

Research Article

## The olfactory response of *Phytoseiulus persimilis* on *Tetranychus urticae* infested bean and cucumber leaves

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**Abstract:** While searching for food, predators use herbivore induced plant volatiles (HIPV), host plant volatiles and those related to con/heterospecifics to find their prey. Not only the volatile components vary among plant species, but also the predator perception of these components might differ among species and samples. Here, we compared the olfactory response of two samples (Turkey and University of Tehran) of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) when received herbivore induced plant volatiles (HIPV) from *Tetranychus urticae* Koch (Acari: Tetranychidae) infested cucumber and bean leaves, along with testing the effect of rearing experience of Turkey sample on its olfactory response in our laboratory conditions. Our data showed that *P. persimilis* of both samples significantly moved towards leaf odors (either cucumber or bean) when they received clean air from the alternative arm. For both samples, the predator did not make a preference between clean bean and cucumber leaves. When the predators were offered a choice between *T. urticae* infested bean and cucumber leaves, they significantly moved towards bean leaves in both samples. Rearing experience did not affect the predator choice of host plant species and *P. persimilis* from both samples preferred odors related to clean leaves rather than clean air. The number of experienced predatory mites moved towards *T. urticae* infested bean leaves was significantly higher than that preferred *T. urticae* infested cucumber leaves in both samples. We discussed whether their similar olfactory responses would be related to their experience of previous rearing conditions.

**Keywords:** olfactory response, foraging behavior, predator, experience, *Phytoseiulus persimilis*, heterospecifics

### Introduction

Predators use volatiles to optimize their foraging behavior in order to find their herbivorous prey (Vet and Dicke 1992; Zahedi Golpayegani *et al.*, 2007). The source of these volatiles could either be of the prey or the host plant or the predator con/heterospecific competitors (Dicke *et al.*, 2000; Pallini *et al.*, 1997). To find a rich

foraging site, predators should benefit from volatiles which increase their recognition ability and lead them straight towards a beneficial prey patch (Maeda 2005). The ability of evaluating the prey patch might differ among samples of predator species and even in one species sample (Dicke *et al.*, 2000). Maeda *et al.* (1999, 2001) noted that as different samples might undergo varified food regimes, their olfactory response should have been differently adapted. This appears more important considering that many prey species such as *Tetranychus urticae* Koch are highly polyphagous (Maeda *et al.*, 2000) that makes predators experience volatiles with

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different blends of components. So the predators from different samples might be considered of different potentials in biological control systems. Meada (2010) studied the differences in foraging strategies between samples of the predatory mite *Neoseiulus womersleyi* (Schicha) and reported that a significant positive correlation was found only between the olfactory response and the patch leaving tendency of geographical samples. Maeda *et al.* (2000) investigated the olfactory response of 13 samples related to *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae) to spider mite infested bean volatiles and recorded different responses among the ones collected from different sites. Dicke *et al.* (2000) carried out experiments which showed that the attraction of *Phytoseiulus persimilis* Athias-Henriot to spider mite infested lima beans from a commercial sample decreased after rearing in the laboratory.

Experience could also affect the predator foraging behavior (Maleknia *et al.*, 2014). According to Zhang and Sanderson (1992) *P. persimilis* females that were born and reared on a given (rose or bean) plant species showed no preference for the odors related to other plant species. Dicke *et al.* (1990) have also demonstrated that previous experience could have an important role on the foraging behavior of *P. persimilis*.

The family Phytoseiidae includes the important group of predatory mites that have brought about successful controls on several plants and crops especially on *T.urticae* by *P.persimilis* (Sato *et al.*, 2011). *Phytoseiulus persimilis* is a predator with no visual ability so that using olfactory information to explore its environments and prey patches is of utmost importance (van Wijk *et al.*, 2006). Several studies have demonstrated that this innate olfactory response could be affected by rearing conditions and could change variably from lack of preference towards prey infested plants (Drukker *et al.*, 2000) to varied responses to different infested plant species (Takabayashi and Dicke, 1992).

*T. urticae* is an important pest of many crops in various parts of the world. It is a

polyphagous pest and feeds on a wide range of plants (de Villiers and Pringle, 2011). Because of increasing acaricide resistance, study on the alternative control methods such as biological control is improving (Kasap and Atlihan, 2011). Regarding that the predator-prey system consisting of *T. urticae* and phytoseiid predators is very unstable (Zemek and Nachman, 1998), investigation on the basic factors affecting the interactions among host plant, prey and predator is of importance.

Here, we have investigated the olfactory response of two samples (one from acarology laboratory of University of Tehran (native population: UT) and another collected from Hatay, Turkey in August 2010 on *Phaseolus vulgaris* var. Barbutia) of the predatory mite *P. persimilis* to odors related to *T. urticae* infested cucumber and bean leaves. Our objectives were to find out (1) whether these two samples would respond differently when receiving odors related to prey infested patches on two host plants and (2) whether rearing the imported sample (Turkey sample) in our laboratory conditions for six months, would affect its olfactory responses. The data of this study reveals practical implications worthy on future questions of whether the imported samples of predatory mites would act as effectively as the local ones? The predator behaviors on cucumber and bean leaf patches are discussed.

## Materials and Methods

### Plants:

Bean (*Phaseolus vulgaris* L. Red Alamouti variety) and cucumber (*Cucumis sativus* L. Soltan variety) plants were chosen in Karaj (Iran), respectively. Plants were grown in the climate room at controlled conditions of  $20 \pm 5$  °C,  $50 \pm 10\%$  RH and 16:8h (L: D) photoperiod in Acarology laboratory of University of Tehran in early August 2010. We used perlite (mixed with soil 50: 50) with daily irrigation and 20-20-20 NPK master fertilizer which was added once every two days. Both host plants were reared until they reached four leaf stage and then were used for the experiments.

**Prey:**

*Tetranychus urticae* (identified in the laboratory of Acarology, College of Agriculture, University of Tehran) was used as prey. Four-leaf plants were used for spider mite infestation and were kept in a separate room at  $20 \pm 5$  °C,  $40 \pm 10\%$  RH and 16:8h (L: D) photoperiod. Infestation was performed consistently during the research period to provide sufficient numbers of spider mites and infested leaves for the experiment sets. Detached infested leaves were used for feeding the predatory mite colonies.

**Predator:**

The predatory mites (*P. persimilis*) were reared on spider mite infested bean/ cucumber (depending on the host leaves we would use in the olfactory experiments) leaves in a growth chamber ( $65 \pm 5\%$  RH,  $25 \pm 2$  °C, 16: 8h (L: D) photoperiod). The infested leaves were put on an arena consisting of a water saturated sponge in a plastic water container. A plastic sheet ( $15 \times 30$  cm<sup>2</sup>) was placed on the arena under the infested leaf mass. Narrow wet tissues around the plastic sheet connected to the container water, provided water for the prey and predators. The predators received new fresh infested lima bean/cucumber leaves everyday and old mite-free leaves were removed. The Turkey sample was kept separate from University of Tehran (UT) sample so their behavior was recorded separately. The predators reared on bean and cucumber leaves were used for the olfactory experiments performed on bean and cucumber leaves respectively.

**Olfactometer:**

The Olfactometer consisted of a Y-shaped glass tube (Ø: 4 cm) with a Y-shaped metal wire in the middle of the tube and positioned parallel to the tube walls (Sabelis and van de Baan, 1983). Glass tubes containing the odour sources were connected to the end of each of the two arms. Starved predators were transferred with a small brush to the base of the metal wire in the Y-tube, where they initiated their upwind movements. Each predator was observed until it passed the junction and moved

into one of the arms of the Y-tube. However, if it did not reach the junction within 5 minutes, the experiment was stopped and the outcome of the experiment was scored as a case of non-preference.

**Olfactory experiments:**

The same aged female predators were used for experiments as searching predators. First, we performed three olfactory experiments on each of the host plants (bean and cucumber) with each of the samples (total six experiments) and four experiments with the partnership of both host plants. Twenty experiments with both samples were performed immediately after importing the Turkey sample to the laboratory (UT) as follows:

In the olfactory experiments, *P. persimilis* (UT sample) received odors related to clean bean leaves from one arm vs. clean air from another at first. Second, we replaced clean leaves by spider mite infested leaves so that the predator received odors related to infested leaves from one arm vs. clean air from another. In the third experiment, *P. persimilis* received odors related to spider mite infested leaves from one arm vs. clean leaves from the other. The tests with UT sample were exactly repeated on cucumber leaves. All three experiments were repeated by Turkey sample on bean and cucumber leaves. Afterwards, we tested the olfactory response of the predatory mite (UT sample) when receiving odors related to clean cucumber leaf vs. clean bean leaf, infested cucumber leaf vs. infested bean leaf, clean cucumber leaf vs. infested bean leaf and infested cucumber leaf vs. clean bean leaf. All four experiments were exactly repeated for Turkey sample.

The odor sources consisted of 12 same aged leaves (bean/ cucumber) infested by 600 adult females of *T. urticae* for 24 hours. Searching predatory females were kept starved for five hours prior to experiments. All experiments were performed by total 60 female predators in three independent replicates (20 replicates in each).

All experiments were repeated after *P. persimilis* (Turkey sample) spent a duration of 6 months (20 generations) in our laboratory under the conditions mentioned above. We repeated

the olfactory experiments on them to be considered as control for the experiments with Turkey sample after six months. Statistical analysis were done using a replicated G-test, which includes a test for heterogeneity among replicate experiments (Sokal and Rohlf, 1995).

**Results**

**Olfactory experiments immediately after importing the samples to the laboratory**

Olfactory response of *P. persimilis* (UT sample) receiving bean leaf odors:

when *P. persimilis* received odors related to clean bean leaves from one arm vs. clean air from another, all 60 predators moved into either of the arms, 75% of which preferred clean bean leaves (P < 0.01) significantly. Replacing clean bean leaves by spider mite infested ones, 59 predators moved into the arms, 85% of which preferred infested bean leaves significantly (P <

0.01). When the odor sources consisted of spider mite infested leaves vs. clean ones, 57 out of 60 predators moved into the arms, 70% of which preferred infested leaves (P = 0.001). The results of replicated G test are shown in table 1.

Olfactory response of *P. persimilis* (Turkey sample) receiving bean leaf odors:

When *P. persimilis* received odors related to clean leaves from one arm vs. clean air from another, 51 predators moved into either of the arms, 69% of which preferred clean bean leaves (P < 0.01) significantly. Replacing clean bean leaves by spider mite infested ones, 58 predators moved into the arms, 83% of which preferred infested bean leaves significantly (P < 0.01). When the odor sources consisted of spider mite infested leaves vs. clean ones, 52 out of 60 predators moved into the arms, 69% of which preferred infested leaves (P = 0.004).The results of replicated G test are shown in table 2.

**Table 1** Data of replicated G-test for the response of *Phytoseiulus persimilis* (UT sample) on bean plants when receiving odors related to clean air vs. clean leaves, clean air vs. infested leaves and clean leaves vs. infested leaves.

Odor source	$G_p$	P-value	$G_h$	P-value	$G_t$	P-value
Clean air vs. clean leaves	15.7	< 0.01**	0.53	0.76 <sup>ns</sup>	16.23	0.001**
	<b>22.15</b>	< <b>0.01**</b>	<b>1.18</b>	<b>0.91</b> <sup>ns</sup>	<b>22.33</b>	< <b>0.01**</b>
Clean air vs. infested leaves	31.4	< 0.01**	1.11	0.35 <sup>ns</sup>	33.5	< 0.01**
	<b>35.43</b>	< <b>0.01**</b>	<b>0.28</b>	<b>0.87</b> <sup>ns</sup>	<b>35.7</b>	< <b>0.01**</b>
Clean leaves vs. infested leaves	9.55	0.001**	0.17	0.91 <sup>ns</sup>	9.72	0.021*
	<b>15.47</b>	< <b>0.01**</b>	<b>0.192</b>	<b>0.9</b> <sup>ns</sup>	<b>15.67</b>	< <b>0.001**</b>

$G_p$ ,  $G_h$  and  $G_t$ : indicate the significance of the pooled data, heterogeneity among replicate experiments and overall data, respectively. Values in bold represent the data after 20 generations.

ns: non significant, \* and \*\*: indicate significant difference at 0.05 and 0.01, respectively.

**Table 2** Data of replicated G-test for the response of *Phytoseiulus persimilis* (Turkey sample) on bean plants when receiving odors related to clean air vs. clean leaves, clean air vs. infested leaves and clean leaves vs. infested leaves.

Odor source	$G_p$	P-value	$G_h$	P-value	$G_t$	P-value
Clean air vs. clean leaves	7.25	0.007**	0.23	0.90 <sup>ns</sup>	7.25	0.06*
	<b>17.80</b>	< <b>0.01**</b>	<b>1.13</b>	<b>0.29</b> <sup>ns</sup>	<b>18.94</b>	< <b>0.0002**</b>
Clean air vs. infested leaves	27.10	< 0.01**	1.85	0.40 <sup>ns</sup>	28.93	< 0.01**
	<b>35.34</b>	< <b>0.01**</b>	<b>0.685</b>	<b>0.709</b> <sup>ns</sup>	<b>31.02</b>	< <b>0.01**</b>
Clean leaves vs. infested leaves	7.9	0.004**	0.25	0.90 <sup>ns</sup>	8.15	0.043 *
	<b>29.3</b>	< <b>0.01**</b>	<b>0.80</b>	<b>0.67</b> <sup>ns</sup>	<b>30.10</b>	< <b>0.01**</b>

$G_p$ ,  $G_h$  and  $G_t$ : indicate the significance of the pooled data, heterogeneity among replicate experiments and overall data, respectively. Values in bold represent the data after 20 generations.

ns: non significant, \* and \*\*: indicate significant difference at 0.05 and 0.01, respectively.

Olfactory response of *P. persimilis* (UT sample) receiving cucumber leaf odors:

receiving odors from clean cucumber leaves from one arm vs. clean air from another, 45 predators moved into either of the arms, 56% of which preferred clean leaves (P = 0.01). When we replaced clean leaves by spider mite infested ones, all 60 predators moved into one of the arms, 82% of which preferred infested ones (P = 0.001). When *P. persimilis* received odors related to infested cucumber leaves from one arm and the clean ones from another, all 60 predators moved into one of the arms, 72% of which preferred infested leaves (P = 0.002). The results of replicated G test are shown in table 3.

Olfactory response of *P. persimilis* (Turkey sample) receiving cucumber leaf odors:

Receiving odors from clean cucumber leaves from one arm vs. clean air from another, 48 predators moved into either of the arms, 67% of which preferred clean leaves (P = 0.02). When we replaced clean leaves by spider mite infested ones, 48 predators moved into one of the arms, 67% of which preferred infested ones (P = 0.003). When *P. persimilis* received odors related to infested bean leaves from one arm and the clean ones from another, 51 predators moved into one of the arms, 67% of which preferred infested leaves (P = 0.02). The results of replicated G test are shown in table 4.

Olfactory response of *P. persimilis* (UT sample) receiving bean vs. cucumber odors:

Receiving odors related to clean cucumber vs. clean bean leaves, 55 predators moved into

either of the arms, none of which showed a significant preference towards each of the arms (P = 0.78). When the predatory mite received odors related to infested cucumber vs. infested bean, 56 mites moved into one of the arms, 64% of which preferred infested bean leaves (P = 0.03). Replacing the arms by clean cucumber vs. infested bean, 53 predators moved into one of the arms, 75% of which preferred infested bean leaves (P < 0.01). When *P. persimilis* received odors related to infested cucumber from one arm and clean bean from another, 50 predators moved into one of the arms, 68% of which preferred infested cucumber leaves (P = 0.01) (Table 5).

Olfactory response of *P. persimilis* (Turkey sample) receiving bean vs. cucumber odors:

Receiving odors related to clean cucumber vs. clean bean leaves, 55 predators moved into either of the arms, none of which showed a significant preference towards each of the arms (P = 0.5). When the predatory mite received odors related to infested cucumber vs. infested bean, 55 mites moved into one of the arms, 66% of which preferred infested bean leaves (P = 0.02). Replacing the arms by clean cucumber vs. infested bean, 56 predators moved into one of the arms, 65% of which preferred infested bean leaves (P = 0.02). When *P. persimilis* received odors related to infested cucumber from one arm and clean bean from another, 51 predators moved into one of the arms, 67% of which preferred infested cucumber leaves (P = 0.02) (Table 6).

**Table 3** Data of replicated G-test for the response of *Phytoseiulus persimilis* (UT sample) on cucumber plants when received odors related to clean air vs. clean leaves, clean air vs. infested leaves and clean leaves vs. infested leaves.

Odor source	$G_p$	P-value	$G_h$	P-value	$G_t$	P-value
Clean air vs. clean leaves	6.584	0.01*	0.077	0.96 <sup>ns</sup>	6.66	0.083 <sup>ns</sup>
	<b>15.25</b>	< <b>0.01</b> **	<b>0.199</b>	<b>0.905</b> <sup>ns</sup>	<b>15.45</b>	<b>0.00146</b> **
Clean air vs. infested leaves	10.75	0.001**	0.082	0.96 <sup>ns</sup>	10.84	0.0012*
	<b>20.35</b>	< <b>0.01</b> **	<b>0.53</b>	<b>0.765</b> <sup>ns</sup>	<b>20.88</b>	<b>0.00011</b> **
Clean leaves vs. infested leaves	9.3	0.002**	0.27	0.87 <sup>ns</sup>	9.57	0.022*
	<b>24.06</b>	< <b>0.01</b> **	<b>0.196</b>	<b>0.9</b> <sup>ns</sup>	<b>24.26</b>	< <b>0.01</b> **

$G_p$ ,  $G_h$  and  $G_t$  indicate the significance of the pooled data, heterogeneity among replicate experiments and overall data, respectively. Values in bold represent the data after 20 generations.

ns: non significant, \* and \*\*: indicate significant difference at 0.05 and 0.01, respectively.

**Table 4** Data of replicated G-test for the response of *Phytoseiulus persimilis* (Turkey sample) on cucumber plants when receiving odors related to clean air vs. clean leaves, clean air vs. infested leaves and clean leaves vs. infested leaves.

Odor source	$G_p$	P-value	$G_h$	P-value	$G_t$	P-value
Clean air vs. clean leaves	5.4	0.02**	0.42	0.9 <sup>ns</sup>	5.85	0.118*
	<b>16.95</b>	< <b>0.01</b> **	<b>0.055</b>	<b>0.97</b> <sup>ns</sup>	<b>17</b>	< <b>0.01</b> **
Clean air vs. infested leaves	8.6	0.0033**	0.08	0.96 <sup>ns</sup>	8.67	0.033 *
	<b>14.65</b>	<b>0.0001</b> **	<b>0.945</b>	<b>0.62</b> <sup>ns</sup>	<b>15.6</b>	< <b>0.01</b> *
Clean leaves vs. infested leaves	5.78	0.02*	0.17	0.92 <sup>ns</sup>	5.95	0.114*
	<b>25.08</b>	< <b>0.01</b> **	<b>0.2</b>	<b>0.904</b> <sup>ns</sup>	<b>25.28</b>	< <b>0.01</b> **

$G_p$ ,  $G_h$  and  $G_t$  indicate the significance of the pooled data, heterogeneity among replicate experiments and overall data, respectively. Values in bold represent the data after 20 generations.

ns: non significant, \* and \*\*: indicate significant difference at 0.05 and 0.01, respectively.

**Table 5** Data of replicated G-test for the response of *Phytoseiulus persimilis* (UT sample) when receiving odors related to clean bean leaves vs. clean cucumber leaves, spider mite infested cucumber leaves vs. spider mite infested bean leaves, spider mite infested bean leaves vs. clean cucumber leaves, spider mite infested cucumber leaves vs. clean bean leaves.

Odor source	$G_p$	P-value	$G_h$	P-value	$G_t$	P-value
Clean bean leaves vs. clean cucumber leaves	0.074	0.785 <sup>ns</sup>	0.733	0.70 <sup>ns</sup>	0.80	0.85 <sup>ns</sup>
	<b>0.076</b>	<b>0.780</b> <sup>ns</sup>	<b>0.736</b>	<b>0.70</b> <sup>ns</sup>	<b>0.80</b>	<b>0.85</b> <sup>ns</sup>
Mite infested cucumber leaves vs. infested bean leaves	4.635	0.031*	0.52	0.77 <sup>ns</sup>	5.15	0.16 <sup>ns</sup>
	<b>5.140</b>	<b>0.023</b> *	<b>0.05</b>	<b>0.975</b> <sup>ns</sup>	<b>5.20</b>	<b>0.16</b> <sup>ns</sup>
Mite infested bean leaves vs. clean cucumber leaves	14.42	<0.01**	0.16	0.92 <sup>ns</sup>	14.60	<0.01**
	<b>7.920</b>	<b>0.005</b> **	<b>0.49</b>	<b>0.78</b> <sup>ns</sup>	<b>8.41</b>	<b>0.038</b> *
Infested cucumber leaves vs. clean bean leaves	6.62	0.010*	0.63	0.73 <sup>ns</sup>	7.26	0.06 <sup>ns</sup>
	<b>10.62</b>	<b>0.001</b> **	<b>0.66</b>	<b>0.72</b> <sup>ns</sup>	<b>11.30</b>	<b>0.01</b> **

$G_p$ ,  $G_h$  and  $G_t$  indicate the significance of the pooled data, heterogeneity among replicate experiments and overall data, respectively. Values in bold represent the data after 20 generations.

ns: non significant, \* and \*\*: indicate significant difference at 0.05 and 0.01, respectively.

**Table 6** Data of replicated G-test for the response of *Phytoseiulus persimilis* (Turkey sample) when receiving odors related to clean bean leaves vs. clean cucumber leaves, spider mite infested cucumber leaves vs. spider mite infested bean leaves, spider mite infested bean leaves vs. clean cucumber leaves, spider mite infested cucumber leaves vs. clean bean leaves.

Odor source	$G_p$	P-value	$G_h$	P-value	$G_t$	P-value
Clean bean leaves vs. clean cucumber leaves	0.455	0.50 <sup>ns</sup>	1.99	0.37 <sup>ns</sup>	2.44	0.50 <sup>ns</sup>
	<b>0.074</b>	<b>0.79</b> <sup>ns</sup>	<b>2.80</b>	<b>0.25</b> <sup>ns</sup>	<b>2.86</b>	<b>0.40</b> <sup>ns</sup>
Mite infested cucumber leaves vs. infested bean leaves	5.340	0.02*	0.47	0.79 <sup>ns</sup>	5.80	0.12 <sup>ns</sup>
	<b>4.477</b>	<b>0.03</b> *	<b>0.40</b>	<b>0.82</b> <sup>ns</sup>	<b>4.87</b>	<b>0.18</b> <sup>ns</sup>
Mite infested bean leaves vs. clean cucumber leaves	5.34	0.02*	0.57	0.75 <sup>ns</sup>	5.91	0.10 <sup>ns</sup>
	<b>14.65</b>	<b>0.00012</b> **	<b>0.25</b>	<b>0.88</b> <sup>ns</sup>	<b>14.9</b>	<b>0.0019</b> **
Infested cucumber leaves vs. clean bean leaves	5.55	0.018*	0.35	0.84 <sup>ns</sup>	5.90	0.11 <sup>ns</sup>
	<b>6.7</b>	<b>0.001</b> **	<b>0.52</b>	<b>0.77</b> <sup>ns</sup>	<b>7.22</b>	<b>0.065</b> <sup>ns</sup>

$G_p$ ,  $G_h$  and  $G_t$  indicate the significance of the pooled data, heterogeneity among replicate experiments and overall data, respectively. Values in bold represent the data after 20 generations.

ns: non significant, \* and \*\*: indicate significant difference at 0.05 and 0.01, respectively.

**Olfactory experiments after 6 months of rearing in the laboratory conditions**

Olfactory response of *P. persimilis* (UT sample) receiving bean leaf odors:

Receiving odors from clean bean leaves from one arm vs. clean air from another arm, 56 predators moved into either of the arms, 80% of which preferred clean leaves ( $P < 0.01$ ). When we replaced clean leaves by spider mite infested ones, 56 predators moved into one of the arms, 88% of which preferred infested ones ( $P < 0.01$ ). When *P. persimilis* received odors related to infested cucumber leaves from one arm and the clean ones from another, 57 predators moved into one of the arms, 76% of which preferred infested leaves ( $P < 0.01$ ) (Table 1).

Olfactory response of *P. persimilis* (Turkey sample) receiving bean leaf odors:

When *P. persimilis* received odors related to clean leaves from one arm vs. clean air from another, 57 predators moved into either of the arms, 77% of which preferred clean bean leaves ( $P < 0.01$ ) significantly. Replacing clean bean leaves by spider mite infested ones, 58 predators moved into the arms, 85% of which preferred infested bean leaves significantly ( $P < 0.01$ ). When the odor sources consisted of spider mite infested leaves vs. clean ones, 57 out of 60 predators moved into the arms, 84% of which preferred infested leaves ( $P < 0.01$ ) (Table 2).

Olfactory response of *P. persimilis* (UT sample) receiving cucumber leaf odors:

Receiving odors from clean cucumber leaves from one arm vs. clean air from another, 54 predators moved into either of the arms, 76% of which preferred clean leaves ( $P < 0.01$ ). When we replaced clean leaves by spider mite infested ones, 57 predators moved into one of the arms, 79% of which preferred infested ones ( $P < 0.01$ ). When *P. persimilis* received odors related to infested cucumber leaves from one arm vs. the clean ones from another, 58 predators moved into one of the arms, 81% of which preferred infested leaves ( $P < 0.01$ ) (Table 3).

Olfactory response of *P. persimilis* (Turkey sample) receiving cucumber leaf odors:

When *P. persimilis* received odors related to clean leaves from one arm vs. clean air from another, 55 predators moved into either of the arms, 77% of which preferred clean bean leaves ( $P < 0.01$ ) significantly. Replacing clean bean leaves by spider mite infested ones, 56 predators moved into the arms, 75% of which preferred infested bean leaves significantly ( $P = 0.0001$ ). When the odor sources consisted of spider mite infested leaves vs. clean ones, 56 out of 60 predators moved into the arms, 82% of which preferred infested leaves ( $P < 0.01$ ) (Table 4).

Olfactory response of *P. persimilis* (UT sample) receiving bean vs. cucumber odors:

Receiving odors related to clean cucumber vs. clean bean leaves, 52 predators moved into either of the arms, none of which showed a significant preference towards each of the arms ( $P = 0.78$ ). When the predatory mite received odors related to infested cucumber vs. infested bean, 57 mites moved into one of the arms, 64% of which preferred infested bean leaves ( $P = 0.02$ ).

Replacing the arms by clean cucumber vs. infested bean, 57 predators moved into one of the arms, 69% of which preferred infested bean leaves ( $P < 0.01$ ). When *P. persimilis* received odors related to infested cucumber from one arm vs. clean bean from another, 56 predators moved into one of the arms, 71% of which preferred infested cucumber leaves ( $P < 0.01$ ) (Table 5).

Olfactory response of *P. persimilis* (Turkey sample) receiving bean vs. cucumber odors:

Receiving odors related to clean cucumber vs. clean bean leaves, 54 predators moved into either of the arms, none of which showed a significant preference towards each of the arms ( $P = 0.79$ ). When the predatory mite received odors related to infested cucumber vs. infested bean, 51 mites moved into one of the arms, 65% of which preferred infested bean leaves ( $P = 0.03$ ). Replacing the arms by clean cucumber vs. infested bean, 53 predators moved into one of the arms, 75% of which preferred infested

bean leaves ( $P < 0.01$ ). When *P. persimilis* received odors related to infested cucumber from one arm vs. clean bean from another, 50 predators moved into one of the arms, 68% of which preferred infested cucumber leaves ( $P = 0.001$ ) (Table 6).

## Discussion

Our results showed that the bean and cucumber plants (varieties Red Alamouti and Soltan, respectively) by themselves are attractant host plants for *P. persimilis* as the predator preferred them over clean air in both samples. This is important in comparison with researches which have shown acaricidal effects of some plants or their extracts for mites. Antonious *et al.* (1997) have reported repellent activities of hot pepper extracts against *Tetranychus urticae*. Yanar *et al.* (2011) have investigated the acaricidal effect of *Chenopodium album* L. which exhibited a significant adult mortality in spider mites. Snoeren *et al.* (2010) provided evidence for HIPV components that made the host plant less attractive for the parasitoid wasp, *Diadegma semiclausum* (Hymenoptera: Ichneumonidae). Schröder and Hilker (2008) reported that non-induced plant compounds could also help predator to locate the prey-infested host plant. Kobayashi and Yamamura (2003) reported that plants that were free of herbivores could also attract predators when they were close to herbivore infested plants. Our results are consistent with Kobayashi *et al.* (2006) who noted that even when the neighbours were not attacked by herbivores, the plants could benefit from attracting predators. Dicke *et al.* (2003) discussed that the costs of producing such attracting volatiles might outweigh the benefits of being protected.

Natural enemies of the herbivores may use HIPV as signals of prey presence and locate their prey. Volatile components are known to vary among plant species. It has been demonstrated that some predators prefer a specific HIPV without any previous experience but other studies have shown that previous experience with a specific HIPV might be

necessary to trigger the preference of that volatile (Sznajder *et al.*, 2010). Our experiments showed that *P. persimilis* of both samples was able to recognize the spider mite induced volatiles of bean leaves and was significantly attracted towards infested leaves when the alternative arm was occupied by either clean air or clean bean leaves. This was obvious for UT sample because of its prolonged previous rearing experience on *T. urticae* infested beans, but as a same preference was recorded from naïve Turkey sample, it could be considered that a same previous experience was not a necessity for prey finding on bean for this sample. In other words, probably the close relation between the host plant varieties (Barbunia and Red Alamouti) and infesting prey (the red and green forms of *T. urticae*) has led to similar HIPVs qualitatively and quantitatively.

In the experiments on cucumber leaves, similar attraction towards infested leaves was recorded in both samples but Turkey sample preference to spider mite infested cucumber leaves rather than clean leaves was less visible ( $P = 0.02$ ) despite significance. It seems that although cucumber leaf odor is attractant for the predator by itself (discussed above), its HIPV is not as well recognized as bean leaf HIPV yet. We interpret this behavior as our Turkey sample of *P. persimilis* seems to still be more dependent to a previous experience on cucumber (Soltan variety) to be able to recognize its volatiles as accurate as UT sample. This interpretation is supposed to be more logical when the same experiment by Turkey sample was repeated after 20 generations of rearing ( $P < 0.01$ ).

Our olfactory experiments showed that both samples of the predatory mite, *P. persimilis* preferred bean leaf var Red Alamouti odours over those of cucumber var Soltan. This was true not only for clean leaf odours but also for the HIPV emitted from leaves infested by *T. urticae*. Kappers *et al.* (2011) compared the attractiveness of eight cucumber varieties to *P. persimilis* and reported the most attractive variety attracted twice as many as the least



attractive one. Although our cucumber variety (Soltan) is not considered as the most attractive one for the predatory mite, but its rapid rate of growth and high productivity has made it the most suitable variety used in the greenhouses, fields and laboratory experiments. Similarly, Tatemoto and Shimoda (2008) investigated the olfactory response of the predatory mite, *Neoseiulus cucumeris* on cucumber plants and reported no significant preference for volatiles from clean cucumber leaves, artificially damaged cucumber leaves and infested ones.

Koveos and Broufas (1999) discussed the feeding history of *Typhlodromus kerkirae* that affected its response to volatiles of spider mite infested beans but it does not sound like feeding history is the main factor that causes *P. persimilis* prefer bean leaves in our experiments as even after 20 generations rearing on cucumber, the predator still preferred bean volatiles in both samples. As the predator preferred spider mite infested cucumber over clean bean leaves, it seems that HIPV plays a more important role in comparison with non-infested plant odor in predator foraging behavior.

Our results confirmed that the olfactory response of *P. persimilis* did not differ between some common Iranian usual varieties of cucumber and bean except some lower preference records for native Turkey sample. Further investigations are needed to compare the predation rate of both samples in the laboratory and greenhouse.

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پاسخ بویایی *Phytoseiulus persimilis* روی گیاهان لوبیا و خیار آلوده به *Tetranychus urticae*

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**چکیده:** شکارگرها در حین جستجو برای غذا از مواد فرار بویایی ناشی از گیاهان آلوده به گیاه‌خواران، بوی گیاه و مواد فرار متعلق به افراد هم‌گونه و غیر هم‌گونه خود به‌منظور پیدا کردن شکار استفاده می‌کنند. نه‌تنها ترکیبات این مواد بویایی در بین گیاهان مختلف بسیار متفاوت است، بلکه درک شکارگر از این مواد هم با توجه به گونه شکارگر و نمونه جمعیتی آن دچار تغییر می‌شود. در این پژوهش پاسخ بویایی دو نمونه (ترکیه و دانشگاه تهران) از کنه شکارگر *Phytoseiulus persimilis* Athias- Henriot (Acari: Phytoseiidae) با دریافت مواد فرار ناشی از گیاهان لوبیا و خیار آلوده به *Tetranychus urticae* Koch (Acari: Tetranychidae) و همین‌طور اثر تجربه پرورش بر نمونه متعلق به کشور ترکیه بر پاسخ بویایی آن در شرایط آزمایشگاه بررسی شده است. داده‌ها حاکی از این بود که *P. persimilis* هر دو نمونه در شرایطی که از سوی مقابل، هوای پاک را دریافت می‌کرد، به‌طور معنی‌داری به سمت برگ‌های آلوده (لوبیا یا خیار) حرکت می‌کرد. در هر دو نمونه، کنه شکارگر قادر نبود بین گیاه خیار و لوبیای سالم تمایزی قائل شود. در هر دو نمونه هنگامی که شکارگر امکان انتخاب بین برگ‌های خیار و لوبیای آلوده به کنه تارتن را داشتند، به‌طور معنی‌داری به سمت لوبیای آلوده حرکت کردند. تجربه پرورش هیچ‌گونه تأثیر معنی‌داری روی انتخاب شکارگر نگذاشت و *P. persimilis* در هر دو نمونه، بوی مربوط به برگ‌های سالم را نسبت به هوای پاک ترجیح دادند. تعداد کنه‌های شکارگر باتجربه‌ای که به سمت برگ‌های لوبیای آلوده حرکت کردند، در هر دو نمونه، بیش‌تر از تعدادی بود که به سمت برگ‌های خیار آلوده حرکت کردند. در مورد ارتباط پاسخ‌های بویایی مشابه کنه‌های شکارگر باتجربه پیشین آن‌ها بحث شده است.

**واژگان کلیدی:** پاسخ بویایی، رفتار کاوشگری، شکارگر، تجربه، *Phytoseiulus persimilis*، غیرهم‌گونه