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Research Article

The toxic effect of camphor vapour against *Aphis craccivora* Koch (Hemiptera: Aphididae) and some of its natural enemies

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Abstract: Fumigant toxicity of camphor was studied against the aphid *Aphis craccivora* Koch and three associated natural enemies, i.e. *Coccinella undecimpunctata* L., *Aphelinus albipodus* Hayat & Fatima and *Aphidius colemani* Viereck. *Aphis craccivora* was the most tolerant one compared with all tested natural enemies as the recorded LC_{50} values were 12.71, 6.33, 1.16 and 0.48 mg camphor/liter space for the above mentioned insects, respectively. Subjecting newly emerged adults of *A. craccivora* to LC_{25} of camphor vapor significantly reduced female longevity from 17.6 to 6.45 days and reduced the female daily progeny from 4.44 to 1.93 nymph / female, which resulted in a reduction in productivity as finite rate of increase decreased from 1.57 to 1.14 female / female / day. Aphids that survived after subjection to LC_{50} were found to have significantly higher amount of acid phosphatase and G. S-transferase than non-treated aphids. Inversely, Survived aphids were found to have significantly less amount of β-esterases and alkaline phosphatase than non-treated aphids; while no significant difference was found in case of α-esterases. Camphor fumigant can be a candidate as a control agent against *A. craccivora* but with restriction because of its drawbacks on natural enemies.

Keywords: Camphor, *Aphis craccivora, Aphelinus albipodus*, *Aphidius colemani, Coccinella undecimpunctata*

Introduction

Aphids infect many greenhouse plants casing a great damage to plants through; loss of sap by sucking, reaction of plant tissues stimulated by aphid saliva, excreting viscous honeydew on which sooty-moulds usually develop and finally by transmission of viral diseases to plants. The intensification of pesticide treatments has shown that the aphids cannot be eradicated but, on the contrary, resistant populations have appeared which exhibit even more vitality than the original sensitive strains (Shotkoski *et al*., 1990). Also, using traditional pesticides should result in loss of the yield that is expected during the pre harvest interval (PHI). Since usually valuable crops are planted in greenhouses, this loss is magnified. Moreover, high-density plantation in greenhouses may complicate traditional application of pesticides. High-density plantation also provides shelters for pests which may prevent the pesticides from reaching all the pest individuals. So using natural fumigants might be suitable for controlling aphids in greenhouses, because of its safety and capability to reach every point in high density plantation. Fumigant toxicity of several volatile oils has been investigated against different pests by many authors

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(Stamopoulos, 1991; Weaver *et al*., 1994; Don-Pedro, 1996; Reddy and Singh, 1998; Sammataro *et al*., 1998). In order to overcome the above problems, current work has been carried out to investigate the fumigant toxicity of camphor against the cowpea aphid *Aphis craccivora* Koch as one of the main greenhouse pests, and three associated natural enemies; the predator *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae), and the parasitoids; *Aphelinus albipodus* Hayat and Fatima (Hymenoptera: Aphelinidae) and *Aphidius colemani* Viereck (Hymenoptera: Braconidae).

Materials and Methods

Insects

Aphids were reared on faba bean plants *Vicia faba* L infested with *A. craccivora* and continued by placing weekly fresh seedlings beside the old ones in the rearing cages. *A. albipodus* and *A. colemani* were reared in cloth cages by releasing mated females on seedlings infested with the aphid species. After 5-6 days, formed mummies were collected, using a soft brush and kept in vials until emergence of parasitoid adults. Parasitoid adults were provided with droplets of honey, to serve as food, Stary (1970). Parasitoids' rearing continued to obtain sufficient population. *C. undecimpunctata* was reared by placing 4-5 adult pairs into plastic jars, covered with muslin, and supplied with faba bean. Seedlings infested with *A. craccivora* as a food and left to deposit eggs and reproduce. Freshly hatched larvae were daily provided with adequate numbers of aphids as food until pupation. Then pupae were kept until adult emergence. Rearing of aphid, predator and parasitoids were carried out under laboratory conditions at 25 ± 1 ^{o}C, 50-70% R.H. and 16:8 (L: D) h photoperiod.

Camphor

Camphor, produced by Kien Chung Camphor Mfg Co Ltd, was purchased as white crystals 95.22% purity, according to GC MS analysis done in Central Agricultural Pesticide Laboratory, Agricultural Research Center, Egypt. Then it was dissolved in acetone in order to facilitate applying small amounts of camphor.

Fumigant insecticidal activity

The fumigant toxicity of camphor was evaluated against *A. craccivora* adults and some associated natural enemies, i.e. the predator *C. undecimpunctata* and the parasitoids *A. albipodus* and *A. colemani*. Airtight containers (5 liters size) were used as a test chamber in our experiment.

Ten adults of *A. craccivora* were transferred to faba bean seedlings. Seedlings were placed in cups covered with muslin. Four cups were prepared, as four replicates, and were placed together in one test container. Serial concentrations of camphor acetonic solution were transferred to clean Petri dishes using pipette then the dishes were left until acetone was evaporated leaving the intended amount of camphor as a pure layer. Each Petri dish contains the certain amount camphor was placed on the bottom of the test container. Concentrations were calculated as mg camphor per one liter container space. Three or four concentrations were prepared for each insect. Blank treatment was applied as a control. The containers were closed and incubated under constant temperature of 25 ± 0.5 °C for 24h, then containers were opened and exposed to fresh air for two hours. The mortality was counted. Individuals that showed no response to brush was considered dead.

The above procedures were repeated for *A. albipodus* and *A. colemani*; 10 adults of each were transferred to a cup which was supplied with honey droplets as nutrition. Ten adults of *C. undecimpunctata* were transferred to a cup, as one replicate, and were supplied with sufficient number of aphids as nutrition. Four cups were used as replicates for each treatment. Then cups were closed with muslin and exposed to camphor vapor, as described above, then mortality was counted as described above.

Mortality percentage was corrected by Abbott's formula (1925). LC_{50}, slope and Chi square values were calculated according to

Finney (1971) using "LdP Line"® software. Tolerance ratio was calculated as LC_{50} of intended insect / LC_{50} of the aphid.

Changes in biological aspects of *A. craccivora* were studied after subjecting newly emerged adults to LC_{25} concentration (9.26 mg / l) for 24h, using the same procedures as described above. More than 40 aphids were subjected to LC_{25} concentration then the 20 survived aphids were transferred separately each to a clean bean seedling and were incubated at 25 ± 0.5 °C, then the number of progeny and aphid mortality were recorded daily until the death of the last female. Similar blank treatment was applied as a control. Data were analyzed using T test in order to examine the differences between treated-survived and non treated aphids. Finite rate of increase (a life table parameter) was calculated according to Birch (1948) for both treated-survived and non treated aphids.

Biochemical analysis

Faba bean *V. faba* seedlings carrying a sufficient amount of *A. craccivora* were placed in Petri dishes covered with muslin and were subjected to LC_{50} concentration of 12.712 mg / l for 24 h in test containers as it was described above. Blank treatment was considered as a control. After treatment, dead aphids were eliminated and live aphids were collected and frozen at-20 ^{o}C until biochemical analysis. Three samples were collected as replicates, from both treated-survived and non-treated aphids.

α-esterases and β-esterases were determined according to Van Asperen (1962) using α-naphthyl acetate and β-naphthyl acetate as substrates, respectively. They were measured as µg α-naphthol/ min/mg protein or µg β-naphthol χ 10^3/min/mg protein, respectively. Glutathione-S-transferase was measured by detecting S-(2,4-dinitro-phenyl)-L-glutathione which resulted from the conjugation of 1-chloro 2,4-dinitrobenzene and reduced glutathione (GSH), by the enzyme. It is expressed as n mole substrate conjugated/mg protein; according to the method described by Habig *et al.* (1974). Acid phosphatase and alkaline phosphatase activity were expressed by unit (U). Where 1 unit hydrolyzes 1.0 µ mole of p-nitophenyl phosphate per minute at 37 °C, and pH 10.4 and 4.8 for alkaline and acid phosphatases, respectively, according to the method described by Powell and Smith (1954).

Results and Discussion

Results in table 1 show that *A. craccivora* was the most tolerant one to camphor vapor with LC_{50} of 12.71 mg camphor/liter space. Unfortunately, all tested natural enemies were less tolerant to camphor vapor. According to tolerance order, *C. undecimpunctata* came after aphid with LC_{50} of 6.33 mg / l that was nearly 50% less tolerant. The two parasites, *A. albipodus* and *A. colemani* were even less tolerant as their LC_{50} values were 1.16 and 0.48 mg/l, respectively. They were 0.091 and 0.038 less tolerant than aphid, respectively.

Results illustrated in Fig. 1 show that population of treated-survived aphids declined rapidly compared with non-treated aphids. Starting with 20 adults, the population nullified within 19 and 27 days in treated-survived and non-treated aphids, respectively. A significant reduction in female longevity was recorded, as the averages of female longevity were 6.45 and 17.6 days, in treated-survived and non-treated aphids, respectively, (Table 2). Besides reducing female longevity, reduction of daily progeny per female was recorded in treated-survived aphids. As it is exhibited in Fig. 2 and tabulated in table 2, treated-survived aphids laid significantly fewer progeny than non-treated aphids as the averages of daily progeny per one female were 1.93 and 4.44 nymphs for treated-survived and non-treated, respectively. The average total number of progeny produced by one female during its life span was 12.85 and 62.1 nymphs, respectively. Both mentioned factors, i.e. shortness of female longevity and reduced numbers of produced progeny are reflected in a reduction in the finite rate of increase as it was reduced from 1.57 female / female/day in non-treated aphids to 1.14 female/female/day in treated-survived aphids (Table 2).

Table 1 Toxicity line parameters of camphor vapour on *Aphis craccivora* and associated natural enemies *Coccinella undecimpunctata*, *Aphelinus albipodus* and *Aphidius colemani*.

Insects	LC_{50} (95% FL)[1] (mg / l)	Tolerance ratio[2]	Slope	Chi square
A. craccivora	12.71 (10.95 - 14.06)	1.00	4.90	1.90
C. undecimpunctata	6.33 (4.70 - 8.13)	0.498	3.14	0.10
A. albipodus	1.16 (1.00 - 1.31)	0.091	4.93	0.42
A. colemani	0.48 (0.30 - 0.63)	0.038	2.39	1.08

1. Fiducial limits at 95%.
2. Tolerance ratio: LC_{50} of intended insect / LC_{50} of aphid.

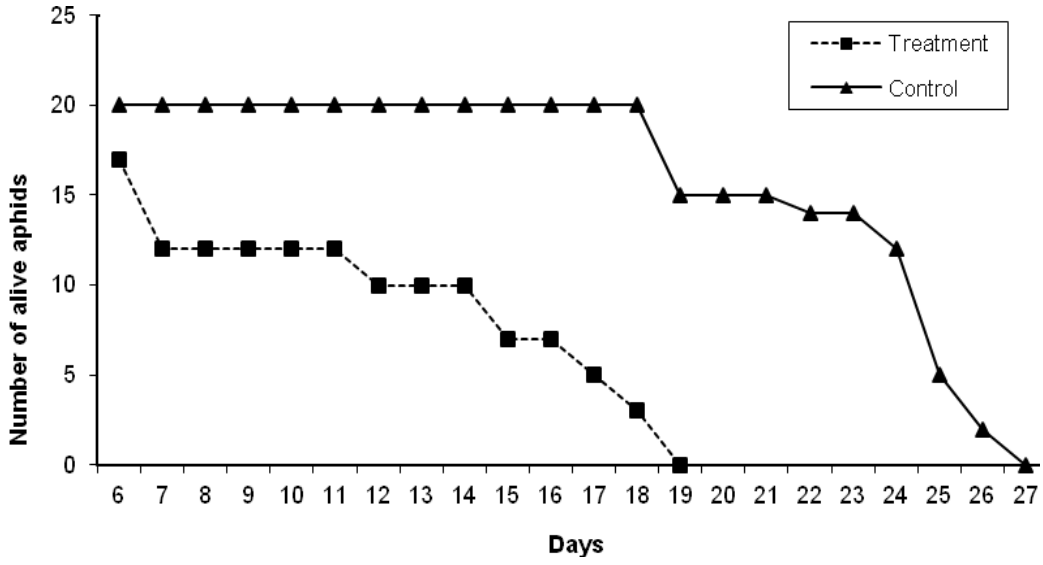

Figure 1 Population decline of *Aphis craccivora* after exposure to LC_{25} camphor vapour.

Table 2 Effect of LC_{25} of camphor vapour on the biological aspects of *Aphis craccivora.*

Treatments	Immature stages (days)	Female longevity (days)	Progeny average/female	Daily progeny per female	Finite rate of increase
Treated	5	6.45	12.85	1.93	1.14
Control		17.6	62.1	4.44	1.57
P[1]		> 0.01	> 0.01	0.013	
		**	**	*	

1: P: probability of t-student test.
* and ** indicate significant differences at $P < 0.05$ and $P < 0.01$.

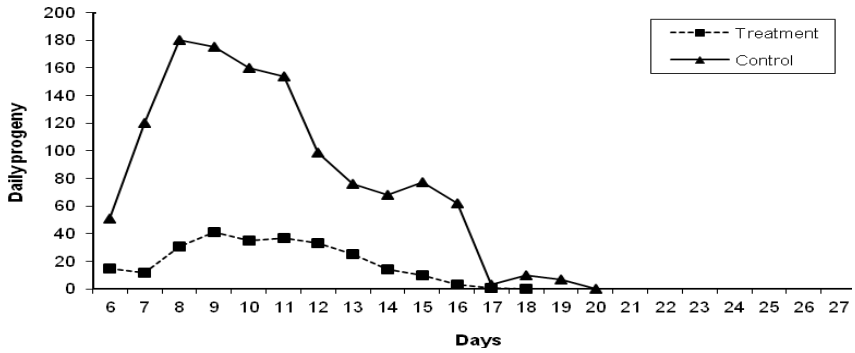

Figure 2 Daily productivity of *Aphis craccivora* resulted from 20 treated-survived and non treated aphids.

In order to study the effect of camphor on biochemical changes, concentration of some enzymes in survived adult female aphids subjected to LC_{50} concentration (12.712 mg / l) were studied. α-esterases analysis revealed no significant difference between treated-survived and non-treated aphids. While the concentrations of acid phosphatase and G. S-transferase were found to be significantly higher in treated-survived than non-treated aphids. Acid phosphatase was measured as 833.89 and 559.33 U χ 10^6 / mg protein in treated-survived and non-treated aphids, respectively. The concentration of G. S-transferase was assessed as 80.19 and 57.70 n mole substrate conjugated/mg protein in both sets, respectively. Inversely, treated-survived aphids were found to have significantly less amount of β-esterases and alkaline phosphatase than non-treated aphids. β-esterases was measured as 1690.56 and 2829.78 µg β-naphthol χ 10^3 / min / mg protein in treated-survived and non-treated aphids respectively. Alkaline phosphatase was estimated as 1599.78 and 2023.33 U χ 10^6 / mg protein in treated-survived and non-treated aphids, respectively (Table 3). Two possibilities can be mentioned to explain the difference of enzyme contents between treated-survived and non-treated aphids. First one is, this difference may have resulted from selection pressure. That occurs when tolerance strength depends on the enzyme level in the individual. It means, high enzyme content may cause the tolerance as in the case of acid phosphatase and G. S-transferase. Contrastingly, in the cases of β-esterases and alkaline phosphatase, low enzyme content may have caused the tolerance. Consequently, some of the aphids survived because of their enzyme content level, while others died. It resulted in a selection pressure toward high or low enzyme content. The second possible explanation would be that the treatment might have, directly or indirectly, induced or suppressed the enzyme.

Detoxification role of nearly all of above enzymes have been reported by many authors (Mukanganyama *et al.*, 2003; Francis *et al.*, 2005, 2006,; Li *et al.*, 2007; Marco *et al.*, 2010; Sprawka *et al.*, 2011; Silva *et al.*, 2012.

This is not the first proof of camphor toxicity against pests. Its pesticidal efficiency, as an emulsifiable concentrate, was proved against the cotton aphid, *Aphis gossypii* Glover and the spider mite, *Tetranychus urticae* Koch by Mousa (2003). Camphor has also been used successfully against different pests such as the beetles, *Sitophilus granarius* L., *Sitophilus zeamais* Motschulsky, *Tribolium castaneum* (Herbst) and *Prostephanus truncatus* Horn (Obeng-Ofori, *et al.,* 1998) and against the rice weevil *Sitophilus oryzae* L. (Dayal *et al.,* 2003). Camphor was also introduced in commercial product against *Varroa jacobsoni* Oudemans (Rickli *et al.,* 1991; Mutinelli *et al.,* 1993). Fumigant toxicity of camphor was previously proved against *T. urticae* (LC_{50} 3.36 mg/l) and its predators *Neoseiulus californicus* (McGregor) (LC_{50} 3.48) and *Phytoseiulus persimilis* (Athias-Henriot) (LC_{50} 3.97) by Bakr (2013).

Table 3 Intensity of some enzymes in aphids survived from LC_{25} of camphor vapour and non treated aphids.

Treatment	A-esterases[1]	β-esterases[2]	Alkaline phosphatase[3]	Acid phosphatase[4]	G. S-transferase[5]
Treated	3.67	1690.56	1599.78	833.89	80.19
Control	3.44	2829.78	2023.33	559.33	57.70
P	0.09	< 0.01	< 0.01	< 0.01	< 0.01
		**	**	**	**

1: µg α-naphthol/min/mg protein.
2: µg β-naphthol χ 10^3/min/ mg protein.
3: U χ 10^6/mg protein.
4: U χ 10^6 mg/protein.
5: n mole substrate conjugated/mg protein.
P: Probability of t-student test.
**: significant at $p < 0.01$.

Conclusion

Camphor fumigant can be suggested as a control agent against *A. craccivora,* but it must be applied before releasing natural enemies (*C. undecimpunctata*, *A. albipodus* and *A. colemani*) because of its negative impact on them.

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تأثیر سمی بخارکافور روی شته اقاقیا *Aphis craccivora* Koch (Hemiptera: Aphididae) و برخی دشمنان طبیعی آن

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چکیده: سمیت تنفسی کافور روی شته اقاقیا یا شته سیاه یونجه *Aphis craccivora* Koch و سه گونه دشمنان طبیعی آن شامل کفشدوزک هفت نقطه ای *Coccinella undecimpunctata* L.، زنبورهای پارازیتوئید *Aphelinus albipodus* Hayat & Fatima و *Aphidius colemani* Viereck. مورد بررسی قرار گرفت. شته سیاه یونجه نسبت به دشمنان طبیعی بسیار مقاوم‌تر بود به‌طوری‌که LC_{50} آن برای حشرات ذکر شده به‌ترتیب ۱۲/۷۱، ۶/۳۳، ۱/۱۶ و ۰/۴۸ میلی‌گرم کافور در هر لیتر هوا بود. در معرض قرار دادن حشرات کامل ماده تازه ظاهر شده شته اقاقیا در برابر مقادیر LC_{25} بخار کافور طول عمر حشرات کامل را از ۱۷/۶ روز به ۶/۴۵ روز کاهش داد. هم‌چنین تولید مثل روزانه از ۴/۴۴ به ۱/۹۳ پوره به‌ازای هر حشره ماده تقلیل یافت. به‌علاوه نرخ متناهی افزایش جمعیت از ۱/۵۷ به ۱/۱۴ پوره به‌ازای هر حشره ماده در روز کاهش یافت. شته‌هایی که در معرض مقادیر LC_{50} قرار گرفته بودند به‌طور معنی‌داری آنزیم اسید فسفاتاز و گلوتاتیون اس ترانسفراز بالاتری نسبت به شاهد نشان دادند. درحالی‌که شته‌های زنده مانده آنزیم بتا استراز و آلکالین فسفاتاز کم‌تری نسبت به حشرات شاهد داشتند، اما اختلاف معنی‌داری در میزان آنزیم آلفا استراز مشاهده نشد. بنابراین سمیت تنفسی کافور می‌تواند گزینه مناسبی برای کنترل شته اقاقیا به حساب آید اما به‌خاطر تأثیر مضر آن روی دشمنان طبیعی یک عامل محدودکننده است.

واژگان کلیدی: کافور، *Aphis craccivora*، *Aphelinus albipodus*، *Aphidius colemani*، *Coccinella undecimpunctata*