CONTROL OF KHAPRA BEETLE BY LEAF EXTRACT OF *Melia azedarach* AND IDENTIFICATION OF POSSIBLE INSECTICIDAL COMPOUNDS THROUGH GC-MS ANALYSIS

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ABSTRACT

The leaf extract of *Melia azedarach* L. was obtained by soaking plant material in ethanol for one week and its toxic effect was assessed on the third instar larvae of khapra beetle (*Trogoderma granarium* Everts). Four concentrations of the extract (2, 4, 6 & 8 µL mL^{-1} acetone) were applied through contact method in Petri plates. Mortality was recorded after 12, 24 and 36 h. All the concentrations showed toxic effects against the larvae to variable extents. The highest mortality i.e. 72% was recorded at 8 µL mL^{-1} acetone concentration after 36 h incubation period. At the same concentration, the mortality was 61.67% and 48.34% after 24 and 12 h, respectively. Minimum mortality (23.33%) was observed at 2 µL mL^{-1} acetone concentration after 12 h. GC-MS analysis of ethanolic leaf extract of parthenium revealed the phytol as the predominant compound with 72.25% peak area. Other compounds were 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- (6.30%), 1-(3,6,6-trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone (6.11%), methyl stearate (4.60%), (+/-)-phytone (4.38%), hexadecanoic acid, methyl ester (3.24%), and hexadecanoic acid, ethyl ester (3.12%). It is concluded that an 8 µL mL^{-1} acetone concentration of leaf extract of *M. azedarach* can kill up to 72% larvae of *T. granarium* in 36 hours.

Keywords: Chinaberry, Leaf extract, *Melia azedarach,* Natural insecticides, *Trogoderma granarium*.

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INTRODUCTION

Trogoderma granarium is a devastating pest of stored grains (Morrison *et al*., 2020). Due to its host range and cosmopolitan nature, it has become an important quarantine pest and has been categorized as A_2 quarantine organism by EPPO (Honey *et al*., 2017). It has the ability to infest wheat, maize, barley, sorghum, groundnut, rice, cotton, cowpea, millet and sesame (Singh *et al*., 2017). The pest reduces the grain to fine powder thus reducing its nutritional value, which ultimately leads to economic loss. The pest also has anthropogenic importance as the contact with the skin of larvae can cause dermatitis and allergic reaction if swallowed (Ahmedani *et al.,* 2007). For its control, fumigation with methyl bromide and phosphine is generally carried out (Honey *et al.,* 2017). However, the classification of methyl bromide as ozone depleting substances has led various countries to impose ban on its usage (Carter *et al*., 2005). Chemicals like fluvalinate, bifenthrin, fenvelerate cypermethrin, chlorpyriphos-methyl, monocrotophos, mathacrifos, spinosad, malathion, pirimiphos-methyl, celite, endosulfan and carbaryl are effective against *T. granarium*. Most of these chemicals have contact mode of action so their effectiveness is reduced because the pest has the ability to hide in cracks and crevices (Khalique *et al.,* 2018; Shahbazi *et al.,* 2022). Due to repeated use of chemical insecticides, the phenomenon of insecticidal resistance is increasing due to which the efficacy of various in practice

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chemicals has reduced (Sarwar and Salaman, 2015). Exploitation of chemical insecticides can negatively impact environment as well as living organisms (Yadav, 2017). Persistent effect of pesticides on human health includes skin allergies, chronic bronchitis, neurological disorders and cancers (Malik *et al*., 2014). Only 1% of the applied insecticides reach the target pest while more than 99% dissipate and cause pollution of different components of environment (Ansari *et al.,* 2014).

Due to limitations of insecticides, researchers are inclined towards discovery of alternative control methods that are more efficient and environment friendly. Plant produce secondary metabolites as a defense against weeds (Javaid et al., 2020; Javaid and Khan, 2020), pathogens (Javaid and Khan, 2016; Jabeen *et al.,* 2021) and insect pests (Zafar *et al.,* 2022). These metabolites can be extracted and used as botanical insecticides (Pavela, 2007; Ahmad *et al.,* 2022). There are limited reported studies related to their toxicity against off-target organisms (Zahu *et al*., 2022). Extracts of garlic, onion, sunflower, peppermint, parthenium and olive (Younes *at el*., 2011; Ahmad *et al.,* 2022).

Different parts of *M. azedarach* have been used as a traditional medicine (Sharma and Paul, 2013). It has been reported to show various biological properties including antibacterial (Kaneria *et al*., 2009), antifungal (Khan and Javaid, 2013; Javaid and Khan, 2016) and antioxidant (Nahak *et al*., 2010). It is also known to have potent toxic effect on public health related pests including the vectors of dengue (*Aedes aegypti*) and malaria (*Anopheles stephensi*), ticks (*Boophilus micoplus*), and the human lices (*Pediculus humanus capitis*) (Al Rubae, 2009). Its extract was also found very effective against stored grain pests including *Tribolium castaneum* (Sabiha *et al*., 2016) and *Chinensis macaculatus* (Kosma *et al.,* 2014). This study aimed to investigate the possible toxic effect of *M. azedarach* leaf extract against khapra beetle.

MATERIALS AND METHODS

Procurement and maintenance of *T. granarium*

T. granarium was acquired from University of Agriculture Faisalabad. Different life stages of the beetle in the culture were identified with magnifying glass. All the life stages of *T. granarium* were transferred to glass jar containing 20 g flour and 100 g wheat. A piece of muslin cloth was tied on the jar to prevent the escape of the insect. The culture was maintained at the optimum temperature of 30±2 ºC by air conditioner and humidity at 70±5% by humidifier. The duration of photoperiod was maintained as 13/11 h of light/dark.

Preparation of *M. azedarch* leaf extract

The leaves were collected from a mature plant of *M. azedarach* at fruiting stage from Lahore. Leaves were washed and left to dry for seven days. After proper drying, leaves were lightly crushed with the help of pestle and mortal. In a conical flask, 100 mL ethanol and 20 g powdered leaves were added, shaked well for 10 min, and tightly covered with a plastic sheet for 3 days for extraction of the compounds. Thereafter, the extract was filtered through Whatman's filter paper and stored in glass tubes at 4 °C in the refrigerator.

Insecticidal bioassay

Four volumes of the extract (2, 4, 6, and 8 µL) were applied directly in the Petri plates with the help of a micro-pipette. A volume of 1 mL acetone was applied to spread the extract evenly. The same quantity of the acetone was added to the control plates but without *M. azedarach* extract. Third instar larvae were used in the bioassay. A total of twenty larvae were transferred to each Petri dish by using forceps. Experiment was done in a completely randomized design with three replications. Temperature and humidity were maintained at 30±2 °C and 70±5%, respectively. The Petri plates were covered with the mesh cloth with the help of rubber band to provide proper aeriation and to prevent escape of larvae. The mortality was recorded after 12, 24 and 32 h intervals (Zafar *et al.,* 2022).

GC-MS analysis

GC-7890B and MS-5977A models of Agilent Technologies were used for GC-MS study. The column used had the dimensions 30 m × 0.25 µm × 0.25 µm. Helium was injected as carrier gas with the split less mode. Rest of the conditions was same as reported by Ahmad *et al.* (2022).

Statistical analysis

The data regarding percentage mortality of the beetle in different treatments of leaf extract of *M. azedarach* were subjected to 2-way analysis of variance followed by application of Tukey's HSD test using Statistix 8.1 software.

RESULTS AND DISCUSSION

Insecticidal activity of *M. azedarach* extract

ANOVA shows that the effects of concentration (C) and incubation period (T) were significant (P≤0.001) for mortality of khapra beetle. Similarly, the interactive effect (C × T) was also significant (P≤0.01). After 12 h of incubation, there was no mortality in the control while there was 23, 33, 45 and 48% mortality due to 2, 4, 6 and 8 µL extract, respectively. With the increase in incubation period, beetle mortality was gradually increased. At 24 and 36 h incubation, there was a a little mortality of 2 and 5% in control, respectively. On the other hand, there was 335, 47, 58 and 62% mortality after 24 h, and 47, 58, 68 and 72% mortality after 36 h due to 2, 4, 6 and 8 µL extract, respectively (Fig. 1). Earlier, there are many reports of insecticidal properties of *M. azedarach* against other insect pests (Padrón *et al.,* 2003). Its acetone extract significantly suppressed the growth of *Spodoptera littoralis* larvae (Farag *et al.,* 2011). Nanoencapsulated leaf extract of *M. azedarach* showed pronounced toxicity to *Trialeurodes vaporariorum* adults (Khoshraftar *et al.,* 2020). A number of effects have been associated with extracts of *M. azedarach* including antifeedant, repellency, delayed development, ecdysis halting, fertility reduction, and physiological and behavioral changes, which lead to mortality of the insects (Defagó *et al.,* 2006; Bullangpoti *et al.,* 2012). Insecticidal properties of *M. azedarach* are generally attributed to limonoids of the terpenes class present in all parts of this plant, with limonoids of C-secoskeleton ring as the most active insecticidal compound (Bohnenstengel *et al.,* 1999).

Table 1. ANOVA showing the effect of leaf extract of *Melia azedarach* on mortality (%) of *T. granarium*.

Source of variance	DF	SS	MS	F	P
Concentration (C)	4	19786	4969	526	0.000**
Time (T)	2	3004	1502	159	0.000**
C × T	8	434	54.31	5.75	0.002*
Error	30	283	9.44		
Total	44	23598			

*, ** show significant difference at P≤0.01 and 0.001, respectively.

GC-MS analysis

GC-MS analysis of leaf extract exhibited the occurrence of 7 compounds as presented in Table 1 and Fig. 2. Phytol was the predominant compound with 72.25% peak area. It is a diterpene alcohol and has been found in different plant species such as leaves of *Chenopodium quinoa* (Khan and Javaid, 2022), flowers of *Ageratum conyzoides* (Ferdosi *et al.,* 2021a) and whole plant of *Euphorbia prostrata* (Ferdosi *et al.,* 2021b). It has a number of biological activities. It has been reported very effective against *Metopolophium dirhodum,* an aphid that attacks cereals (Benelli *et al.,* 2020). In addition, it has

antifungal and antibacterial activities against *Candida albicans, Aspergillus niger* and *Escherichia coli* (Ghaneian *et al.,* 2015). Moreover, it is also known as an antidiabetic, immunostimulatory, antidiuretic, anti-inflammatory, anticancer and antioxidant agent (Santos *et al.,* 2013). The remaining six compounds were 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- (6.30%), 1-(3,6,6-trimethyl-1,6,7,7a-tetrahydrocyclo penta[c]pyran-1-yl) ethanone (6.11%), methyl stearate (4.60%), (+/-)-phytone (4.38%), hexadecanoic acid, methyl ester (3.24%), and hexadecanoic acid, ethyl ester (3.12%). In addition to these compounds, a number of other compounds have also been reported in *M. azedarach* with insecticidal properties (Lin *et al.,* 2022). Many earlier studies have shown that compounds belonging to triterpenoids class are responsible for insecticidal properties of *M. azedarach* (Abd El-Ghany *et al.,* 2012). Four compounds namely meliacarpinin A, B, C and D identified in *M. azedarach* showed insecticidal properties against *Spodoptera exigua* (Munehiro *et al.,* 1995). Likewise, a number of other insecticidal compounds namely 12-O-acetyltrichilin B (Huang *et al.,* 1995); 1,12-di-O-acetyltrichilin B; azedarachin A and C; aphanastatin; trichilin B, D and H; meliatoxin A2 (Munehiro *et al.,* 1995), toosendanin (Chen *et al.,* 2010) and 12-hydroxia-moorastatin (Carpinel *et al.,* 2003) have been identified from *M. azedarach.*

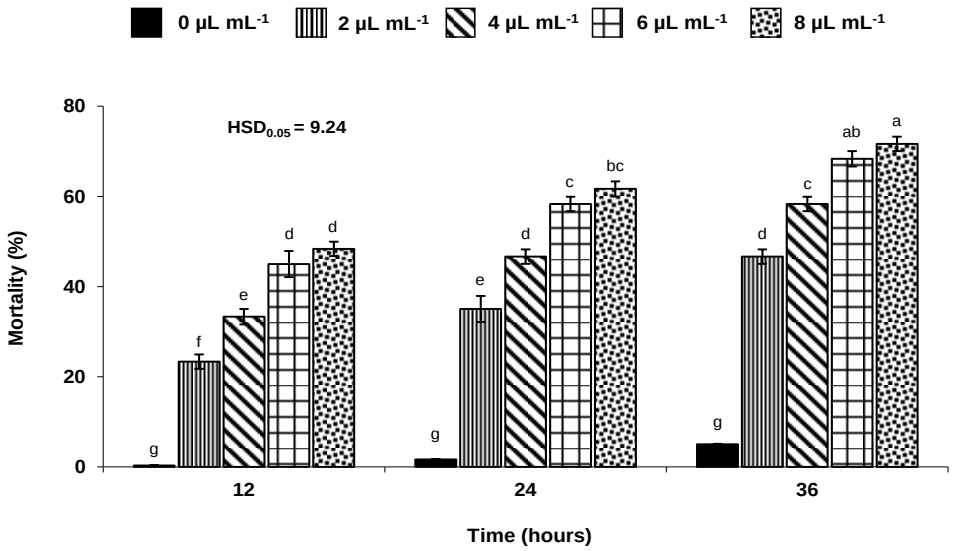
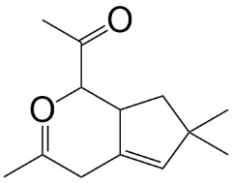


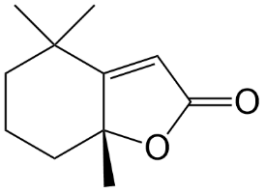
Figure 1. Effect of different concentration of ethanolic leaf extract of *Melia azedarach* on mortality of khapra beetle.

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

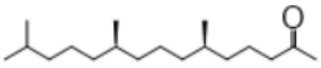
Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone	$C_{13}H_{18}O_2$	206.28	11.74	6.11
2	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	$C_{11}H_{16}O_2$	180.24	14.22	6.30
3	(+/-)-Phytone	$C_{18}H_{36}O$	268.47	19.45	4.38
4	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	20.77	3.24
5	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.47	21.84	3.12
6	Phytol	$C_{20}H_{40}O$	296.53	23.62	72.25
7	Methyl stearate	$C_{19}H_{38}O_2$	298.50	23.87	4.60



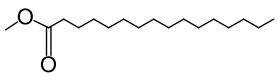
1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone



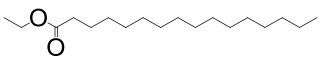
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-



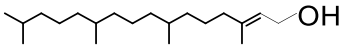
(+/-)-Phytone



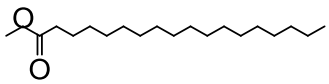
Hexadecanoic acid, methyl ester



Hexadecanoic acid, ethyl ester



Phytol



Methyl stearate

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

CONCLUSION

Leaf extract of *M. azedarach* was found highly toxic and lethal against larvae of *T. granarium.* An 8 µL mL^{-1} acetone concentration of ethanolic leaf extract of *M. azedarach* can cause up to 72% mortality of larvae of this beetle after 36 h of incubation. Phytol was identified as the predominant compound in the extract.

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