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INDUCTION OF RESISTANCE IN COMMON BEAN PLANTS "*Phaseolus vulgaris* L." USING DIFFERENT PLANT ELICITORS AGAINST SPIDER MITE "*Tetranychus urticae* KOCH" INFESTATION

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ABSTRACT

The common bean plants "*Phaseolus vulgaris* L." is frequently attacked by the two spotted spider mite (TSSM) *Tetranychus urticae*, causing a several decrease in bean plant growth and yield. Therefore, for commercial production of bean in the field, controlling mite infestation is necessary. Foliar application of salicylic acid (SA) or methyl jasmonate (MeJA) on common bean plants before or after two spotted spider mite infestation proved to be effective in reducing infestations. In most concentrations these elicitors significantly improved common bean plant growth i.e. had a positive effect on plant height, number of branches, shoot dry weight and leaf area per plant and bean yield. SA at 100 mg/L had the strongest positive effect. Moreover, application of elicitors significantly altered leaflet anatomical characters i.e. increased thickness of leaflet blade, thickness of palisade and spongy parenchyma as well as thickness of midrib region of the leaflet and changed the dimension of vascular bundles, resulting in mite infestation had strongly decreased on these leaflets of new anatomical characters. We conclude that both elicitors, in particular, 100 mg/L SA could be used for controlling *T. urticae* infestation, to improve plant growth and to improve bean yield and its quality in the field.

Keywords: Salicylic, Methyl Jasmonate, Two spotted spider mite (TSSM), Bean, Leaf Anatomy.

INTRODUTION

Common bean (*Phaseolus vulgaris* L.) is one of the most popular vegetable crops and hence a fundamental source of proteins for human consumption. For example, in Egypt, common bean export reached about 24704 tones in 2006 (FAO, 2009). The *two spotted spider mite (TSSM), Tetranychus urticae* Koch (Acari: Tetranychidae) is considered a major pests on different agricultural crops including bean plants. Globally, around 1,200 species of spider mites are known to be pest on different crops (Zhang, 2003), but TSSM is the most polyphagous of all and has been reported on over 150 host plant species of economic value (Zhang, 2003; Wilkerson *et al.*, 2005). TSSM feeds by puncturing cells with its stylets and draining the contents, thereby producing a characteristic yellow specking of the leaf surface. This chlorotic damage reduces the plant's ability to build carbohydrates via photosynthesis which consequently results in reduction of the total yield of vegetable crops. Moreover, following two spotted spider mite infestation secondary infestation by pathogens often causes substantial additional damage while, finally, it has been reported that TSSM outbreaks cause occupational allergic disease in greenhouse workers (Jee *et al.*, 2000).

A major obstacle in controlling two spotted spider mite is the fact that it rapidly evolves resistance to pesticides (Geoghiou, 1990). Greenhouse pest management of two spotted spider mite is still largely based on chemical control since biological control has limited efficacy against higher mite population densities. During the last few decades, the need for sustainable vegetable production has increased to minimize the damage to environment and health as a consequence of the use of acaricides. Moreover, acaricides may also interfere with biological control since natural enemies often are similarly sensitive to them as their herbivorous prey, while getting exposed to these products directly or indirectly after ingesting pesticide-contaminated prey (Picone and Tassel, 2002). Thus, it is important to develop pest control methods that are better compatible with biological control, using agents like elicitors at low concentrations natural inducible plant resistance, from heron referred to as 'elicitors' (Bi *et al.*, 1997). Such elicitors often are plant hormones or synthetic analogs. Ozawa *et al.* (2000) reported that spider mite feeding on lima bean plants elevated the expression of acidic and basic pathogenesis-related genes which could also be induced by the salicylic acid derivative methyl salicylate (MeSA) and Jasmonic acid (JA) suggesting that the SA- and JA- related signaling pathway are induced by spider mite feeding. In addition they found that both pathways are involved in the regulation of spider mite, induced Lima been volatiles that are attractive to the carnivorous natural enemies' i.e. predatory mites, of spider mite.

An elicitor can be any naturally occurring plant compound as well as synthetic substance that initiates induced plant defense responses in the tissue it is applied a fashion comparable to natural induction by herbivores or pathogens. Hence elicitors can be used to 'mimic' natural induction without causing (mechanical) damage while increasing the plant's resistance to real herbivores or pathogens (Farouk *et al.*, 2008). Before the discovery of chemical elicitors, induced resistance was not a practical tool for use in agriculture because pre-inducing plants with biotic elicitors (eg actual herbivores) to protect against future, possibly more harmful attackers was too complicated to be feasible (Karban *et al.*, 1997). However, to use induced plant defense responses as an effective tool in pest management, it is necessary to carefully evaluate their effects on plant performance and yield in a horticultural and/or agricultural setting.

Jasmonic acid (JA) and Salicylic acid (SA) are two endogenous signaling molecules implicated in regulation of plant resistance to herbivores and pathogens. JA is found in many plant species and is involved in regulating diverse plant functions, not only herbivore and pathogen resistance but also e.g. senescence (Creelman and Mullet, 1997). JA can be used to elicit plant defense. In tomato, application of JA results in induction of proteinase inhibitors and polyphenol oxidase and in a decrease in the preference and abundance of many common herbivores in the field (Thaler *et al.*, 1996, 1999). Induction of JA-related responses is often obtained via application of its methyl ester methyl jasmonate (MeJA).

Salicylic acid (SA) (o-hydroxybenzoic acid), is a plant phenolic, is widely distributed throughout the plant kingdom. It is a hormone-like substance, which plays an important role in the regulation many aspects of plant growth and development (Raskin, 1992, Farouk, 2005). However, it is especially famous for its ability to induce systemic acquired resistance (SAR) in plants (Ryals et al, 1996) i.e. resistance in induced but also uninduced distal leaves of the same plant.

The present work threw light on the effectiveness of the elicitors MeJA or SA for controlling TSSM infestation under field condition and also tested their effects on common bean plant growth and yield as well as leaflet structure.

MATERIALS AND METHODS

Two field trials were conducted in Faculty of Agriculture, Mansoura University in two successive seasons 2006/2007 to study the impact of MeJA and SA on *Tetranychus urticae* Koch infestation on common bean plants, and on plant growth, yield and leaflet structure. The obtained results represented the average number of the data of the two successive season 2006 /2007.

Seeds of common bean cv. Bronco were sown on March 12 and 9 in the two successive seasons respectively. Seeds were sown in hills 10 cm apart on one side of ridges 3 m long and 60cm width. All agricultural practices were carried out according to the recommendation of Ministry of Agricultures, Egypt. The plants were divided into ten treatment groups within a complete randomized block design consisting of four plots as replicates per treatment each one 2.5 × 3 m^2. Treatment one treated with distilled water and used as check while treatment two artificially infested with 50 adult females of *T. urticae* per plant which dispersed over the two trifoliate leaves of 15 days-old plants. The other Treatments from 3 -6 were infested by the same way as treatment two then sprayed with one of the following solutions; 50, 100 mg/L SA, and $5x10^{-5}$, 10^{-5} M MeJA, two weeks after the infestation. Spraying with plant elicitors were conducted first time when the age of plants was 35 days and repeated again every five days after adding 1 % tween 20 as a wetting agent. The treatments from 7 -10 were sprayed by the same way in the previous treatments and then infested artificially with mites after the third spray. All plants were covered with mite-proof mesh bag. The densities of all stages of two spotted spider mite were estimated each every 10 days starting when plants were 50 days old. At each sampling date, 25 leaflets were randomly selected from each of the treatments and counting was conducted on plant leaves using a stereo-binocular microscope.

At full blooming stage (70 DFS) (days after sowing) a random sample of 5 plants from each experimental plot was taken for determining morphological characters, and photosynthetic pigments content of the third upper leaves. Chlorophyll was extracted by methanol after adding traces of sodium carbonate for 24 hours at room temperature. Chlorophyll concentration was determined spectrophotometrically according to Lichtenthaler and Wellburn (1985).

At the stage green pods could be harvested (85 DFS) the following data were recorded; number of green pods per plant, green pod yield per plant as well as the fruit composition with respect to ascorbic acid, total protein, total carbohydrates, acidity and total soluble solid. In short, total carbohydrates and the ascorbic acid as well as total acidity and total soluble solid of fruit were estimated using the anthrone method and 2.6-Dichlorophenol indophenole as described by Sadasivam and Manickam (1996) and AOAC (1980) respectively. Protein was extracted from the fruit and estimated by the method of Bradford (1976). For potassium and phosphorus determination, dry common bean pods were digested with $HClO_3/H_2SO_4$ until a sample has become clear cooled and made up to 50 ml using deionized water. Potassium was determined by flame photometer (Kalra, 1998), and phosphorous using ammonium molybdate and ascorbic acid (Cooper, 1977) .

Leaflet structure: Small pieces from the midrib region of the 3rd upper leaflet (second season) were taken when plants were 70 days old. The samples were fixed in formalin aceto alcohol for 48 h, then dehydrated via n-butanole series and embedded in paraffin wax (52-54 °C melting points). Sections were prepared using a rotary microtome at 15-17 µm thickness and stained with safranin/light green and finally mounted in Canada balsam (Johanson, 1940). Selected sections were examined using microscope to determining the anatomical changes in leaves

The data was evaluated using One-way ANOVA to compare the effectiveness of elicitors on the population of two spotted spider mite, and common bean growth as well as yield and its quality. Means were compared at P=0.05 to separate means (Norman and Streiner, 2003) using MSTAT-C v.1.2 software.

RESULTS

I. Population of immature stages of spider mite *T. urticae*

A. Egg stage

Data listed in Table (1) showed that the lowest Average number of *T. urticae* eggs was 182.66 obtained due to application of elicitors SA 100 mg/L when applied after infestation. While, the highest one was 853.33 when application of elicitors MJ 5x10-5 before infestation after 50 days. Moreover, after 60 days the lowest Average number of *T. urticae* eggs was 202.00 obtained due to application of elicitors SA 100 mg/L when applied after infestation. On the other hand the highest one was 675.33 obtained from artificial infestation. After 70 days the average number of *T. urticae* eggs was 288.66 at application of elicitors SA 100 mg/L when applied after infestation while artificial infestation gave 747.66 eggs . After 80 days the high average number of *T. urticae* eggs was 817.00 at artificial infestation while the lowest was 340.33 eggs recorded at application of elicitors SA 100 mg/L when applied after infestation. From the previous results, it was observed that the overall means of *T. urticae* eggs were 290.49, 708.49, 462.41, 384.41, 598.08 and 253.41at natural infestation, artificial infestation, MJ 10-5, MJ 5x10-5, SA 50 mg/L and SA 100 mg/L %, respectively. while, , 449.33, 630.08, 353.16 and 433.24 at application of elicitor MJ 10-5, MJ 5x10-5, SA 50 mg/L and SA 100 mg/L, respectively.

Table 1: Average number of *T. urticae* eggs during the two successive seasons.

Treatment / Sample		After 50 days	After 60 days	After 70 days	After 80 days	Overall means
Natural infestation		145.66± 11.21c	215.33± 76.70d	348.33± 82.15cd	452.66± 63.57bc	290.49± 68.47c
Artificial infestation		594.00± 29.68ab	675.33± 25.77 a	747.66± 39.84a	817.00± 22.67a	708.49± 47.88a
Infested and spraying	MJ 10-5	451.66± 107.76bc	407.00± 24.41bc	452.66± 18.62cd	538.33± 49.65bc	462.41± 27.45b
	MJ 5x10-5	375.33± 39.75bc	331.00± 24.00cd	393.00± 29.02cd	438.33± 27.44bc	384.41± 22.20bc
	SA 50 mg/L	574.00± 200.32 ab	541.33± 77.05ab	667.66± 149.23ab	609.33± 135.39b	598.08± 27.03a
	SA 100 mg/L	182.66± 44.45c	202.00± 11.59d	288.66± 9.38d	340.33± 14.49c	253.41± 37.02c
Spraying then infested	MJ 10-5	546.66± 152.27 ab	428.33± 71.42bc	377.33± 30.02cd	445.00± 26.72bc	449.33± 35.49b
	MJ 5x10-5	853.33± 112.88 a	516.00± 53.40b	542.66± 52.71bc	608.33± 66.98b	630.08± 76.90a
	SA 50 mg/L	295.00± 5.19bc	325.00± 10.11cd	374.33± 6.48cd	418.33± 14.67bc	353.16± 27.18bc
	SA 100 mg/L	398.33± 38.98 bc	418.33± 44.84 bc	411± 54.14cd	505.33± 63.57bc	433.24± 24.37b

Means in columns by different letters are significantly different, $P < 0.05$ by (Duncan's Multiple Range Test). ± SE

B. Larval stage

On the other hand, data in Table (2) showed that the lowest average number of *T. urticae* larvae was 43.33 obtained due to application of elicitors SA 100 mg/L after infestation, while, the highest was 227.66 at at artificial infestation after 50 days. Moreover, after 60 days the lowest average number of *T. urticae* larvae was 67.00 obtained due to application of elicitors SA 100 mg/L after infestation while, the highest one was 256.66 get from artificial infestation. After 70 days, the lowest average number of *T. urticae* larvae was 116.00 at application of elicitors SA 100 mg/L after infestation while artificial infestation gave 334.33 larvae of *T. urticae*. After 80 days the highest average number of *T. urticae* larvae was 390.00 at artificial infestation while the lowest average number was 200.33 recorded at application of elicitors SA 100 mg/L applied after infestation. From the previous results, it was observed that the overall means of *T. urticae* larvae were 113.99, 302.16, 156.08, 122.08, 196.99and 106.66at natural infestation, artificial infestation, MJ 10-5, MJ 5x10-5, SA 50 mg/L and SA 100 mg/L %, respectively. while, 141.99, 192.16, 112.91and 158.24when applying of elicitors MJ 10-5, MJ 5x10-5, SA 50 mg/L and SA 100 mg/L, respectively.

Table 2: Average number of *T. urticae* larvae during the two successive seasons.

Treatment / Sample		After 50 days	After 60 days	After 70 days	After 80 days	Overall means
Natural infestation		39.66 ± 11.21 e	83.66 ± 5.36 c	127.66 ± 18.77 bc	205.00 ± 22.71 b	113.99± 35.25b
Artificial infestation		227.66 ± 20.49 a	256.66 ± 18.74 a	334.33 ± 18.44 a	390.00 ± 30.17 a	302.16± 36.93a
Infested and spraying	MJ 10-5	97.00 ± 6.11 cd	127.66 ± 16.02 bc	178.33 ± 23.31 bc	221.33 ± 15.64 b	156.08± 27.46b
	MJ 5x10-5	66.00 ± 10.06 de	94.00 ± 12.76 c	136.00 ± 11.01 bc	192.33 ± 11.140 b	122.08± 27.48b
	SA 50 mg/L	87.66 ± 23.72 cde	139.33 ± 12.11 bc	257.33 ± 98.88 ab	303.66 ± 97.43 ab	196.99± 50.24b
	SA 100 mg/L	43.33 ± 8.17 e	67.00 ± 14.29 c	116.00 ± 15.69 c	200.33 ± 8.08 b	106.66± 34.69b
Spraying then infested	MJ 10-5	76.00 ± 25.11 cde	128.66 ± 54.63 bc	164.66 ± 46.84 bc	198.66 ± 35.04 b	141.99± 26.23b
	MJ 5x10-5	155.33 ± 17.38 b	186.33 ± 14.43 ab	189.33 ± 35.48 bc	237.66 ± 33.92 b	192.16± 17.00b
	SA 50 mg/L	59.66 ± 6.35 de	88.33 ± 16.69 c	128.66 ± 18.81 bc	175.00 ± 11.93 b	112.91± 25.07b
	SA 100 mg/L	122.66 ± 5.89 bc	117 ± 40.10 bc	174.33 ± 38.17 bc	219.00 ± 41.06 b	158.24± 24.00b

Means in columns by different letters are significantly different, $P < 0.05$ by (Duncan's Multiple Range Test). ± SE

C. Nympal stage

Table (3) showed that the lowest average number 63.00 of *T. urticae* nymphs was obtained due to application of elicitors MJ 10-5 before infestation. While, it was 336.66when application of elicitors SA 50 mg/L after infestation after 50 days. After 60 days, the lowest average was 101.66 nymphs due to application of elicitors SA 100 mg/L after infestation while, the highest was 336.66 get from artificial infestation. After 70 days the average number of *T. urticae* nymphs was 167.66 at application of elicitors SA 100 mg/L applied after infestation while, artificial infestation gave 428.66 nymphs. After 80 days highest average of *T. urticae* nymphs was 499.00 at artificial infestation while the lowest 241.33 at application of elicitors SA 100 mg/L applied after infestation. The overall means of *T. urticae* nymphs were 146.58, 393.33, 286.24, 175.24, 379.83 and 145.74 at natural infestation, artificial infestation, MJ 10-5, MJ 5x10-5, SA 50 mg/L and SA 100 mg/L %, respectively. while, being 188.41, 311.83, 208.74 and 301.16 at application of elicitors MJ 10-5, MJ 5x10-5, SA 50 mg/L and SA 100 mg/L, respectively

II. Population of spider mite adult stage

Table (4) showed that the lowest Average number of *T. urticae* adult was 79.66 and 30.66 obtained due to application of elicitors SA 100 mg/L when applied after infestation and natural infestation , respectively., while, the highest one was 513.33 at artificial infestation after 50 days. Moreover, after 60 days the lowest Average number of *T. urticae* adults was 111.33 and 139.00 obtained due to application of elicitors SA 100 mg/L applied after infestation and natural infestation , respectively while, the highest was 562.00 get from artificial infestation.

Table 3: Average number of *T. urticae* nymphs at during the two successive seasons.

Sample / Treatment		After 50 days	After 60 days	After 70 days	After 80 days	Overall means
Natural infestation		35.66 ± 4.05 e	115.33 ± 20.28 c	186.00 ± 25.02 de	249.33 ± 19.15 d	146.58± 45.99d
Artificial infestation		309.00 ± 9.86 a	336.66 ± 10.80 a	428.66 ± 26.73 a	499.00 ± 34.94 a	393.33± 43.52a
Infested and spraying	MJ 10-5	209.33 ± 12.87 b	243.66 ± 13.28 b	322.00 ± 28.02 abc	370.00 ± 34.96 bc	286.24± 36.54abc
	MJ 5x10-5	112.66 ± 10.17 c	145.66 ± 15.89 c	232.00 ± 42.71 cde	210.66 ± 7.21 d	175.24± 27.79cd
	SA 50 mg/L	336.66 ± 27.23 a	318.33 ± 6.33 a	409.33 ± 12.46 ab	455.00 ± 13.11 ab	379.83± 31.84a
	SA 100 mg/L	72.33 ± 8.66 de	101.66 ± 20.49 c	167.66 ± 20.25 e	241.33 ± 23.87 d	145.74± 37.58d
Spraying then infested	MJ 10-5	63.00 ± 8.96 de	147.66 ± 22.19 c	225.33 ± 31.88 cde	317.66 ± 15.07 cd	188.41± 54.35bcd
	MJ 5x10-5	242.66 ± 8.95 b	277.00 ± 8.38 ab	295.66 ± 20.99 cd	432.00 ± 23.35 ab	311.83± 41.53ab
	SA 50 mg/L	100.33 ± 9.02 cd	170.00 ± 37.87 c	246.00 ± 65.62 cde	318.66 ± 83.59 cd	208.74± 47.19bcd
	SA 100 mg/L	312.00 ± 13.57 a	285.33 ± 44.03 ab	300.33 ± 46.69 bcd	307.00 ± 13.79 cd	301.16± 5.79abc

Means in columns by different letters are significantly different, P < 0.05 by (Duncan's Multiple Range Test). ± SE

Table 4: Average number of *T. urticae* adults at 2006 and 2007 seasons.

Sample / Treatment		After 50 days	After 60 days	After 70 days	After 80 days	Overall means
Natural infestation		30.66 ± 3.52 e	139.00 ± 50.84 d	238.66 ± 82.00 cd	401.00 ± 56.02 b	202.33± 78.67c
Artificial infestation		513.33 ± 15.21 a	562.00 ± 25.79 a	659.66 ± 39.73 a	718.33 ± 31.97 a	613.33± 46.37a
Infested and spraying	MJ 10-5	149.33 ± 14.33 cde	218.33 ± 31.97 cd	308.00 ± 23.06 bcd	371.00 ± 21.03 bc	261.66± 48.81bc
	MJ 5x10-5	167.33 ± 11.83 cde	194.33 ± 19.93 cd	296.66 ± 15.45 bcd	352.00 ± 16.86 bc	252.58± 43.28bc
	SA 50 mg/L	254.33 ± 129.95 bcd	276.66 ± 128.99 bcd	347 ±110.96 bcd	382.00 ± 107.78 bc	314.99± 29.81bc
	SA 100 mg/L	79.66 ± 14.85 e	111.33 ± 8.37 d	195.66 ± 10.10 d	231.00 ± 12.16 c	154.41± 35.36c
Spraying then infested	MJ 10-5	192.00 ± 60.25 bcde	191.66 ± 6.96 cd	266.66 ± 22.48 bcd	488.66 ± 24.31 b	284.74± 70.22bc
	MJ 5x10-5	275.33 ± 63.1 bc	379.66 ± 40.88 b	371.66 ± 23.91 bc	660.00 ± 66.18 a	421.66± 82.90b
	SA 50 mg/L	104.66 ± 15.01 de	308.00 ± 22.05 bc	367.33 ± 14.05 bc	483.33 ± 29.07 b	315.83± 79.24bc
	SA 100 mg/L	338.66 ± 26.59 b	386.33 ± 39.87 b	415.66 ± 53.16 b	469.66 ± 43.47 b	402.57± 27.41b

Means in columns by different letters are significantly different, P < 0.05 by (Duncan's Multiple Range Test). ± SE

After 70 days, the average number of *T. urticae* adult was 195.66 at application of elicitors SA 100 mg/L after infestation while artificial infestation gave 659.66 adults of *T. urticae*. After 80 days the highest average number of *T. urticae* adult was 718.33 at artificial infestation while the lowest was 231.00 recorded at application of elicitors SA 100 mg/L after infestation. From the previous results, it was observed that the overall means of *T. urticae* adult were 202.33, 613.33, 261.66, 252.58, 314.99and 154.41 at natural infestation, artificial infestation, MJ 10-5, MJ 5x10-5, SA 50 mg/L and SA 100 mg/L %, respectively. while, being 284.74, 421.66, 315.83 and 402.57 at application of elicitors MJ 10-5, MJ 5x10-5, SA 50 mg/L and SA 100 mg/L, respectively.

The data represented in the tables (1- 4) revealed that after artificial infestation number of all *T. urticae* stages increased significantly at 50, 60, 70 and 80 days of plant age compared to natural infestation. In contrast, application of SA or MeJA at all concentration either before or after artificial infestation resulted in significant lower number of eggs, larvae, nymphs and adult *T. urticae* compared to the artificial infestation. Notably in most cases application of 100 mg/L SA after artificial infestation significantly decreased the number of eggs, larvae, nymphs and adults compared to the same treatment on the naturally infested plants.

The highest rate of increase of *T. urticae* adults was 14.36 as observed after artificial infestation while the lowest rate of increase was 4.62 and was observed after application of 100 mg/L salicylic acid to artificially or naturally infested plants (Figure 1). The rates of increase after application of MeJA irrespective of the concentration or of 50 mg/L SA were 7.42, 7.04, and 7.64 respectively.

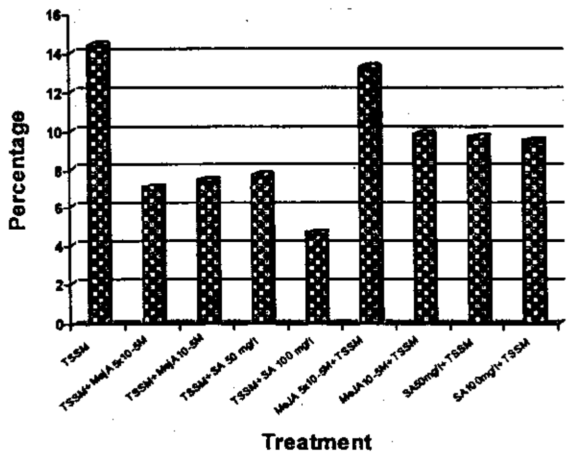

Figure 1: The total increase in Spider mite adults on plants after treatment with exogenous SA or MeJA in two concentrations during the second season.

However, when these same elicitor concentrations had been applied before the artificial infestation the rates of increase were 9.77, 13.2, 9.6 respectively and 9.39 after 100 mg/L salicylic acid

Growth Characteristics:

Spider mite infestation caused chlorotic lesions at feeding sites and bronze-discoloration of infested leaves. Moreover, females TSSM produce webbing on the infested leaflet surface in which eggs are deposited. In these webs also dust aggregates hence affecting respiration and photosynthesis.

Plants infested with spider mite were significantly smaller, had significantly fewer branches and fewer leaves that were significantly smaller while shoot fresh and dry weight was significantly lower compared to uninfected plants (Table 5). In contrast, application of SA or MeJA prior to infestation had the exact opposite effect resulting in plants that were significantly bigger and heavier than the untreated infested plants but also than the untreated uninfected control plants. Here application of elicitors before infestation resulted in bigger plants than application after infestation with *T.urticae* SA at 100 mg/L was the most effective treatment for increasing plant growth and had the strongest effect when applied before infestation (Table 5).

Photosynthetic pigment:

Chlorosis is a typical symptom of plants infested with *T. urticae* and may result from loss in chlorophyll due to mite feeding in combination with necrosis-like cell death.

Accordingly we observed a significant reduction in chlorophyll a, b, and total chlorophyll content as well as total carotenoids in the artificially infested common bean leaves compared to clean healthy leaves (figure, 2a). In contrast, the same pigments had increased significantly after exogenous application of SA or MeJA, either before or after infestation compared to infested or healthy plants with the strongest effect obtained by 100 mg/L SA (Figure 2b).

Finally the $Chl_{a:b}$ ratio increased significantly by *T. urticae* infestation but decreased significantly when also SA or MeJA had been applied as compared to healthy untreated control plants while application of SA at 100 mg/L prior to the infestation had the strongest effect.

Leaflet structure:

The leaves of the bean plant as shown in figure (3) are dorsiventral, that is, have a palisade parenchyma localized on the upper surface of the blade (adaxile) and a spongy parenchyma on the lower surface (abaxial). The palisade parenchyma is made up of elongated cells, densely packed and arranged perpendicularly to the epidermis.The spongy parenchyma cells show different forms and constitute the major part of the mesophyll cells arranged in an irregular fashion, with a smaller number of chloroplasts compared to the palisade cells, as well as large intercellular spaces

Application of SA or MeJA at all concentrations caused an increase in the thickness of midrib region of common bean leaflet with the strongest effect of SA at 100 mg/L (Table 5 and Figure 3). This increase is coincided with the increase in length and width of the main vascular bundle (Table 6).

Table 5: Growth characteristics of 70-days old common bean plants infested with *T.urticae* and treated with or without SA or MeJA during the two growing seasons.

Treament		Plant height (cm)		No of branches per plant		No. of leaves per plant		Shoot fresh weight (g)		Shoot dry weight (g)		Leaf area (cm2)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control		57.3	57.3	3	3	14.3	12	65.4	66.4	2.68	2.73	479.21	434.26
Infested		37.6	41.6	2.6	2.6	8.6	9.3	46.7	49.5	2.40	2.27	435.91	372.88
Infested and sprayed	MeJA 5x10^{-5}M	64.3	62.6	4.3	4	15.3	15	85.6	83.2	3.41	3.34	653.74	639.29
	MeJA10^{-5}M	58.3	58.6	4	3.6	14.3	13.6	76.3	74.8	2.99	3.00	535.05	581.72
	SA 50 mg/L	64.3	63.3	4.3	4	16.6	15.6	86.9	85.1	3.77	3.64	685.34	656.61
	SA 100 mg/L	66.6	67	5	5	20.3	18.3	98.1	93.3	4.53	4.27	769.72	726.30
Sprayed then infested	MeJA 5x10^{-5}M	66	64.6	4.3	4.3	19.3	16.6	89.1	88.7	4.19	3.95	728.95	700.02
	MeJA10^{-5}M	63	61.3	4	4	14.3	14	83.6	80.2	3.16	3.10	624.22	616.67
	SA 50 mg/L	70.6	68	5.3	5.6	22	19.6	111.3	105.3	5.07	4.81	801.18	778.64
	SA 100 mg/L	73.6	71	5.6	6	26	22.3	114.9	112.9	5.36	5.20	885.01	877.72
LSD at 5%		3.79	1.76	0.56	0.44	1.14	0.95	4.03	3.44	0.23	0.13	22.777	19.997

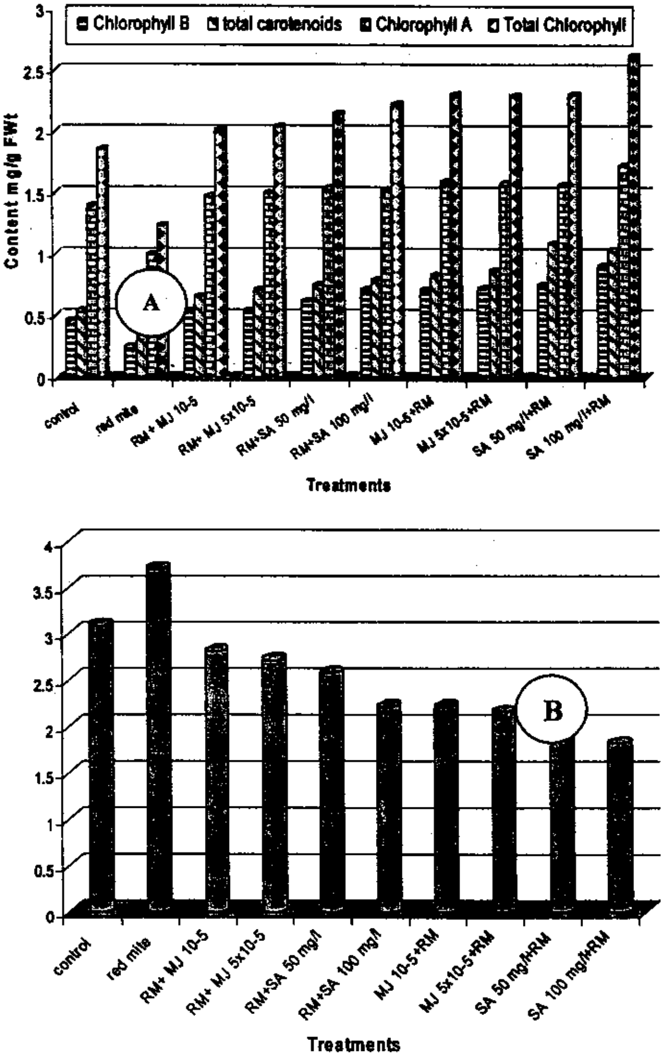

Figure 2: Photosynthetic pigments content (A) and chl a/b ratio (B) of 70 days old common bean leaves with or without *T. urticae* infestation and with or without SA or MeJA treatment during second season

Application of the higher MeJA concentration (10^{-5} M) decreased the width of vascular bundle specially when applied after infestation, meanwhile when used before infestation it gave the same width as healthy plants.

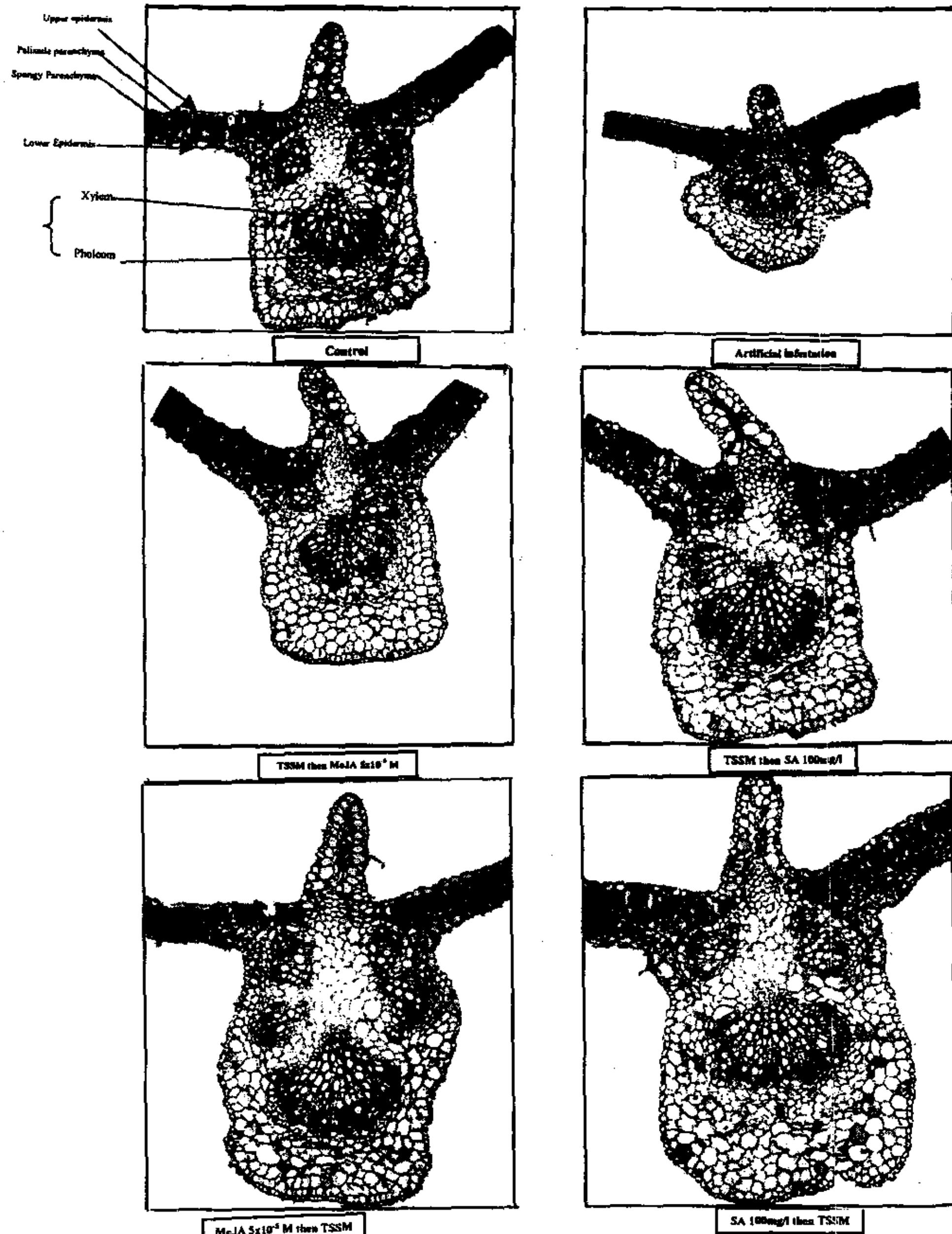

Figure 3: Common bean leaflet internal structure as affected by elicitors under *T. urticae* infestation after 70 days from sowing during second season.

Table 6: Common bean leaflet internal structure as affected by elicitors with or without T. urticae infestation after 70 days from sowing during second season

Treatment		Leaf blade thickness (µ)		Thickness of upper epidermis (µ)		Palisade parenchyma thickness (µ)		Spongy parenchyma thickness (µ)		Thickness of lower epidermis (µ)		Main vascular bundle dimension (µ) length		Main vascular bundle dimension (µ) width		Thickness of xylem tissue (µ)		Thickness of phloem tissue (µ)		Thickness of midrib region (µ)	
Control		31	100%	2	100%	12	100%	15	100%	2	100%	51	100%	80	100%	41	100%	10	100%	352	100%
Infested		20	64	2	100	7	58	9	60	2	100	33	64	46	57	25	60	8	80	248	70
Infested and sprayed	MeJA $5x10^{-5}$M	33	106	3	150	13	108	15	100	2	100	63	123	98	122	49	119	14	140	468	132
	MeJA10^{-5}M	29	93	3	150	12	100	14	93	2	100	56	109	71	88	42	102	14	140	408	115
	SA 50mg/L	41	132	3	150	17	141	19	126	2	100	67	131	94	117	51	124	16	160	512	145
	SA100 mg/L	46	148	3	150	18	150	22	146	3	150	71	139	102	127	56	136	15	150	544	154
Sprayed then infested	MeJA $5x10^{-5}$M	42	135	4	200	16	133	18	120	4	200	67	131	102	127	51	124	16	160	472	134
	MeJA10^{-5}M	34	109	5	250	11	91	15	100	3	150	53	103	80	100	41	100	12	120	480	136
	SA 50 mg/L	46	148	4	200	16	133	22	146	4	200	81	158	109	136	64	160	17	170	580	164
	SA100 mg/L	49	158	5	250	19	158	21	140	4	200	85	166	113	141	66	160	19	190	604	171

Application of SA or MeJA at all concentrations increased the thickness of leaflet blade in comparison to healthy or infested plants. This coincided with an increase in the thickness of the layers of palisade- and spongy-parenchyma as well as that of the upper and lower epidermis. In contrast, the higher concentration MeJA when applied after infestation caused the leaflet blade to become thinner. In most cases SA at 100 mg/L resulted in the strongest increase in leaflet blade thickness compared to natural or artificial infestation. Finally, leaflets infested with *T. urticae* had thinner leaflet blades and a thinner main vascular bundle

Yield and fruit quality

The bean yield and its components i.e. pod number per plant, pod yield/plant and pod length as well as yield quality (expressed as total protein, ascorbic acid, total carbohydrates contents and the total percentage of soluble solid, total acidity percentage, phosphorous percentage and potassium percentage) was significantly lower after artificial TSSM infestation compared to uninfected untreated controls. In contrast, application of elicitors, in particular, 100 mg/L SA significantly increased yield and its components as well as yield quality compared to infested plants. The highest yield and good quality was obtained after application of elicitors especially after 100 mg/L SA when applied before infestation which increased pod number per plant, pod yield per plant, pod length, TSS%, protein, and ascorbic acid, carbohydrates, total acidity percentage, phosphorous percentage and potassium percentage compared to natural or artificial infestations (Table 7a, b).

Table 7a: Yield of common bean plant as affected by elicitors under *T. urticae* infestation after 70 days from sowing during the two growing seasons.

Treament		Pod number per plant		Green pod yield per plant (g)		Pod length (cm)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Control		25.3	25.0	91.96	96.20	10.6	10.6
Infested		19.3	20.0	65.80	70.70	8.3	8.6
Infested and sprayed	MeJA $5x10^{-5}$M	39.0	33.0	128.3	114.6	13.3	12.0
	MeJA10^{-5}M	26.0	26.3	96.93	99.80	12.0	11.3
	SA 50 mg/L	41.6	37.6	128.9	122.5	14.0	13.0
	SA 100 mg/L	42.6	40.3	138.9	133.7	14.0	13.3
Sprayedthen infested	MeJA $5x10^{-5}$M	42.3	39.6	138.86	127.93	14.0	13.0
	MeJA10^{-5}M	28.3	28.3	112.4	105.6	13.0	11.3
	SA 50 mg/L	42.6	42.0	141.0	140.2	15.0	14.0
	SA 100 mg/L	44.0	44.0	141.3	141.8	15.3	14.3
LSD at 5%		3.58	1.59	5.74	1.76	0.58	0.51

Table 7b: Yield quality of common bean plant as affected by elicitors under *T. urticae* infestation after 70 days from sowing during the two growing seasons.

Treament		TSS %		Protein %		Ascorbic acid mg/100 g FW		Carbohyd-rates %		Acidity%		Phosphorous %		Potassium %	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control		2.68	2.70	17.9	17.2	25.38	25.23	46.78	46.97	7.19	7.24	0.486	0.476	2.88	2.94
Infested		2.58	2.46	17.12	16.3	22.95	22.08	43.99	43.46	6.13	6.16	0.474	0.462	2.68	2.76
Infested and sprayed	MeJA $5x10^{-5}$M	2.96	2.93	18.9	18.7	27.50	26.96	49.94	49.67	7.85	7.70	0.504	0.495	3.11	3.07
	MeJA10^{-5}M	2.76	2.83	18.33	18.26	26.38	26.36	48.35	48.09	7.57	7.54	0.494	0.483	2.95	3.03
	SA 50 mg/L	3.02	2.96	19.2	19.0	28.34	27.31	50.98	50.39	8.20	7.94	0.515	0.503	3.15	3.13
	SA 100 mg/L	3.21	3.12	19.7	19.5	29.55	29.09	52.89	52.36	8.66	8.45	0.524	0.516	3.28	3.21
Sprayed then infested	MeJA $5x10^{-5}$M	3.14	3.07	19.4	19.3	28.57	28.56	51.27	51.03	8.39	8.26	0.515	0.506	3.19	3.17
	MeJA10^{-5}M	2.82	2.86	18.4	18.3	27.01	26.70	49.40	49.00	7.72	7.61	0.502	0.498	3.06	3.05
	SA 50 mg/L	3.40	3.27	20.9	20.4	30.90	30.54	55.46	53.89	9.16	8.73	0.539	0.534	3.49	3.38
	SA 100 mg/L	3.60	3.52	21.5	21.2	32.50	31.91	57.02	56.20	9.57	9.30	0.566	0.548	3.68	3.53
LSD at 5%		0.126	0.040	0.261	0.316	0.562	0.372	0.999	0.405	0.107	0.116	0.007	0.007	0.065	0.098

DISCUSSION

World wide crop losses without the use of pesticides and other non-chemical control strategies is estimated to be about 70% of crop production, amounting to 400 billion US Dollars. Moreover, pests often adapt to pesticides via natural selection just as they adapt to natural plant resistance. The use of chemical pesticides not only results in rapid build-up of resistance but their non-selectivity often affects the balance between pests and their natural predators which are also often used in biological control. Although using 'natural' plant elicitors can not abolish herbivores to become resistant they can be much more environmental friendly since they are active in relative low amounts and do not likely interfere with the well-being of carnivorous insects. The results of the present study showed that treatment of bean plants in the field with SA or MeJA improved plant growth, yield and yield-quality profoundly and decreased natural and artificial spider mite infestations.

Infestation with spider mite decreased significantly plant growth and yield. These decreases may be due to the inhibition of photosynthesis of infested leaves and accelerated transpiration, leading to the drying out and premature fall of the leaves (Flechtman, 1979; Cheong *et al.*, 2002). In damaged plant tissues there is an induction of ethylene biosynthesis that, in turn, has been associated with the induction and acceleration of abscission (Taiz and Zeiger, 1998). For this reasons, it would be important to certify the effect of damages caused by the spider mite on ethylene production of common bean plants in the future studies. Moreover, the infestation with *T. urticae* resulted in increasing the production of reactive oxygen species (ROS) which destroy membrane permeability, and proteins as well as nucleic acid which decreased plant growth and yield. Moreover, the infestation leads to decreasing the content of nitrogen and phosphorous due to its effect of ion uptake which results from destroy membrane permeability (unpublished data). The stylest of mite penetrates the adaxial epidermis, reaching the palisade layer. They fed on the cell contents, destroying the chloroplast in the area restricted to where feeding takes place leading to the appearance of chlorotic areas (Flechtman, 1979).

In the present study the data indicate that application of elicitors decreased significantly spider mite population on the leaves. This reduction may be due to activation of plant induced systematic resistance (Repka, 2001). Generally elicitors may be served as a stimulatory effect on secondary metabolites production and accumulation (Gundlach *et al.*, 1992), i.e. soluble phenolic compounds in (unpublished data). This accumulation may be due to inhibition catalase activity (unpublished data), which in turn induces PAL gene expression and synthesis of phenolic compounds (Vermerris and Nicholson, 2006). Yet total phenols have long been considered as important defense-related compounds whose levels are naturally high in resistant varieties of many crops (Gogoi *et al.*, 2001). Results also proved that application of both elicitors, in particular, SA increased significantly ion contents i.e., N, P, K and Ca in common bean shoot which reflect to increasing plant growth and induced resistance to pests due to its role in plant metabolism, such as promote the development of thicker outer walls and stability of plant membrane in epidermal cells, thus preventing pests attack (Marschner, 1986). In this concern, increasing of nitrogen in plant stimulate synthesis of phenols and lignin content. All these are parts of the defense system of plant against infection (Marschner, 1986).

The application of both elicitors increased the total chlorophyll content of common bean leaves (Figure, 2a). This increment may be due to stimulating pigment formation and enhancing the efficacy of photosynthetic apparatus with a better potential for resistance and decrease in photophosphorylation rate usually occurring after infection (Amaresh and Bhatt, 1998). Elicitors, in particular, SA was found to increase potassium content (Farouk *et al.*, 2008) in plant tissue, which may increase the number of chloroplast per cell, number of cell per leaf and consequently leaf area (Possingham, 1980). The stimulating effect of elicitors on chlorophyll content may be due to stabilizing the active site of enzymes and photosynthetic reactions (Rahman *et al.*, 2002), and increased nitrogen accumulation in plant shoot (unpublished data) which might have served in enhancing chlorophyll synthesis. Finally both elicitors activated the synthesis of carotenoids which protect chlorophyll from oxidation and finally increased chlorophyll content as reported in the present study. In addition, the stimulating effect of elicitors on chlorophyll pigment may be due to enhancing cytokinin, auxin, GAs content. These results are in agreement with those obtained by Türkyılmaz *et al.* (2005) on bean, Gharib (2006) on sweet basil and marjoram, and El-Mergawi and Abdel-Wahed, (2007) on maize plants who found that salicylic acid caused significant increased in photosynthetic pigments content in leaves.

In addition, application of both elicitors decreased $chl_{a:b}$ ratio which play a curricular role in stabilizing photosynthetic processes which reflect to increasing photoassimilate and pod yield. The physiological significance of the decreased the ratio of $Chl_{a:b}$ during leaf infestation with *T. uricae*, it is still unclear due to contradictive results from different studies. Bjo¨rkman, (1981) suggested that the decrease in Chl $_{a:b}$ ratio can improve the capture of far-red radiation and helps to maintain an optimal energy balance between PSI and PSII. Therefore, the decrease in $Chl_{a:b}$ ratio may be required for *T. uricae* infested plants to readjust their photosynthetic balance while under stress.

Foliar application with elicitors in both season showed in most cases a significant increase in common bean growth parameters, in particular, 100 mg/L SA. These increases may be attributed to elicitor's effect on physiological processes in plant such as ion uptake, cell elongation, cell division, enzymatic activation, and protein synthesis. In this concern, salicylic acid enhanced growth of wheat (Shakirova *et al.*, 2003), pea (Farouk, 2005) and cucumber (Farouk *et al.*, 2008) plants. Moreover, El-Bahay (2002) reported that salicylic acid has the potentiality to exert a suppressive or simulative impact on various growth aspects of lupine seedlings through their direct interference with the enzymatic activities responsible for biosynthesis and/or catabolism of growth promoting and inhibiting substances.

The increase in common bean yield may be due to role of elicitors in stimulation of physiological processes which reflected on improving vegetative growth (Table, 1) that followed by active translocation of the photoassimilate from source to sink in common bean plant due to increasing leaf blade thickness as well as dimension of vascular bundles as a results of elicitors application (Table 2, Fig 5). In this concern, salicylic acid might be regulating plant growth by increasing enzyme activity as α-amylase and nitrate reductase, which accelerate the sugar translocation from the leaves to developing fruit (Sharma *et al.*, 1986). Moreover, application of both elicitors lead to increased potassium content (unpublished data), which enhancing photosynthetic pigments and capacity, and subsequently dry matter accumulation (Umar and Bansal, 1995). In addition, application of salicylic acid inhibits ethylene production leading to increasing fruit number and consequently increased fruit yield per plant (Leslie and Romani, 1986). Generally, plants treated with salicylates or methyl jasmonate had higher yield in many plant species, cultivated either in greenhouse or in open conditions (Abdel-Wahed *et al.*, 2006; Iqbal and Ashraf, 2006; Farouk *et al.*, 2008).

Concerning yield quality, it is noted that application of both elicitors at both concentration improved yield quality as compared to infested or control plants, these results are agreement with those obtained by Sarangthem and Singh (2003) who found that the level of N, proteins and nitrate reductase activity were increased in *Phaseolus vulgaris* by foliar application of SA at 0.1%. Similarly, Haroun *et al.* (1998) found that low dose of salicylic acid (2.5 mM) significantly increased total carbohydrate content in seed lupine. Moreover, Farouk (2005) and Farouk *et al.*, (2008) indicated that, under greenhouse and open field conditions, application of both SA or MeJA increased significantly pea and cucumber fruit quality respectively as regard to the content of ascorbic acid, protein, carbohydrates and some ions.

In conclusion, it may be possible to replace conventional chemical pesticides with any of the tested elicitor's specially Salicylic acid at 100 mg/L due to its safety for human and environment and thus provided both economical and ecological efficacy. In addition to the effectiveness using elicitors in controlling TSSM population and increase yield and quality of common bean under field conditions.

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تأثير المستحثات النباتيه على نمو وانتاجيه نبات الفاصوليا فى وجـود الإصـابة بالعنكبوت الاحمر

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يعد العنكبوت الاحمر من أخطر وأهم آفات نبات الفاصوليا والذى يؤثر سلبيًا علـى نمـو وإنتاجية نبات الفاصوليا بالتالى فمقاومتة تحت ظروف الإنتاج التجارى للفاصوليا أمرًا ضـروريا لتحسين النمو وزيادة الإنتاجية. إستخدام أى من المستحثات المستخدمه (حامض السالسيلك والميثيل جاسمونات) قبل أو بعد الإصابة بالعنكبوت الأحمر يقلل من نسبه الاصابه بالعنكبوت الأحمر والذى يكون متبوعاً بتحسين نمو وزيادة إنتاجية نبات الفاصوليا متمثلاً فى زيادة طـول النبـات، وعـدد الأفرع،والوزن الجاف، و المساحة الورقية للنبات بالإضافة إلى زيادة المحصول وتحسين صـفات جودتة. حامض السالسيلك بتركيز ١٠٠ ملليجرام/لتر هو الأكثر فاعلية فى تحسين النمـو وزيـادة المحصول وتحسين صفات جودته بالإضافه إلى إنخفاض نسبة الإصابة بالعنكبوت الأحمر.

أدى إستخدام المستحثات إلى إحداث تغيرات ملحوظة فى التركيب الداخلي للأوراق، والتى تمثلت فى زيادة سمك النصل، زيادة سمك كل من البارنكيما العمادية والأسفنجية بالإضافة لـسمك منطقة العرق الوسطى بجانب زيادة أبعاد الحزم الوعائية. علـى النقـيض مـن ذلـك فالإصـابة بالعنكبوت الأحمر تؤدى إلى نقص معنوى فى معظم الصفات التشريحية المدروسة ممايِنعكس على نقص النمو والمحصول وصفات الجودة للثمار.

نخلص مما سبق الى إمكانيه إستخدام أى من المـستحثات المدروسـه خاصـه حـامض السالسيلك بتركيز ١٠٠ ملليجرام/لتر لتقليل الاصابه بالعنكبوت الأحمر بالتالى تحسين نمو وانتاجيه وجودة محصول الفاصوليا تحت ظروف الحقل.

قام بتحكيم البحث

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