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Research Article

Toxicity and repellent effect of essential oils and a major component against *Lipaphis erysimi*

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Abstract: Essential oils (EOs) extracted from aerial parts of *Aster indamellus* Grierson, *Calamintha umbrosa* Benth. and *Erigeron annuus* (L.) Pres. were analyzed by GC, GC/MS. The major acetylenic constituent (*cis*-lachnophyllum ester) of *E. annuus* was isolated and characterized by ^{1}H and ^{13}C-NMR spectra. Their toxicity and repellent effect against *Lipaphis erysimi* was tested. Oils of *E. annuus* and *C. umbrosa* exhibited higher toxicity on direct spray and by fumigation. LC_{50} value of *E. annuus* oil was 0.43 mg/ml as direct spray. By fumigation, LC_{50} value for *E. annuus* was 1.29 ml/l air, while for *C. umbrosa*; it was 1.00 ml/l air. With acetylenic ester, about half of *L. erysimi* were killed at 10 mg/ml approximately within 13.25 h, while with *E. annuus* oil the LT_{50} value was approximately 8.89 h. In conclusion, the EO of *E. annuus* and its acetylenic constituent have potential as biopesticide for economically important crop pests.

Keywords: Essential oil, *cis*-lachnophyllum ester, *Lipaphis erysimi*, toxicity, repellence

Introduction

Mustard is one of the important cruciferous oilseed crops and constitutes major source of edible oil for human consumption and as cake for animals. Efforts are being made to raise the productivity of these crops by adopting modern agricultural practices to meet growing demand but the attack of insect pests has proved major limiting factor in the successful cultivation of mustard crop. Among various insects Mustard aphid, *Lipaphis erysimi* (Order: Hemiptera; Family: Aphididae) is one of the most serious pests of *Brassica juncea* (Rouf and Kabir, 1997). Both the adults and nymphs cause damage to mustard plants by sucking sap from seedling to maturity stage. Maximum damage is caused at flowering stage. Aphids suck the sap from new shoots, lower surface of leaves, inflorescence and developing seliqua resulting in yield loss to the extent of 90% with 15% loss of oil contents (Verma and Singh, 1987).

Chemical insecticides have been effective to suppress the pest population under pest management strategies, but their periodic and excessive use results in adverse effects on non-target organisms as well as agro-ecosystem (Foster *et al*., 2007; Furk and Hines, 1993; Lowery and Smirle, 2003; Whalon *et al.,* 2008). Furthermore, this causes adverse effects leading to toxic residues in food, soil, ground water and air (Bughio and Wilkins, 2004). Consequently, there is need for developing economic and environment friendly strategy for the control of pest population. As an alternative, naturally occurring, biologically active plants and their chemical constituents can play prominent role in the development of new commercial pesticides for increasing productivity and safety

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of the environment and public health (Bhathal and Singh, 1993; Ceferino *et al.,* 2006; Nerio *et al*., 2009; Singh *et al.,* 1980).

Plants produce secondary metabolites with diverse chemical structures. Terpenoids, constituents of essential oils (EOs), cause plant protection against micro-organisms and insect herbivores (Choi *et al.,* 2004; Kazana *et al.,* 2007). As compared to commercial pesticides, EOs are generally safer to mammals, birds and fish (Isman, 2006). Plant EOs with their bioactive constituents generally possess broad-spectrum activity against pest insects and plant pathogenic fungi ranging from insecticidal, fumigant effects, antifeedant, repellent, ovipositor, deterrent and growth regulator activities. Biodegradable property shows their potential as future insecticides (Cox, 2004; Kubo, 2006; Misra *et al.,* 1996; Sampson *et al.,* 2005). Essential oils of Asteraceae and Lamiaceae usually possess biologically active acetylenic compounds besides mono- and sesquiterpenoids. Their antiseptic (bactericidal, viricidal and fungicidal) and medicinal (e.g., antioxidant and anticancer) properties (Bakkali *et al.*, 2008; Dorman and Deans, 2000; Kumar *et al.*, 2014; Mathela and Mathela, 1986; Shah *et al*., 1992; Saxena and Mathela, 1996; Zu *et al*., 2010) have been documented.

As a part of strategy for safer pest management and crop protection, EOs and their chemical constituents have potential as insecticidal, fumigant and repellent. The aim of the present investigations was to evaluate the toxicity and repellent activity of three EOs viz. *Erigeron annuus*, *Aster indamellus* and *Calamintha umbrosa* and acetylenic constituent (*cis*-lachnophyllum ester) of *E. annuus* against mustard aphid, *L. erysimi*. Toxicity of EOs against aphid species of different hosts has been documented but to best of our knowledge activity of oils from these species and acetylenic constituent against *L. erysimi* is being reported for the first time.

Materials and Methods

Materials

Aster indamellus Grierson, *Calamintha umbrosa* Benth. and *Erigeron annuus* (L.) Pres. were collected at flowering stage and identified from Botanical Survey of India, Dehradun.

Extraction method

The fresh aerial parts of species (2-3 kg) were subjected to steam distillation and organic phase was separated with n-hexane followed by dichloromethane to ensure complete extraction of chemical constituents. The n-hexane and dichloromethane extracts were combined together, dried over anhydrous Na_2SO_4 and the solvent was distilled off in a rotary vacuum evaporator to obtain residual oil which was stored in a dark vial at 4 ºC until use.

GC-EIMS analyses

Qualitative and quantitative analysis of EOs from *E. annuus, A. indamellus* and *C. umbrosa* were done by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The GC was carried out on a Nucon 5765, India GC-equipped with Rtx-5 non-polar fused silica capillary column (30 m × 0.32 mm, film thickness: 0.25 µm). The oven temperature (60-210 °C) was programmed at 3 °C/ min and N_2 as the carrier gas at 4 kg/ cm^2. The injector and detector temperatures were 210 °C each and the injection volume 0.5 µL of 10% solution of the oil in *n*-hexane. The GC/ MS was conducted on a Thermo Quest Trace GC 2000 (Thermo Quest/Finnigan, Germany) interfaced with a Finnigan MAT Polaris Q Ion Trap mass spectrometer. Helium was used as the carrier gas at 1.0 mL/min. The injection volume was 0.10 µL and the split ratio was 1:40. The MS were taken at 70 eV with a mass range of 40-450 amu. Other operating parameters were the same as for GC. Characterization of components of the EOs was done by comparing their retention indices (RI), published data (Adams, 2007) and NIST and WILEY MS library searches. The relative percentage of the oil constituents was expressed as percentage by FID response in GC.

Isolation and characterization of acetylenic compound

The EO (2 mL) of *E. annuus* was subjected to silica gel column chromatography (230-400

mesh, Merck, 20g) with hexane:diethyl ether (99: 1 to 85: 15) as eluent. The fractions showing similar TLC pattern were mixed and further analyzed by GC for their purity. Fractions 13-16 were mixed and run on a separate silica gel column (230-400 mesh, Merck, 20g) with hexane: Et_2O (99: 1-95:5) as eluent to obtain pure compound (90 mg; > 98% purity). It was characterized as *cis*-lachnophyllum ester on the basis of its MS, ^1H and ^{13}C-NMR spectra and comparison with reported data (Albuquerque *et al.,* 2004; Kumar *et al.,* 2014).

Insect cultures

Lipaphis erysimi were collected together with the infested leaves and flowers of *B. juncea* from the oilseed crop field located at Crop Research Centre, GBPUA&T, Pantnagar, India and maintained on *B. juncea* plants grown in polyhouse. Adults of same size of 4-5 days old were used in bioassays carried out at 20 ± 2 ^oC. Monocrotophos; a toxic organophosphate insecticide was used as standard for comparison purpose under identical conditions.

Insecticidal activity

Insecticidal activity of EOs and acetylenic constituent were determined against *L. erysimi* by the direct spray and indirect spray methods. A series of dilutions of EOs and acetylenic constituent (0.50-10.00 mg/ml) were prepared using dimethyl sulphoxide (DMSO) as solvent with addition of Tween-20 (0.05%) as emulsifier. In the direct spray *L. erysimi* was released on 4-6 weeks old *B. juncea* plants using camel hairbrush and after 24 h, 5 mL of each solution was sprayed separately using atomizer. Controls were treated with DMSO. Ten same size adults of *L. erysimi* were used for each concentration and control, and the experiment was replicated three times. Mortality was recorded after 24 h of the treatment and calculated by using Abbott's formula (Abbott, 1925) and the LC_{50} value were calculated according to Finney (1971). Insects incapable of moving after slight touch with fine brush were considered as dead. Another experiment was designed in order to determine the toxicity of oils and the exposure time required to kill 50% insects (LT_{50}) at 10 mg/ml. Replication and other conditions were the same as described for the previous experiment. Mortality was recorded after 8, 24, 30 and 48 h of exposure to the EOs. Time-mortality data for each experiment were analyzed via the method developed by Finney (1971) with time as the explanatory variable.

Fumigant activity

Fumigant toxicity of three EOs and acetylenic constituent were calculated against *L. erysimi* based on Pascual-Villalobos and Robledo (1998). *L. erysimi* (10 same size adults) were transferred from stock colony to mustard leaf with petioles (warped with moist cotton) placed in petri dish (9 cm diameter) and allowed to settle for half an hour before being exposed to EOs. Aliquots of 0.20 mL of the EO dilutions (0.50-10.00 ml/l air) were applied on filter paper (3 cm^2, Whatman #1) and air dried for half an hour. The impregnated filter paper was then attached to the undersurface of the petri dish. Plates were then sealed with parafilm, which contained ten adults of *L. erysimi.* Each treatment was replicated thrice. Control having no EO was also used. All the petri dishes were kept under the identical experimental conditions. Mortality data were recorded after 2, 4, 6 and 24 h of exposure to the EOs. Mortality data were subjected to Probit analysis (Finny 1971) to calculate the LC_{50} & LT_{50} values.

Repellent activity

To evaluate repellency, mustard leaf (3 cm^2) was treated with 0.20 mL acetonic solution of each concentration (0.50 mg/ml) of oils and acetylenic constituent. Leaf treated with acetone without any EO was used as control. Control and treated leaves were placed over wet Whatman #1 filter paper in the same petri dish and the tested aphids were placed at an equal distance from either leaf after half an hour for solvent evaporation. Three replicates were maintained for each concentration. After 24 h,

number of insects were counted on treated and control leaf separately and insects that did not settle on any leaves were discarded from calculation in both assays. The percentage repellency (PR) was calculated as follows: PR = [(C-T)/(C + T)] × 100, where C = numbers of insects on control leaf and T= numbers of insects on treated leaf (Pascual-Villalobos and Robledo, 1998; Nerio *et al*., 2009).

Statistical analysis

Data were subjected to one-way Analysis of Variance (ANOVA) and compared by Duncan Multiple Range tests at a level of significance of $p < 0.05$. Probit analysis (Finney, 1971) was conducted to estimate the lethal concentration (LC_{50}) and lethal time (LT_{50}) with their 95% fiducial limit. Analysis was done using SPSS 16.0 statistical software.

Results

Essential oils

EO yields from fresh aerial parts of *E. annuus*, *A. indamellus* and *C. umbrosa* were in the range of 0.10-0.20% (v/w). The GC and GC/MS analyses of EOs characterized mono- and sesquiterpene hydrocarbons, their oxygenated sesquiterpenoids and acetylenic compound. Chemical composition of EOs of *E. annuus* oil showed presence of germacrene D (10.35%), β-caryophyllene (1.39%) and β-eudesmol (1.35%) besides an acetylenic ester (*cis*-lachnophyllum ester; 68.09%) as the most abundant constituent. The chemical constituents (>2%) in *C. umbrosa* EO were β-caryophyllene (11.66%), germacrene D (9.81%) and spathulenol (9.44%), β-pinene (7.47%), terpinolene (6.86%), α-cubenol (5.01%), α-copaene (4.08%), α-cadinol (3.65%), linalool (3.40%), δ-cadinene (3.02%) and piperitenone (2.65%). Essential oil of *A. indamellus* was found to contain α-muurolol (18.15%), germacrene-B (6.16%), 10-*epi*-γ-eudesmol (5.72%), α-cubenol (4.92%), germacrene-D-4-ol (4.19%), humulene epoxide-II (3.90%), carvacrol (3.42%). borneol (2.94%), β-myrcene (2.15%), carvacrol acetate (2.03%) and ledol (2.59%).

Insecticidal activity

Toxicity of essential oils and acetylenic constituents against *L. erysimi* data are given in Table 1. On the basis of LC_{50} values, *L. erysimi* was significantly more sensitive to the oil of *E. annuus* than to the *C. umbrosa* and *A. indamellus*. Lowest LC_{50} value (0.43 mg/ml) was recorded in case of *E. annuus*, followed by *C. umbrosa* (1.07 mg/ml) and *A. indamellus* (3.53 mg/ml). In case of *cis*-lachnophyllum ester LC_{50} was 0.85 mg/ml. The lethal time values decreased significantly with increase in the concentration. The LT_{50} values at 10 mg/ml concentration of oils and *cis*-lachnophyllum ester from *E. annuus* oil are presented in Table 1. Lowest LT_{50} value was recorded in *E. annuus* (8.89 h) as compared to synthetic insecticide where the LT_{50} was 7.19. The lower LC_{50} and LT_{50} values of *E. annuus* and *cis*-lachnophyllum ester indicate significant insecticidal potential against *L. erysimi*.

Fumigant activity

The toxicity to *L. erysimi* and mortality depended on nature of EOs as well as their concentration and duration of exposure (Table 2). Among the three oils, the *C. umbrosa* was found to be most potent oil against *L. erysimi* with the LC_{50} of 1 ml/l air, while the least potent was *A. indamellus* oil with LC_{50} of 7.45 ml/l air, as compared to the synthetic insecticide where the LC_{50} was 0.71 ml/l air. *cis*-Lachnophyllum ester showed slightly lower insecticidal activity (LC_{50} = 1.49 ml/l air) than *E. annuus* oil (LC_{50} = 1.29 ml/l air). While EO of *A. indamellus* showed the lowest toxicity (LC_{50} = 7.45 ml/l air) as compared to other EOs. When the insects were fumigated with *E. annuus* oil and *cis*-lachnophyllum ester at concentration of 0.50 ml/l air, the lower and upper 95% fiducial limits of the LT_{50} value were 18.99 and 82.79 h, and 14.98 and 51.66 h, respectively. The 95% fiducial limits of the LT_{50} value for the concentration tested (0.50 ml/l air) was 17.17 and 79.42 h, and 29.65 and 98.94 h, respectively for the oils of *C. umbrosa* and *A. indamellus* (Table 2).

Table 1 Contact toxicity of essential oils, *cis*-lachnophyllum ester and standard insecticide against *Lipaphis erysimi.*

Sample	LC_{50} (95% FL) (mg/ml)	Slope ± SE	$\chi 2$	LT_{50} (95% FL) (h) at 10 mg/ml	Slope ± SE	$\chi 2$
E. annuus	0.43 (0.11-0.79)	1.11 ± 0.27	11.34	8.89 (6.57-10.99)	4.04 ± 0.75	9.81
C. umbrosa	1.07 (0.52-1.68)	1.12 ± 0.25	7.20	11.51 (9.26-13.79)	4.62 ± 0.70	4.03
A. indamellus	3.53 (2.40-5.76)	1.29 ± 0.29	9.24	17.10 (11.14-22.76)	1.93 ± 0.44	9.84
Lachnophyllum ester	0.85 (0.32-1.41)	1.02 ± 0.24	7.56	13.25 (9.99-16.29)	3.24 ± 0.53	10.49
Monocrotophos	0.28 (0.06-0.49)	1.72 ± 0.47	4.63	7.19 (3.47-8.29)	7.41 ± 2.66	2.01

LC: lethal concentration (mg/ml); LT: lethal time (h); FL: fiducial limit; $\chi 2$: chi square.

Table 2 Fumigant toxicity of essential oils, *cis*-lachnophyllum ester and standard insecticide against *Lipaphis erysimi*.

Sample	LC_{50} (95% FL) (ml/l air)	Slope ± SE	$\chi 2$	LT_{50} (95% FL) (h) at 0.5 ml/l air	Slope ± SE	$\chi 2$
E. annuus	1.29 (0.42-2.37)	0.78 ± 0.23	14.97	39.42 (18.99-82.79)	1.21 ± 0.35	11.14
C. umbrosa	1.00 (0.00-2.27)	0.48 ± 0.22	10.28	30.45 (17.17-79.42)	1.46 ± 0.36	13.31
A. indamellus	7.45 (3.22-41.84)	0.58 ± 0.23	5.32	70.82 (29.65-98.94)	1.42 ± 0.46	6.72
Lachnophyllum ester	1.49 (0.37-3.15)	0.66 ± 0.23	11.32	28.82 (14.98-51.66)	1.70 ± 0.38	15.50
Monocrotophos	0.71 (0.47-0.94)	2.22 ± 0.39	8.17	21.44 (10.92-36.77)	0.94 ± 0.94	7.36

LC: lethal concentration (ml/l air); LT: lethal time (h); FL: fiducial limit; $\chi 2$: chi square.

Repellent activity

The EOs and *cis*-lachnophyllum ester possessed moderate repellent activity to *L. erysimi* (Fig. 1). The repellent activity of the oils and the compound were influenced by the concentration applied and the exposure time. *L. erysimi* was particularly more sensitive to the EOs of *E. annuus* and *C. umbrosa*. Repellency noticed was 36.50% and 42.06%. *A. indamellus* oil was found to be less repellent (20.53%) than the other two oils. *cis*-Lachnophyllum ester (26.50%) showed lower repellent activity compared to *E. annuus* oil.

Discussion

The GC and GC/MS of *E. annuus* oil revealed *cis*-lachnophyllum ester (68.09%) as major constituent besides germacrene D (10.35%), β-caryophyllene (1.39%) and β-eudesmol (1.35%) which were comparable with the previous report by Kumar *et al.,* (2014) except for minor compositional changes. β-Caryophyllene (11.66%), germacrene D (9.81%) and spathulenol (9.44%) were characterized in the oil from *C. umbrosa*, while in a previous report (Shah *et al.,* 1992) *cis*-piperitone oxide (63.05%) and piperitenone oxide (17.98%) had been reported. The difference in chemical composition of *C. umbrosa* oil could be attributed to existence of different chemotypes within *C. umbrosa*. The *A. indamellus* oil was characterized by the dominant presence of sesquiterpenoid alcohols *viz*. α-muurolol (18.15%), 10-epi-γ-eudesmol (5.72%), α-cubenol (4.92%), germacrene-D-4-ol (4.19%) besides germacrene-B (6.16%) and carvacrol (3.42%).

Bioactive organic compounds produced by plants include repellents, growth inhibitors and toxins that form an extensive chemical defense against invading pathogens and insects. The essential oils have insecticidal activity due to the presence of terpenoids (Isman, 2006; Viegas, 2003). Various terpenoids found in the essential oils exert toxicity against aphids through various mechanisms *viz*., deterring the penetration by aphid into host plant, disturbing feeding behavior, reducing reproduction in parthenogenetic individuals and inhibiting oviposition in sexually reproducing individuals (Tewary *et al*., 2005). The rapid action against some pests is indicative of a neurotoxic mode of action of essential oil and there is evidence for interference with the neuromodulator octopamine by some oils and with GABA-gated chloride channels by others (Kostyukovsky *et al*., 2002; Priestley *et al*., 2003).

Essential oil from *E. annuus* showed high activity followed by *cis*-lachnophyllum ester and *C. umbrosa* oil. We believe that the strong activity

of *E. annuus* oil could be due to *cis*-lachnophyllum ester, an acetylenic non-terpenoid constituent of the oil (>60%). Sesquiterpenoid alcohol constituents of *A. indamellus* oil appear to be much less active than the acetylenic constituent of *E. annuus*. From the stand point of pest control, important property of the EOs is their fumigant activity. When *L. erysimi* were fumigated for 24 h with EO from *A. indamellus*, a concentration of 7.45 ml/l air caused 50% mortality (LC_{50}), while the oil of *C. umbrosa* caused equal mortality with a concentration of 1 ml/l air. Fast insecticidal activity by the vapor of oil from *C. umbrosa* also became evident from the medium lethal time values (LT_{50}).

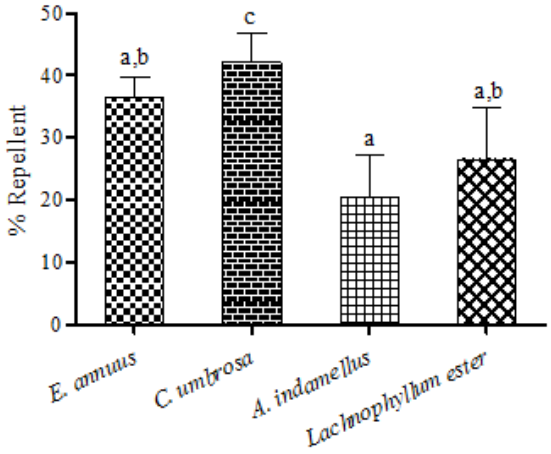

Figure 1 Repellent activity of essential oils and acetylenic ester at 0.50 mg/ml against *Lipaphis erysimi* after 24 h. Bars with different letters (a-c) are statistically different at the level of $p < 0.05$ according to the Duncan Multiple Range Test.

In conclusion, this is the first study demonstrating repellency and toxicity activity of EOs from *E. annuus, cis*-lachnophyllum ester, an acetylenic ester constituent of *E. annuus* and *C. umbrosa* showed potential against *L. erysimi* for pest control while the oil from *A. indamellus* was much less active.

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اثرات سمی و دورکنندگی اسانس‌های گیاهی با یک ترکیب اصلی آن روی شته خردل *Lipaphis erysimi*

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چکیده: اسانس‌های گیاهی از اندام‌های هوایی گیاهان *Aster indamellus* Grierson، *Calamintha umbrosa* Benth. و *Erigeron annuus* (L.) Pres. استخراج و توسط GC و GC/MS مورد شناسایی قرار گرفت. سپس ترکیب استیلنی *cis*-lachnophyllum ester از گیاه *E. annuus* جداسازی شد و طیف آن توسط دستگاه NMR مورد بررسی قرار گرفت. در این مطالعه اثرات سمی و دورکنندگی ترکیبات فوق روی شته خردل بررسی شدند. اسانس‌های گیاه *E. annuus* و *C. umbrosa* اثرات تماسی مستقیم و تدخینی بالایی نشان دادند. مقدار LC_{50} اسانس *E. annuus* در تماس مستقیم معادل ۰/۴۳ میلی‌گرم در میلی‌لیتر و در تماس غیرمستقیم معادل ۱/۷۶ میلی‌گرم در لیتر بود. اما مقدار LC_{50} برای اسانس *E. annuus* در حالت تدخینی معادل ۱/۲۹ میلی‌گرم در لیتر و برای اسانس *C. umbrosa* معادل ۱ میلی‌گرم در لیتر بود. مدت زمان لازم برای مرگ‌ومیر ۵۰ درصد از جمعیت (LT_{50}) توسط استر استیلنی در غلظت ۱۰ میلی‌لیتر بر لیتر معادل ۱۰ ساعت و برای اسانس *E. annuus* معادل ۱۳ ساعت بود. به‌طور کلی اسانس گیاه *E. annuus* و ماده استیلنی آن به‌عنوان یک آفت‌کش گیاهی روی آفات زراعی مهم مؤثر است.

واژگان کلیدی: اسانس، استر سیس لاکنوفیلوم، شته خردل، سمیت، دورکنندگی