



## FUMIGANT TOXICITY OF *ARTEMISIA ABSINTHIUM* ESSENTIAL OIL TO COMMON STORED PRODUCT PESTS

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### ABSTRACT

Essential oil of *Artemisia absinthium* (L.) a herbaceous perennial plant widely grown Kashmir valley was extracted by steam distillation method. The chemical composition of the extracted essential oil showed a total of 17 components, majority being  $\beta$ -thujone (17.25%), p-cymene (13.19%), camphor (12.42%),  $\alpha$  terpineol (12.27%), eugenol (12.20%) and terpinen-4-ol (10.10%). The essential oil exhibited fumigant toxicity against the 1-7 days adults of *Corcyra cephalonica* (Stainton), *Tribolium castaneum* (Herbst) and *Callosobruchus chinensis* (L.). The LC<sub>50</sub> values in case of *C. cephalonica*, *T. castaneum* and *C. chinensis* were 42.45, 48.63 and 58.11  $\mu\text{L L}^{-1}$  air after 24 hr and 18.23, 34.32 and 41.16  $\mu\text{L L}^{-1}$  air after 72 hr of exposure, respectively.

**Key words:** *Artemisia absinthium*, fumigant toxicity, *Corcyra cephalonica*, *Tribolium castaneum*, *Callosobruchus chinensis*, LC<sub>50</sub>, essential oil, extraction, GLC, components

During storage in granaries and store houses the stored products are infested by number of insect pests (Tanya et al., 2020), and cause approximately 10- 30% crop losses (Ferry et al., 2004). Control of stored product insects by pesticides has its drawbacks (Rkia Moutiq et al., 2020; Andrea Veres et al., 2020). Fumigation has proved to be promising in their IPM (Isman, 2004). Methyl bromide and phosphine are the major fumigants used worldwide (Jemimah et al., 2020). As many insect pests have now developed resistance to phosphine, its continuous use is questionable. Methyl bromide use is also under scanner due to its ozone depleting properties (Pimentel et al., 2009). It is necessary to make safe, better, effective and biodegradable alternatives. Some plant and their products are known to be active towards many pests of stored grains (Isman, 2006). Essential oils have tremendous contact and fumigant toxicity, repellent and antifeedant activity and development and growth inhibitory activity against insects (Abdelgaleil et al., 2009). *Artemisia* spp. are used widely in folk and modern medicine, cosmetics and pharmaceutical industry (Abad et al., 2012; Reddy, 2019). Essential oils have aromatic components that impart characteristic odour and flavour to plants (Koul et al., 2008). Essential oils have also been used as fumigants against stored food products (Ebadollahi et al., 2010). Considering the insecticidal properties of essential oil of *Artemisia*

spp. the present study evaluates the fumigant toxicity of the essential oil of *Artemisia absinthium* (L.) against three major storage product insect pests.

### MATERIALS AND METHODS

Three storage pests namely *Corcyra cephalonica* (Stainton), *Tribolium castaneum* (Herbst) and *Callosobruchus chinensis* (L.) were mass cultured in plastic jars (5 l) containing whole rice, wheat flour/ crushed maize and bean grains, respectively (20 $\pm$  1°C. 60 $\pm$  5% RH) in the Bio control laboratory, Division of Entomology She-r-e-Kashmir University of Agricultural Sciences and Technology Shalimar, Srinagar. Adult insects used in the fumigation toxicity tests were 1 to 7 days old. Series of cultures of respective insect species were maintained continuously. The aerial parts of *A. absinthium* were collected from different locations in Kashmir Valley when the plants were in flowering stage. After collection, the plant material was shade dried for 5-7 days in the laboratory (23-24 °C) with proper ventilation. The dried material was then stored under refrigeration until the extraction. The essential oil was extracted using steam distillation in a Clevenger<sup>®</sup> apparatus. Distillation process was carried out for 4 hr and the essential oil obtained was dried over anhydrous sodium sulphate and stored in airtight glass vials under refrigeration at 4 °C. The certified reference material

of the individual constituents present in the essential oil of *A. absinthium* was procured from Sigma Aldrich Laramie WY USA.

The composition of the essential oil of *A. absinthium* was analysed on a Varian 450 (Walnut Creek, CA, USA) gas chromatograph (GLC) coupled with a flame ionization detector (FID). The capillary column used for the analysis was CP SIL 8 CB (30 m x 0.25  $\mu$ m ID x 0.25 $\mu$ m film thickness of 5% diphenyl and 95% dimethylpolysiloxane). The operating parameters of the instrument were as follows: initial oven temperatures 80°C hold for 2 min and then 1<sup>st</sup> ramp @ 5°C min<sup>-1</sup> to 130 °C hold for 2 min, 2<sup>nd</sup> ramp @ 5°C min<sup>-1</sup> to 200 °C hold for 2 min, 3<sup>rd</sup> ramp @ 5°C min<sup>-1</sup> to 230°C hold for 2 min, 4<sup>th</sup> ramp @ 3°C min<sup>-1</sup> to 260°C hold for 2 min and 5<sup>th</sup> ramp @ 10°C min<sup>-1</sup> to 280°C hold for 2 min. Carrier gas was nitrogen used at a flow of 1 ml min<sup>-1</sup>. The temperature of the injector port was 250 °C. The detector was maintained at 300°C. The gas flows to the detector were 30 ml min<sup>-1</sup>, for hydrogen, 29 ml min<sup>-1</sup> for make-up gas and 300 ml min<sup>-1</sup> for zero air. The total run time was 54 min. Galaxy chromatography data system version 1.9.302.530 was used for data processing and instrument control. The method devised by Keita et al. (2000) was used with slight modification for fumigant bioassay. Different doses of essential oil (10, 20, 30, 40 & 50  $\mu$ l/ l air) and control (without any essential oil) were used. Each treatment was replicated four times for each insect pest species. Mortality was recorded after 6, 12, 24, 48 and 72 hr after treatment. When no antennal or leg movement was observed when probed, the insects were counted as dead. The % mortality data were corrected by using Abbott's (1925) formula and the probit regression analysis was carried out to estimate the LC<sub>50</sub> values as per Finney (1971).

## RESULTS AND DISCUSSION

The chemical characterization of the essential oil of *A. absinthium* revealed the presence of 17 compounds. The compounds were  $\beta$ -thujone (17.255%), p-cymene (13.194%), camphor (12.428%),  $\alpha$ -terpineol (12.277%), eugenol (12.202%), terpinen-4-ol (10.105%),  $\alpha$  pinene (9.905%), farnesol (5.807%), myrecene (4.546%),  $\alpha$ -thujone (1.629%), and  $\beta$ -pinene (0.545%) (Table 1). The essential oil of *A. absinthium* exhibited fumigant toxicity to adults of *C.cephalonica*, *T.castaneum* and *C.chinensis*. Mortality increased with concentration of the oil from 10- 50  $\mu$ l/ l of air and exposure periods ranging from 6-72 hr. *A. absinthium* oil was relatively more toxic to adults of *C.cephalonica* than *T.castaneum*

Table 1. Chemical composition and relative proportion of essential oil of *A. absinthium*

Compound	Retention Time (min)	Composition (%)
Camphor	12.61	12.428
$\alpha$ -pinene	13.15	9.905
$\beta$ -pinene	13.42	0.545
P-cymene	14.37	13.194
$\alpha$ -thujone	16.37	1.629
$\beta$ -thujone	17.37	17.255
$\alpha$ -terpineol	19.70	12.277
Terpinen 4 ol	20.23	10.105
N.I	20.58	0.001
N.I	21.17	0.001
N.I	23.08	0.044
N.I	23.52	0.011
N.I	23.67	0.039
Eugenol	25.17	12.202
N.I	25.25	0.011
Farnesol	33.74	5.807
Myrecene	34.33	4.546

and *C. chinensis*. The lowest concentration (20 $\mu$ l/ l) of the oil yielded maximum mortality of *C. cephalonica* after 72 hr exposure, as compared to *T.castaneum* and *C.chinensis* at the same concentration and exposure time (Figs. 1-4). It was observed that with an increase in oil concentration and exposure period, % mortality also increased. Probit analysis revealed that *C.cephalonica* was more susceptible than *T.castaneum* and *C.chinensis* with LC<sub>50</sub> values of 42.45, 48.63 and 58.11 and 18.23, 34.32 and 41.16  $\mu$ l/l after 24 and 72 h of exposure, respectively (Table 2). The fumigant toxicity was maximum with *C. cephalonica*, and it was dose dependent and enhanced with the increase in exposure time (Haouas et al., 2012). The present results are in agreement with those of earlier workers on the fumigant activity of essential oils from *Artemisia* spp. against stored product insects. Negahban et al. (2006) observed this with *A. sieberi* against *C. maculatus*, *S. oryzae* and *T. castaneum*; similar was the case with *A. aucheri* (Shakarami et al., 2004). Sharifian et al. (2012)

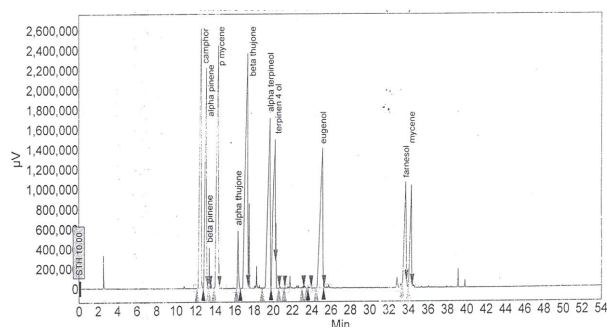


Fig. 1. Compounds in the essential oil of *A. absinthium*

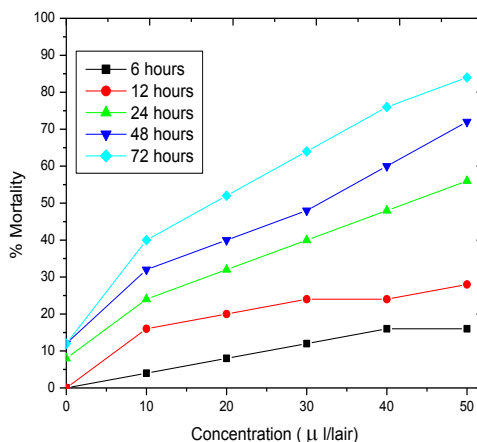


Fig. 2. Fumigant toxicity of *A. absinthium* oil against *C. cephalonica*

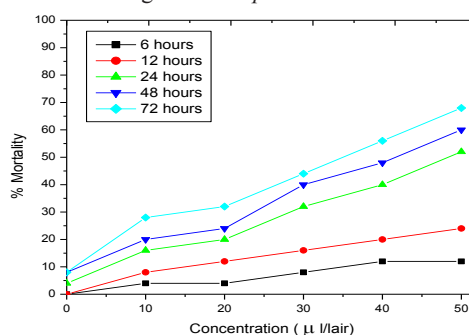


Fig. 3. Fumigant toxicity of *A. absinthium* oil against *T. castaneum*

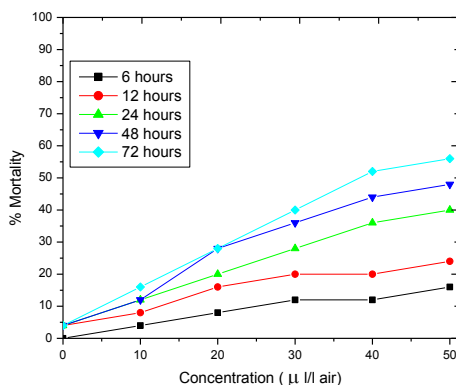


Fig. 4. Fumigant toxicity of *A. absinthium* oil against *C. chinensis*

reported fumigant toxicity of *A. herba-alba* against *T. castaneum*, *C. maculatus* and *R. dominica*. Hashemi and Safavi (2012) investigated fumigant toxicity of essential oil of *A. khorassanica* against *T. confusum*. Bachrouch et al. (2015) studied the fumigant toxicity of the essential oil of two *Artemisia* species (*A. herba-alba* and *A. absinthium*) against *O. surinamensis* and *T. castaneum*.

GC/FID analysis of the essential oil of *A. absinthium* yielded 17 components among which  $\beta$ -thujone (17.25%) was the most dominant followed by p-cymene (13.19%), camphor (12.42%),  $\alpha$ -terpineol (12.27%), eugenol (12.20%), terpinen-4 ol (10.10%) and  $\alpha$ -pinene (9.90). These results agree with those of Bachrouch et al. (2015) that essential oil of *A. absinthium* from Tunisia is rich in camphene (2.37%),  $\beta$ -thujone (22.72%), 1, 8 cineole (5.47%) and camphor (16.71%). Lopez-Lutz et al. (2008). Khangholi and Rezaeinodehi (2008) reported that  $\beta$ -pinene and  $\beta$ -thujone were the main components of Iranian wormwood essential oil. Thus, the essential oil of *A. absinthium* possesses fumigant toxicity and potential as natural fumigant against stored product pests viz., *C. cephalonica*, *T. castaneum* and *C. chinensis*. The essential oils of *Artemisia* spp. exhibited important insecticidal activities that depend upon the type and nature of constituents and their individual concentration levels (Koul et al., 2008). Fumigant toxicity of *A. absinthium* is attributed to the dominant presence of component  $\beta$ -thujone.

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Table 2. LC<sub>50</sub> values of *A. absinthium* against stored product pests

Pest	Time (hr)	LC <sub>50</sub> (µl/l air)	F L	$\chi^2$	p-value	SE
<i>Coreyra cephalonica</i>	24	42.45	(30.6787-54.2276)	0.15	0.98	5.94
	72	18.23	(10.2877-26.1748)	0.29	0.96	4.01
<i>Tribolium castaneum</i>	24	48.63	(39.3219-57.9544)	0.61	0.89	4.70
	72	34.32	(25.1305-43.5115)	0.82	0.84	4.64
<i>Callosobruchus chinensis</i>	24	58.11	(47.0797-69.1493)	0.04	0.99	5.57
	72	41.16	(32.4538-49.8860)	0.08	0.96	4.40

F L- Fiducial limits; LC<sub>50</sub>= Concentration that causes 50% mortality of a sample population; S E = Standard error;  $\chi^2$  value refers to the goodness of fit test at p > 0.05.

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