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ASSESSMENT OF TOXICITY OF *PARTHENIUM HYSTEROPHORUS* L. EXTRACT AGAINST LARVAE OF *TROGODERMA GRANARIUM*

^aShahbaz Ahmad, ^aRabia Zafar, ^bIqra Haider Khan, ^bArshad Javaid, ^cAzeem Intisar

^a Department of Entomology, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.

^b Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.

^c School of Chemistry, University of the Punjab, Quaid-i-Azam Campus, Lahore, Pakistan.

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ABSTRACT

The leaves of parthenium (*Parthenium hysterophorus* L.) were extracted in ethanol and toxicity of the extract was examined on khapra beetle (*Trogoderma granarium* Everts) larvae. Four concentrations (2, 4, 6 and 8 $\mu\text{L mL}^{-1}$ acetone) were applied directly on the larvae in Petri dishes. Toxicity was recorded after 12, 24 and 36 h. All the concentrations had considerable effect on the mortality of larvae. At the highest concentration (8 $\mu\text{L mL}^{-1}$), the mortality observed was 57%, 72% and 78% after 12, 24 and 36 h, respectively. The minimum mortality was observed to be 43% at the concentration of 2 $\mu\text{L mL}^{-1}$ acetone after 12 h. GC-MS analysis of the parthenium leaves showed 7 compounds in the extract including phytol (38.68%), β -cubebene (11.98%), hexadecanoic acid, methyl ester (11.22%), caryophyllene (10.37%), 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (9.98%), methyl stearate (9.01%), and hexadecanoic acid, ethyl ester (8.74%). The study concluded that an extract of 8 $\mu\text{L mL}^{-1}$ acetone concentration is highly effective against larvae of khapra beetles that can cause 78% mortality after 36 h of direct exposure to the larvae.

Corresponding Author: Shahbaz Ahmad

 Email: shahbaz.iags@pu.edu.pk

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INTRODUCTION

Trogoderma granarium is a notorious pest of stored grains (Kulkarni et al., 2015; Shahbazi et al., 2022). It has been listed among A2 quarantine organisms by European and Mediterranean Plant Protection Organization (Honey et al., 2017). It is a polyphagous and cosmopolitan pest (Kulkarni et al., 2015), which has the ability to infest crops including barley, cotton, cowpea, groundnut, maize, millet, rice, sorghum, sesame, and their stored products (Singh et al., 2017). This pest is also of public health concern (Ahmedani et al., 2017). Fumigation with methyl bromide is the most extensively used practice to control this pest (Khalique et al., 2018). It has the ability to block the spiracle of insects and thus

cause death by suffocation. The classification of methyl bromide as a major ozone depleting substance by Environmental Protection Agency has led many countries to impose ban on its usage. Fumigation with phosphine is also a custom method for the control of *T. granarium*, but its efficacy has been reduced as insects have acquired resistance against this chemical. An advanced method is the use of phosphine gas with CO₂ (Gourgouta et al., 2020). Use of CO₂ can increase the chances of inhalation of phosphine gas by insect thus causing more mortality. Contact insecticides like malathion, chlorprifos and pyrethrins are also used as chemical control but the insects have the ability to hide in cracks and crevices so their effectiveness is reduced.

The insects have also started to acquire resistance against these chemicals (Honey et al., 2017).

Chemical insecticides have high persistency in the environment (Gavrilescu et al., 2005; Wang et al., 2022). They can cause a lot of neurological problems in humans (Cassereau et al., 2017). They can also increase the chance of cancer development (Sun et al., 2020). Insecticides can also cause mortality in non-target organisms that could be beneficial for the mankind (Devici et al., 2021). The phenomenon of insecticidal resistance is also on the rise (Sparks and Nauen, 2015). These factors have induced the researchers to find new control methods that are effective, environment friendly and target oriented. Botanical insecticides are a suitable alternative as they are more effective, have less persistency in the environment and there are very limited reported cases of effect on non-subjected organisms (Pavela, 2016; Zafar et al., 2022). Botanical extracts have been found very effective in controlling various pests including insects (Muhammad et al., 2020), fungal pathogens (Javaid et al., 2020a; Khan et al., 2020) and weeds (Javaid and Khan, 2020; Javaid et al., 2020b). These were also found effective against *T. granarium* (Arivudainambi and Singh, 2003; Ali et al., 2022). Besides this the effect of various plant extracts including garlic (*Allium sativum*), onion (*Allium cepa*) camphor (*Eucalyptus globulus*), sunflower (*Helianthus annuus*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), and olive (*Olea europaea*) were also effective against khapra beetle (Younes et al., 2011).

Parthenium hysterophorus is a noxious invasive weed (Devkota and Sahu, 2017; Javaid et al., 2022). It possesses many biological activities including insecticidal, antifeedant, antiviral, antifungal, antimalarial and antibacterial (Kushwaha and Maurya, 2012). The extract of *P. hysterophorus* showed promising toxicity on diamond backmoth (*Plutella xylostella*) and cowpea aphid (*Aphis craccivora*) (Reddy, 2018). Toxic effect of *P. hysterophorus* powder was also observed on pulse beetle (*Callosobruchus chinensis*) as reported by Tesfu and Eman (2013). It has also been reported that the extracts of *P. hysterophorus* inhibit cholinesterase of cockroach brain (Pandey, 2009). Due to the phytochemical profile of *P. hysterophorus* and its biological activities, it was hypothesized that its extract may exhibit toxic effect on the *T. granarium*. The present study was conducted to test this hypothesis.

MATERIALS AND METHODS

Procurement and maintenance of khapra beetle

The culture containing different life stages of khapra beetle was obtained from University of Agriculture Faisalabad. To identify different stages, magnifying glass was used. Glass jar was filled with 100 g wheat and 20 g flour. All the stages were transferred to the jar. For aeration and preventing the escape of insect, the jar was tightly covered with the piece of muslin cloth. Optimum conditions were maintained at 30 ± 2 °C and $70 \pm 5\%$ humidity. Temperature was maintained by using air conditioner while humidity was maintained by using humidifier. The photoperiod included 13 h light and 11 h dark.

Preparation of extract of parthenium weed

The aerial parts of *P. hysterophorus* were collected from University of the Punjab, Lahore. To remove impurities including debris, dirt and pathogens the plant parts were washed thoroughly with distilled water. The plant material was allowed to dry for 5 days. The dried leaves were crushed lightly with pestle and mortar. Electric blender was used to convert the plant material into fine powder. Ethanol of 95% purity was used for extraction. A conical flask about half filled with 100 mL ethanol and 20 g parthenium powder was added to it. The flask was continuously shaken for 10 min. A plastic wrap was tightly placed at the open end of the flask with the help of a rubber band. The apparatus was then left overnight for 2 days for proper extraction of chemical compounds from the plant material. Thereafter, the filtration of the extract was done through Whatmas filter paper. After that, the extract was transferred to glass tubes and stored at 4 °C in the refrigerator.

Insecticidal bioassay

Contact method of application of the extract was used in this study. In sterilized Petri dishes, the plant extract (2, 4, 6, 8 µL) was directly applied by using micro-pipette and spread evenly by applying 1 mL acetone. The experimental conditions were set at 30 ± 2 °C temperature and $70 \pm 5\%$ relative humidity. The third instar larvae were used in the experiment. A total of twenty larvae were released in each Petri dish. Mesh cloth was tied with the help of rubber band on the open end of Petri dish for proper aeration and prevention of escape. In control, 1 mL of acetone was used only. The experimental design was completely randomized with three replicates. Mortality of the larvae was recorded after 12, 24 and 36 h of exposure.

GC-MS analysis

GC-7890B: MS-5977A of Agilent Technologies were the models used for GC-MS analysis. The column used was of 30 m × 0.25 μm × 0.25 μm dimensions. Carrier gas was helium and the split less mode was used. The injection volume of sample was 1 μL. Initial temperature of oven ramping was 50°C that was increased by 10 °C after each minute until it reached to 290 °C. The scan range for MS program was 50-500 m/z with solvent delay time of 3 min. Inlet line temperature was 280 °C, source temperature was 230 °C and quadrupole temperature 150 °C. The ionization voltage was set at 70 eV. The total run time was 25 min. The library used for comparison was mass hunter/NIST version 2017.

Statistical analysis

All the data were analyzed by 2-way ANOVA followed by Tukey's HSD test to determine the treatment means at 5% level of significance. Software Statistix 8.1 was used to analyze the data.

RESULTS AND DISCUSSION

Insecticidal activity of parthenium extract

Analysis of variance showed a significant ($P \leq 0.05$) effect of extract concentration (C), incubation time (T) and $C \times T$, on mortality of *T. granarium* (Table 1). After 12 h of incubation, there was not any mortality in control while there was 23, 33, 45 and 48% mortality due to 1, 2, 3 and 4 μL mL⁻¹ extract of parthenium, respectively. After 24 h incubation, there was 2% mortality of the larvae in control. On the other hand, a significantly higher mortality i.e. 35, 47, 58 and 62% due to 1, 2, 3 and 4 μL mL⁻¹ extract, respectively. The insecticidal property of the extract was the highest after 36 h of incubation. At this time period, there was just 5% mortality of the larvae in control that showed the natural death. By contrast, 1, 2, 3 and 4 μL mL⁻¹ concentrations of the extract caused a significantly higher mortality than control i.e. 47, 58, 68 and 72%, respectively (Figure 1).

Table 1. Two-way analysis of variance regarding percentage mortality of khapra beetle (*Trogoderma granarium*) due to different concentrations of the extract of *Parthenium hysterophorus*.

Source of variance	DF	SS	MS	F	P
Concentration (C)	4	23761	5940	428	0.000**
Time (T)	2	1921	961	69	0.000**
C × T	8	329	41.11	2.96	0.014*
Error	30	417	13.89		
Total	44	26428			

*,** show significant different at $P \leq 0.01$ and 0.001, respectively.

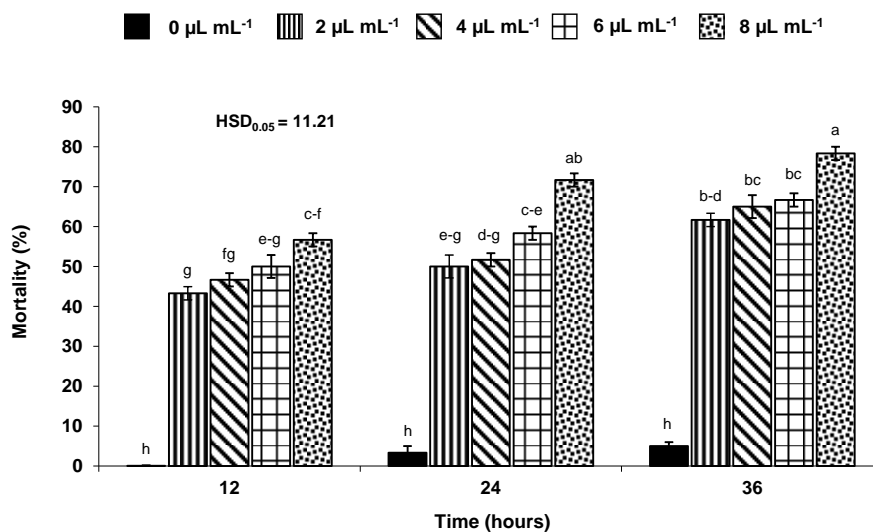


Figure 1. Effect of different concentration of ethanolic leaf extract of *Parthenium hysterophorus* on mortality of khapra beetle. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey's HSD Test.

Many previous studies have shown insecticidal properties of parthenium against other insect species. A 50 mg methanolic extract of parthenium significantly reduced larval survival rate and various digestibility indices of *Spodoptera litura* from 12.3 to 80.4% (Irfan Ullah et al., 2022). Likewise, methanolic extract of parthenium caused 59.1% mortality of *Nilaparvata lugens*, the paddy brown plant hopper (Hiremath, 1997). Similarly, fresh aqueous leaf extract of parthenium of 200 g 500 mL⁻¹ concentration caused 100% of 2nd and 3rd instar larvae of *Aedes aegypti* (Amir et al., 2017). A sesquiterpene lactone allelochemical namely parthenin has been reported in parthenium that was found moderately antifeedant against larvae of *S. litura* (Datta and Saxena, 2001).

This active ingredient also showed insecticidal properties against *Dysdercus koenigii*, *Callosobruchus chinensis*, *Tribolium castaneum*, and *Phthorimaea operculella* (Sharma and Joshi, 1977). In addition, parthenin also exhibited repellent activity to *Aphis craccivora* and *Plutella xylostella* (Reddy et al., 2018).

GC-MS analysis

GC-MS study of the leaves of parthenium exhibited the presence of 7 compounds in the extract. The predominant compound was phytol with 38.68% peak area. This compound is known for its antifungal activity against *Aspergillus niger* (Ghaneian et al., 2015), and therapeutic potential of phytol towards *Trypanosoma congolense* (Saad et al., 2020). Phytol was also found very effective against cereals aphid *Metopolophium dirhodum* (Benelli et al., 2020). The remaining compounds included β -cubebene (11.98%), hexadecanoic acid, methyl ester (11.22%), caryophyllene (10.37%), 9, 12, 15-octadecatrienoic acid, ethyl ester, (Z, Z, Z)- (9.98%), methyl stearate (9.01%), and hexadecanoic acid, ethyl ester (8.74%) as shown in Table 2. Earlier, β -cubebene and caryophyllene have been identified in flowers of *Ageratum conyzoides* (Ferdosi et al., 2021). These compounds are known for their antimicrobial activity (Dakah et al., 2019; Moo et al., 2020).

Table 2. Compounds identified in ethanolic leaf extract of *Parthenium hysterophorus* through GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak Area (%)
1	Caryophyllene	C ₁₅ H ₂₄	204.35	12.289	10.37
2	β -Cubebene	C ₁₅ H ₂₄	204.35	13.386	11.98
3	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.45	20.774	11.22
4	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284.47	21.850	8.74
5	Phytol	C ₂₀ H ₄₀ O	296.5	23.622	38.68
6	Methyl stearate	C ₁₉ H ₃₈ O ₂	306.48	23.880	9.01
7	9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)-	C ₂₀ H ₃₄ O ₂	306.48	24.465	9.98

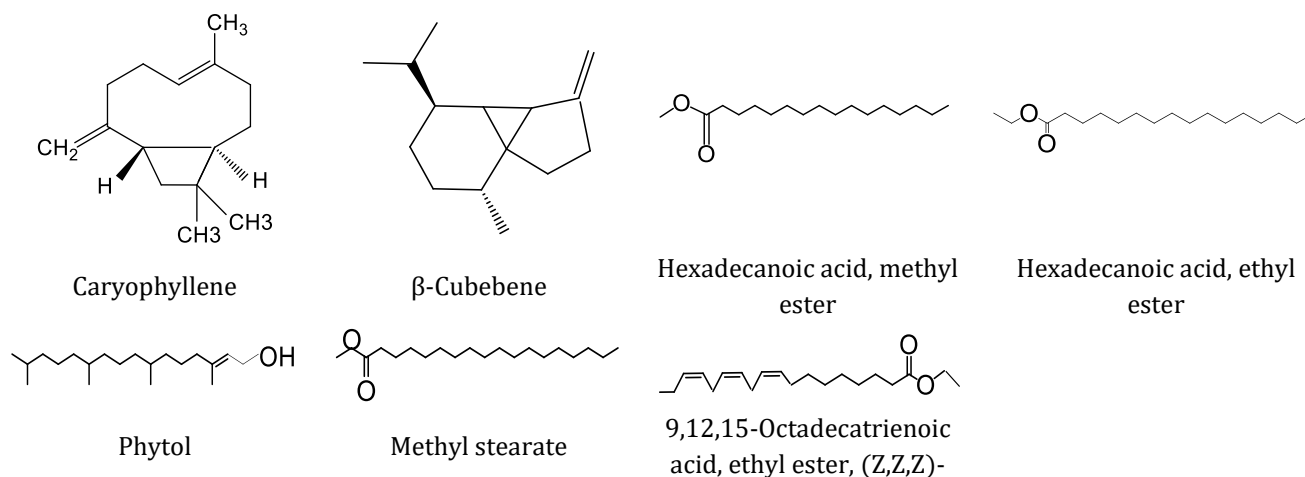


Figure 2. Structures of compounds identified in ethanolic leaf extract of *Parthenium hysterophorus* through GC-MS analysis.

CONCLUSION

Ethanol extract of parthenium was highly mortal to the larvae of khapra beetle. An 8 $\mu\text{L mL}^{-1}$ acetone extract caused up to 78% mortality of the target organisms. Major compound in this study was phytol that might be responsible for mortality of the larvae. In addition to that, parthenin has been identified as active ingredient in parthenium in many previous studies having insecticidal potential.

AUTHORS' CONTRIBUTION

SA supervised the work, RZ did experimental work and wrote a part of paper, AJ co-supervised the work, prepared graphs, carried out statistical analysis, participated in manuscript writing and did final editing of the work, IHK constructed structures of compounds, AI helped in GC-MS analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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