

## Effecacy of Soaking Soybean Seeds Within Three Plant Resistance Inducers under the Stress of *Melioidogyne incognita* Infection

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**Abstarct:** A greenhouse experiment was conducted to evaluate the efficacy of soaking soybean seeds at three rates (5, 10 and 20 mg L<sup>-1</sup>) of plant resistance inducers i.e., salicylic acid (SA), ascorbic acid (AA) and potassium silicate (PS) on soybean plant cv. Giza 82 growth parameters under the stress of *M. incognita* infection. results indicated that all treatments at the tested three rates improved plant growth parameters and reduced nematode criteria as well. Treatments showed a positive correlation between plant growth improvement and increasing plant resistance inducer concentration. Plant resistance inducer rates showed significant (P<0.05) protection performance in soybean plants against *M. incognita* infection in terms of reduction percentage of the number of juveniles in soil, developmental stages, root galling, females and egg masses in the soybean root system. The high level of 20 mg L<sup>-1</sup> ranked first in diminishing final nematode population, galls and egg masses number with values of 92.8, 93.7 and 95.95%; 88.7, 71.8 and 94.3%; 88.1, 84.7 and 97.1% for salicylic acid, ascorbic acid and potassium silicate, respectively. All tested treatments achieved a remarkable increase in N, P, K and total phenol concentration with different degrees exceeding that of nematode alone. The application of salicylic acid at the high level (20 mg L<sup>-1</sup>) accomplished the highest percentage increase value of N (70.2%), P (44.8%), K (52.5%) and total phenol contents (78.5%) compared to nematode alone respectively.

**Key words:** Soaking • Soybean • Seeds • Salicylic acid • Ascorbic acid • Potassium silicate • *M. incognita* • Resistance inducers • Concentration

### INTRODUCTION

Soybean, *Glycine max* L. is the most important food crop and remains an important source of high protein for people. Because soybeans are a legume, soybeans have lessened the need for nitrogen fertilizers. Soybeans contain Rhizobium bacteria in their roots that convert gaseous nitrogen into nitrogenous compounds. Because of the magnitude of yield losses caused by the root streptococcus nematode of soybean crops, it is necessary to minimize crop damage by adopting appropriate management methods available. The use of chemical nematicides does not always ascertain to be effective and economical [1]. In addition, the poor target specificity of the chemicals poses toxicological risks to the environment and humans. Therefore, environmentally friendly alternatives are needed to combat nematodes. Biological control is one of the possible safe alternatives to

pesticides for disease management and is likely to be free of residual tainted effects. There are several antimicrobials for root-knot nematodes and their application leads to a significant reduction in nematode numbers [2]. Plant seed soaking is one such strategy that enhances plant natural defense mechanisms that have recently relied on natural participation in plant defense responses and cause plant resistance to pests and diseases [3]. Salicylic acid (SA) is a natural compound that has been instrumental in certain physiological processes and plant defense responses [4]. SA originates in the leaves and inflorescences of thermophilic plants exposed to pathogen infection [5]. Pre-treatment of rice seeds with salicylic acid inhibited catalase activity, improved hydrogen peroxide levels and improved rice resistance to cadmium stress [6]. SA application indicated a positive increase in tomato root resistance to *Fusarium oxysporum* infection [7]. Salicylic acid is the first plant-derived

phenolic compound that stimulates plant resistance [8]. It is concerned with a variety of physiological processes and is included in a new group of plant growth regulators [5]. Soaking seeds in SA for irrigation or spraying with a SA solution has shown efficacy in many plant species against abiotic stresses [9, 10]. Several researchers have recorded that SA promoted germination under saline stress conditions, as in pea and tomato seeds [11, 12]. Preparation of cucumber seeds with SA increases germination level and enzyme activity under salinity stress [13]. Soaking treatment with on conazole significantly downsized seedling height with a good germination rate and promoted efficient photosynthesis [14]. Soaking the seeds in certain bioagents has been recommended as an economical, easy and environmentally friendly method for root-knot nematode management [15]. Because of the literature on the role of some growth regulators regarding soybean growth and nematode growth, efforts have been made to study the effect of soybean seed soaking. Giza 82 in some growth regulators and potassium silicate in controlling *M. incognita* and enhancing plant growth under greenhouse conditions.

## MATERIALS AND METHODS

**Preparation of *M. incognita* Inocula:** Meloidogyne incognita (j2) was extracted from the roots of infected Coleus plants (*Coleus blumei*) by incubating egg masses in distilled water. Second-stage juveniles (J2) were obtained from a pure culture assembled from a single egg mass of *M. incognita* heretofore identified according to perineal pattern characteristics [16] and cultured on coleus plants in a greenhouse. Nematology Research Unit, Faculty of Agriculture, Mansoura University, where this experiment was conducted.

**Growth Regulators Used in this Work:** Salicylic acid (IAA), ascorbic acid (GA<sub>3</sub>) and Potassium silicate (Ps) were used at three rates 5, 10 and 20 mg L<sup>-1</sup> according to the design of the experiment.

**Soybean Seeds Priming:** Soybean seed variety. Giza 82 was obtained from the Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. The seeds were placed in a dish containing a small amount of washing-up liquid drops in water for 5 min and then rinsed several times with distilled water. The seeds were then disinfected with a 1% commercial bleach solution (15 min), rinsed with distilled water and dried on filter paper.

The seeds were soaked in 5, 10 and 20 mg l of salicylic acid, ascorbic acid and potassium silicate for 24 h while the other seeds were soaked in distilled water and the other seeds were left without soaking to serve as controls. Soybean seeds were immediately sown in experimental pots containing one kilogram of sterile soil.

**Experimental Design:** Sixty-two (15 cm-day) plastic containers filled with 1000 g of sterilized sandy clay soil (1:1) (v:v) were used in this experiment. The experiment was conducted under greenhouse conditions with four replicates for 11 treatments of which 10 treatments received soybean seeds (one plant/pot) that were soaked when tested with three concentrations of salicylic acid, ascorbic acid and potassium silicate each (5, 10 and 20 mg l<sup>-1</sup>). Two treatments containing seeds soaked in distilled water, as well as seeds without soaking, served as control. Two weeks after germination, a nematode inoculum (1000 J2) of *M. incognita* was introduced according to the experimental design. The pots were arranged in a random whole block design on a bench of a partially controlled greenhouse at 27 ± 5°C. The plants were watered regularly and agricultural treatments were as recommended. Plants were harvested 45 days after nematode inoculation.

**Assessment Parameters:** All plants were uprooted and both vegetative and root systems were utilized as fresh and dried tissues for the following efficacy estimate analyses.

**Plant Growth Parameters:** Soy bean plant morphology parameters including; plant length (shoot and root); fresh shoot and root weights; and shoot dry weight were measured and recorded. On the other hand, plant roots were stained with acid fuchsin in lactic acid and counted for females and egg masses [17].

**Determination of Nematode Reproduction:** Nematodes were extracted from 250 g soil using sieving and modified Baermann technique from soybean plant roots [18]. The nematode suspensions were examined in a Hawksely counting slide with a dissecting microscope to quantify the numbers of juveniles. Roots were stained with acid fuchsin in lactic acid [17] and counted for Juveniles and egg masses.

**Biochemical Analyses:** Fresh soybean leaves of each replicate/treatment were taken for the assessment of the amount of chlorophyll according to Goodwine methodology [19]. The dried leaves of soybean plants

were ground and wet digested for determination of nitrogen, phosphorus, potassium contents, according to Kjeldahl methods [20] described by Pregl [21]; Jakson [22] and John [23]. The total phenol contents were extracted and calculated at 520nm via spectrophotometer by chatichole as standard [24].

**Statistical Analyses:** Results were statistically analyzed using ANOVA [25] and means were compared according to Duncan multiple range tests [26].

## RESULTS AND DISCUSSION

**Morphological Characters:** Data in Table (1) and Figure (1) affirmed the impact of soaking soybean seeds in three rates (5, 10 and 20 mg L<sup>-1</sup>) of plant resistance inducers i.e., salicylic acid (SA), ascorbic acid (AA) and potassium silicate (PS) on soybean plant cv. Giza 82 growth parameters under the stress of *M. incognita* infection reached with oxamyl under greenhouse conditions (27±5°C). Results indicated that all treatments at the tested three rates improved plant growth parameters and reduced nematode criteria as well. In the meantime, most treatments showed a positive correlation between plant growth improvement and increasing plant resistance inducer concentration. The application of soaked soybean seeds at three plant resistance inducers i.e., salicylic acid, ascorbic acid and potassium silicate at the rate of 20 mg L<sup>-1</sup> ranked first and achieved the maximum increment values that averaged 100.3, 196.97, 236.73, 146.34 and 75.47%; 75.8, 239.4, 257.14, 174.39 and 69.81%; 68.5, 266.7, 308.16 and 206.4% for plant length, total plant fresh weight, shoot dry weight and number of leaves/plant respectively, as compared to nematode alone. On the other hand, the tested three plant resistance inducers at the moderate rate (10 mg L<sup>-1</sup>) recorded considerable values in this respect with values that amounted to 96.1, 133.3, 165.31, 133.23 and 50.94%; 69.9, 227.3, 226.53, 165.24 and 56.60%; 68.0, 224.2, 246.94, 104.27 and 32.08% for the same parameters, respectively. Moreover, Soybean seeds treated by all plant resistance inducers at the low level (5 mg L<sup>-1</sup>) represented the least position with the minimum values of plant length (79.2, 58.7 and 51.1 %); total plant fresh weight (130.3, 216.7 and 212.1%); and shoot dry weight (165.31, 216.33 and 216.33%) and numbers of leaves (122.56, 138.72 and 66.16%), for salicylic acid, ascorbic acid and potassium silicate, respectively. Moreover, oxamyl (0.3ml/plant) as a nematicide gave a considerable percentage increase in values for plant length (27.8%) total plant fresh weight

(39.4%) and shoot dry weight (44.90%) and the number of leaves (67.68%), respectively. Meanwhile, plants free of nematode and untreated with any tested materials showed reasonable percentage increase values that averaged 29.2, 87.9, 79.59 and 81.40% for plant length, total plant fresh weight, shoot dry weight and the number of leaves respectively, comparing to nematode alone, (Table 1 and Fig. 1).

**Nematode Criteria:** Data in Table (2) and Figure (2) showed the nematicidal activities of soaking soybean seeds cv. Giza 82 at three rates of three plant resistance inducers under *M. incognita* infection under greenhouse conditions. Data revealed that the tested plant resistance inducer rates showed significantly (P<0.05) protection performance in soybean plants against *M. incognita* infection in terms of reduction percentage of the number of juveniles in soil, developmental stages, root galling, females and egg masses on the soybean root system. Among levels of the tested materials, the high level of 20 mg L<sup>-1</sup> ranked first in diminishing final nematode population, galls and egg masses number with values of 92.8, 93.7 and 95.95%; 88.7, 71.8 and 94.3%; 88.1, 84.7 and 97.1% for salicylic acid, ascorbic acid and potassium silicate, respectively (Table 1 & Fig. 1). Moreover, soaking plant seeds with salicylic acid, ascorbic acid and potassium silicate at the moderate rate of 10 mg L<sup>-1</sup> ranked second in diminishing final nematode population, galls and egg masses number with values 88.0, 88.6 and 91.6%; 80.0, 71.5 and 93.5%; 65.3, 77.0 and 78.7%, respectively, comparing with nematode alone (Table 2). Moreover, plant seeds treated with all materials at the low level of 5 mg L<sup>-1</sup> represented the least position in diminishing final nematode population, galls and egg masses number with values of 82.6, 86.5 and 91.6%; 76.1, 65.2 and 82.3%; 48.2, 31.1 and 57.5%, respectively comparing with nematode alone (Table 1 & Fig. 1). Meanwhile, nematode reproduction factor (RF) under the application of at three rates of three plant resistance inducers were adversely affected. Such rates ranged from 1.31 to 0.18 vs. 2.52 for nematode alone. Namely, the treatment containing salicylic acid 20 mg L<sup>-1</sup> had the lowest rate of reproduction (0.18) whilst that of potassium silicate 1ml/pot showed clearly the highest rate (1.31), respectively. It is worthy to note that oxamyl as a systemic nematicide recorded the highest, percentage reduction in the final nematode population (88.2%), number of galls (89.8%) and egg masses (96.4%), respectively with the least value of RF (0.30) comparing with nematode alone.

Table 1: Impact of soaking Soybean seeds cv. Giza 82 at three rates of three plant resistance inducers under *M. incognita* Infection at Greenhouse Conditions (27±5°C)

Treat.	Conc.	Plant growth response											
		Plant Length (cm)				Plant Fresh weight (g)				Shoot dry			
		Shoot	Root	Total	Inc. %	Shoot	Root	Total	Inc. %	Wg. (g)	Inc. %	No. of leaves	Inc. %
Ascorbic acid	5	35.50 <sup>e</sup>	21.00 <sup>abcd</sup>	56.50 <sup>cd</sup>	58.70	14.40 <sup>ab</sup>	6.50 <sup>abc</sup>	20.90 <sup>a</sup>	216.70	3.10 <sup>f</sup>	216.33	78.30 <sup>bc</sup>	138.72
	10	38.80 <sup>de</sup>	21.80 <sup>abc</sup>	60.50 <sup>bc</sup>	69.90	14.00 <sup>ab</sup>	7.60 <sup>a</sup>	21.60 <sup>a</sup>	227.30	3.20 <sup>e</sup>	226.53	87.00 <sup>ab</sup>	165.24
	20	42.60 <sup>c</sup>	20.00 <sup>abcd</sup>	62.60 <sup>bc</sup>	75.80	15.70 <sup>a</sup>	6.70 <sup>ab</sup>	22.40 <sup>a</sup>	239.40	3.50 <sup>b</sup>	257.14	90.00 <sup>ab</sup>	174.39
Potassium silicate	5	35.80 <sup>e</sup>	18.00 <sup>bcd</sup>	53.80 <sup>d</sup>	51.10	15.40 <sup>a</sup>	5.20 <sup>abcd</sup>	20.60 <sup>a</sup>	212.10	3.10 <sup>g</sup>	216.33	54.50 <sup>e</sup>	66.16
	10	40.00 <sup>cd</sup>	19.80 <sup>abcd</sup>	59.80 <sup>bcd</sup>	68.00	14.30 <sup>ab</sup>	7.10 <sup>ab</sup>	21.40 <sup>a</sup>	224.20	3.40 <sup>c</sup>	246.94	67.00 <sup>cde</sup>	104.27
	20	35.50 <sup>e</sup>	24.50 <sup>a</sup>	60.00 <sup>bcd</sup>	68.50	17.80 <sup>a</sup>	6.40 <sup>abc</sup>	24.20 <sup>a</sup>	266.70	4.00 <sup>a</sup>	308.16	100.50 <sup>a</sup>	206.40
Ascorbic acid	5	35.50 <sup>e</sup>	21.00 <sup>abcd</sup>	56.50 <sup>cd</sup>	58.70	14.40 <sup>ab</sup>	6.50 <sup>abc</sup>	20.90 <sup>a</sup>	216.70	3.10 <sup>f</sup>	216.33	78.30 <sup>bc</sup>	138.72
	10	38.80 <sup>de</sup>	21.80 <sup>abc</sup>	60.50 <sup>bc</sup>	69.90	14.00 <sup>ab</sup>	7.60 <sup>a</sup>	21.60 <sup>a</sup>	227.30	3.20 <sup>e</sup>	226.53	87.00 <sup>ab</sup>	165.24
	20	42.60 <sup>c</sup>	20.00 <sup>abcd</sup>	62.60 <sup>bc</sup>	75.80	15.70 <sup>a</sup>	6.70 <sup>ab</sup>	22.40 <sup>a</sup>	239.40	3.50 <sup>b</sup>	257.14	90.00 <sup>ab</sup>	174.39
Oxamyl	0.3	26.80 <sup>f</sup>	18.80 <sup>bcd</sup>	45.50 <sup>e</sup>	27.80	5.50 <sup>d</sup>	3.70 <sup>de</sup>	9.20 <sup>cd</sup>	39.40	1.42 <sup>k</sup>	44.90	55.00 <sup>e</sup>	67.68
N alone		19.30 <sup>g</sup>	16.40 <sup>cd</sup>	35.60 <sup>f</sup>	---	4.00 <sup>d</sup>	2.60 <sup>e</sup>	6.60 <sup>d</sup>	---	0.98 <sup>l</sup>	---	32.80 <sup>f</sup>	---
Plant free of N & any treatment		26.30 <sup>f</sup>	19.80 <sup>abcd</sup>	46.00 <sup>e</sup>	29.20	8.90 <sup>c</sup>	3.40 <sup>de</sup>	12.40 <sup>bc</sup>	87.90	1.76 <sup>j</sup>	79.59	59.50 <sup>de</sup>	81.40

N=1000 (J2) of *M. incognita*

\* Each figure is the mean of four replicates.

\*\* Increase % = (Treatment -N alone)/N alone ×100.

Means in each column followed by the same letter(s) did not differ at P<0.05 according to Duncan's multiple-range test.

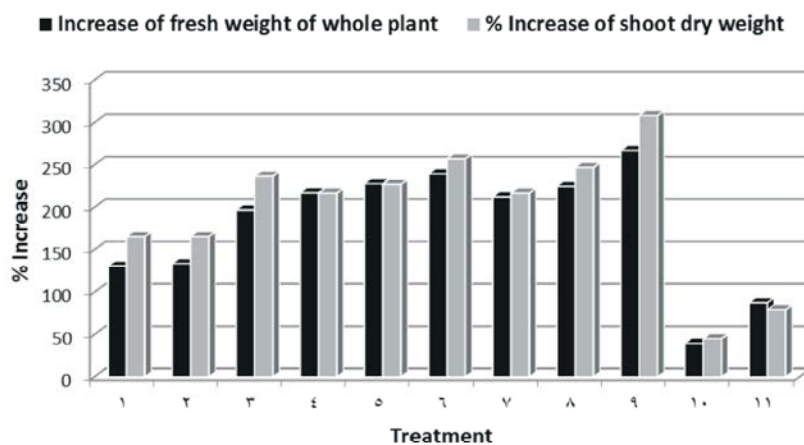


Fig. 1: Efficacy of soaking Soybean seeds cv. Giza 82 at three rates of three plant resistance inducers under *M. incognita* Infection at Greenhouse Conditions (27±5°C)

- Salicylic acid (5 mg L<sup>-1</sup>).
- Salicylic acid (10 mg L<sup>-1</sup>).
- Salicylic acid (20 mg L<sup>-1</sup>).
- Ascorbic acid (5 mg L<sup>-1</sup>).
- Ascorbic acid (10 mg L<sup>-1</sup>).
- Ascorbic acid (20 mg L<sup>-1</sup>).
- Potassium silicate (5 mg L<sup>-1</sup>).
- Potassium silicate (10 mg L<sup>-1</sup>).
- Potassium silicate (20 mg L<sup>-1</sup>).
- Oxamyl (0.3ml).
- Plant free of N & any treatment.

Table 2: Nematode parameters of *M. incognita* infecting soybean as affected by seed soaking at three rates of three plant resistance inducers on controlling *M. incognita* under greenhouse conditions (27±5°C)

*Nematode parameters													
Nematode population in													
Treatment	Level (ml)	Soil/pot J <sub>2</sub>	D.V. stage	Females	Total	**RF	***Red. %	No. of galls	****Red. %	***RGI	No. of egg masses	****Red. %	***EI
Ascorbic acid	5	432.1 <sup>d</sup>	131.9 <sup>a</sup>	38.0 <sup>b</sup>	601.9 <sup>d</sup>	0.60	76.1	38.6 <sup>c</sup>	65.2	4	18.5 <sup>cd</sup>	82.3	3
	10	382.0 <sup>d</sup>	74.5 <sup>a</sup>	46.8 <sup>b</sup>	503.2 <sup>de</sup>	0.50	80.0	31.6 <sup>cd</sup>	71.5	4	6.8 <sup>c</sup>	93.5	2
	20	190.5 <sup>d</sup>	54.5 <sup>a</sup>	39.0 <sup>b</sup>	283.9 <sup>de</sup>	0.28	88.7	31.2 <sup>cd</sup>	71.8	4	6.0 <sup>c</sup>	94.3	2
Potassium silicate	5	937.0 <sup>b</sup>	247.0 <sup>a</sup>	121.4 <sup>a</sup>	1305.4 <sup>b</sup>	1.31	48.2	76.3 <sup>b</sup>	31.1	4	44.5 <sup>b</sup>	57.5	4
	10	622.8 <sup>c</sup>	218.6 <sup>a</sup>	32.2 <sup>b</sup>	873.6 <sup>c</sup>	0.87	65.3	25.5 <sup>cd</sup>	77.0	3	22.3 <sup>c</sup>	78.7	3
	20	280.5 <sup>d</sup>	0.0 <sup>a</sup>	19.0 <sup>b</sup>	299.5 <sup>de</sup>	0.30	88.1	17.0 <sup>cd</sup>	84.7	3	3.0 <sup>c</sup>	97.1	2
Salicylic acid	5	253.3 <sup>d</sup>	170.3 <sup>a</sup>	14.7 <sup>b</sup>	430.5 <sup>de</sup>	0.43	82.9	15.0 <sup>cd</sup>	86.5	3	8.8 <sup>de</sup>	91.6	2
	10	241.3 <sup>d</sup>	43.4 <sup>a</sup>	7.0 <sup>b</sup>	301.8 <sup>de</sup>	0.30	88.0	12.6 <sup>cd</sup>	88.6	3	8.8 <sup>de</sup>	91.6	2
	20	142.1 <sup>d</sup>	25.8 <sup>a</sup>	17.1 <sup>b</sup>	182.6 <sup>e</sup>	0.18	92.8	7.0 <sup>d</sup>	93.7	2	4.3 <sup>c</sup>	95.9	2
Oxamyl	0.3	288.0 <sup>d</sup>	3.5 <sup>a</sup>	5.3 <sup>b</sup>	296.8 <sup>de</sup>	0.30	88.2	11.3 <sup>cd</sup>	89.8	2	3.8 <sup>c</sup>	96.4	2
N alone		2333.0 <sup>a</sup>	65.0 <sup>a</sup>	121.0 <sup>a</sup>	2519.0 <sup>a</sup>	2.52	---	110.8 <sup>a</sup>	---	5	104.8 <sup>a</sup>	---	5

N=1000 (J<sub>2</sub>) of *M. incognita*

Each value is a mean of four replicates.

\* Initial population (Pi) = 1000 egg of *M. incognita*

\*\* Rate of build-up (RF) =  $\frac{\text{Final population (pf)}}{\text{Initial population (pi)}}$  \*\*\*Reduction % = (N alone - Treatment) / N alone × 100

\*\*\* Root gall index (RGI) or Egg mass index (EI): 0 = no galling or egg masses; 1= 1-2 galls or egg masses; 2= 3-10 galls or egg masses; 3= 11-30 galls or egg masses; 4=31-100 galls or egg masses; 5= more than 100 galls or egg masses.

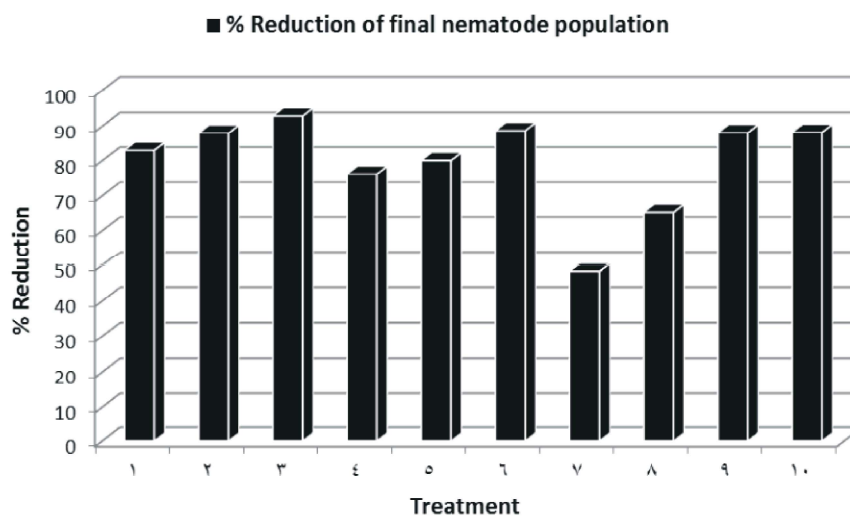


Fig. 2: % Reduction of final nematode population of *M. incognita* infection as affected by seed soaking at three rates of three plant resistance inducers on controlling *M. incognita* under greenhouse conditions (27±5°C)

- Salicylic acid (5 mg L<sup>-1</sup>).
- Salicylic acid (10 mg L<sup>-1</sup>).
- Salicylic acid (20 mg L<sup>-1</sup>).
- Ascorbic acid (5 mg L<sup>-1</sup>).
- Ascorbic acid (10 mg L<sup>-1</sup>).
- Ascorbic acid (20 mg L<sup>-1</sup>).
- Potassium silicate (5 mg L<sup>-1</sup>).
- Potassium silicate (10 mg L<sup>-1</sup>).
- Potassium silicate (20 mg L<sup>-1</sup>).
- Oxamyl (0.3ml).

Table 3: Nitrogen, phosphorus and potassium concentrations, total chlorophyll and phenol contents in leaves of soybean infected with *M. incognita* as affected by seed soaking at three rates of three plant resistance inducers comparing with oxamyl under greenhouse conditions (27±7°C)

* Chemical components of leaves													
Treatment	Level (ml)	N% mg/g	Inc.%	P% ppm	** Inc.%	K% ppm	**Inc.%	Chlorophyll content mg/g F.WT.			Red. %	Total phenol mg/g	** Inc.%
								A mg/g	B mg/g	Total mg/g			
Ascorbic acid	5	36.4	26.0	0.235	21.1	18.49	12.7	0.663 <sup>a</sup>	0.532 <sup>b</sup>	1.195 <sup>c</sup>	37.5	315.4 <sup>a</sup>	35.0
	10	38.5	33.2	0.261	34.5	19.12	16.6	0.844 <sup>d</sup>	0.435 <sup>c</sup>	1.279 <sup>d</sup>	47.2	361.1 <sup>d</sup>	54.5
	20	44.7	54.7	0.256	32.0	20.17	23.0	0.873 <sup>c</sup>	0.517 <sup>b</sup>	1.390 <sup>e</sup>	60.0	364.2 <sup>d</sup>	55.8
Potassium silicate	5	38.9	34.6	0.214	10.3	18.17	10.8	0.516 <sup>i</sup>	0.385 <sup>d</sup>	0.901 <sup>f</sup>	3.7	281.3 <sup>b</sup>	20.4
	10	47.4	64.0	0.249	28.4	19.53	19.1	0.792 <sup>e</sup>	0.315 <sup>e</sup>	1.107 <sup>f</sup>	27.4	335.3 <sup>f</sup>	43.5
	20	48.3	67.1	0.269	38.7	22.03	34.3	0.951 <sup>b</sup>	0.587 <sup>a</sup>	1.538 <sup>b</sup>	77.0	364.0 <sup>d</sup>	55.8
Salicylic acid	5	37.6	30.1	0.214	10.3	18.77	14.5	0.625 <sup>h</sup>	0.394 <sup>cd</sup>	1.020 <sup>g</sup>	17.4	331.9 <sup>f</sup>	42.0
	10	45.3	56.7	0.228	17.5	19.47	18.7	0.745 <sup>f</sup>	0.370 <sup>d</sup>	1.115 <sup>f</sup>	28.3	348.8 <sup>e</sup>	49.3
	20	49.2	70.2	0.281	44.8	25.01	52.5	1.05 <sup>a</sup>	0.553 <sup>b</sup>	1.607 <sup>a</sup>	84.9	417.1 <sup>a</sup>	78.5
Oxamyl	0.3	41.9	45.0	0.226	16.5	18.62	13.5	0.946 <sup>b</sup>	0.433 <sup>c</sup>	1.379 <sup>e</sup>	58.7	417.1 <sup>a</sup>	78.5
N alone		28.9	---	0.194	---	16.4	---	0.513 <sup>i</sup>	0.355 <sup>d</sup>	0.869 <sup>i</sup>	---	233.7 <sup>i</sup>	---
Plant free of N & any treatment		47.3	63.7	0.251	29.4	18.46	12.6	0.535 <sup>i</sup>	0.435 <sup>c</sup>	0.969 <sup>b</sup>	11.5	391.5 <sup>c</sup>	67.5

N=1000 (J2) of *M. incognita*

\*Each value is a mean of three replicates.

Means in each column followed by the same letter(s) did not differ at p<0.05 according to Duncan's multiple-range test.

N= Nitrogen, P= Phosphorus, K= Potassium,

\*\* Increase % =  $\frac{\text{Treatment} - \text{N alone}}{\text{N alone}} \times 100$

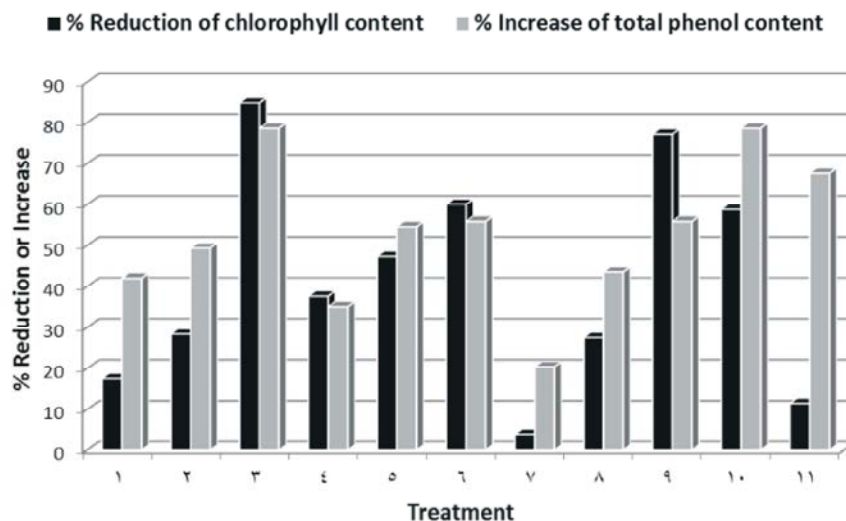


Fig. 3: Nitrogen, phosphorus and potassium concentrations, total chlorophyll and phenol contents in leaves of soybean infected with *M. incognita* as affected by seed soaking at three rates of three plant resistance inducers in comparison with oxamyl under Greenhouse Conditions (27±7°C)

- Salicylic acid (5 mg L<sup>-1</sup>).
- Salicylic acid (10 mg L<sup>-1</sup>).
- Salicylic acid (20 mg L<sup>-1</sup>).
- Ascorbic acid (5 mg L<sup>-1</sup>).
- Ascorbic acid (10 mg L<sup>-1</sup>).
- Ascorbic acid (20 mg L<sup>-1</sup>).
- Potassium silicate (5 mg L<sup>-1</sup>).
- Potassium silicate (10 mg L<sup>-1</sup>).
- Potassium silicate (20 mg L<sup>-1</sup>).
- Oxamyl (0.3ml).
- Plant free of N & any treatment.

**Biochemical Analysis:** Data in Table (3) and Figure (3) illustrate the influence of soaking soybean seeds in ascorbic acid, potassium silicate and salicylic acid at three levels (5, 10 and 20 mg L<sup>-1</sup>) on nitrogen, phosphorus and potassium concentrations, total chlorophyll and phenol contents under the infection with *M. incognita* in comparison with oxamyl under greenhouse conditions (27±5°C). It is interesting to note that all tested treatments achieved a remarkable increase in N, P, K and total phenol concentration with different degrees exceeding that of nematode alone (Table 3). The application of salicylic acid at the high level (20 mg L<sup>-1</sup>) accomplished the highest percentage increase value of N (70.2%), P (44.8%), K (52.5%) and total phenol contents (78.5%) compared to nematode alone respectively. On the other hand, total chlorophyll content (a + b) reduction percentage values for the previous application were also superior to other treatments with values of 84.9%. In the meantime, The application of low level (5 mg L<sup>-1</sup>) of the tested three plant resistance inducers achieved the lowest increase percentage values of N (26.0, 34.6 and 30.1%%), P (21.1, 10.3 and 10.3%%), K ( 12.7, 14.5 and 10.8%) and total phenol contents (35.0, 42.0 and 20.4%%) as well as, the lowly reduction percentage values of total chlorophyll content (a+b) with values of 37.5, 17.4 and 3.7% respectively, as compared to nematode alone. It is worthy to note that oxamyl as a systemic nematicide showed a remarkable percentage increase value of total chlorophyll, total phenol contents, N, P and K concentrations that amounted to 67.5%, 45.0%, 16.5%, 13.5% and 39.15% comparing to nematode alone. Meanwhile, plants free of nematode and any treatment recorded a reasonable percentage increase in values of the tested items compared to nematode alone (Table 3).

Present value of application moderate concentration (10 mg L<sup>-1</sup>) of the tested plant resistance inducers indicated the highest values of the tested plant growth criteria, while higher (20 mg L<sup>-1</sup>) indicated the highest nematode reproduction rate for salicylic, ascorbic acids and potassium silicate, respectively. Moreover, soaking plant seeds in the same compounds significantly increased the contents of nitrogen, phosphorus, potassium, phenol and chlorophyll in soybean leaves with variable values. The defensive effect of these substances may be due to their chemical composition. These chemicals are either absorbed by the seeds or formed by chain reactions catalyzed by the elicitor or activator contained in such substances. Moreover, usage is a moderate concentration. (10 mg L<sup>-1</sup>) of tested resistance inducers showed the highest values for improvement of the tested parameters of plant growth,

while a higher concentration (20 mg L<sup>-1</sup>) registered less plant improvement compared to the previous concentration. These results were in agreement with those of Shakirova *et al.* [27], who displayed that salicylic acid showed activation in peroxidase that has an important role in the biosynthesis of the embryo and suberin that are involved in cell stress, thus protecting the plant from infection by pests and pathogens. Seeds grown with salicylic acid supplemented medium showed rich levels of isocitrate lyase and malate synthase [28, 29]. Concentration factors based on seed quality response and 125 ppm concentration of silica nanoparticles led to higher seed quality criteria such as rice seed germination and plant morphology criteria [30]. The good effect of potassium silicate on the tested soybean parameters may be due to the increased cell division within the apical meristem of seedlings [31]. Environmental stress led to the increased formation of reactive oxygen species that oxidize nucleic acids, membrane lipids, dyes and photoproteins [32]. Silicon treatments under drought stress increased lipid peroxidation in lentil seedlings which reduced the accumulation of O<sub>2</sub>O<sub>2</sub> or H<sub>2</sub>O<sub>2</sub> and lipid peroxidation. The use of silicon significantly increased the activity of antioxidant enzymes that may play a role in maintaining low levels of H<sub>2</sub>O<sub>2</sub> in cells under environmental stress [32]. Thus, the results suggest that the enhanced activities of the antioxidant enzymes resulting from the addition of silicon may protect plant tissues from membrane oxidative damage which can contribute significantly to improved stress tolerance. Moreover, planting seeds in the field is one of the most important cropping stages, due to sensitivity to harmful abiotic and biotic factors. Thus, studies looking at this point, aimed at evaluating products that can be used to enhance crop yield as well as protect the plant from pest and pathogen infection through an environmentally friendly and inexpensive strategy, should be encouraged. However, at the moment, it is not known how the plant “remembers” the applied seed activator that leads to an increase in the defensive reaction induced when attacked by pathogens.

## REFERENCES

1. Pakeerathan, K., G. Mountain and N. Thrashing, 2009. Eco-friendly management of root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood using different green leaf manures on tomato under field conditions. *American Eurasian J. Agric. Environ. Sci.*, 6: 494-497.

2. Khan, M.R., 2007. Prospects of microbial control of root-knot nematodes infecting vegetable crops, In: Biotechnology, Plant Health Management (N. Sharma, H.B. Singh, ed.). International Book Distributing Co., Lucknow, India, pp: 643-665.
3. Worrall, D., G.H. Holroyd, J.P. Moore Glowacz, M. Croft, P. Taylor, J.E. Paul and M.R. Roberts, 2012. Treating seeds with activators of plant defense generates long-lasting priming of resistance to pests and pathogens. *New Phytol.*, 193(3): 770-778.
4. Shi, Q. and Z. Zhu, 2008. Effects of exogenous salicylic acid on manganese toxicity, element contents and antioxidant system in cucumber. *Environ Exper Bot.*, 63(1-3): 317-326.
5. Castro, P.R.C. and E.L. Vieira, 2001. Aplicac,~oes de reguladores vegetais na agricultura tropical. Gua\_1ba: Editora Agropecu\_aria, pp: 132.
6. Guo, B., Y. Liang and Y. Zhu, 2009. Does salicylic acid regulate antioxidant defense system, cell death, cadmium uptake and partitioning to acquire cadmium tolerance in rice. *J Plant Physiol.*, 166(1): 20-31.
7. Mandal, S., N. Mallick and A. Mitra, 2009. Salicylic acid-induced resistance to *Fusarium oxysporum* F. sp. *Lycopersici* in tomato. *Plant Physiol Biochem.*, 47(7): 642-649.
8. Araujo, J.S.P., K.S., Gonc,alves, B.C. Oliveira, R.L.D. Ribeiro and J.C. Polidoro, 2005. Efeito do acibenzolar-S-methyl sobre murcha-bacteriana do tomateiro. *Hortic Bras.Brasileira*, 23(1): 5-8.
9. El-Tayeb, M.A., 2005. Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul.*, 45(3): 215-224.
10. Szepesi, A., J. Csiszar, K. Gemes, E. Horvath, F. Horvath, M.L. Simon and I. Tari, 2009. Salicylic acid improves acclimation to salt stress by stimulating abscisic aldehyde oxidase activity and abscisic acid accumulation and increases Na<sup>+</sup> content in leaves without toxicity symptoms in *Solanum lycopersicum* L. *J Plant Physiol.*, 166(9): 914-925.
11. McCue, P., Z. Zheng, J.L. Pinkham and K. Shetty, 2000. A model for enhanced pea seedling vigor following low pH and salicylic acid treatments. *Proc. Biochem.*, 35(6): 603-613.
12. Szepesi, A., J. Csiszar, S. Bajkan, K. Gemes, F. Horvath, L. Erdei, A.K. Deer, M.L. Simon and I. Tari, 2005. Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt- and osmotic stress. *Acta Biol Szegediensis.*, 49: 123-125.
13. Zhang, Z. and Q. Shang, 2010. Promoting effect of salicylic acid and chitosan on germination of cucumber seeds under NaCl stress. *China Vegetables*, 1(8): 26-29.
14. Ahmad, I., M. Kamran, S. Ali, B. Bilegjargal, T. Cai, S. Ahmad, X.P. Meng, S.U. WN, T. Liu and Q.F. Han, 2018. Uniconazole application strategies to improve lignin biosynthesis, lodging resistance and production of maize in semiarid regions. *Field Crops Res.*, 222: 66-77.
15. El-Nagdi, W.M.A. and M. Youssef, 2004. Soaking faba bean seed in some bio-agents as prophylactic treatment for controlling *Meloidogyne incognita* root-knot nematode infection. *J Pest Sci.*, 77(2): 75-78.
16. Taylor, A.L. and J.N. Sasser, 1978. Biology, identification and control of root-knot nematode (*Meloidogyne* spp.) Raleigh: North Carolina state Univ. Graphics.
17. Byrd, D.W., T. Kirkpatrick and K. Barker, 1983. An improved technique for clearing and staining plant tissues for detection nematodes. *J. Nematol.*, 15(3): 142-143.
18. Goodey, J.B., 1957. Laboratory methods for work with plant and soil nematodes. Tech. Bull. No. 2 Min. Agric. Fish Ed. London, pp: 47.
19. Goodwine, T.W., 1965. Distribution of carotenoids In: Goodwin, T. W., editor. Chemistry and biochemistry of plant pigments. Cambridge, MA: Academic Press, pp: 127-142.
20. Anonymous, 1980. Official methods of analysis. 12<sup>th</sup> ed. Washington. DC: Published by the Association of Official Analytical Chemists, Benjamin, France Line Station.
21. Pregl, E., 1945. Quantative organic micro-analysis 4<sup>th</sup> ed. J. Chundril. London.
22. Jakson, M.L., 1967. Soil chemical analysis. Prentice. Hall of India, New Delhi, pp: 498.
23. John, M.K., 1970. Colorimetric determination of phosphorus in soil and plant material with ascorbic acid. *Soil Sci.*, 109(4): 214-220.
24. Simons, T.S. and A.F. Ross, 1971. Changes in Phenol Metabolism Associated with Induced Systemic Resistance. *Phytopathology*, 61(10): 1261-1265.
25. Gomez, K.A. and A.A. Gomez, 1984. Statistical procedures for Agricultural Research. 2<sup>nd</sup> Ed., John Wiley & Sons: Inc., New York.
26. Duncan, D.B., 1955. Multiple range and multiple, F-test. *Biometrics*, 11: 1-42.



27. Shakirova, F.M., A.R. Sakhabutdinova, M.V. Bezrukova, R.A. Fatkhutdinova and D.R. Fatkhutdinova, 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.*, 164(3): 317-322.
28. Eastmond, P.J. and I.A. Graham, 2001. Re-examining the role of the glyoxylate cycle in oilseeds. *Trends Plant Sci.*, 6(2): 72-78.
29. Rajjou, L., K. Gallardo, C. Job and D. Job, 2006. Proteome analysis for the study of developmental processes in plants. In: C Finnie, ed. *Plant proteomics*. Oxford: Blackwell Publishing, pp: 151-184.
30. Patil, N.B., H. Sharanagouda, S.R. Doddagoudar, C.T. Ramachandra and K.T. Ramappa, 2018. Effect of Rice Husk Silica Nanoparticles on Rice (*Oryza sativa* L.) Seed Quality. *Int. J. Curr. Microbiol. App. Sci.*, 7(12): 3232-3244.
31. Janmohammadi, M. and N. Sabaghnia, 2015. Effect of pre-sowing seed treatments with silicon nanoparticles on germinability of sunflower (*Helianthus annuus*). *Botanica Lithuanica*, 21(1): 13-21.
32. Sajitha, B., F. Sigfredo and G. Dorin, 2017. Silicon improves seed germination and alleviates drought stress in lentil crops by regulating osmolytes, hydrolytic enzymes and antioxidant defense system. *Plant Physiol. Biochem.*, 119: 250-264.