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Fumigant toxicity of essential oils from *Artemisia annua* L. and the synergistic effect of acetone against three most important stored pests

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Abstract
Many plants have compounds with insecticidal properties and due to the high damage pests and adverse effects of pesticides, using of these compounds and essential oils is the best way to control pests. This study was carried out to determine the insecticidal activity of essential oil *Artemisia annua* L. against adult of three most important stored pests namely, *Tribolium castaneum* Herbst., *Sitophilus granarius* L. and *Callosobruchus maculatus* F. The experiments were planned in three replicate with completely randomized design under 27±1 °C, 60±5 R.H. under dark condition. The result was evaluated after 24 and 48h. The results indicated that the fumigant toxicity of essential oil against these pests. LC_{50} values for *A. annua* treatments on *T. castaneum, S. granarius and C. maculatus* were 75.528, 59.356 and 56.916 µl/l air and for Acetone 161.564, 138.733 and 123. 119 µl/l air, respectively, and LC_{25} values of *A. annua* and Acetone treatments on *T. castaneum, S. granarius* and *C. maculatus* were 45.808, 37.282 and 32.903 µl/l air and 113.614, 91.806 and 80.862 µl/l air, respectively. Then Acetone synergistic effects were tested on *A. annua* essential oil lethality. For this purpose, LC_{25} value of Acetone with a combination of LC_{25} value of Artemisia essential oil were tested against adult of three pests and results showed Acetone have synergistic effect on fumigant toxicity of *A. annua*.

Keywords: Acetone, *Artemisia annua*, essential oil, fumigant toxicity, stored pests

1. Introduction

Stored product pests are one of the most important problems in agriculture products storage that cause to great damage to stored products after harvesting until consumption. High reproduction rate, cosmopolite and oligophagity of these pests are the major cause high damage as in the store houses of the traditional terms, the amount of damages has been reported 10-80% [11]. Control of stored product insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as environmental disturbances, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms, in addition to direct toxicity to users [3]. Due to the high damage of stored product pests and adverse effects of chemical pesticides, using combination of plants such as essential oils are the best way of storage pests control [13]. Essential oils and their derivatives are recognized as an alternate means of controlling many harmful insects which are rapidly degradable in the environment and harmless to non-target organisms [14]. Essential oils have broad spectrum and are used against various pests [2]. Various essential oils have been tested against pests. These compounds have low toxicity in mammals and create less pollution in the environment [8]. Different types of aromatic plant preparations such as powders, solvent extracts, essential oils and whole plants are being investigated for their insecticidal activity including their action as repellents, antifeedants and insect growth regulators [2, 23]. *Artemisia* species (Asteraceae) are widely used medicinal plants in folk medicine. Some species such as *A. absinthium* L., *A. annua* L. or *A. vulgaris* L. have been incorporated into the pharmacopoeias of several European and Asian countries [15]. Tripathi *et al*. (2000) [20] had reported toxicity of *A. annua* on red flour beetle. Synergists also are widely used to overcome resistance and help to control different species of pests on the farm and specially stored grains [9]. Acetone is one of the metabolites in the human body and a number of mammals and there is in their blood and urine. This chemical compound is absorbed from the human digestive system and distributed throughout the body, and if 300 ml of pure acetone enter the body just causes paralysis of the central nervous system [22].

In the present study, we investigated fumigant toxicity of *A. annua* essential oil and its combination with acetone on three most important stored pests namely, *Tribolium castaneum* Herbst. *Callosobruchus maculatus* F. and *Sitophilus granarius* L.

2. Materials and Methods

2.1 Insect rearing

Preliminary population was obtained from laboratory stock cultures maintained at the Entomology department, Urmia University, Iran and the culture was maintained in the dark in an incubator set at 26±1 °C and 60±5% RH. Flour, wheat grains and mung bean grains were employed to rear *T. castaneum*, *S. granarus* and *C. maculatus* respectively. Adult insects, 1-7 days old, were used for fumigant toxicity tests. All experiments were carried out under the same conditions.

2.2 Essential oil preparation

Aerial parts include branches and leaves of *Artemisia annua* were collected during the flowering stage in October 2015. The air dried of the branches and leaves of the plant were subjected to hydro-distillation for 3 h using a Clevenger apparatus type and oil obtained was collected and dried over anhydrous sodium sulphate and stored in screw capped glass vials in a refrigerator at 4-5 °C.

2.3 Bioassay Tests

Bioassay trials carried out following Kita *et al*., technique [4]. For testing, glass vials with a cap that was impervious to the air were selected as protective fumigation. Twenty insects (1-7 days old adults) were used for each treatment with three replications. For each experiment, control treatment was used for data reconciliation and reliability of tests. In all cases, treatment time was 24 and 48 hours. In the other case, the number of dead insects was recorded up 48h in control and treatments % mortality calculated with Abbot formula. Adults were placed in film bones with 5cm length and 3 cm width and covered with ventilated lids (fabric with 40 mesh netting), Then were placed in glass vials. *A. annua* essential oil and acetone in pure form and without solvent are poured on a filter paper with 2 cm diameter by micro sampler and for uniform distribution of oil, filter paper was placed inside the cap of glass vials. To investigate synergistic effect, LC_{25} value of essential oil and acetone in mixture form were used on three pest beetles according to the former method. Treatments were kept at 27 ± 1 °C in the dark condition for 24 and 48 hours and adult mortality was counted at the end of trial. In this experiment the insects that couldn't move their legs and antenna by using hot needles were dead postulated. The mortality in control was observed in any of the experiments. This research was carried out on completely random plot. The data were analyzed using the Probit procedures with SPSS16 software and LC_{25} and LC_{50} values calculated.

3. Results and Discussion

3.1 LC_{50} and LC_{25} *A. annua* on three adult pests

According to the results, *A. annua* essential oil has good insecticidal effect against stored pests and has reduced acceptable these insect populations. The results of the Probit analysis, LC_{25} and LC_{50} values in 24 and 48 h are shown in Table 1.

Table 1: Fumigation Toxicity of *A. annua* essential oil against three stored insect adults.

Insect	Time(h)	Slope±SE	Chi-square	Lethal concentration	
				LC_{25} (Upper and lower confidence limits)	LC_{50} (Upper and lower confidence limits)
T. castaneum	24	-0.740±0.838	2.958	48.920(58.218-37.155)	82.117(93.423-71.244)
	48	-0.830±0.821	4.430	45.808(54.516-34.959)	75.528(85.539-65.369)
C. maculatus	24	0.281±0.713	6.684	34.356(51.486-6.187)	61.967(91.916-33.518)
	48	0.026±0.719	10.188	32.903(51.961-1.417)	59.356(90.381-18.513)
S. granarus	24	-1.161±0.814	10.165	40.847(61.160-5.787)	64.449(92.962-26.922)
	48	0.923±0.777	11.436	37.282(57.759-3.281)	59.356(88.047-19.671)

3.2 LC_{50} and LC_{25} Acetone on three adult pests

The results of the Probit analysis and LC_{25} and LC_{50} values in 24 and 48 h are shown in Table 2.

Table 2: Fumigation Toxicity of Acetone against three stored insect adults.

Insect	Time(h)	Slope±SE	Chi-square	Lethal concentration	
				LC_{25} (Upper and lower confidence limits)	LC_{50} (Upper and lower confidence limits)
T. castaneum	24	-0.256±1.947	6.453	119.048(153.440-7.008)	166.663(227.704-82.534)
	48	0.259±1.813	5.903	113.614(147.062-15.752)	161.564(212.254-91.451)
C. maculatus	24	1.989±1.163	10.227	84.844(122.369-5.344)	127.620(182.776-44.283)
	48	2.278±1.084	11.785	80.862(119.919-2.341)	123.119(184.445-32.458)
S. granarius	24	2.121±1.279	9.366	94.807(133.991-3.083)	143.187(213.200-50.288)
	48	2.058±1.216	8.413	91.806(128.154-12.118)	138.733(194.874-67.145)

The adult stages of *C. maculatus* are more susceptible to both agent controls compared with *S. granarius* and *T. castaneum*. Results showed that percentage mortality of adult pests in same concentration to both agents *A. annua* essential oil was lethality in compared Acetone.

3.3 Combined effects of *A. annua* and Acetone on adult stages of *T. castaneum*

Effects of LC_{50} *A. annua*, LC_{50} Acetone, combined of LC_{25} *A. annua* with LC_{25} Acetone on *T. castaneum* was evaluated and counted the percentage mortality after 24 and 48 hours (figure 1). The results showed that there was a significant difference between LC_{25} (*A. annua*) + LC_{25} (Acetone) with separate application LC_{50} (*A. annua*) and LC_{50} (Acetone) with 95% confidence in two time (df= 3 & 8, P<0.05, F= 272.250, Sig= 0.001 (df= 3 & 8, P<0.05, F= 230.667, Sig=0.001), respectively. The result showed that combined effect of treatment (LC_{25} *A. annua* + LC_{25} Acetone) were more lethality in compared other treatments.

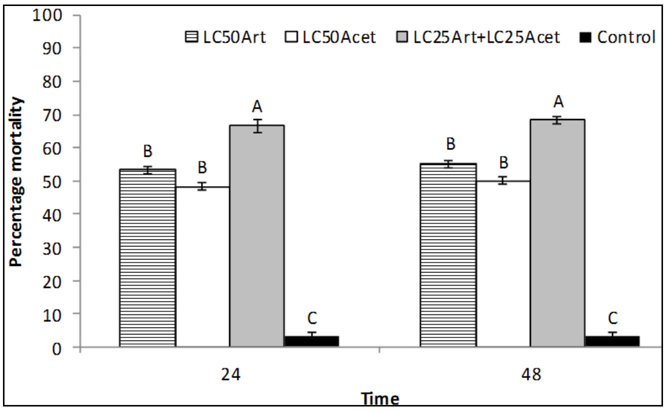

Fig 1: Compare mortality rate of essential oil, Acetone and synergistic effects of them on *T. castaneum* adults after 24 and 48 and hours. (Compare mean of treatments in three time independents from each other).

3.4 Combined effects of *A. annua* and Acetone on adult stages of *S. granarius*

Effects of LC_{50} *A. annua*, LC_{50} Acetone, combined of LC_{25} *A. annua* with LC_{25} Acetone on S. *granarius* was evaluated and counted the percentage mortality after 24 and 48 hours (figure 1). The results showed that there was a significant difference between LC_{25} (*A. annua*) + LC_{25} (Acetone) treatment with separate application treatments LC_{50} (*A. annua*) and LC_{50} (Acetone) with 95% confidence in two time (df= 3 & 8, $P<0.05$, F= 241.583, Sig= 0.001 (df= 3 & 8, $P<0.05$, F= 266.250, Sig=0.001), respectively. The result showed that combined effect of treatment (LC_{25} *A. annua* + LC_{25} Acetone) were more lethality in compared other treatments.

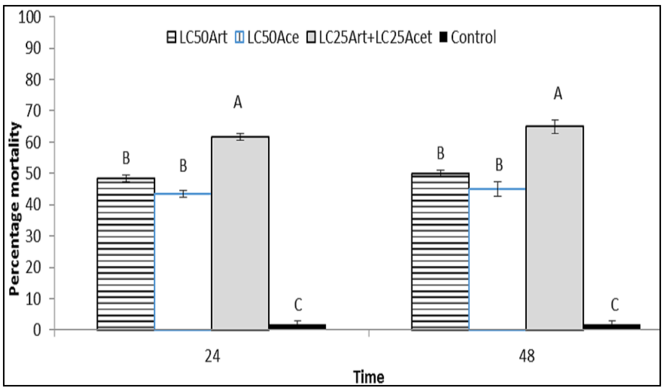

Fig 2: Compare mortality rate of essential oil, Acetone and synergistic effects of them on adults *S. granarius* after 24 and 48 and hours. (Compare mean of treatments in three time independents from each other).

3.5 Combined effects of A. annua and Acetone on adult stages of *C. maculatus*

Effects of LC_{50} *A. annua*, LC_{50} Acetone, combined of LC_{25} *A. annua* with LC_{25} Acetone on *C. maculatus* was evaluated and counted the percentage mortality after 24 and 48 hours (figure 1). The results showed that there was a significant difference between LC_{25} (*A. annua*) + LC_{25} (Acetone) treatment with separate application treatments LC_{50} (*A. annua*) and LC_{50} (Acetone) with 95% confidence in two time (df= 3 & 8, $P<0.05$, F= 214.667, Sig= 0.001 (df= 3 & 8, $P<0.05$, F= 328.000, Sig=0.001), respectively. The result showed that combined effect of treatment (LC_{25} *A. annua* + LC_{25} Acetone) were more lethality in compared other treatments.

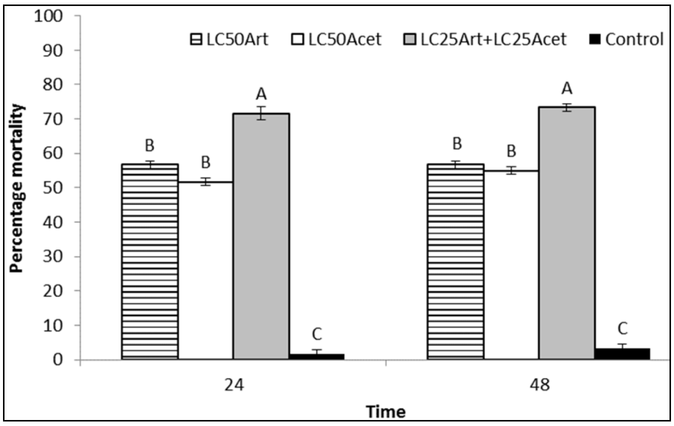

Fig 3: Compare mortality rate of essential oil, Acetone and synergistic effects of them on adults *C. maculatus* after 24 and 48 and hours. (Compare mean of treatments in three time independents from each other).

Natural compounds from plants could be efficient alternatives to conventional fumigants because of their low toxicity to mammals, fast degradability properties, and regional availability [16]. Our results on fumigant toxicity of *A. annua* on *T. castaneum*, *S. granarius*, and *C. maculatus* indicated that this essential oil had good toxicity on these pests by fumigation. Among the examined pests, *C. maculatus* was the most sensitive. Negahban *et al.* (2007) [12] reported that, the essential oil of *Artemisia sieberi* demonstrated fumigant toxicity to *C. maculatus*, *S. oryzae* and *T. castaneum*. The insecticidal activity varied with insect species, concentrations of the oil and exposure time. The results showed higher mortality rates in *C. maculatus* than in *S. oryzae* and *T. castaneum*. In the test of LC_{25} value of essential oil and acetone in combination form was observed high lethality that represents synergistic effect of two materials on each other and the mortality rate increased. Container size used in this study was 310 ml that has conformity with Lee *et al*. (2004) [6] research. Obviously due to diffusion and low pressure steam power essential oils, with increase of fumigation chamber volume, used essential oil and LC_{50} and LC_{95} values will rise exponentially [19]. The research conducted by the Safavi and Mobki (2012) [17] respiratory toxicity of tangerine peel essential oil was examined against adult *T. castaneum* calculated LC_{50} was 38.2 µl/l air that compared with studied essential oils have shown high respiratory toxicity. In research conducted by Mobky *et al.* (2015) [10] synergistic effect of acetone in lethality of tangerine peel essential oil was found against adult *C. maculatus*. Also in Khodabande *et al.* (2014) [5] researches fumigant toxicity of juniper fruits essential oil and acetone in mixture form were examined on adult *Rhyzopertha dominica* F. that acetone has synergistic effect in toxicity of these essential oils that has conformity with our research results. These results suggested that *A. annua* oil in combination with acetone may have potential as a control agent against *C. maculatus*, *S. granarius* and *T. castaneum*.

Results of this study clearly illustrated that insects varied in their susceptibility to different essential oils, which probably refers to the insecticidal ability of their active constituents; however, there is no difference between the time of high mortality for all essential oils and insects.

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