



## Toxicity of some medicinal plant extracts to *Pieris brassicae* and combined effects with Proteus® against *Brevicoryne brassicae*

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### Abstract

*Brevicoryne brassicae* (Homoptera: Aphididae) is a considerable pest of cabbage. *Pieris brassicae* (Lepidoptera: Pieridae) exist in most countries where cabbages are cultivated. In this research, we were examined the synergistic interaction between *Satureja hortensis* L., *Trachyspermum ammi* I. Sprague, *Ziziphora tenuior* L., *Cuminum cyminum* L. and *Foeniculum vulgare* Mill. methanolic extracts with Proteus® against *B. brassicae* adults and toxicity of mentioned extracts to eggs and second instar larvae of *P. brassicae* under laboratory conditions. Probit analysis of extracts showed that the most effective extracts were ziziphora and savory which both exhibited LC<sub>50</sub> values on adults of *B. brassicae* equivalent to 26.66 ppm. Methanolic extracts (except fennel) synergized the performance of Proteus®. Treated eggs of *P. brassicae* by methanolic extract of ziziphora elicited the lowest percentage of hatching rate. Studying of toxicity of extracts to 2<sup>nd</sup> instar larvae of *P. brassicae* demonstrated that ammi, cumin and savory 5% methanolic extracts exhibited the highest efficacy to the pest.

**Keywords:** cabbage pests, botanical insecticide, insecticidal activity, synergistic effect.

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## Introduction

The cabbage aphid, *Brevicoryne brassicae* L. is one of the most important pests of cabbage and canola in the world such as Iran (Mousavi Anzabi et al., 2013). The pest causes severe damage via transfer of viral diseases that could be seriously led to product destruction. Large cabbage butterfly, *Pieris brassicae* L. has been recorded as a serious pest of cabbage, cauliflower and broccoli where cabbages are grown (Hassan & Ansari, 2010). In addition to the direct damage of large larvae, they are polluting and cause the product become unusable by their feces (Pfiffner, 2009). Public concern related to pesticides and their residues in, and on the foods prompted consumer interest in organically produced foods. There have been growing efforts to detect and introduce suitable plant compounds that they have insecticidal properties. Since resistance of *B. brassicae* and *P. brassicae* to common chemical insecticides is well documented, we examined the effect of methanolic extracts of ammi, cumin, fennel, savory and ziziphora on eggs and 2<sup>nd</sup> instar larvae of *P. brassicae* and *B. brassicae* adults under laboratory conditions at 25±5°C, 60±10% RH and photoperiod of 16:8 (L: D). We also surveyed synergistic/antagonistic interaction between these plants with Proteus® against *B. brassicae* adults under mentioned conditions. These plants were selected because they have medicinal properties, and they are safe for human and environment. Proteus® is a contact and systemic insecticide with two active ingredients (thiacloprid and deltamethrin) with a different mode of actions that making it as a proper management tool. Since the combined application of control agents can be more effective in an Integrated Pest Management (IPM)

program, we assayed combined effects of methanolic plant extracts with Proteus® against *B. brassicae*.

## Materials and methods

**Insects:** The colonies of cabbage aphid and large white butterfly were reared on cabbage under laboratory conditions at 25±5°C, 60±10% RH and photoperiod of 16: 8 (L: D). Experiments were carried out during summer of 2016 in the laboratory of Department of Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran.

**Plant material:** *S. hortensis*, *T. ammi*, *Z. tenuior*, *C. cyminum* and *F. vulgare* was purchased from the local market of Urmia province. Methanolic extracts were obtained by using a soxhlet extractor.

**Insecticide:** The insecticide used was Proteus® (110 OD, 100 g/l thiacloprid and 10 g/l deltamethrin, Bayer CropScience, New Zealand) that is a broad spectrum insecticide.

**Methanolic extracts:** The toxicity of botanical extracts was carried out according to Prabhaker et al. (1988) technique. Cabbage leaves contain 20 adult aphids were dipped in different concentrations of methanolic extracts (20 seconds) that determined by the preliminary dose setting experiments. After solvent evaporating, cabbage leaves were placed in Petri dishes. Mortality rates were recorded after 24 h. The sensitivity of large cabbage butterfly eggs was carried out according to Prabhaker et al. (1989) technique, so a

group of 1-day-old eggs were dipped in mentioned 5% methanolic extracts. Mortality rates were recorded after hatching eggs. Mentioned extracts were assayed for toxicity to 2<sup>nd</sup> instar larvae of *P. brassicae* by cabbage leaf-dipping in 5% extracts. When solvent evaporated, 20 numbers of 2<sup>nd</sup> instar larvae were placed on leaves. Mortality rates were recorded after 24 h. Each trial was replicated three times.

**Methanolic extracts combined with Proteus®:** Sub-lethal concentrations (LC<sub>25</sub>) of extracts were combined with LC<sub>25</sub> of Proteus® to investigate whether there was a synergistic or antagonistic interaction between extracts with Proteus®. Mortality surveyed after 24 h. Each treatment was replicated three times.

**Data analysis:** In order to determine LC<sub>50</sub> and LC<sub>25</sub> values, the data were analyzed using the probit procedures with SPSS for Windows® release 20. The percentage data were transformed into  $\arcsin\sqrt{x}$  before statistical analysis. To determine synergistic/antagonistic interactions, experiments were conducted following Tallarida (2000). The

relationship between data was assayed by analysis of variance (ANOVA) and correlation analysis. The means were separated by using the Tukey test.

## Results and Discussion

According to table 1, the most effective methanolic extracts were ziziphora and savory which both exhibited LC<sub>50</sub> values on *B. brassicae* adults, equivalent to 26.66 ppm. As demonstrated in Table 1, Proteus® exhibited the highest toxicity to *B. brassicae* (LC<sub>50</sub>= 0.59 ppm). The inclusion of all methanolic extracts (except *F. vulgare*) with Proteus® was led to synergistic interaction to *B. brassicae* (Table 2). Results presented in table 3 shows that *Z. tenuior* methanolic extract had the highest effect on *P. brassicae* eggs (hatching rate = 5%). But the larvae hatched from the eggs that treated with *T. ammi*, *C. cuminum* and *S. hortensis* extracts showed zero percent survival. According to table 4, ammi, cumin and savory extracts exhibited the highest toxicity to 2<sup>nd</sup> instar larvae of *P. brassicae* which elicited 99.01, 98.27 and 97.96% mortality, respectively. There was no mortality in the control (untreated) of all experiments.

Table 1: Probit analysis of toxicity of some methanolic extracts and Proteus® on adults of *Brevicoryne brassicae* adults after 24 h.

Extract	$\chi^2$	Slope $\pm$ SE	LC <sub>25</sub> (ppm)	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)
ammi	4.25	4.34 $\pm$ 0.61	33.33	146.66	306.66
cumin	1.89	4.81 $\pm$ 0.64	20	33.33	60
fennel	4.17	2.19 $\pm$ 0.34	20	33.33	133.33
savory	3.87	4.76 $\pm$ 0.61	20	26.66	46.66
ziziphora	3.52	4.64 $\pm$ 0.27	13.33	26.66	160
Proteus®	1.64	2.10 $\pm$ 0.31	0.28	0.59	2.39

Table 2: Synergistic and antagonistic interactions between some methanolic extracts and Proteus® against *Brevicoryne brassicae* adults.

P + methanolic extract	% mortality $\pm$ S. E.		interaction
	Expected	Observed	
P + ME of ammi	57 $\pm$ 1.98	75 $\pm$ 2.57	synergism
P + ME of cumin	56.69 $\pm$ 2.05	80 $\pm$ 4.03	synergism
P + ME of fennel	46.62 $\pm$ 2.25	40 $\pm$ 0.98	antagonism
P + ME of savory	76.73 $\pm$ 2.95	82 $\pm$ 4.03	synergism
P+ ME of ziziphora	76.72 $\pm$ 2.25	80 $\pm$ 3.00	synergism

P= Proteus®, ME= methanolic extract.

Table 3: Effect of some methanolic extracts (5%) on one-day-old eggs of *Pieris brassicae*.

Plant extract	Hatching rate (%) $\pm$ S. E.	L1 survival (%) $\pm$ S. E.
ammi	98.2 <sup>a</sup> $\pm$ 2.23	0 <sup>b</sup>
cumin	97.85 <sup>a</sup> $\pm$ 2.03	0 <sup>b</sup>
fennel	98.00 <sup>a</sup> $\pm$ 2.92	90 <sup>a</sup> $\pm$ 2.18
savory	20 <sup>b</sup> $\pm$ 0.23	0 <sup>b</sup>
ziziphora	5 <sup>c</sup> $\pm$ 0.09	89 <sup>a</sup> $\pm$ 2.00
Control	100 <sup>a</sup> $\pm$ 3.01	99.8 <sup>a</sup> $\pm$ 2.99

Means in column with the same letter are not significantly different ( $p < 0.05$ ) ( $F_{(5, 20)}=270.24$ ).

Table 4: Toxicity of methanolic extracts (5%) to second instar larvae of *Pieris brassicae* after 24 h.

Plant extract	Mortality (%) $\pm$ S. E.	Concentration (%)
ammi	99.01 <sup>a</sup> $\pm$ 2.59	5
cumin	98.27 <sup>a</sup> $\pm$ 2.76	5
fennel	10 <sup>b</sup> $\pm$ 0.09	5
savory	97.96 <sup>a</sup> $\pm$ 3.01	5
ziziphora	9.2 <sup>b</sup> $\pm$ 0.02	5
Control	0 <sup>b</sup>	-

Means in column with the same letter are not significantly different ( $p < 0.05$ ) ( $F_{(5, 20)}=252.5$ ).

*B. brassicae* adults were sensitive to all methanolic extracts, but savory and ziziphora extracts had the highest toxicity to the pest (according to  $LC_{50}$  values and slopes). However, Proteus® was the most effective to the cabbage aphid. Application of botanical sources like plant extracts could be a low-risk way for controlling pests. Pavela (2013) assayed efficacy of essential oils of *Lavandula angustifolia*, *Rosmarinus officinalis*, *Nepeta cataria* and *Origanum majorana* against *B. brassicae* and reported that the essential oils of *N. cataria* and *L. angustifolia* were the most toxic and oils extracted from *O. majorana* and *R. officinalis* were less effective. Khorrami and Soleymanzade (2016) demonstrated that methanolic extracts of ziziphora and cumin were potential agents against

*Callosobruchus maculatus* F. adults. Pavela (2008) surveyed larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* larvae (Diptera: Culicidae) and demonstrated that *S. hortensis* extract caused 100% mortality of the pest larvae. Combination of sub-lethal doses of pesticides and non-chemical agents can result in improving insect management. In this research, results showed that the combination of all extracts (except fennel) with Proteus® led to synergistic interaction against cabbage aphid adults. Almasi et al. (2016) studied various stages of *Hippodamia variegata* that were exposed to the sub-lethal concentration ( $LC_{30}$ ) of Proteus® and pymetrozine. They concluded that sub-lethal concentration of both insecticides affected the insect at

a demographic level. Our results showed that the most effective extract was ziziphora that significantly decreased hatching rate of large cabbage butterfly eggs. But ammi, cumin and savory extract inhibited survival of 1<sup>st</sup> instar larvae of the pest. All these three extracts caused to high mortality of 2<sup>nd</sup> instar larvae of *P. brassicae*. Sharma et al. (2011) were tested repellent efficacies of aqueous and ethanol extracts of some plant species against *P. brassicae*. They noted that both extracts of *Azadirachta indica* A Juss and *Melia azedarach* showed higher repellency for 2<sup>nd</sup> instar larvae of the pest. Since pesticides are irreducible components of agricultural productions, application of sub-lethal doses of pesticides can be very effective in reducing their risks. Therefore, integration sub-lethal of these dangerous compounds with sub-lethal of safe compounds such as botanical sources can be useful and efficient.

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