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Larvicidal & Development retarding effects of hexane crude extract of Otostegia limbata on 3rd instar larvae of Drosophila melanogaster meign (Diptera: Drosophilidae)

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Abstract
The toxicological evaluation of Otostegia limbata was done against the third instar larvae of Drosophila melanogaster (Meigen) for 24hours of exposure. One ml of Otostegia limbata extract having concentrations of 2%, 3%, 4%, 5% and 6% was mixed with 1 gram of overripe banana fed to Drosophila melanogaster which caused 12%, 24.6%, 44%, 68%, 89% mortality and showed 100% death at higher concentration. While the percent pupation observed were 88%, 73.7%, 60.3%, 48%, and 23.3% respectively at 0.5%, 1%, 1.5%, 1.8% and 2%of extract. The Pupal inhibition and high mortality rate revealed that O. limbata has deleterious effects on insect growth. This is the first work to find the toxicological effects of O. limbata although the other members (plants) of the same species have been evaluated for toxicity, these findings provide the evidence that O. limbata is a good medicinal plant for further investigation against medically important insect pests.

Keywords: *Otostegia*, Drosophila, Toxicology, Plant extracts, Mortality, Pupal inhibition

1. Introduction
In Pakistan among the fruit pests, *Drosophila melanogaster* has caused injury to different types of pulpy fruits particularly guava and banana. They feed on pulps thus rendering the fruits worthless. Occasionally in summer, their population raises much above the expectation, and cause an awful deteriorating of fruits Yasmin *et al*. (1995) [1]. Hassan *et al* reported that during a survey done by FAO, loss done annually by insects and rodent pests is 10 to 25% of world harvested food. In Pakistan, fruit flies cause a loss of around 7 million rupees to growers annually Khan *et al*. (1999) [2]. Therefore, the control of these flies and other insect pests is an important economic and health concern.
For the control of insect pests many researchers have reported on the effectiveness of plant extracts or essential oils against Mosquitoes and *Drosophila melanogaster*. More than 2,400 plant species have been tested for pest-control properties Arun *et al*. (2009) [3]. Among these medicinal plants, the *Lamiaceae* family is extensively used for biological control. In Pakistan, only three species of the *Lamiaceae* plants have been found very effective against the pests till the end of 2009. The main species found are *Otostegia aucheri*, *O. persica* and *O. limbata* (BENTH.). The species *O. limbata* is vernacularly called "spin aghzai" or "chitibooti." that has shown larvicidal activities against several insects. The purpose of the present study was to explore the larvicidal activity of *O. limbata* plant against the 3rd instars larvae of *D. melanogaster* so that the extract potentials might be used for insect control instead of artificial insecticides which have caused multiple adverse effects on the biota of earth.

2. Materials and Methods
2.1. Test Insect
Adult flies of Drosophila melanogaster were collected (10 pairs) from banana fruits at Peshawar fruit market during January to May 2009. All flies were reared under controlled laboratory conditions in the department of Zoology/Toxicology at Kohat University of Science and Technology (KUST), KPK Pakistan. The flies were reared in the glass bottles of size 10x7 cm on medium containing overripe banana which is natural diet for these flies. The open mouths of the bottles were covered with a piece of clean cloth tied by means of rubber bands.

The laboratory temperature was maintained at 25 ^{0}C to 28 ^{0}C with relative humidity of 60 +_5%.

2.2 Larvicidal and pupal bioassays:

Hexane crude extract of *Otostegia limbata* was prepared by Dr. Ijaz Ahmad of Chemistry department, Kohat University of Science and Technology (KUST). Nine different concentrations (0.5%, 1%, 1.5%, 1.8%, 2%, 3%, 4%, 5%, 6%) of the extract were prepared and each mixed with one gram of overripe banana as larval food. Ten 3rd instar larvae of uniform size and age were released in glass Petri dishes, All Petri dishes were marked with respective concentrations of extract. Each concentration was replicated fifteen times. The control batches consisted of only 1 gram of banana without extract while the second batch for solvent toxicity (check) consisted of banana with 1 ml of hexane solvent as the plant extraction was made in hexane and were run parallel with these experiments. If in any experiment, the control and check mortality was more than 10%, the experiment was discarded and repeated again. The mortality of D. *melanogaster* larvae feeding on the medium with and without (control) extract was recorded after 24 hours. The whole experiment was repeated three times for each concentration. Minor observations on the pupa formation/ inhibition were also recorded for 24 hours of larval exposure to the compound.

3. Results

3.1 Mortality Ratio

The percent mortality of 3rd instar larvae of *D. melanogaster* after 24 hours of exposer is presented in table 1 and figure 1, at 6% concentration of extract the mortality was 90% with LC_{50} at 4.5%. The decrease in dose concentration resulted in low larval mortality such that at 5% the percent mortality reduced to 68% while at 4% percent it reduced further to 44%. Gradual decrease in mortality was observed with decrease in dose concentration, such as at 3% concentration the mortality observed was 25% while at 2% concentration the mortality became 12% and at 1% the percent mortality becomes zero. The mortality in control and check batches were less than 10% during all experiments.

3.2 Percent Pupation Ratio

Table 2 and figure 2 reflect the inhibitory properties of the extract indicating the plant antagonistic effect on pupal development because after exposure to the extract the larvae did not convert into pupae. Almost all the mortality was caused at larval stages. The pupal inhibition was inversely proportional to the concentration variation, greater the dose concentration the less pupal formation was observed, such as at 0.5% the pupal formation was highest (88%) and at 1% the pupal formation was 73.7%. When the dose concentration increased the pupal formation further decreased. At a dose concentration of 1.5%, 1.8%, and 2% the pupal formation were 60.3%, 48%, and 23.3% respectively. While pupal formation in the control and checks were high, scoring to more than 90%.

Table 1: Mortality of 3rd instar larvae of *Drosophila melanogaster* after 24 hours feeding on banana media with various concentrations of *Otostegia limbata*

S. NO.	Dose (%)	Average Mortality (%)	S.D	S.E	Confidence limit	
					95%	99%
1.	**2**	**12**	**4**	**1.03**	(9.7,14.2)	(8.93,15.06)
2.	**3**	**24.6**	**4.98**	**1.28**	(21.8,27.5)	(20.78,28.41)
3.	**4**	**44**	**4.89**	**1.26**	(41.19,46.8)	(40.24,47.75)
4.	**5**	**68**	**6.18**	**1.59**	(65.12,72.2)	(63.26,72.73)
5.	**6**	**89.3**	**5.73**	**1.48**	(86.04,92.64)	(84.89,93.71)

Table 2: Percent pupation of *Drosophila melanogaster* after 24 hours feeding on banana media with various concentrations of *Otostegia limbata*

S. No.	Dose (%)	Average Mortality (%)	S.D	S.E	Confidence limit	
					95%	99%
1.	0.5	88	4	1.03	(85.70,90.29)	(84.9,91.1)
2.	1	73.7	5.15	1.33	(70.78,76.68)	(69.7,77.65)
3.	1.5	60.3	3.39	0.87	(58.38,62.28)	(57.71,62.88)
4.	1.8	48	10.45	2.69	(42.00,53.99)	(39.99,56.00)
5.	2	23.3	10.11	2.61	(17.70,28.89)	(15.53,31.1)

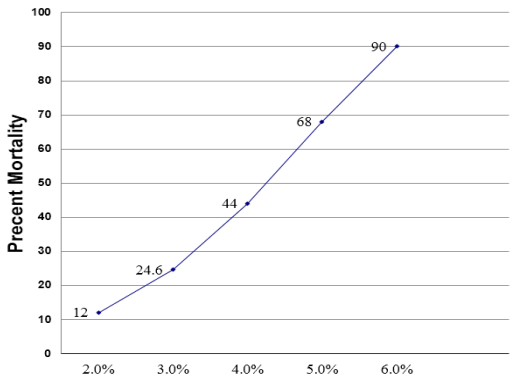

Fig 1: Mortality rate of *Drosophila melanogaster* after 24hours of feeding on banana media with various concentrations of *Otostegia limbata*

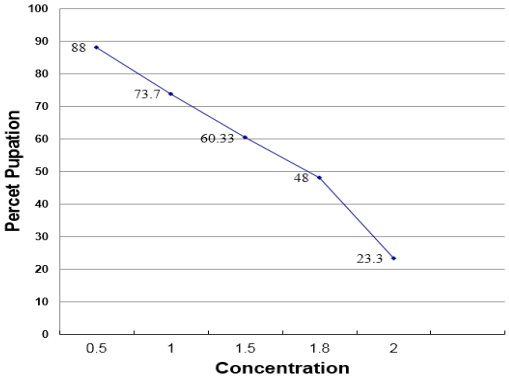

Fig 2: Percent pupation of *Drosophila melanogaster* after feeding on banana media with various concentrations of *Otostegia limbata*

4. Discussion

In the present investigation the hexane crude extraction of *Otostegia limbata* gave noticeable mortality of more than 90% at very low concentration like 6%. Fifty percent (LC_{50}) of the mortality was recorded at 4.5% of the extract which shows that this compound is very effective for the control of *Drosophila* larvae, on the basis of present findings it may be suggested to use this plant for the control of other dipterous insect pests of medical importance such as mosquitoes and houseflies which cause many serious diseases to human being as well as other animals. A number of workers have carried out the toxicological studies using *Lamiaceae* plants. Among such workers (Anees, 2008) [4] evaluated the toxicity of acetone, chloroform, ethyl acetate, hexane, and methanol leaf and flower extracts of *Ocimum sanctum* against fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus*. They found highest larval mortality in leaf extract of *O. sanctum* against *A. aegypti* and *C. quinquefasciatus* larvae. The LC_{50} values were 425.94, 150.40, 350.78, 575.26, and 175.67ppm against the larvae of *A. aegypti* and 592.60, 93.92, 212.36, 76.61, and 82.12 ppm, against the larvae of *C. quinquefasciatus*. The Anees's [4] report is in agreement with the current results because the lethal effects of the hexane crude extract of *O. limbata* against the 3rd instar larvae of *D. melanogaster* observed were 4.5% after 24 hours of exposure. The difference in the LC_{50} of the two studies may be due to the difference in insect species or extract solvent used.

Aroiee *et al*. (2005) [5] evaluated the insecticidal activity of essential oils of some medicinal plants of *Lamiaceae* (fennel, caraway and rosemary) against whitefly. The essential oils of fennel at 7.5 ppm showed 89.82 % mortality, and caraway at 5 ppm caused 82.95% mortality and thus showed highest effect on whitefly fatality, while the essential oils of rosemary at 5 ppm were in second grad with 72.7 % killing effect of whiteflies. These results are similar to the present observations, because *O. limbata* also caused 90% death of the *Drosophila* larvae, but in our research the dose concentration is high (6%).

Palacios *et al*. (2009) evaluated the insecticidal activity of nine essential oils (EOs) from medicinal plants belonging to *Lamiaceae* family against the house fly (*Musca domestica*) and recorded LC_{50} in 30 min from 46.9 mg/dm^3. The essential oils from *M. verticillata* was found as the most potent insecticide (LC_{50}=0.5 mg/dm^3) followed by EOs from *H. multiflora* (LC_{50}=1.3 mg/dm3) and *A. annua* (LC_{50}=6.5 mg/dm^3). These observations are close to the findings of our bioassays because the LC_{50} of *Otostegia limbata* against the 3rd instar larvae of *D. melanogaster* is 4.5% which is in proximity to the above determined values for other species of the same family (*Lamiaceae*), the difference may be due to the method of application.

(Cetin and Yanikoqlu, 2006) [7] examined the insecticidal activity of essential oils of two species of Lamiaceae; (*Origanum onites*, *O. minutiflorum*) against 3rd and 4th instar larvae of mosquito, *Culex pipiens* and found LC_{50} of 24.8 and 61.3 ppm. Their findings are generally in accordance to the present investigated results of *O. limbata* against the 3rd instar larvae of *Drosophila* with the LC_{50} at 4%. Our results are also in agreement with the work of Arabi *et al*. (2008) [8] who reported the toxicological effects of the essential oil from *Perovskia abrotanoides* (Lamiaceae) against *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). The toxicity investigated by them was against 1- to 7-day-old adults of *S. oryzae* and *T. castaneum.* The compound caused considerable mortality, and rate of mortality increased by increasing the concentrations from 32, 161, 322, 483 and 645 µl/l air and with exposure time from 2 to 15 hrs. The lowest concentration (32 µl/l air) of the oil induced 100% mortality of *S. oryzae* and *T. castaneum* after 15 and 8hrs exposure, respectively. The oil at 322 µl/l air caused 100% mortality for *S. oryzae* and *T. castaneum* within 13 and 7 h exposure, respectively. At 645 µl/l air, the LT_{50} values (lethal time for 50% mortality) were 8 and 2.84 h for *S. oryzae* and *T. castaneum***,** respectively, these reported observations are similar to the present work because the crude extract of *Otostegia limbata* at 6% of concentration caused 100% fatality of *Drosophila* larvae after 24 hours of treatment with LC_{50} at 4% extract concentration. Like the above reported description the present research also showed the dependence of mortality rate on the dose concentration as well as time of exposure, when the concentration decreased the reduction in death rate also reduced such as at 2% the death rate became 10%, but when the time of exposure increased the death rate also increased the difference may be due to the species.

The essential oils (EOs) extracted by Pavlidou *et al*. (2004) [9] from *Salvia fruticosa* and *Mentha pulegium* (Mint) were tested for insecticidal effects on the olive fruit fly *Bactrocera oleae* and *Drosophila.* They reported that Mint essential oil and its main constituents were the most effective insecticides against *B.* oleae and *D. melanogaster* and concluded that these oils may be used for the control of other dipterous medical important insects like Mosquito.

The larvicidal activity of plant *O. limbata* has been carried out by many researchers and reported that its larvicidal activity is due to the presence of compounds such as tricyclic cis-clerodane type diterpenoids known as limbatolide A(1), B(2), C(3), oleanic acid and b –sitosterol. They reported that the compounds 1-3 displayed inhibitory potential in a concentration-dependent manner against acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) enzymes, respectively (Viqar *et al*. 2009; Menzoni *et al*. 2004) [10, 11].

In relation to above discussion the similar reports have been given that the constituents of *Lamiaceae* plants caused jerking symptoms that suggest a neurotoxic mode of its action, in this way a monoterpenoid linalool isolated from *O. limbata* was demonstrated to act on the nervous system affecting ion transport and the release of acetylcholine esterase in insects Re *et al.* (2000). According to another report on the mode of action of monoterpenoids isolated from *O. limbata* which indicated the inhibition of acetylcholinesterase enzyme activity as the major site of action Rajendran *et al*. (2008) [13].

Beside mortality, the extract of *O. limbata* also caused an inhibitory effect on pupal development and prevented the larvae to become pupa. Swevers *et al*. (2003); Sawada *et al*. (2003) [14, 15] reported that *Lamiaceae* plants have many ecdysone inhibiting compounds known as ecdysteroid mimetics for controlling the larval growth and inflicting toxicity against various pest species of Diptera and Lepidoptera, they also observed 174 new dibenzoylhydrazine derivatives and other potent compounds against *D. melanogaster.* Other reports from Smagghe *et al*. (1995); Suresh *et al.* (2002) on the ecdysteroid mimetics from *lamiaceae* species in *Drosophila melanogaster* showed growth inhibitory, antifeedant, antioxidant and antiproliferative activities of diterpenes.

5. Conclusion

Investigations on the insecticidal bioassay of crude extract of *O. limbata* (Spin aghzai) against third instars larvae of *D. melanogaster* showed toxicity and pupal inhibition. The results of present study in comparison with other researchers showed

ecdysone inhibiting mechanism of Lamiaceae plants extracts especially *O. limbata* against *D. melanogaster* larvae. The toxicity of crude fraction of Chitibooti against third instar larvae of *Drosophila* in the form of high mortality and pupal delation revealed that these activities may be due to the presence of toxic and ecdysone inhibiting compounds in the extract as reported by researchers who worked on specific compounds like Limonoids ecdysone mimickers present in these plants. It is apparent from the current results and the literature record that essential oils and extracts of *O. limbata* (chitibooti) have strong potential in the production of new safe and environment friendly larvicides and adulticidal compounds that will pose little risk to human health. Products based on *Otostegia limbata* extracts may thus contribute greatly to the reduction of environmental hazards and to an overall reduction in the population density of some insect pests of fruits and others vectoring pathogens of human disease.

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7. Author Contributions

Conceived and designed the experiments: SIA. Performed the experiments: JK. Analyzed the data: JK IK FR. Contributed reagents/materials/analysis tools: SIA. Wrote the paper: JK. Provided suggestions and comments on the manuscript: IK AK.

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