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EFFECT OF SEED SOAKING IN AQUEOUS SOLUTIONS OF ASCORBIC ACID, INDOLE ACETIC ACID, LYSINE AND THIAMINE ON *Meloidogyne javanica* INFFCTED SOYBEAN

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ABSTRACT

Response of *M. javanica* infected soybean plants to seed soaking in aqueous solutions of ascorbic acid, indole acetic acid, lysine and thiamine, at 500 and 1000 ppm for five hours before sowing was studied in a pot experiment under greenhouse conditions at 27±5°C. Results indicated that the tested chemicals obviously suppressed galling and reproduction of *M. javanica*, since number of galls and egg-masses on roots and infective juveniles in soil as well as rate of nematode reproduction were significantly decreased in soaked seed treatments. Seed soaked in 1000 ppm gave relatively higher effect compared to 500 ppm. With respect to the effect on certain plant growth parameters, results clearly indicated that all treatments alleviated the inhibitory effect of *M. javanica* and inhanced growth of soybean plants.

Regarding to chemical analysis , it was found that as the concentration of the tested compounds increased from 500 to 1000 ppm, free phenols were increased, except in thiamine treatment, while amount of total phenols did not show certain trend. On the other hand, increasing concentration obviously increased reducing sugars, except in indole acetic acid treatment which showed slight increase in reducing sugars. However, opposite trend was noticed with total amino acids, since increasing concentration decreased total amino acids in all treatments.

Keywords: *Meloidogyne javanica* , soybean , seed soaking , ascorbic acid , indole acetic acid , lysine, thiamine.

INTRODUCTION

The soybean, *Glycine max* L. is the most world's foremost provider of protein and oil. It's cultivation is very important for needs of fast growing population in Egypt. Several nematode genera and species have been reported to attack soybean (Schmitt & Barker, 1981; Robbins, 1982, Schmitt&Noel, 1984 and Salem *et al.*,1994). Root-knot nematodes *Meloidogyne* spp. are ranked among the principal pathogens reducing soybean production (Sasser, 1980). In the southern USA, soybean growers annually lose 25 to 30 million dollars of the soybean crop as a result of root-knot nematode damage (Sciumbato, 1993).

The use of chemical nematicides for controlling root-knot nematodes is an expensive tool and have several problems due to their toxic effect on some beneficial microorganisms, environmental pollution and disturbance of natural balance. On the other hand, crop rotation and developing nematode-resistant cultivars are among the most widely used practices to control root-knot nematodes. These practices are faced with some constrains that oppose their complete application. Crop rotations may not succeed if the chosen non-host crops are exposed to populations to which they are susceptible. Similarly, repeated use of resistant cultivars may select virulent biotypes that break resistance (Rodriguez-Kabana *et al.*, 1992 and Whitehead, 1999).Therefore, scientists turned their view to search for another alternative

methods to control plant parasitic nematodes. Generally, the number of management tools used against nematodes has increased drastically in the last years. Among these tools, is the application of growth regulators and resistance inducers (Osman &Viglierchio, 1981; Farahat, 1989; Nandi *et al.*, 2000 ; Abdel-Momen *et al.*, 2005 and Saeed *et al.*, 2005).

Some plant growth regulating substances showed tremendous effects on nematode populations, either direct and indirectly which make the plant more resistant to nematodes as well as improve plant growth (Sawheny & Webster, 1975; Osman *et al.*, 1984 ; Abdel-Momen *et al.*, 2005 and Saeed *et al.*, 2005). On the other hand, some amino acids have been found to reduce nematode populations and consequently damage on different hosts (Arrigoni *et al.*, 1979; Osman & Viglierchio, 1981; Al-Sayed & Thomason, 1988 and Al-Sayed, 1990).

The objective of the present study is to perceive possibility of inducing resistance in soybean against the root-knot nematode, *Meloidogyne javanica* through seed soaking in aqueous solutions of ascorbic acid, indole acetic acid, lysine and thiamine. Moreover, growth of soybean and some chemical components in healthy and infected plants were also determined.

MATERIALS AND METHODS

1.Effect of seed soaking in aqueous solutions of certain chemicals on *Meloidogyne javanica* infecting soybean under greenhouse conditions:

Seeds of soybean cv Giza 21 were soaked for five hours in 500 or 1000 ppm aqueous solutions of ascorbic acid, indole acetic acid, lysine and thiamine. Also seeds of the control treatment were soaked in distilled water for the same period. Seeds were planted in clay pots (15-cm diam.) filled with steam sterilized sandy loam soil at the rate of five seeds per pot. After germination plants were thinned to one seedling per pot and inoculated with 1000 infective juveniles (J2) of *M. javanica*. The inoculum was obtained from available pure culture formerly prepared and propagated in the greenhouse. Second stage juveniles(J2) were obtained by incubating egg-masses in Petri dishes containing distilled water. Newly hatched juveniles were collected using micropipette.

Each treatment was replicated three times. All pots were arranged in a randomized complete block design on a bench in the greenhouse at 27± 5°C and watered as needed. Ninety days after inoculation, plants were carefully uprooted and roots of each pot were gently rinsed with tap water to remove adhering soil particles. Length as well as fresh weight of shoot and root systems were recorded. Numbers of galls and egg-masses per root system and infective juveniles per 250 gm soil were counted. Nematodes were extracted from soil by sieving and modified Baermann technique (Goodey, 1957). Rate of nematode reproduction was calculated by dividing the final count of nematode by the initial number used in soil infestation. Data were statistically analyzed using ANOVA and means were compared by L.S.D. at 5%.

2. Chemical determination of sugars, phenolic compounds and amino acids:

A representative samples (one gram each) were collected from fresh roots of all treatments at the end of the experiment. These roots were cut into small pieces and immediately plunged into 95 % boiling elthanol for 10 minutes to kill the tissues. The extraction was then resumed in a soxhelt apparatus using 75 % ethyl alcohol for about 8-10 hours until the perolate was colorless. Alcoholic extracts were filtered and evaporated to near dryness on mild water bath at 60°C. The dried residue was re-dissolved in 50 ml of 50 % isopropanol. These extracts were then used for determination of sugars, phenols and amino acids.

2.1. Total and reducing sugars:

Total and reducing sugars were measured spectrophotometerically by picric acid method as described by Thomas & Dutcher (1924). Sugar contents were determined as mg glucose/gm fresh weight of roots. Values were obtained from a standered curve prepared for glucose. The color density was detected by using spectrophotometer in the presence of a blank as a control treatment at wave length of 540 nm.

2.2. Phenolic compounds:

Phenolic compounds were determined using the colorimetric method of Folin-Denis reagent described by Snell & Snell (1953). The color intensity was recorded using spectrophotometer in the presence of a blank (containing all reagents without the extract) at the wave length of 560 nm. The concentrations of free and total phenols were calculated as mg caticol/gm fresh root weight. Values were obtained from standard curve constructed for caticol in an identical way.

2.3. Amino acids:

The total free amino acids were calculated as mg leucine/gm fresh weight by modified colorimetric ninhydrin method used by Rason (1959). The color density was immediately recorded using spectrophotometer in the presence of a blank at wave length of 570 nm. Values were obtained from standard curve prepared for leucine.

RESULTS

1. Effect on galling and reproduction of *M. javanica:*

Soaking seeds of soybean in aqueous solutions of ascorbic acid, indole acetic acid, lysine and thiamine at 500 or 1000 ppm for five hours before sowing significantly (P=0.05%) suppressed development and reproduction of *M. javanica* as compared to the check treatment which soaked in distilled water only. Since, numbers of galls and egg-masses per root system in these treatments at 500 ppm were 25.7(14.3), 120(103.3), 60(53.7) and 127.7 (114.0) respectively (Table 1).These values were noticebly decreased at 1000 ppm to reach 22.7(10.2); 96.7 (81.7); 44.3 (32.3) and 70.0 (62.0), respectively, while the parallel values for the control treatment were 240 and 206.1, respectively (Table 1). On the other hand, numbers of infective juveniles in 250 gm soil for the abovementioned treatments at 500 and 1000 ppm were 480 (400), 820 (500) ,400(200) and

840(400) respectively, compared to 1280 in the control treatment. Rate of nematode reproduction was obviously decreased in treated plants. Seed soaking in 1000 ppm gave relatively higher effect compared to seed soaking in 500 ppm. Since rate of *M. javanica* reproduction in treatments of ascorbic acid, indole acetic acid, lysine and thiamine at 500 and 1000 ppm were 4.54 (0.2), 3.38 (1.86), 3.8 (2.5) and 4.42 (2.5), respectively, while rate of nematode reproduction in treatment of distilled water was 107.34(Table 1).

Table (1): **Effect of seed soaking in aqueous solutions of ascorbic acid, indole acetic acid, lysine and thiamine on galling and reproduction of *M. javanica* infecting soybean.**

Treatments (ppm)	Number of second stage juveniles in 250 gm/soil	Number of galls per roo system	Number of egg-masses per root system	Rate of nematode reproduction (PF/Pi)
Ascorbic acid				
500 ppm	480	25.7	14.3	4.54
1000 ppm	400	22.7	10.2	0.20
Indole acetic acid				
500	820	120.0	103.3	3.38
1000	500	96.7	81.7	1.86
Lysine				
500	400	60.0	53.7	3.80
1000	200	44.3	32.3	2.50
Thiamine				
500	840	127.7	114.0	4.42
1000	400	70.0	62.0	2.50
Control	1280	240	206.1	107.34
L.S.$D_{0.05}$	13.22	59.22	7.25	6.29

2. Effect on plant growth parameters:

Irrespective to the effect on certain plant growth parameters data in Table (2) clearly indicated that all treatments were significantly superior as compared to the control treatments. At 500 ppm the highest shoot length was obtained with lysine treated seeds (38 cm) followed by ascorbic acid (36 cm) then thiamine (33.3 cm) and indole acetic acid (31.3 cm). Whereas, at 1000 ppm the parallel values were 45.7, 37.7, 37.3 and 37.0 cm, respectively. On the other hand, in non-treated seeds shoot length of infected plants was 19.0 cm, while it was 21.3 cm in the non-treated free-nematode plants.

As for shoot fresh weight for plants treated with 500 ppm, it was found that thiamine gave the highest fresh weight (10.3 gm), followed by ascorbic acid (10.0 gm), then indole acetic acid (9.3 gm) and lysine (7.7 gm) respectively (Table 2). At 1000 ppm, lysine had the highest effect on fresh weight with 28gm, followed by indole acetic acid (13.3 gm), then ascorbic acid (13.0 gm) and thiamine (10.7 gm). On the other hand, shoot weight of infected non-treated plants and healthy onces were 3.6 and 6.8 gm, respectively (Table 2).

Table (2): Effect of seed soaking in aqueous solutions of ascorbic acid, indole acetic acid, lysine and thiamine on certain plant growth parameters of soybean infected with *M. javanica*.

Treatments (ppm)	Shoots		Roots	
	Length (cm)	Fresh weight (gm)	Length (cm)	Fresh weight (gm)
Infected				
Ascorbic acid				
500 ppm	36.0	10.0	35.3	9.3
1000 ppm	37.7	13.0	38.32	13. 4
Indole acetic acid				
500	31.3	9.3	30.3	9.3
1000	37.0	13.3	33.7	18.0
Lysine				
500	38.0	7.7	37.7	21.3
1000	45.7	28.0	41.7	29.0
Thiamine				
500	33.3	10.3	30.3	17.7
1000	37.3	10.7	31.3	19.7
Control	19.0	3.6	18.7	4.0
Untreated	21.3	6.8	24.3	5.0
$L.S.D_{0.05}$	5.73	3.93	7.06	2.26

Concerning length and weight of roots, it was clear that soaking seeds of soybean in aqueous solution of the tested materials significantly alleviated the inhibitory effect of *M. javanica* infection and inhanced root growth more than non-infected and infected plants. As concentration increased from 500 to 1000 ppm insignificant effect on root length of treated plants which were measured with range of 30.3 to 41.7 cm was resulted, while root length of non-treated infected plants was 18.7 cm as compared to 24.3 cm for not-treated free nematode plants (Table 2). On the other hand, the highest root fresh weight at 500 ppm was detected with lysine (21.3 gm) followed by thiamine (17.7 gm) and similar results for both indole acetic acid and ascorbic acid which gave the lowest effect (9.3 gm). At 1000 ppm lysine gave the highest effect (29 gm) followed by thiamine (19.7 gm),then indole acetic acid (18.0 gm) and the lowest one was ascorbic acid (13.4 gm) as compared to 4 and 5 gm for control and untreated free nematode plants, respectively (Table 2).

3. Effect on phenolic, sugar and total amino acid contents:

Influence of the tested compounds on phenolic, sugar and total amino acid contents was measured. Data in Table (3) clearly showed that, soaking seeds in aqueous solution of the tested chemicals remarkably increased free phenols as compared to infected and healthy untreated plants. As the concentration increased from 500 to 1000 ppm, free phenols were also increased, except in thiamine treatment. Slight increase was noticed with ascorbic acid (2.752 and 2.781 mg/gm root at 500 and 1000 ppm, respectively), while pronounced increase was detected with lysine (0.237 and 4.511 at 500 and 1000 ppm, respectively. On the other hand, increasing concentration of ascorbic acid and indole acetic acid from 500 to 1000 ppm slightly decreased total phenols, fro.. 5.444 and 5.370 to 4.802 and 5.123 mg/gm root, respectively (Table 3). While opposite trend with marked

increase was noticed with lysine and thiamine at 500 (3.279 and 2.543 *mg/gm) and 1000 ppm (7.994 and 5.419 mg/gm roots) respectively.*

Table (3): Effect of seed soaking in aqueous solutions of ascorbic acid, indole acetic acid, lysine and thiamine on some chemical components of soybean roots infected with *M. javanica*.

Treatment	Free phenols	Total phenols	Reducing sugars	Total sugars	Total amino acids
Infected					
Ascorbic acid					
500 ppm	2.752	5.444	0.904	123.494	0.057
1000 ppm	2.781	4.802	4.518	170.783	0.037
Indole acetic acid					
500	1.102	5.370	3.313	75.904	0.057
1000	1.363	5.123	3.916	91. 566	0.029
Lysine					
500	0.237	3.279	0	6.931	0.094
1000	4.511	7.994	1.119	58.955	0.009
Thiamine					
500	0.413	2.543	0.602	86.145	0.055
1000	0.272	5.419	5.120	102.108	0.017
Control	0.143	4.354	3.213	98.193	0.049
Untreated	0.248	3.860	4.418	50.803	0.001

*** Each value is the concentration (mg / gm) root fresh weight.**

Increasing concentration of the tested chemicals markedly increased total and reducing sugars. The only exception was found with indole acetic acid which showed slight increase in reducing sugars with values 3.313 and 3.916 mg/gm root at 500 and 1000 ppm, respectively. The highest amounts of total and reducing sugars (170.783 and 5.120 mg/gm root) were measured with ascorbic acid and thiamine at 1000 ppm, respectively. While the lowest values (6.931 and 0 mg/gm root) were detected in plants treated with lysine at 500 ppm, respectively. Amounts of total and reducing sugars in healthy untreated roots were 50.803 and 4.418 mg/gm root while the parallel values in infected untreated plants were 98.193 and 3.213 mg/gm root, respectively.

Regarding total amino acids, it was found that increasing concentration of the tested chemicals, decreased total amino acid contents in all treatments. The highest amount (0.094 mg/gm root) was measured with lysine at 500 ppm while the lowest once (0.009 mg/gm root) was obtained with the same compound at 1000 ppm. On the other hand, control plants free from nematodes and not treated with chemicals gave 0.001 mg/gm roots. This value was increased to 0.049 mg/gm root in infected plants not treated with chemicals.

DISCUSSION

The obtained results are in harmony with many investigators who indicated that the tested chemicals reduced *Meloidogyne* infection and increased growth of treated plants. For instances, some amino acids have been found to inhibit egg hatch, juvenile survival and root galling of *M. incognita* (Reddy *et al.*, 1975). Increasing the amount of ascorbic acid in the

roots of susceptible tomato induced resistance to nematodes. The role played by ascorbic acid in biological defense mechanisms was postulated to be control of the cyanide-resistance respiration in plant tissues (Arrigoni *et al.* 1979). Spraying tomato foliage with ascorbic acid significantly decreased *M. incognita* infection and increased tomato fruit yield, while thiamine had little effect in this respect (Al-Sayed & Thomason, 1988 and Al-Sayed, 1990). Soaking seeds of sunflower for five hours in certain chemicals including ascorbic acid, indole acetic acid, lysine and thiamine at 1000 or 2000 ppm suppressed reproduction of *M. javanica* and increased plant growth parameters (Abdel-Momen *et al.*, 2005).Conversely, obtained results disagree with those reported by Al-Sayed & Montasser (1986) who showed that ascorbic acid gave negative effect on growth of tomato. Moreover, Badra *et al.* (1980) showed that the application of the plant growth regulator, gibrillic acid on guava plants infected with *M. incognita* increased the number of second stage juveniles as compared with the untreated plants.

Roots infected with plant parasitic nematodes contained higher levels of phenols as compared to healthy ones (Bajaj &Mahagan,1977). Positive correlations between the concentrations of phenolic compounds in plants and resistance to *Meloidogyne* spp. have been reported (Masood & Husain, 1978 and Narayana & Reddy, 1980). The increase of free phenols by increasing concentrations of certain applied chemicals could be attributed to the increase of plant defense against *Meloidogyne* spp. (Mahgoob & Zaghlool, 2002 and Abdel-Momen *et al.*,2005).Accumulation of amino acids in tissues invaded by nematodes is the result of amino acids secreted by nematodes or accumulation of amino acids released from host plant proteins through proteolytic nematode enzymes (Myers, 1963). The decrease in the total amino acid by increasing concentration of the tested chemicals could be attributed to suppression of nematode infection and consequently lower replenish of proteins from the plant cells that adjacent to the infected ones (Nandi *et al.*, 2003).

The present results are in harmony with those reported by Abdel-Momen *et al.* (2005) who showed that increasing concentration of the used chemicals including ascorbic acid, lysine, thiamine and indole acetic acid increased total and reducing sugars. This increase could be attributed to enhancing the metabolism and accumulation of metabolities that contain sugars. Another possibility is that, the increase of concentration resulted higher nematode suppression and consequently less consumption on nutrients including sugars.

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تأثير نقع البذور في المحاليل المائية لحمض الأسكوربيك و حمض الخليك الإندولى و الليسين و الثيامين على فول الصويا المصاب بنيماتودا تعقد الجذور (ميلودوجينى جافنيكا)

مرفت حسن إبراهيم
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يعتبر فول الصويا من أهم المحاصيل الزيتية التي تزرع في مساحات كبيرة على مستوى العالم ومصر . و تحظى نيماتودا تعقد الجذور بالاهتمام الأكبر لما تحدثه من ضرر بالغ ليذه النباتات . ويهدف هذا البحث إلى دراسة تأثير نقع بذور فول الصويا لمدة ٥ ساعات في المحاليل المائية لحمض الأسكوربيك و حمض الخليك الإندولى و الليسين و الثيامين بتركيزات ٥٠٠&١٠٠٠ جزء من المليون على نمو النباتات و الإصابة بنيماتودا تعقد الجذور للنوع ميلودوجينى جافنيكا ثم زرعت هذه البذور تحت ظروف الصوبة وأضيفت العدوى (الطور اليرقى الثاني بمعدل ١٠٠٠ يرقة / نبات) وبعد حوالي ثلاثة شهور من العدوى أوضحت النتائج المتحصل عليها ما يلى:-

١- زيادة في الطول و الوزن الطرى للمجموع الجذري و الخضري للنباتات التي نقعت بذورها في المحاليل السابقة الذكر بالمقارنة بالنباتات التي لم ينقع بذورها.
٢- قلة أعداد العقد الجذرية و كذلك أكياس البيض المتكونة على جذور النباتات التي نقعت بذورها في التركيبات السابقة مع انخفاض معنوي في معدل تكاثر النيماتودا.
٣- أعطت البذور التي تم نقعها في التركيز ١٠٠٠ جزء في المليون تأثير أعلى بالمقارنة بالتركيز ٥٠٠ جزء في المليون.
٤- أما بالنسبة للتحليلات الكيماوية لجذور نباتات فول الصويا المعاملة فقد وجد انه بزيادة التركيز تزداد كمية الفينولات الحرة في جميع المعاملات ماعدا في المعاملة بالثيامين في حين لم تظهر الفينولات الكلية اتجاه واضح و أيضا لوحظ زيادة في السكريات المختزلة ماعدا المعاملة بحمض الخليك الإندولى أما بالنسبة للأحماض الأمينية الكلية فقد وجد أنه بزيادة التركيز تقل كمية الأحماض الأمينية الكلية في النباتات المعاملة.