

# The Biological Control of Two Spotted Spider Mites using the Predatory Mite on Bean Grown under Greenhouse Conditions

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## Abstract

This study was carried out to investigate the effect of applying biological control program by using *Phytoseiulus persimilis* to control *Tetranychus urticae*, and the response of 'Aya' runner bean cultivar's growth, quality and quantity. The gained results showed that the predator controlled the pest after seven weeks of releasing, and the predation efficacy reached 100%. Additionally, using the predator improved the leaf area and the total chlorophyll concentration, compared with it in the prey treatment whereas the leaf area was 11106 cm<sup>2</sup>, and the total chlorophyll concentration 2.62 mg/g, in the predator treatment while they were 7911cm<sup>2</sup>, and 1.23 mg/g, in the prey treatment. Also the predator treatment exceeded the prey treatment significantly ( $p < 0.05$ ) regarding fruit set ratio, it was 80% in the predator treatment and 27% in the prey treatment. The unit rate production was multiplied 5 times in the predator treatment compared with the prey treatment. Likewise, the obtained results exhibited the negative impact of *T. urticae* on the pod's quality, whereas it decreased pod's components as soluble solids, fibers, dry matter, protein, vitamin C, and increased nitrates content. On the contrary, releasing *P. persimilis* on the infected plants; improved pod's quality.

**Key words** - Greenhouse, Biological control, *Tetranychus urticae*, *Phytoseiulus persimilis*, Runner Bean, Growth, Quality, Quantity.

## I. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) belong to family Fabaceae, is an annual herbaceous plant; it is widespread in fields and greenhouses, because of its nutritional and medical importance. That it is the most important source of proteins and carbohydrates, and the major and cheaper source for it compared to animal proteins in many countries. It is very important for agricultural rotation because of its ability to fix nitrogen into the soil by *Rhizobium* [35],[7],[14].

*Tetranychus urticae* Koch (Acari: Tetranychidae) is considered to be the most economically important pest of the family, it infects

field crops, greenhouses, fruit trees and ornamental plants [11],[25]. It is the major mite pest on beans [28]. The reason behind being a very dangerous pest is its females ability to produce new generations without mating [4]. It feeds by sucking the contents of plant cells, and damage includes webbing, fine stippling, leaf yellowing, leaf drop, and even plant death; occur due to direct feeding damage in severe infestation [24],[23]. Indirect effects of feeding include decreases in photosynthesis and transpiration [26]. Chemical pesticides have mostly been relied upon controlling *T. urticae* populations; however, their intensive and continuous usage has also resulted in pesticide resistance [38],[37]. Besides the problem of pest resistance, there is also concern about the high toxicity of common pesticides to humans, animals, and environment. Concerns about the usage of agrochemical control measures have led to search for alternative control measures to suppress *T. urticae* populations, including biological control, using the predatory mites which are worldwide common [22], particularly by applying predatory phytoseiid mites *Phytoseiulus persimilis*, which has been shown to be the most efficient in the biological control of *T. urticae* [17]. This predatory mite is specialized predator of web-spinning spider mites such as the two spotted spider mite [9],[21], it feeds on all life stages of the pest (egg, larvae, protonymph, deutonymph, and adult), besides that its life cycle is shorter than the pest [15]. the proper numbers and timing of predatory mite release is essential to achieve adequate control of *T. urticae* [38]. Successful control of mites by use of *P. persimilis* was reported also on tomatoes, strawberry, cucumber, eggplant, ornamental plants and bean [5], [8][12],[6],[3],[39].

The present investigation was designed to control *T. urticae* population using the predatory mite *P. persimilis*, and evaluate its efficacy, and its effect on 'Aya' climbing bean cultivar parameters under greenhouse conditions .

## II. MATERIALS and METHODS

### A. Plant material and mite inoculation

*Phaseolus vulgaris* L. var. Aya produced by Makrogen Tohumculuk Turkish company, was used

in this study, which is: Climbing cultivar, middle-ripening, white flowers, pod's shape sword-like, without fibers, good for cooking and manufacturing

Bean seeds, were planted in a greenhouse (400 m<sup>2</sup>) at Lattakia center for rearing natural enemies, in the middle of February during both seasons of 2017 and 2018. Good agricultural practices (GAP) were followed (i.e. well-ploughing, adding organic fertilizer as 4 kg/m<sup>2</sup> and recommended doses of mineral fertilization). Plantation was conducted using double-row per bed. The planting consisted of four beds, the width of each one 85 cm, the distance between rows was 60 cm, and between seeds was 40 cm at the same row. Plants density was 2.95 plant/m<sup>2</sup>. Plants were irrigated 2-3 times weekly using drip irrigation. The daily temperature and humidity during the experiment changes were recorded using a thermometer installed inside the greenhouse.

The individual *T. urticae* colonies used in the experiment were obtained from a culture maintained at Lattakia center for rearing natural enemies on bean plants *Phaseolus vulgaris* L, in a controlled glasshouse (26°C; RH 65%, 12 h light), plants were grown in pots (200 mm diameter and 200 mm deep) placed in a gravel bed. These mites are pure strain that it was reared for 12 years; mites were transferred to bean plants experiment, that adult females were artificially inoculated onto plants at transplanting by placing a bean leaf disc, containing 10 mites (When bean seedlings reached about 20 days old.), onto the youngest fully expanded leaf of each plant. The number of mites on each leaf disc was counted under a dissecting microscope prior to inoculation.

The predatory mite: *P. persimilis* colonies derived from a culture maintained on artificial arenas made of plastic dishes (30×20×10 cm) on mulberry leaves laid on wet cotton wool in these dishes fed *T. urticae*, under a laboratory conditions (26°C; RH 65%, 16 h light) using a strain of *P. persimilis*, which had brought to Syria from Egypt since 2005, and this strain tolerated high temperature. *P. persimilis* transferred to bean plants predator treatment after 10 days of transferring *T. urticae*, at a rate of 1:10 (one predator to 10 preys).

### B. Experimental design

There were three treatments:

- 1- Control: unharmed plants
- 2- Prey treatment: plants infected by *T. urticae*.
- 3- Predator treatment: plants infected by *T. urticae* and inoculated by *P. persimilis*.

We used the Randomized Block Design with three treatments separated from each other by using fine mesh. each treatment was replicated four times, and there was 30 plants in each replication (360 the total number of plants in the experiment). Five plants were took out from each replication to measure the

leaf area and the net assimilation rate, and productivity was measured by taking the production's average of 10 plants of each replication. Ten plants were chosen to investigate the population development of the prey and predator. The data were analyzed using ANOVA (GenStat, 12), difference test at a 5% level of probability, and the correlation between prey and predator were examined.

Bean plants were monitored and the population dynamics of the prey and predator were recorded at weekly intervals, starting after 10 days of releasing the pest and it continued for 11 weeks, by counting the numbers of motile stages (adults) of the predator and prey, on the both surfaces of studied plant's leaves, using a hand lens (10x). The ratio predator: prey was measured to determine the time in which the predator controls the prey. Henderson and Telton equation [16], was used to estimate the predator efficacy.

## III. RESULTS and DISCUSSION

### A. Population development of *T. urticae* on bean plants in the prey treatment

Results showed that the numbers of *T. urticae* was multiplied 7 times in week 2 after spreading the pest *T. urticae* on plants, it multiplied from 0.2 to 1.4 individuals/leaflet, which is very close to the limit of economic damage (2-3) individuals/leaflet [1], an average of two weeks is sufficient to produce a new generation of *T. urticae* in the normal conditions [31]. Starting from week 3 the numbers of *T. urticae* exceeded the extent of the economic damage and reached 4.2 individuals/leaflet, in week 4 *T. urticae* reached 33 individuals/leaflet, which is sufficient to cause significant damage to the lowest parenchymal layer and chlorophyll function, and this direct feeding damage; affect negatively the growth of plants [15],[24],[23]. In week 5, there was 95 individuals/leaflet and a part of the leaf area was damaged in coincidence with temperature ranging between 15-45°C and RH between 25-72%. In week 6, it reached 107.8 individuals/leaflet, then it increased and reached 1037.04 individuals/leaflet. In week 8, this breakout was taken place because of the hatched eggs, the evolution of the resulting offspring, and the appropriate weather conditions, that the host plant's resistance to the pest became very weak [20],[32]. in week 9 *T. urticae* reached 1055 individuals/leaflet, in coincidence with temperature ranging between 19-35°C and RH between 23-71%, at the last week the inspection result was zero because of the severe damage of the plant that leaves dried and died, and the pest started searching for new resource for food to continue its life cycle, and this corresponds with [19] that the daily increase of mites numbers may reach 40%, and exponential population growth usually ends abruptly due to overexploitation of the host plant.. Fig 1.

From the above-mentioned results, it is obvious that the plant continued its growth and improvement until the fourth week, then since the fifth week the growth appeared to decline and this was due to the damage taken place and no formation

for new leaves. Hence, *T. urticae* can destroy the plant completely after nine weeks, and this corresponds with [28] results that concerns *T. urticae* damage on bean.

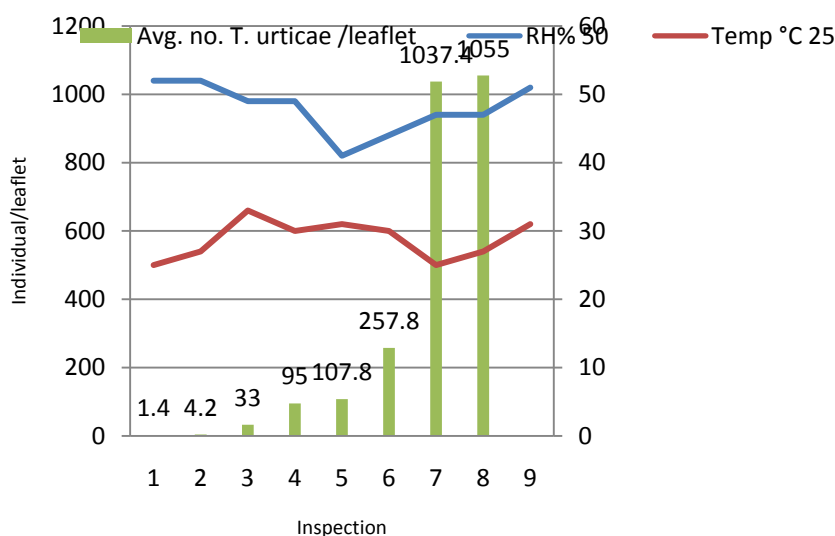


Fig 1. Population development of *T. urticae* in the prey treatment against inspection time(week).

**B. Population development of *P. persimilis* and the suppression of the target pest *T. urticae* on bean plants in the presence in the predator treatment**

Results presented in Table 1. declare the changes of the predator: prey ratio to the advantage of the predator. The predator *P. persimilis* was inoculated when the average of *T. urticae* was  $7.6 \pm 2.4$  individuals/leaflet, which is above the limit of economic damage. The population of released *P. persimilis* grew gradually, where the predator began its activity after two weeks, and its efficacy reached 69%, in week 3 and predator: prey ratio was 1: 108, in week 4 the average of *T. urticae* was  $245.4 \pm 28.2$  individuals/leaflet because of the hatched eggs, and the suitable conditions, that the temperature ranged between 16-44°C, RH between 22-65%. Raising of temperature above 30°C; increased the predator consumption of the prey [20],[36], and the predator efficacy decreased to 47%, while the ratio was 1:17 (predator: prey), in week 5 the prey population declined faster and the predator efficacy reached 89%, the ratio was 1:9, at this stage the damage to new-grown bean leaves was ,considerably, lower and

new leaves initiated. In week 6, the efficacy reached 91%, and the ratio was 1:2. Finally, in week 7 the predator managed to control the prey and its efficacy was 100%. In week 8 *T. urticae* was reduced to zero density by *P. persimilis* . Through the last three weeks the predator managed to attain the level of the prey constant, in order to ration the prey consume and save its life for further time, but when its numbers increased and its need for food increased too, so the prey decreased until it vanished at the end of this study, all of this coincident with continuing of the plant's growth like those plants at control treatment. This finding corresponds with [13].

So, the presented results indicated that the predator managed to control the prey after four weeks of its releasing on the infected plants and its efficacy was 89%, then it reached 100% after seven weeks and it could control the pest completely. The correlation between the prey and the predator in the predator treatment was negative and strong ( $r = - 0.96$ ) that the numbers of *T. urticae* decreased while *P. persimilis* numbers increased.. Fig 2.

TABLE 1. 1  
Population development of *T. urticae* and *P. persimilis* versus in the predator treatment

Inspection date	Avg. no. mites/leaflet ± S.E.		Predator : prey ratio	Predator efficacy %	Weekly average	
	<i>T. urticae</i> /leaflet *	<i>P. persimilis</i> /leaflet *			Temp °C	RH%
20/3	$1.4 \pm 0.5$	-	-	-	11-38	22-78
28/3	$2.2 \pm 1$	-	-	-	13-36	26-77

4/4	7.6 ± 2.4	-	-	-	14-40	33-71
11/4	7 ± 1.5	0	-	-	23-42	33-64
18/4	31.8 ± 6.7	0	-	-	15-45	25-72
25/4	64.6 ± 21.5	0.6 ± 2.3	1:108	69	23-38	29-53
2/5	245.4 ± 28.2	14.4 ± 10.4	1:17	47	16-44	22-65
9/5	209.2 ± 5.3	22.8 ± 8.7	1:9	89	21-29	25-68
16/5	169.4 ± 3.8	76.8 ± 19.2	1:2	91	19-35	23-71
23/5	175 ± 4.8	106 ± 17.1	1:2	100	22-40	31-70
30/5	0	210 ± 11.8	1:00	100	17-40	24-68

\*: motile stages

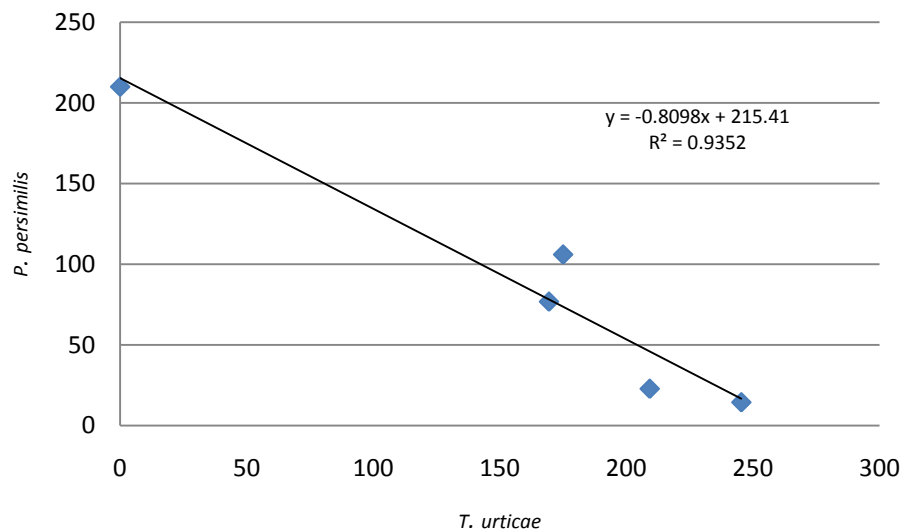


Fig 2: The correlation between *T. urticae* and *P. persimilis* numbers in the predator treatment

**B. The effect of the prey *T. urticae* on the concentration of chlorophyll, the leaf area, its index, and on the net assimilation rate in the presence of the predator *P. persimilis* on bean plants:**

The leaf area (cm<sup>2</sup>/plant), was measured after 40 days of transferring *T. urticae* to the plants, using disks way [41]. Also the index of the leaf (m<sup>2</sup>/m<sup>2</sup>) was measured according to [2], besides the net assimilation rate (mg/cm<sup>2</sup>/ day), was measured twice, after 30 days and 40 days of transferring *T. urticae* [30]. Results showed that the control and predator treatments exceeded the prey treatment significantly ( $p < 0.05$ ) in the value of: leaf area, leaf index, and net assimilation rate; that the leaf area reached 11910, 7911, 11106 cm<sup>2</sup>, in the control, prey, predator treatments, respectively, so the difference between the predator leaf area average, prey, predator treatments, respectively, so the difference between the predator leaf area average, and the prey was 3195 cm<sup>2</sup>, and between the control one and the predator was 804 cm<sup>2</sup>. The leaf index results showed that these values were ideal in the control and predator treatment 3.5, 3.27 m<sup>2</sup>/m<sup>2</sup>, consecutively, and those exceeded the prey treatment significantly ( $p < 0.05$ ) that the average of it was 2.3 m<sup>2</sup>/m<sup>2</sup>. This, also, reflected on the total net assimilation rate. The results of Table. 2 demonstrated that the average of

net assimilation of rate reached 0.65, 0.18, 0.36 mg/cm<sup>2</sup>/day, in the control, prey, and predator treatments, each in turn. The control and predator treatments exceeded the prey one significantly ( $p < 0.05$ ), also the control exceeded the predator treatment. This decline in the prey treatment may be taken place due to the damage caused by the prey *T. urticae* during sucking the contents of plant cells, leaf deformation, leaf yellowing, leaf drop, and plant death at sever infestation [24],[23].

The greater the leaf area is, the more chlorophyll the leaf contains, and the higher the photosynthate will be. The photosynthate is used as reserved food, respiration, and growth. Good photosynthesis supports plant growth development [10]. So the total chlorophyll concentration ( mg/g), was measured by using Spectronic20 colorimeter [33]. Results presented in Table 2 manifested that the presence of *T. urticae* caused a decrease of leaf content of chlorophyll, with a significant differences compared to the control and predator treatment, also the control treatment exceeded the predator one, that it recorded 2.86, 1.23, 2.62 mg/g, in the control, prey, predator treatments, in series. This decrease of chlorophyll is due to the activity of *T. urticae* that it feeds on leaf cells' content and damages plant's tissues, so it loose its physiological activities [40],[23],[26].

**Table2. The effect of *T. urticae* on the leaf area, leaf index, the net assimilation rate, and on the total concentration of chlorophyll, in the presence of *P. persimilis* (the average of two seasons) comparing with the control and the prey treatments.**

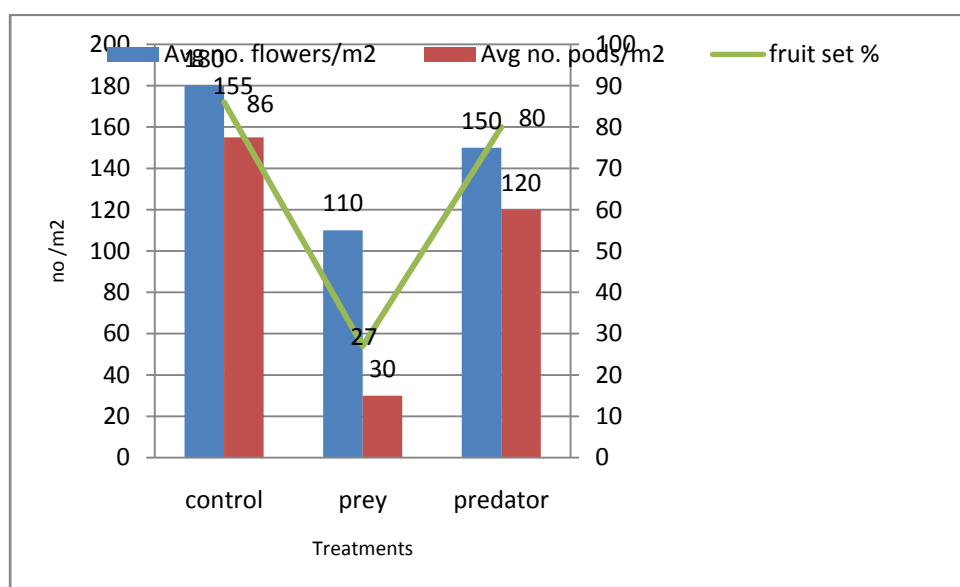
The treatment	The average of Leaf area cm <sup>2</sup>	The average of Area index m <sup>2</sup> /m <sup>2</sup>	The average of Net assimilation rate mg/cm <sup>2</sup> /day	The average of Total chlorophyll concentration mg/g
Control	11910	3.5	0.65	2.86
Prey	7911	2.3	0.18	1.23
Predator	11106	3.27	0.36	2.62
LSD 5%	83.4	0.19	0.052	0.013
CV	3.2	2.9	5.8	0.3

**C. The effect of *T. urticae* on the number of flowers, pods /m<sup>2</sup>, and on the fruit set in the presence of *P. persimilis* on bean plants:**

The presented results in Fig. 3 demonstrate that the control and predator treatments exceeded the prey one significantly ( $p < 0.05$ ), that it recorded 180, 110, 150 flower/m<sup>2</sup>, in the control, prey, predator treatments, in sequence, the increase was 70, and 40 flower/m<sup>2</sup> from its value in the prey and predator treatment, respectively, also the control treatment exceeded the predator one by an increase of 30 flower/m<sup>2</sup>. On the other hand the control treatment exceeded significantly the prey and predator treatments by the numbers of pods/ m<sup>2</sup>, with an increase of 125, 35 pod/m<sup>2</sup> in the prey, predator treatments, respectively, followed by predator treatment which exceeded the prey treatment with an increase of 90 pod/m<sup>2</sup>, and the fruit set recorded 86, 27, 80 pod/m<sup>2</sup> for the control, prey, predator treatments, respectively. The increase of the fruit set in the prey treatment is due to the damage of the foliage, its chemical contents, the shortage of the leaf area, and the chlorophyll concentration, that affected photosynthesis, the activity of pollen, and the amount of plant growth regulators [29],[20].

**D. The effect of *T. urticae* on the average of the total and standard production in the presence of *P. persimilis* on bean plants:**

The gained results showed that the control and predator treatment exceeded the prey treatment significantly ( $p < 0.05$ ), that the average of the area unit production was 1.62, 0.24, 1.26 kg/m<sup>2</sup>, in the control, prey, predator treatments, respectively. The control treatment exceeded the predator one by an increase of 0.36 kg/m<sup>2</sup>, and the percentage of production increase reached 85, 80% , in the control and predator treatments, in order. The damage caused by *T. urticae* reflected on the amount of the standard production that the numbers of the deformed pods increased, and the standard production percentage in the prey treatment was 25%, while it reached 96.9 and 96.8 in the control and predator treatments, sequentially. This reduction of productivity is due to the damage caused by *T. urticae* and its feeding on cells' contents, which affected the chlorophyll concentration, consequently it affected the leaf area and the net assimilation rate, which led to decrease in photosynthesis, thereby it caused decrease of the growth, pods numbers, and pods deformation [18][34][40]. Fig.4.



**Fig 3. The effect of *T. urticae* on the of flowers' numbers, pods /m<sup>2</sup>, and on the fruit set %, in the presence of *P. persimilis* (the average of two seasons)**



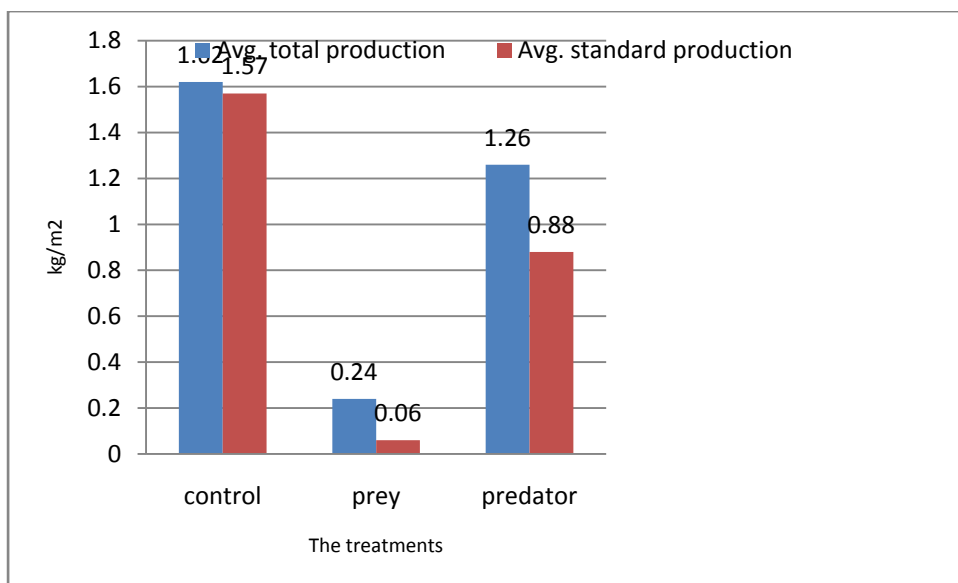


Fig 4. The effect of *T. urticae* on average of the total and standard production in the presence of *P. persimilis* (the average of two seasons)

LSD 5%: Total production=0.01; Standard production=0.01

**E. The effect of *T. urticae* on pods' content in the presence of *P. persimilis* on bean plants:**

The attained results showed that the control and predator treatment exceeded significantly the prey treatment in the pods contents of dry matter rate, soluble solid substance rate, and vitamin c amount, that it recorded: 13.5, 10, 13.5% for the dry matter, 5.5, 3.7, 5.3% for the soluble solid substance, and 29.04, 27.28, 29.04 mg% for vitamin c, in the control, prey, predator treatments, respectively. Also, the predator treatment exceeded the prey one significantly, in its content of fiber and protein, the values which recorded was 1.47, 0.15, 0.62% for the fibers, and 4.92, 0.78, 2.06% for protein in the

control, prey, predator treatments, respectively. The decrease of the pods contents in the prey treatment was because of the damage caused by *T. urticae* that it decreased photosynthesis process, and the component that formed by this process, while using *P. persimilis* controlled this pest and improved plants growth and pods component [18],[34] Nitrates were higher in the pods of the prey treatment 107.3%, because of the negative effect of *T. urticae*, while the presence of the predator *P. persimilis* contributed to decrease this rate that it was 102.3% while it recorded 95.3% at control treatment, although of this increase it still lower than the permitted limits in bean's pods which is 300 mg/kg [27].

Table4. The effect of *T. urticae* on the pods content in the presence of *P. persimilis* (the average of two seasons)

The treatment	Avg. dry matter %	Avg. soluble substance %	Avg. Vitamin c mg%	Avg. fiber %	Avg. protein %	Avg. nitrates %
Control	13.5	5.5	29.04	1.47	4.92	95.3
Prey	10	3.7	27.28	0.15	0.78	107.3
Predator	13.5	5.3	29.04	0.62	2.06	102.3
LSD 5%	0.34	0.36	0.32	0.05	1.09	3.46
C.V.	1.2	3.3	0.5	3.5	19.4	1.5

**IV. CONCLUSIONS**

The present experiment reveals that *T. urticae* increased exponentially up to the ninth week on controlled plants (prey treatment). But on the plants where *P. persimilis* were released. The *T. urticae* population was checked effectively due to predation, that *T. urticae* population reduced to the minimum number, until the disappearance up to the seventh week. The results, also, showed that the infection reduced the leaf area and its index, photosynthesis efficacy, and chlorophyll concentration, this reflected negatively on the numbers of flowers, pods, fruit set,

and the amount of production, beside increasing the rate of deformed pods, which its content of dry matter, soluble solid substance, vitamin c, fibers, and protein, was low. Releasing the predatory mite *P. persimilis* caused an increase in the leaf area and its index, photosynthesis efficacy, chlorophyll concentration, also it increased the numbers of flowers, pods, fruit set, production and the marketable production, also it improved pods' contents compared with the prey treatment.

From the previous results we can recommend to release the predatory mite *P. persimilis* on beans plants to control the two spotted spider mite *T.*

*urticae* as a method of biological control instead of using chemicals.

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