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Abstract

The study was conducted to determine the insecticidal and physiological activity of essential oil from *Artemisia annua* L. on Indian meal moth *Plodia interpunctella* (Hübner) in laboratory condition. The yellowish oil of wormwood *Artemisia annua* L. was diluted in acetone and different concentrations (15%, 11%, 8%, 5.5% and 4%) were assayed on 17 days old larvae. LC$_{25}$, LC$_{50}$, and LC$_{75}$ were estimated 5.96%, 8.4% and 11.3% respectively after 24 h. The sub lethal doses showed that essential oil reduced adult emergence, longevity of male and female insects, fecundity and fertility of females. Evaluation of toxic vapors on adult insect was also considered and LC$_{25}$, LC$_{50}$, and LC$_{75}$ were estimated to be 6.35%, 8.13% and 10.45%. There was no difference in mortality on either sex. The protein, carbohydrate and lipid contents of treated larvae were significantly reduced compared with the controls.

Keywords: Worm-wood; Indian meal moth; Reproduction; Protein; Carbohydrate; Lipid

Introduction

For many years, there has been a long and persistence fight against insects attacking the food and food products. Many of the earlier pesticides were of plant origin and were used locally and traditionally before, during and after food production [1]. Synthetic or fumigant pesticides used for plant protection and pests controlling in stores usually bring about resistance in these pests [2,3].

The persistent use of synthetic pesticides in agriculture, silviculture and even animal husbandry has created several difficulties on public health. However, even today the pesticides constitute one of the major parts in PCM for controlling insect pests. Plant origin pesticides are trustworthy, economical and safe for ecosystems and can control various ranges of pests. These insecticides- due to their little side effect on natural enemies, little toxicity on vertebrates and fast degradation in environment - are of special importance [4,5]. Hence, it seems that plant origin insecticides are safe alternatives to traditional insect control measures [6,7]. Plant products used for pest control, essential oils are used as fumigant [8,9], contact [10-12], deterrents [13-15] and antifeedants [16]. They may also affect some life parameters like development, longevity, reproduction and as well as sex finding behaviors [2] and finally growth regulatory effect [17]. The insecticidal effect of plant origin insecticides is determined by insect species, the behaviors [2] and finally growth regulatory effect [17]. The essential oils used for stored product insects should have the potential of killing all life stages of insects [2]. Due to this reason the study was undertaken against eggs, larvae and adults of *Plodia interpunctella* (Huebner) for the effect of essential oils [19-22] but its mode of action has not been elucidated. *A. annua* is an indigenous plant growing wild in north of Iran around paddy fields and has shown considerable effect before, during and after food production [1]. Synthetic or fumigant pesticides were of plant origin and were used locally and traditionally in environment - are of special importance [4,5]. Hence, it seems that plant origin insecticides are safe alternatives to traditional insect control measures [6,7].

Material and Methods

Insect

*Plodia interpunctella* (Huebner) was collected from infested rice in stores. The insects were reared in plastic jars (7.5 ×145×18.5 cm), holes (2×2cm) were provided with meshing cloth for aeration. The insects were reared in a controlled room (26±1°C, 14:10 LD and RH% 65±5). They were provided with artificial diet made of 800gms wheat, 160gms yeast, 200cc glycerol and 200cc natural honey [24]. The 17 day old larvae (unsexed) and adults (sexed) of cohort age were used for this study.

Plant essential oil

*Artemisia annua* L. was collected from field; leaves were separated, then washed with water and dried in shade and finally made into powder. Essential oil was extracted by a modified clevenger type apparatus. The oil was subsequently dehydrated by anhydrous sodium sulphate and kept in refrigerator (4°C) until used for experiments.

Bioassay and treatment

The bioassay for 17 day old larvae were carried out on filter paper [25]. The essential oil was diluted in acetone and used in 15%, 11.8%, 5.5% and 4% concentrations after initial bracketing test. The control received pure acetone (Merck, Germany). This test was carried out in four replications for each concentration and one control including 10 larvae (unsexed) of cohort age. Filter papers were impregnated with 1CC of essential oil in acetone and after evaporation of acetone, 10

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lakes were released into each Petri dish. The Petri dishes were closed and tightly covered by Parafilm. After 24h, the number of dead or live insects were counted. The data were analyzed by R-Pc software and LC$_{25}$, LC$_{50}$ and LC$_{75}$ were estimated. Live larvae were separated and were reared separately in plastic jars and food material was provided to them. The parameters like longevity and percent adult emergence of male and female insects were calculated.

The bioassay on adults were performed on male and female based on the presence of claspers in male and after initial bracketing tests which included 4 concentration ranges of 9%, 8%, 6% and 5% along with acetone as a control. This experiment was carried out in 3 replicates and by 32 insects for each replication [25]. For this purpose adults of cohort age were separated and 2 adults were transferred to vials (50 mm high and 29.5 mm width). Filter paper of 15mm width was located on the underside of the cap and was impregnated with essential oil or acetone alone in case of controls. A cloth mesh was also placed between the filter paper and vial cap in order to prevent adults from direct contact with filter paper. After 3 minutes of acetone evaporation the insects were released in the vials and the mortality was recorded after 24h. The mortality was separately recorded for male and female adults and with the help of R-Pc software LC$_{25}$, LC$_{50}$ and LC$_{75}$ was estimated. The insects remaining alive after 24h were transferred to plastic jars 76.3mm width × 187mm height whose lids were partly cut and replaced by meshing cloth for aeration. The daily mortality was recorded and was compared with the controls.

Biochemical analyses

Some important biochemical analyses (proteins, carbohydrates and lipids) were investigated on 17 day old larvae. 24 larvae, treated with LC$_{25}$, LC$_{50}$ and LC$_{75}$ were considered in 3 replicates for male and female insects and one control treated with acetone alone. After 24 h from every replicate 3 larvae were randomly selected from each sex (based on the presence of testis visible under stereomicroscope), homogenized in 2CC double distilled water and then centrifuged for 10 min at 4°C in 10000rpm and the supernatant was preserved at -20°C until the onset of experiments. The Bradford method [26] was adopted for measuring total protein and the absorbance was read at 630nm in an Eliza reader. The method of Van Handel [27] was adopted for analysis of lipid content and the absorbance was read at 630nm. The method of Van Handel et al. and Yuval et al. [27,28] was adopted for measuring carbohydrates and the absorbance was read at 450 nm in the same Eliza reader.

Statistical analysis

The analysis of data was performed using SAS software (1997). For comparing different effects of essential oil in used concentrations, equalized complete randomized design and sometimes unequalized complete randomized were used and also for comparing the effects of essential oil in sex and concentrations, factorial design was used. For biochemical analyses the complete randomized design was adopted. The Tukey test was used for comparing the means. Wherever necessary the percentage data were Arc-Sin transferred.

Results

The toxicity and physiological activity of essential oil on larvae

Dose-response of essential oil on 17 day old larvae of *Plodia interpunctella* is presented in Figure 1. The LC$_{25}$, LC$_{50}$ and LC$_{75}$ values after 24h exposure are shown in Table 1.
The result showed that there were significant differences between percent emergence of adult in control (89.75 ±1.57) and the treatments. The least emergence was recorded for LC$_{75}$ ($F= 19.44$, df= 3, $p<0.01$). The percent adult emergence for male was less than female, but this difference was not statistically significant (Figure 3) ($F= 3.32$, df= 1, $p>0.05$).

The comparison of means of adult longevity of treated larvae in 3 concentrations (LC$_{25}$, LC$_{50}$ and LC$_{75}$) showed that the control having 8.08 ±0.17 days had the maximum mean and stayed first in ranking (Figure 4). Even though the mean for male longevity was less than female insects but the differences were not significant (Figure 5). The analysis variance of the effect of concentration × sex between 3 concentrations was statistically significant ($F= 87.94$, df= 3, $P<0.01$).

The evaluation of the effect of essential oil of A.annua on reproduction showed that both fecundity and fertility were significantly reduced (Figure 6 and Figure 7).

The toxicity effect of essential oil on adults of Plodia interpunctella

Dose-response of essential oil on adults is depicted in Figure 8 and...
in Table 2. The results of toxicity on adults showed the lowest mortality in controls (3.6±0.014) and the highest mortality in 9% concentration (33.36±0.014). Therefore the higher is the dose, the higher will mortality be (Figure 9) (F=37.14, df=4, p<0.01). Analysis of variance in adults does not show any significant difference in male and female (F= 0.28, df= 1, p> 0.05). Similarly, concentration into sex does not show any differences (Figure 10) (F= 2.28, df=4, p>0.05).

The effect of A.annua essential oil on energy reserves of Plodia interpunctella

The effect of different concentrations (LC$_{25}$, LC$_{50}$ and LC$_{75}$) on protein content of treated larvae is shown in Figure 11. The result showed that the mean for protein content of control was 2.9128±0.096 mg/larvae and in concentrations LC$_{25}$, LC$_{50}$ and LC$_{75}$ were 2.6472, 2.6471 and 2.4113 mg/larvae respectively; it also showed significant reduction compared with the control 24 h post treatment (F=7.94, df= 3, p<0.01). The mean for protein content of female (2.635 mg/ larva) was more than male (2.479 mg/ larva) but this difference was not statistically significant (F= 2.38, df= 1, p>0.05).

The effect of different concentrations (LC$_{25}$, LC$_{50}$ and LC$_{75}$) of A.annua essential oil and the control on lipid level of larvae is indicated in Figure 13. While the amount of lipid in control is 22.7881±0.63 mg / larva and for LC$_{25}$, LC$_{50}$ and LC$_{75}$ treated larvae are 22.4800, 16.3728 and 12.2643 mg / larva respectively and therefore show significant reduction after treatment (F= 63.72, df= 3, p<0.01). The amount of lipid in female (16.96 mg/larva) was more than male (17.98.mg / larva) but not statistically significant (F= 2.42, df=1, P> 0.05).

Discussion

Plants are great sources of secondary plant metabolites which compete herbivores and pathogens [22]. Iran has a particular geographic condition and thus varied floras which have not been taken into consideration regarding the issue of plant protection. Therefore it seems reasonable to have a plan for further investigation on insecticidal effects of plants in future [5].

The results of this investigation clearly indicate that by increasing concentrations, the rate of mortality increases; it is similar to results reported on P. interpunctella adults by various essential oils [18].
The results of [22] also showed significant mortality of Tribolium castaneum, when A. annua extract at 0.5% concentration was used.

The species of Artemisia have shown a good effects on mortality of different species; for example the studies of [15] on Xanthogaleruca luteola Mull have shown LC50 values of 48% and 43% after 24 and 48 h of exposure. Another study by [5] on A. sieberi showed 100% mortality by 37μl / air for Callosobruchus maculatus (F), Tribolium castaneum (Herbst), Sitophilus oryzae (L). Similar studies on different species of Artemisia showed fair mortality on different species [30-32]. Camphore, a monoterpinoid in some plants, has been shown to have mortality effect as a fumigant in essential oils [5]. The camphore has been reported in A. annua [33].

The study also showed that different LC values were used to find out whether the adult emergence led to a significant result compared to the controls. This result is indicative of the effect of the essential oil on some morphological characteristics which unable the insect to emerge from pupal shell. In Phutella xylostella L. the Chinnaberry fruit extract showed similar results where, by increasing dosages, the emergence reduced [34]. This effect is definitely due to the metabolites present in either essential oil or in extract [35].

In the present study fecundity and fertility of adults emerging from treated larvae reduced significantly. The tentative answer for such a result may be the reduction in biochemical parameters (Protein and lipid) which has been considerably decreased after larval treatment. [36,37] found similar results in their experiments and concluded that changes in fecundity and fertility may be the result of changes in Protein.

Total protein content of larvae treated with LC25, LC50 and LC75 after 24 h was significantly reduced compared with the controls. The reduction in protein reserves may be due to physiological adoption of the insect to compensate the stress caused by insecticides [38] or due to the production mechanism of lipoprotein for making the cells and tissues. Another possible reason may relatively be the lack of ferric or other essential molecules due to insecticidal or other stresses to some extent [39]. Lipids conservation occurs in larval period since they result in the production mechanism of lipoprotein for making the cells and tissues [40]. As for lipid we can say that hemolymph and fat body lipids could degrade due to the a hormone controlling lipid metabolism might have been affected by exogenous chemicals. Adipokinetic hormone [41] and other hormones controlling the metabolism of lipid or carbohydrates might be affected [42] and therefore the hormone sites may be the site of effect of exogenous chemicals.

The toxicity and sub lethal action of Artemisia annua L. and other species of this genus reveal that this plant may be a good and safe natural toxicant in stored products which deserves further investigation.

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