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The Bio-pesticidal potential of ethyl acetate extract of *Peganum harmala* (Zygophyllaceae) seeds against *Brevicoryne brassicae* (Aphididae) in Pakistan

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SUMMARY

A wide diversity of insect pests harm crops. This is diminishing yearly agricultural yields throughout the planet. Existing pesticides' usefulness is constantly being called into question as resistance to them grows. At the same time, these herbicides pose significant environmental and health risks. There arose a need to develop environmentally friendly insecticides. This study looked at the bio-pesticidal properties of an ethyl acetate extract of Peganum harmala seeds against Brevicoryne brassicae. The extract was administered orally at three different doses (5%, 7.5%, and 10%), and percentage mortality was measured at various time intervals. 100% mortality was recorded at 7.5 percent and ten percent at 18 and 14 hours, respectively. The measured LT50 and LT95 values are 6.1 and 12.1 at 10% extract concentration, respectively. It is determined that the phytochemicals of Peganum harmala are prospective candidates for the development of novel biopesticides.

Keywords Agriculture, *Peganum harmala*, *Brevicoryne brassicae*, Biopesticides, Aphids

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INTRODUCTION

Crop yields are reportedly reduced by 35% globally due to insect pests, which also inflict major damage to crops (Egonyu et al., 2022). Due to the growing global population, we must raise food production by 40% (Cerda et al., 2017). Synthetic pesticides negatively impact both the environment and human health. Synthetic pesticides can be replaced with bio-pesticides, which are also safe and efficient (Asimakis et al., 2022; Hussain and Tanveer, 2023). Aphids belonging to the order Homoptera (Aphididae) are a serious threat to many different crops worldwide since they are extremely destructive pests and significantly reduce crop yields, particularly in wheat crops (Kaur et al., 2017).

Aphids reproduce quickly while having a short lifespan, which causes entire crops to be quickly destroyed. Aphids, over 4,000 species, are found on more than 250 host plants worldwide. The weight of wheat grains is reduced by

50% yearly in Pakistan due to aphid damage to wheat harvests (Khan et al., 2015). These pests cause 35-40% direct damage to crops and 20-80% indirect damage by spreading viral and fungi diseases and directly removing cell sap (Ahmad et al., 2016). Aphids like *Lipaphis erysimi* and *Brevicoryne brassicae* severely impair the yields of Brassica and oilseed Brassicas in India (10-90%) and Pakistan (70-80%), with the risk of entire grain loss at harvest (Khan et al., 2015; Dhillon et al., 2018). Aphids also prevent brassicas used for oilseeds from photosynthesis. Previous research has investigated the use of botanicals in crop management to reduce aphid populations (Shah et al., 2017).

Peganum harmala L. is the only member of the genus Peganum (family: Zygophyllaceae) that is salt-tolerating (Pereira, 2018). *P. Harmala* is commonly found in Iran, North Africa, the Middle East, America, Australia, India, and Pakistan (Khan et al., 2017). In the past times, the seeds of this plant were used to treat asthma, cough, and rheumatism, regulate blood supply, and ease pain (Li et al., 2018). It is reported by many workers that *P. harmala* has a lot of pharmacological and therapeutic attributes. These attributes include antibacterial, antifungal, antiparasitic, antiviral, anti-inflammation, antispasmodic, anti-nociceptive, anti-tumoral, anti-cancer, anti-leishmanial, anti-oxidant, wound healing, leukemic healing, hypoglycemic, immune-modulator, analgesic, vasorelaxant, insecticidal and spontaneous effect (Li et al., 2018). *P. harmala* extract contains a large amount of bioactive material as well as a rich source of primary and secondary metabolites (Li et al., 2017). The complete body of this plant, particularly the seed, and roots, has different types of alkaloids (Bournine et al., 2017). The dry weight of alkaloids present in the seed is about 3.92% (Li et al., 2018). Various workers showed the lethal effect of alkaloids extracted from the *P. harmala* against different insect pests *like Plodia interpunctella, Schistocerca gregaria, Tribolium castaneum, Bemisia tabaciand Spodoptera littoralis* (Salari et al., 2012). Some researchers also investigated the repellent and insecticidal effect of *P. harmala* extract against plant-sucking insects such as *Aphis fabae, Aphis gossypii, Aphis nerii,* and *Myzus persicae* (Salari et al., 2012). Due to all these attributes of *P. harmala*, it is considered the best agent to be a good bio-pesticide to control insect pests that are harmful to wildlife, human beings and cropspesticide.

Moreover, existing literature highlights the damaging impact of aphids and the limitations of current pest management practices, necessitating the exploration of alternative and eco-friendly strategies, such as the potential use of Peganum harmala extract as a bio-pesticide. Therefore, this study was designed to evaluate the bio-pesticidal potential of *Peganum harmala* extract against a devastating pest, aphid.

MATERIAL AND METHODS

Sample collection

The seeds of *Peganum harmala* (Harmal) were purchased from the local market of the district of Lahore and brought to the laboratory of the Department of Zoology, University of Education, Lahore. *Brevicoryne brassicae* (Aphids) were collected by the Hand-picking method from the fields of district Kasur and were kept in the laboratory at 32 ℃ and 60% humidity.

Preparation of *Peganum harmala* extract

Seeds were ground and sieved using a 25-mesh sieve and kept in polythene bags. 10 g of seeds were extracted in 50 ml solvent of water and ethyl acetate using the Soxhlet extraction method for 2, 6, and 12 hours and dried under reduced pressure. The extracts were filtered, condensed, and kept in the refrigerator for further use (Hajji et al., 2020)

Insect bioassays

Adult aphids were divided into four groups and one was designated as a control group and three as treatment groups (n=40) containing 10 insects in each group. Each treatment group was treated with 5, 7.5, and 10% plant extract while the control was treated with the solvent only. The insecticidal activity was measured using the Oral method (Simon, 2014). Ten insects were kept in the Petri plates of 9 cm having food. Insect mortalities were observed and recorded at 0, 6,12, and 18 hours after treatment (HAT).

Statistical analysis

The percentage of mortality was done using Abbott's (1987) formula. = (1-individual alive after treatment/no. of the individual in control) x100.

By using probit analysis (Minitab 14.1) LT$_{50}$ and LT95 values were calculated (Finney, 1971). The statistical analysis of experimental data was done by a completely randomized design using SPSS statistical software (version 22). The data were analyzed by one-way ANOVA followed by Duncan's multiple range test (each group is represented by 10 individuals and each condition was triplicate).

RESULTS

Treatment with P. harmala concentrations (5%, 7.5%, and 10%) resulted in concentration-dependent mortality of B. brassicae. At 6 hours, 5% concentration caused 12.5±5.77% mortality, while 7.5% and 10% concentrations exhibited 30±5.77% and 57±5.77% mortality, respectively as depicts in figure 1. Statistical analysis showed significant differences among all groups (P=0.000, F=809.000) compared to the control.

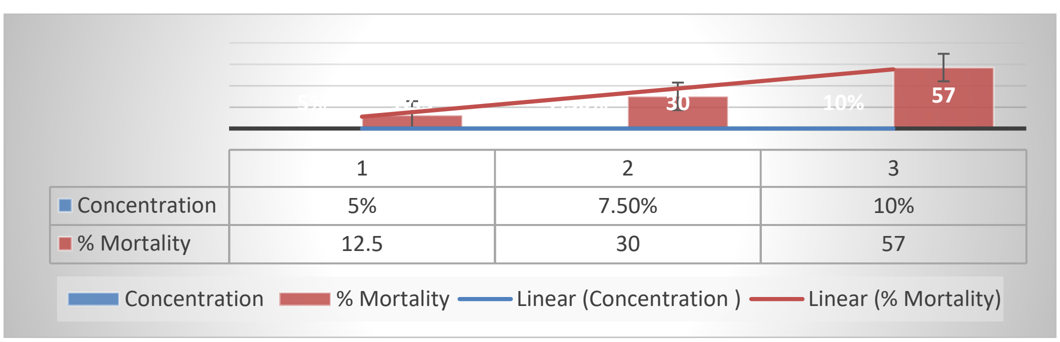

Figure 1: Insecticidal effect of different concentrations of *P. harmala* extract on *B. brassicae* at 6 hours.

After 12 hours, P. harmala concentrations (5%, 7.5%, and 10%) continued to demonstrate concentration-dependent insecticidal effects. Mortality rates were 36.6±5.77%, 73.33±5.77%, and 90±0.0% for 5%, 7.5%, and 10%, respectively as shown in figure 2. Significant differences were found among all groups (P=0.000, F=289.333) compared to the control.

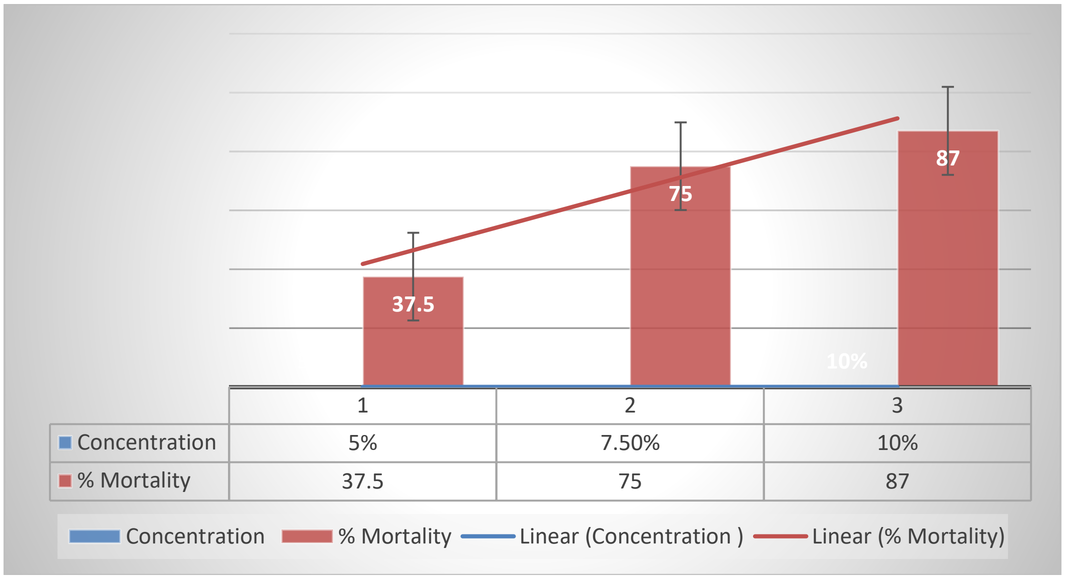

Figure 2: Insecticidal effect of different concentrations of *P. harmala* extract on *B. brassicae* at 12 hours.

Over 18 hours, B. brassicae mortality rates increased with P. harmala concentrations. At 5%, mortality was 76.66±5.77%, while concentrations of 7.5% and 10% achieved 100±0.0% mortality as describes in figure 3. Statistically significant differences were observed among all groups (P=0.000, F=809.000) compared to the control.

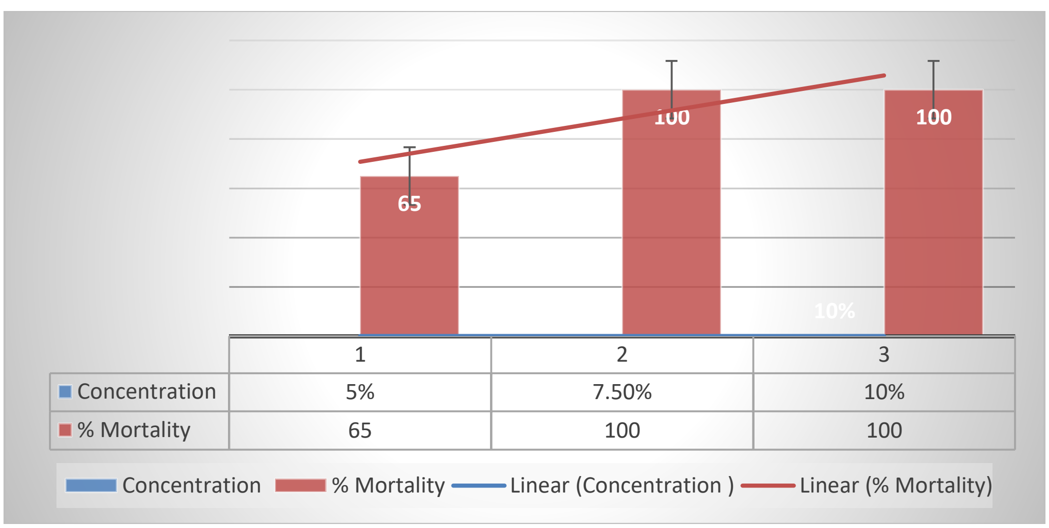

Figure 3: Insecticidal effect of different concentrations of *P. harmala* extract on *B. brassicae* at 18 hours.

The calculated LT_{50} and LT_{95} values for *B. brassicae* are given in Table 1. With 5% concentration, the highest calculated LT_{50} and LT_{95} values for *B. brassicae* were (13.02±1.3) and (21.92±3.08) respectively. It is observed that LT_{50} and LT_{95} values decreased with the increase in extract concentration.

Table 1: Calculated LT_{50} and LT_{95} for *Brevicoryne brassicae* exposed to different concentrations of extract of *Peganum harmala.*

Concentrations	5%	7.5%	10%
LT_{50}	13.1±1.3	9±1.1	6.1±1.10
LT_{95}	21.1±3.8	15.7±2.17	12.1±1.9

Note: The time in the table is in hours. Values after ± in the above table represent the standard error mean.

DISCUSSION

Herbivorous insects can cause substantial damage to food and fiber crops, leading to yield losses ranging from 10% to 90%, with an average impact of approximately 35-40% (Weinberger & Srinivasan, 2009). In Pakistan, aphids have emerged as significant pests, responsible for damaging various crops (Khan et al., 2015). In the realm of pest control, botanical extracts have demonstrated their efficacy against insects, providing a viable alternative to chemical pesticides for managing aphid populations (Khan et al., 2015; Faiz, 2022). Our study focuses on confirming the toxic effects of the seed extract of *P. harmala* on *B. brassicae*. The results show that the mortality of *B. brassicae* was dependent on both the concentration of the extract and the exposure time. The highest mortality rate was observed at a concentration of 10% after 15 hours of exposure, consistent with previous research (Ashraf et al., 2018). The knockdown result of *P. harmala* acetonic seed extract against different species of aphids was also reported in a study (Salari et al., 2012). The variation in insect mortality between *A. gossypii* and *A. nerii* following treatment with the extract may be attributed to differences in detoxification and penetration methods, with *A. gossypii* experiencing maximum mortality immediately, while *A. nerii* exhibited initially moderate mortality that progressively increased over time (Salari et al., 2012). The insecticidal activities of other plant extracts against aphids have also been reported. Işık & Görür investigated 7 essential oils against *B. brassicae* and found rosemary oil as an excellent aphicide to reduce the population of aphids (Işık and Görür, 2009).

Artemisia seiberi oil was reported most effective against woolly apple aphids (Ahmed et al., 2020). A similar result reported that *Artemisia herba-alba* oil was highly toxic against *A. gossypii* with LC_{50} 0.023% and a 90.44% decrease in population (Mohamed et al., 2010). Many previous studies found the same results regarding aphid mortality (PaVela, 2009; Arshad et al., 2010; Iqbal et al., 2011). Our findings are also similar to these studies regarding aphid mortality. Similarly, various researchers have worked on the insecticidal activity of *P. harmala* against different insect pests (Abbasipour et al., 2010; Eltahir and Dahab, 2019). In contrast to our results, aak extract proved ineffective against aphids as it caused only 11% mortality after 4 days of treatment (Singh, 2007). It is also reported that the toxicity of neem, tobacco, and garlic extracts against aphids and they found that garlic extract was ineffective with the lowest mortality percentage (Sohail et al., 2012).

Conclusion

The *P. harmala* extract has demonstrated remarkable efficacy and numerous advantages in terms of aphid control. Its effectiveness stems from its remarkable ability to eliminate the pest through contact toxicity. This study represents an initial investigation to assess the bioactivity of *P. harmala* extract against aphids under laboratory conditions. To comprehensively evaluate its potential, further research is warranted to explore the impact of various extract concentrations on aphids and other pests within field settings.

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