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Toxicity assessment of the methanol extract from *Elaeagnus angustifolia* against larvae of *Drosophila melanogaster* meign (Diptera/Drosophilidae)

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Abstract
The aim of the present study was to evaluate the toxicological potential of the methanol extract of *Elaeagnus angustifolia* plant against the 3rd instar larvae of *Drosophila melanogaster megin* (Diptera/Drosophilidae) by contact method. The larvae were exposed to the treatment of the studied extract for 24, 48, 72, 96 and 120 hours intervals. The mortality rate of larvae, retrieval of pupae and emergence of adults were noted at each interval of treatment. The obtained results indicated that methanol extract of *E. angustifolia* possess toxic potential against *D. melanogaster* larvae. With 1, 2 and 3% concentration of the studied extract, mortality rate was found to be 10, 10, 10 (for 24 hours), 10, 30, 90 (for 48 hours), 10, 50, 90 (for 72 hours), 10, 60, 90 (for 96 hours) and 10, 60, 90 (for 120 hours) respectively. The mortality rate of the larvae indicated that toxicity of the extract increases linearly with the increase of concentration and time period of treatment. This extract can be used against dipterous larvae and for the control of insect pests. The use of botanical extracts as a tool for biological control of pests is environment friendly and has no impaired health risks unlike those associated with chemical control measures. In this way, it is concluded that extract of *E. angustifolia* has toxic potential and can be effectively used for the control of insect pests.

Keywords: Toxicity, *Drosophila melanogaster*, *Elaeagnus angustifolia*, methanol extract, Diptera

1. Introduction
Mosquitoes and flies transmit pathogenic diseases by acting as vectors for the pathogens from one organism to another. Mosquitoes alone transmit various diseases to more than 700 million people annually [1]. Flies have the potential of rapid distribution, high rate of reproduction and good ecological adaptability. Flies are the carriers of more than 100 different types of pathogens and develop diseases in both human beings and animals [2]. *Musca domestica* is responsible for the transmission of protozoan, bacterial, helminthic and viral infections [3], [4].
Fruit flies have a great economic importance by causing damage to fruits and vegetables, especially in the tropical, subtropical and temperate regions of the world. About 81 plant species of family Cucurbitae are affected by fruit flies [5]. In Pakistan, the peach fruit fly has caused 25-30% damage to guava [6]. *D. melanogaster* is the most commonly used experimental insect. Some species of *D. melanogaster* feeds on waste products of animals and humans and on uncooked food thereby acting as a vector for a number of diseases. In Pakistan, *D. melanogaster* causes damage to pulp of different types of fruits, especially guava and banana. In summer season its population surges and posed serious health risks. *D. melanogaster* has great importance in genetics and cell biology and also in the study of toxicology [7]. Life cycle of *D. melanogaster* starts with egg laying inside the fruits, after hatching, the larvae feed on the pulp of fruits and make them useless. Control of the flies is easier at the larval stage particularly just before the pupa formation at the 3rd instar larval stage when they are about to enter in the soil for pupation. This was the reason that 3rd instar larvae were chosen for the toxicity assessment in the present study. The aim of the present work was to evaluate the toxicological potential of methanol extract of *E. angustifolia* against 3rd instar larvae of *D. melanogaster*. Future studies are recommended to ascertain the toxicological and medicinal

aspects of the studied extract. The botanical pesticides are useful against dipterous insects and other pests in respect of minimal risks to environment and biodiversity.

2. Material and Methods

The present study was carried out from March to May, 2016 at the Department of Zoology, Kohat University of Science and Technology, Khyber Pakhtunkhwa, Pakistan. Toxicity assessment in *D. melanogaster* was carried out by contact method. Flies were collected from houses and fruit market of Kohat city and then reared under controlled conditions at the Department of Zoology, Kohat University of Science and Technology (KUST). The flies were reared on its natural diet over ripe banana and guava at room temperature of about 30-35 °C. The flies were placed in glass bottles each of 10cm and 7cm diameter. The mouth of the bottles were covered with a piece of clean cloth by means of rubber bands. The bottles were kept away from direct sun light, heat sources, predator and parasitic organisms.

The methanolic extract of *E. angustifolia* was obtained from the Chemistry Department of KUST. All experiments were conducted on third instar larvae of *D. melanogaster*. Different concentrations of extract such as 1%, 2% and 3% were applied by mixing with one gram of overripe banana which acts as a natural food for rearing, while the dose was kept constant. Then 10 larvae of the same age and size at 3rd instar stage were introduced in separate petri dishes and were marked with respective concentrations of the extract. Each experiment was repeated five times, the control batch for environmental effects and the check batch for solvent toxicity. Readings were taken after 24, 48, 72, 96 and 120 hours interval after treatment with the methanolic extract. With the application of the studied extract at respective concentrations and at each studied time period, readings of the dead larvae were noted and recorded regularly. Recovery of pupae and emergence of adults were also noted regularly for each concentration and studied time period. Each step was repeated five times for respective concentration and given time period. On the basis of the obtained readings, mean mortality rate was calculated for the 3rd instar larval stage of *D. melanogaster*. In a similar way, mean recovery and emergence rates for pupae and adults were also calculated respectively.

3. Results

During laboratory bioassay, the methanolic extract of *E. angustifolia* when mixed with one gram of food (overripe banana) in separate petri dishes, the calculated values of mortality and recovery of pupae indicated that the studied extract possess sufficient insecticidal potential (Table 1, 2). The movement of larvae away from the food in the petri dishes indicated that the subject extract possess sufficient repellent potential as well.

The percent mortality in third instar larvae of *D. melanogaster* at various intervals of treatment and different concentrations of the studied extract is presented in Table 1. The percent recovery of pupae is presented in Table 2 while the emergence of adults is shown in Table 3. The overall results indicate that the methanolic extract of *E. angustifolia* has affected the larval stage of the *D. melanogaster* which ultimately has reduced the recovery of pupae and similarly the emergence of adults. The detailed profile of toxicity of the studied extract against the subject fruit flies in given in the subsequent tables.

Table 1: Toxicity of *Elaeagnus angustifolia* against 3rd instar larvae of *D. melanogaster* at various exposure intervals.

S. No.	Concentration (%)	Mortality of larvae (%) at various intervals of exposure				
		24 Hours	48 Hours	72 Hours	96 Hours	120 Hours
1	1 %	10	10	10	10	10
2	2 %	10	30	50	60	70
3	3 %	10	90	90	90	90

Table 2: Recovery of pupae after application of *E. angustifolia* extract against 3rd instar larvae of *D. melanogaster* at various exposure intervals.

S. No.	Concentration (%)	Recovery of pupae (%) after exposure of larvae to various intervals of treatment				
		24 Hours	48 Hours	72 Hours	96 Hours	120 Hours
1	1 %	20	50	30	30	00
2	2 %	20	20	20	20	10
3	3 %	10	10	10	10	10

Table 3: Emergence of adults after application of *E. angustifolia* extract against 3rd instar larvae of *D. melanogaster* at various exposure intervals.

S. No.	Concentration (%)	Emergence of adults (%) after exposure of larvae to various intervals of treatment				
		24 Hours	48 Hours	72 Hours	96 Hours	120 Hours
1	1 %	00	10	30	60	90
2	2 %	00	00	00	00	10
3	3 %	00	00	00	00	00

4. Discussion

The 3rd instar larval stage of *D. melanogaster* is an ideal stage for the assessment of toxicity and has already studied with Neem extract and chlorpyrifos for melanization and pupation [8]. The rate of mortality and repellent behavior in the present work was dose dependent which can be compared with a previous study [9]. In another study [10], 1% petroleum extract of kuth (*Saussurea lappa*) treated with guava totally inhibited the pupal formation and emergence of fruit flies. The findings can be compared with our present work but the difference lies in the nature of botanical extract used against the fruit flies.

In another study [11], blowfly *Chrysomya megacephala* and housefly *Musca domestica* were subjected to the treatment of neem extracts containing 0.24 percent azadirachtin that reduced larval and pupal survival, adult emergence and pupal weight and caused mortality. In the present work, methanolic extract of *Elaeagnus angustifolia* was used against *Drosophila melanogaster* and produced similar results of larval mortality, adult emergence, pupal recovery as reported in the cited study. The difference lies in the type of the tested

extract and the species of fly under study. Metabolic extract of *Elaeagnus angustifolia* and its several other fractions has been using against bacteria, different fungi and various insects pest as well clarifying the medicinal use of the tested plant [12-17]. The increase in mortality was observed while increase the concentration of the plant extract in the current study but proper investigation of the active compound of the tested plant against insects is needed. Similar reports also have been documented against *Tribolium castaneum* and *Ephestia cautella* [18]. Additionally, the rates of mortalities authenticated the previous findings on the potential activities of *Elaeagnus angustifolia* extract which specifies that this extract can be effectively used for the control of various diseases and pathogens [19].

It has been found that insecticidal, anti-arthritic, antioxidant; wound healing, cardioprotective, hypolipidemic, antinociceptive, antimicrobial anti-inflammatory, antimutagenic and antitumor as well as gastroprotective activities are among the principle natural and pharmacological properties of *Elaeagnus angustifolia* in view of current examinations [21-28]. Thus, it might be considered as a harmless product for human use or mammals when applied for insecticidal or anti-fungal purposes [20].

Conclusion

It can be concluded from the obtained results that methanol extract of *Elaeagnus angustifolia* possess sufficient toxicological potential and can be used effectively against harmful dipterous flies and other insect species. Botanical extracts are environment friendly and don't pose threat to biodiversity and environment so these can be used instead of chemical control measures to secure human beings from the side effects of chemicals. The control of flies at 3rd instar larval stage is easy and effective as compared to pupal and adult stages.

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