45 ± 0.38b	6.40 ± 0.64ab	8.11 ± 0.85a
	T.M μm (F = 17.12; d.f. = 3; P < 0.05)	0.10 ± 0.05c	0.25 ± 0.04bc	0.36 ± 0.05b	0.62 ± 0.07a

constituents i.e. flavonoids, saponins, tannins, steroids, cardiac glycosides, alkaloids, anthraquinones and terpenoid (Table 7). Flavonoids and Alkaloids were found present in extracts of all tested weed plants. Saponins, Tanins and cardiac glycosides were absent in the extract of *P. hermala*, while steroids were not detected in *D. stromium*. Cardiac Glycosides were not found in *P. hermala* and *T. terrestris* extracts respectively. Only *C. murale* showed negative

test for anthraquinones. The terpenoids were not detected in *P. hermala* and *C. murale*.

3.8. Fourier Transform Infrared Spectroscopy (FTIR)

The crude extract of the weed plants are used to determine organic and inorganic compounds by Fourier transform infrared

Table 7
Phytochemical constituents of petroleum ether extract of selected weed plants.

Sr. No.	Weed Plants	Chemical Constituents							
		Fl	Sa	Tn	St	CG	Al	Anth	Ter
1	<i>A. indica</i>	+	+	+	+	+	+	+	+
2	<i>P. hermala</i>	+	-	-	+	-	+	+	-
3	<i>D. stromium</i>	+	+	+	-	+	+	+	+
4	<i>T. terrestris</i>	+	+	+	+	-	+	+	+
5	<i>C. murale</i>	+	+	+	+	+	+	-	-

Fl = flavinoids, Sa = saponins, Tn = tannins, St = steroids, CG = Cardiac glycosides, Al = alkaloids, Anth = anthraquinones, Ter = terpenoids, + = Present and - = Absent.

spectroscopy (FTIR). Generally, the FTIR analysis looks at the vibration of functional groups present in organic molecules and searches the structural alterations as the function of shifts in wave number. So, the resultant peaks specify the functional groups present in organic and inorganic compounds. The FTIR spectroscopic analysis of *P. hermala* extract revealed the presence of various chemical constituents (Fig. 1). The intense absorption bands at 2916.49 cm^{-1} corresponds to CH group stretching due to CH_3 and 1607.54 cm^{-1} represents C=O stretching. The absorption band at 1317.55 cm^{-1} denotes the presence of C–N bond vibration possibly due to NH_2 , $=\text{NH}$, or $\equiv\text{N}$ absorption. Similarly, for *Datura stromium*, the peaks at 2919.69 cm^{-1} merged O–H stretching correspond to C–H stretching, 1634.73 cm^{-1} represents carbonyl (C=O) group, 1323.99 cm^{-1} assigned to C–H group and 1017.97 cm^{-1} to aromatic in-plane C–H bending (Fig. 2). FTIR profile of *Azadirachta indica* is also shown in Fig. 3.

4. Discussion

The synthetic pesticides have been considered better option for the management of insect pests; but however, employment of pes-

ticides poses serious concerns to the environment. The selection of new pesticides that fulfill the requirements of economy, safety, and efficacy highlights the objectivity of farmers. Therefore, now-a-days biopesticides are being used to control different insect pests (Needham et al., 2004; Anjum et al., 2010; Alves et al., 2014). Bio-insecticides, in fact, are pesticides originated from natural resources like plants, bacteria, viruses, animals, and certain minerals. Subsequently, the botanicals has been accepted as the most effective source of chemical compounds and/or products that are used to protect plants against various pests (Isman and Akhtar, 2007).

Neem, *A. indica* was used as a reference to observe their efficacy against house fly. The present study identifies that the weed plants are rich source of various bioactive metabolites that can be degraded into nontoxic compounds which are potentially suitable for integrated pest management (IPM). Similarly, the study also indicates the sensible use of surplus weeds that are commonly eradicated by employing the herbicides because they are regarded as pests in crops (Alves et al., 2014). Since, many scientists as well as entomologists proved the efficacy of plant extracts and essential oils derived from *A.indica*. So, Neem plant was taken as a reference plant for the current study (Sultana et al., 2016).

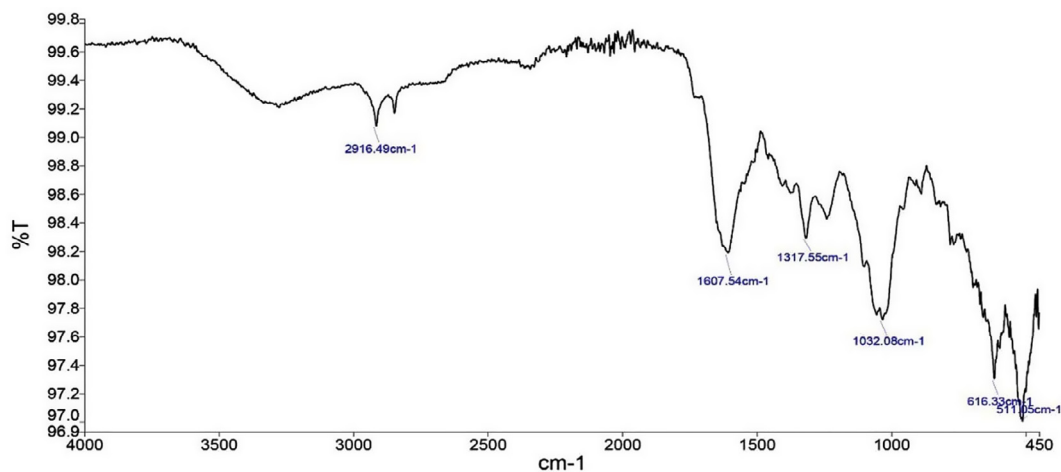


Fig. 1. Full FTIR spectrum of *Peganum harmala*.

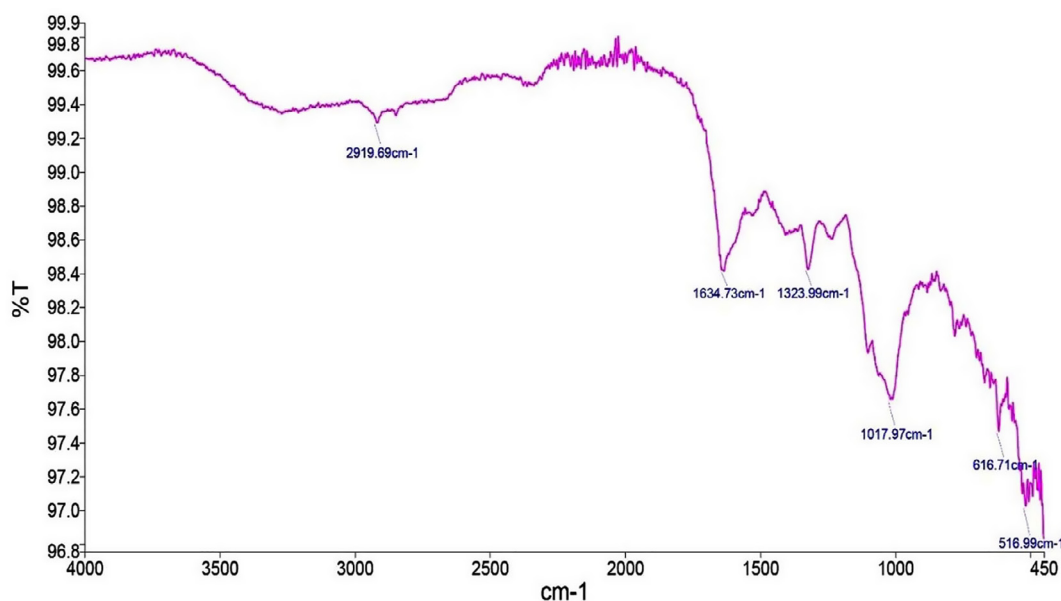


Fig. 2. Full FTIR spectrum of *Datura stromium*.

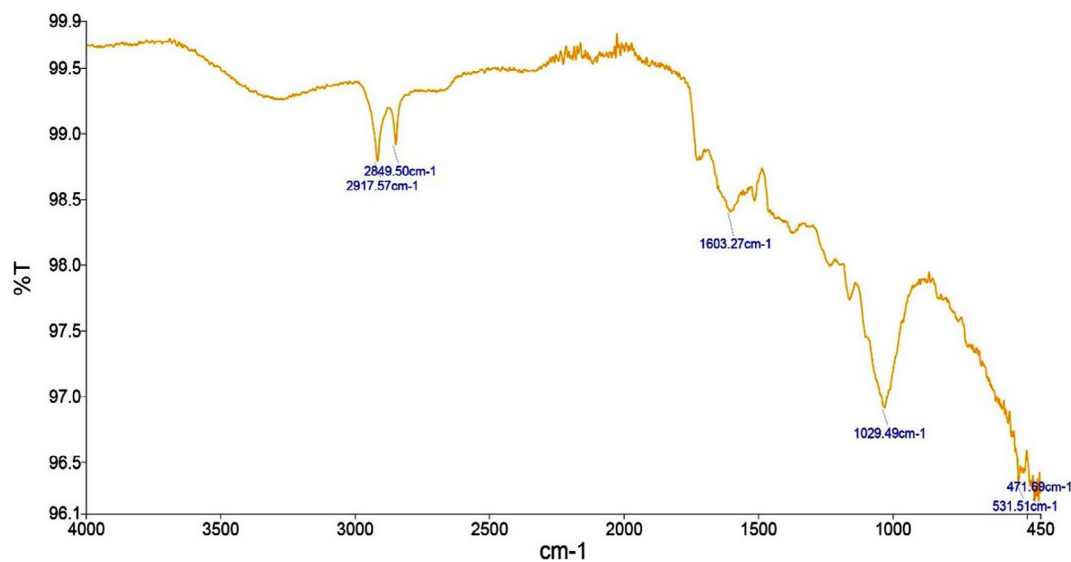


Fig. 3. Full FTIR spectrum of *Azadirachta indica*.

Mortality: The insecticidal activities of extracts obtained from *P. harmala*, *D. stramonium*, *T. terrestris* and *C. murale* were evaluated against 2nd instar larvae of *M. domestica*. *P. harmala* was proved most efficient toxicant with high mortality (63.87%) followed by *D. stramonium* (62.78%), *A. indica* (53.84) and *T. terrestris* (41.86%). Recently, [Riaz et al. \(2018\)](#) investigated the toxicity of some weed plants against *D. melanogaster*. In accordance, the current results showed that mortality of 2nd instar larvae of *Musca domestica* was significantly increased with the increase in dose rate and exposure time. Similar results were obtained by [Caballero-Gallardo et al. \(2012\)](#) who investigated the toxicity of *Cymbopogon flexuosus*, *C. martini*, and *Lippia origanoides* against *T. castaneum*. High mortality rate was recorded at high concentration of extract. Subsequently, the findings are also consistent with the studies of [Lu and Wu \(2010\)](#). They demonstrated that toxicity of *Ailanthus altissima* (Swingle) (Sapindales: Simaroubaceae) bark essential oil against *S. oryzae* adults was increased with exposure time. [Zia et al. \(2013\)](#) described efficacy of citrus peel extracts and revealed that the vapors of essential oil displayed varying toxicity to test insects relying on concentration and exposure duration.

[Mansour et al. \(2011\)](#) compared the toxicity of commercial insecticides with 11 plant extracts against the larval stage of the housefly, *M. domestica*. They described a range of toxicity attributes of the tested plant extracts against the larvae of *M. domestica*. Crude ethanol extracts of *Annona squamosa* and *Calotropis procera* leaves were found toxic and could be used as bio-insecticides against *Musca domestica*. While petroleum-ether extracts of seeds of *Griffonia simplicifolia* and root extract of *Zanthoxylum xanthoxyloides* was found effective repellents and toxicants against the fly with LD₅₀ of 0.28 and 0.35ug/mg, respectively ([Begum et al., 2011](#)).

The current results also showed consistency with the results of [Mamun et al. \(2009\)](#) who evaluated the toxic potential of six botanicals Ghora-neem (*Melia sempervirens*), Bazna (*Zanthoxylum rhetsa*), Hijal (*Barringtonia acutangula*), Mahogoni (*Swietenia mahagoni*), Karanja (*Pongamia pinnatd*), and Neem (*Azadirachta indica*) against *Tribolium castaneum* Herbs. They found that *A. indica* seed extract showed highest toxicity (mortality, 52.50%). [Tripathi et al. \(2002\)](#) investigated the toxic potential of essential oils extracted from turmeric leaves *Curcuma longa* L., against three beetles *Sitophilus oryzae* L., *Rhyzopertha dominica* F., and *Tribolium castaneum* Herbst. It was found that *R. dominica* adults were significantly susceptible to action of *C. longa* leaf oil, having LD₅₀ value of 36.71 ug/mg weight of insect.

The result of current study were lined with [Ahmad et al. \(2015\)](#) who represented the activity of extracts of neem (*Azadirachta indica*), tobacco (*Nicotiana tabacum*), ginger (*Zingiber officinale*), holy basil (*Ocimum sanctum*), jatropha (*Jatropha podagrica*) and turmeric (*Curcuma longa*) on house fly larvae. Their results revealed that the effect of extracts increased with concentration and significantly influenced the life history parameter so that these extracts could be used for the management of housefly. During the present study, growth regulatory impact of weed plant extracts of all the weed plants was observed. *P. harmala* showed longest larval and pupal duration The results obtained was strongly supported by the findings of [Ayvaz et al. \(2010\)](#) and [Alzogaray et al. \(2011\)](#) they suggested that insecticidal potential of the essential oils vary in accordance to species, stage of insect, and the plant origin of the essential oil that would be attributed to the diversified chemical composition of the oils and the interactions in between the individual constituents of the mixture. The insecticidal activity of *N. tabacum*, *A. indica*, *C. citrullus*, *M. azedarachta*, and *E. camaldulensis* was confirmed by [Khan and Marwat \(2004\)](#).

Growth regulation: [Vinuea et al. \(2000\)](#) also investigated that azadirachtin, one of the active metabolite of *A. indica* has disturbing effects on insect development including prolongation of larval or pupal stages and inhibition of moulting. These biochemical metabolites induce elongation of pupation period and adult emergence due to the interference with bioformation of ecdysone hormone by flavonoids which affect cytochrome- P450 involved in control of moulting process in insects. Similar findings were also observed during present study, the longest larval duration (9.33 ± 0.33 days) of *M. domestica* treated with *P. harmala* and *D. stramonium* followed by *A. indica* (8.67 ± 0.33 days). On the other hand, prolonged pupal duration (7.33 ± 0.33 days) was noted in *P. harmala* and *A. indica* as compared to *D. stramonium* (7.00 ± 0.58 days). Whereas *P. harmala* induced maximum development time (17.00 ± 0.58 days) than *D. stramonium* (16.67 ± 0.33 days), *A. indica* (15.67 ± 0.33 days), *T. terrestris* (14.67 ± 0.33 days) and *C. murale* (14.33 ± 0.33 days).

Oviposition deterrence: The results of oviposition deterrence in present work showed that percent deterrence was increased with the increase in concentration of plant extracts. *C. murale* showed highest oviposition deterrence activity (81.88%) followed by *D. stramonium* (79.58%), *A. indica* (74.10%), *P. harmala*, (68.14%) and *T. terrestris* (48.96%). Inhibition of egg-laying and hatchability (99.2% effectiveness at a 0.01% concentration) due to

compound extracted from *Guarea kunthiana* against cattle tick *Rhipicephalus (Boophilus) microplus* is reported by Migueta et al. (2014). *Lantana camara* extract caused significant reduction in number of eggs of *M. domestica* as reported by Elkattan et al. (2011). All of these studies revealed that plant extracts showed oviposition deterrent effect.

Repellency: Of repellent activity, it was found that the repellency potential of extracts was increased as the test concentration was increased. The highest repellent effect was observed after 1st hour at 25% concentration among all used plants. The highest mean repellency at the concentration of 25% after all exposure times was showed by *P. harmala* (76.67%) followed by *D. stromium* (73.16%), *A. indica* (58.55%), *T. terrestris* (48.89%) and *C. murale* (25.82%). The repellent, larvicidal and pupicidal effect of essential oils of plants against *Musca domestica* has already been reported by Kumar et al. (2011). These results were also supported by Sagheer et al. (2013) they investigated acetone extracts of four indigenous plants, *Nicotiana tabacum*, *Pegnum hermala*, *Saussurea costus* and *Salsola baryosma* in different concentrations viz. 2.5, 5, 7.5 and 10% for their repellent and insecticidal effects against *Tribolium castaneum* (Herbst). *P. hermala* imposed significant repellency and mortality of *T. castaneum* (Herbst). Sidra-Tul-Muntaha et al. (2017) also evaluated the repellent and growth regulatory efficiency of five plant extracts (*Pegnum hermala*, *Melia azadirach*, *Azadirachta indica*, *baryosma* and *Zingiber officinale*) and three synthetic insecticides on *Callosobruchus chinensis* populations. *A. indica* was found to be most efficient among plant extracts and showed higher than 90% repellency and upto 80% progeny inhibition at high concentration (20%) level. Current weed plant extracts showed significant repellency and mortality against *M. domestica* larvae and these findings are parallel to the results reported.

Enzyme Activity: These results are related to Riaz et al. (2018) that plant extracts caused reduction in the activities AChE, ACP, AKP, α -Carboxyl, and β -Carboxyl enzymes. The tested weed plant extracts revealed substantial inhibitory impact on target enzymes which was enhanced with increased concentration. Similarly, the enzymes inhibition activity of plant extracts have already reported by Zibae and Bandani (2010) and enhancing the plant extract concentrations enhanced the enzyme inhibition. The present results of enzymes inhibition activity of plant extracts are supported by AChE inhibition activity of *Periplaneta americana* L., at 4 ppm in the cockroach, observed by Shafeek et al. (2004).

Comet assay: The studied weed plants also represented significant DNA damage that are supported by the findings of Dua et al. (2013), reported genotoxicity induced by essential oil of *Psoralea corylifolia* Linn. against *Culex quinquefasciatus*. The observed mean comet tail lengths were $6.2548 \pm 0.754 \mu\text{m}$ and $8.47 \pm 0.931 \mu\text{m}$ and their respective DNA damages were 6.713% and 8.864% compared to control at 0.034 and 0.069 mg/cm². Kumar et al. (2015) also observed genotoxicity of adult flies *Drosophila melanogaster* at 30 and 55 mg/L ethanolic extract of *Acorus calamus*. The mean comet tail length was $4.24 \pm 0.653 \mu\text{m}$ and $6.13 \pm 0.721 \mu\text{m}$ and the respective DNA damage was 5.1% and 7.3% with reference to controls. These findings are parallel to the of present study which showed the mean comet tail lengths of *Penganum harmala*, *Datura stramonium* and *Azadirachta indica* at highest concentration as 10.20 ± 0.49 , 9.20 ± 0.37 and $7.80 \pm 0.49 \mu\text{m}$ while percent DNA damage was 10.56 ± 0.77 , 10.67 ± 1.62 and $8.11 \pm 0.85\%$ respectively. It is also reported that alkaloids are known to cause death of susceptible organisms by their ability to bind to the DNA of the organisms therefore affecting replication and subsequent synthesis.

4.1. Phytochemical screening

The phytochemical current results of petroleum ether extracts of weeds and *A.indica* (Reference plant) revealed the presence of flavo-

noids, alkaloids, cardiac glycosides, saponins, steroids, terpenoids and tannins. Kaur and kalia (2012) evaluated the presence of flavonoids, tannins, saponins and glycosides in phytochemical analysis of petroleum ether extract of arial parts of *C. arvensis*. The concentration of phytochemical compounds in decreasing order was as alcohols > CHCl₃ > petroleum ether. Similarly, Abbas et al. (2012) studied 15 weeds and reported steroids, alkaloids, saponins, flavonoids, glycosides, terpenoids, anthraquinone and tannins from the weed seeds during qualitative phytochemical screening.

FTIR analysis: FTIR analysis was performed for those plants displaying most prominent pesticide potential for the analysis of major secondary bioactive compounds. Mahendra et al. (2016) described that ethanolic extract of *O. corniculata* possess secondary metabolites such as steroids, phenolic, saponins, groups, tannin, flavonoids, coumarins, carbohydrates, alkaloids and terpenoids which were further supported by FTIR analysis. The present results of IR spectra of *P. harmala* are related to the previously reported FTIR analysis. The extract revealed major peaks and possible chemical bonds indicating C—H bending assigned to C=H group, carbonyl (C=O) signifying the ester linkage, C—H group stretching due to alkyl group and aromatic ring having phosphate group. Furthermore, the phytochemical analysis indicated the occurrence of flavonoids, alkaloids and anthraquinones. And these bioactive ingredients can be potentially employed for the management of insect pest (Isman et al., 2007). Based on the current results and supported work reported in the literature; it is suggested that plant-derived bioactive compounds have shown high biological activity against life history attributes of house fly, *Musca domestica*. Thus, the results revealed that weed plants extract can be used as an effective alternative of synthetic pesticides for its control.

Author contributions

MKZ designed and supervised the work. Attaullah performed the experiments. MZ, KR, KS did collection, extraction and helped in chemical as well as data analyses. SQ, MAZ, MSM, HNM helped to perform biochemical assays as well as gave their input during write up. MI gave suggestions and helped during write-up. All authors read and approved the final version of the manuscript

Acknowledgments

The facilities and support provided by the Department of Zoology, Government College University Faisalabad are highly acknowledged.

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