J. Agric. Sci. Mansoura Univ., 30 (2): 1097 - 1103, 2005

SUPPRESSIVE EFFECT OF SOME AMINO ACIDS AGAINST *Meloidogyne incognita* ON SOYBEANS *

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ABSTRACT

The amino acids; L-alanine, DL-aspartic, L-cysteine, L-glutamic, L-hydroxyproline, L-phenylalanine and L-proline were evaluated for their efficacy against the root-knot nematode, *Meloidogyne incognita* , and in improving growth of the infected soybean plants. Foliar spray applications of three concentrations of the tested amino acids revealed their potent effects on the nematode development and reproduction. However, variability in such effect was, obviously, noticed linking with properties of the amino acids and the used concentrations. Conclusively, the nematode final population and its rate of build up were decreased in most amino acid treatments in comparison to those of the check. Also, significant improvement in growth parameters of the infected soybean plants were obtained by most amino acids applications. However, no or little adverse effect on growth parameters was noticed. Hence, it is suggested that amino acids specially L-alanine , L-cysteine, L-glutamic and L-hydroxyproline at their suitable concentrations may affect directly and/or indirectly the invaded nematodes by inducing a variety of resistance to the host.
Keywords: amino acids, *Meloidogyne incognita*, soybean, resistance.

INTRODUCTION

Improving plant conditions and/or inducing resistance to susceptible plants by using safe and beneficial chemicals is a required target for nematode management. Amino acids are one of those materials, which have been used to realize such goal. Spray application of selective amino acids on root-knot-infected plants have proved that they could suppress gall formation, retard nematode development, decrease its reproductive potential, and improve the plant of infected (Overman & Woltz, 1962; Krishna Prasad & Setty, 1974 ; Reddy *et al.* ,1975 ; Osman & Viglierchio, 1981 ; AL- Sayed & Thomason, 1988 ; Kesba, 1999 & 2003 ; Oka *et al.*, 1999 and Vaitheeswaran *et al.*,2003). Obviously, amino acids do its job either directly and / or indirectly in plant defense system. Giebel and Krunz (1975) suggested that some amino acids have the ability to activate proline hydroxyproline by which reverse the suscebtible plant to resistant. Roots of tomato plants treated with beta-amino-n-butyric acid became less attractive to root-knot nematode, and physically the roots became harder to be invaded (Oka *et al.*, 1999). They , also, speculated that some specific substances may be produced in roots inhibiting nematodes or its feeding sites. Also, Oka and Cohen (2001) referred to such amino acid as an effective resistance inducer. Moreover, activities of enzymes were more in nematode-infected plants when treated with the amino acid alanine than in untreated ones (Vaitheeswaran *et al.*, 2003).

*** A part from the doctoral thesis by the senior author submitted to Faculty of Agriculture , Cairo University, Giza, Egypt.**

MATERIALS AND METHODS

The objectives of this study were to determine the effect of selected amino acids on development and reproduction of *Meloidogyne incognita* , and growth of soybean plants under stress of the nematode infection.

Seven amino acids viz; L-alanine , DL-aspartic , L- cystiene , L-glutamic , L-hydroxyproline , L-phenylalanine ,and L-proline were tested under greenhouse conditions for their role in suppressing development and reproduction of *M. incognita* on soybean-plants; and consequently in improving the host growth. Seeds of soybean cv. Giza 35 were sown in clay pots 15 cm in diam. filled with steam sterilized sand clay soil (2:1, v:v). After seeds germination, the seedlings were thinned to one plant / pot . The seedlings were, then, inoculated with 1000 active second stage juveniles of *M. incognita* per pot. Solutions of the amino acids were made by dissolving the molecular weight of each one in one litre distilled water . Dilutions of the stock solutions were made to prepare 0.25 ,0.10 and 0.01 M concentrations. Foliar spray of the materials started just seven days after nematode inoculation Soil surface of each treated pot was covered with aluminum foil during spraying to avoid splashing with the sprayed material. All the treatments as, well as the check were replicated five times, and arranged in a randomized block design on a clean bench in a greenhouse at 30 ± 5c° , receiving water and nutrients as needed. After 60 days from inoculation time, the plants were harvested and data on plant growth on the basis of fresh weights of shoots and roots and lengths of shoots and roots were obtained. Nematode galls/root , juveniles in soil, developmental stages, eggmasses per root, and number of eggs / eggmass were counted. Statistical analysis of the obtained data were carried out using the regular analysis of variance by MSTAT version 4.

RESULTS

Spray applications of the tested amino acids on *M. incognita* – infected soybean cv. Giza 35 have revealed their potent effects on development and reproduction of the nematode (Table, 1). Gall formation, in most cases, was more or less affected by the amino acid applications. Although, number of galls was increasingly decreased as concentration increased (except in L-glutamic and L- phenylalanine), it significantly decreased by the lowest concentration level in case of L-hydroxyproline. Variability in counts of the nematode in soil was noticed among the treatments, regardless, to the concentration level of the amino acids. However, the lowest concentration level of L-hydroxyproline caused considerable reduction in the nematode counts. In roots, nematode counts of the amino acid treatment ,were, mostly, less than those of the check (Table,1). Significant reductions in counts of the nematode developmental stages in amino acids treatments were noticed in comparison to that of the check. Such reductions were positively correlated with the acid concentrations, except in those of L-glutamic and L-phenylalanine. The highest concentration of DL-aspartic acid achieved the highest reduction, while the lowest concentration of L-alanine caused the lowest one. Also, counts of eggmasses were, to some extent, negatively affected by the amino

acids application. The uppermost effect was obtained by L-alanine and L-cysteine at the highest and modest concentration levels. However, treatments of the lowest concentration levels of L-alanine, L-phenylalanine and L-proline had, relatively, higher counts of eggmasses rather than those of the other treatments and check. Also, application of the amino acids has reflected on number of eggs per eggmass. Fecundity of the nematode was affected either negatively or positively according the tested amino acids. For instance, all treatments of L-proline had considerable number of eggs /eggmass which surpassed that of the check. On the other hand , all concentration levels of L-glutamic acid succeeded to suppress eggs production. The most suppressive effect was obtained by L-hydroxyproline at the lowest concentration level . Accordingly, the nematode final population and its rate of build-up were also affected. Although, treatments of most amino acids at different concentration levels had lower rates of build-up than that of the check one, some of which had similar rates (Table,1). L-hydroxyproline at the lowest concentration level (0.01 M) achieved the lowest rate of build-up (1.6), resembling that obtained by the highest concentration level of L-alanine (0.25 M).

With regard to soybean growth response, data in table (2) reveal that most amino acid treatments significantly improved growth parameters of shoot and root lengths and fresh weights. Percentages of plant biomass increment were greatly influenced by the amino acid property and concentration used. Such improvement was more remarkable in root weights of most treatments than in shoot weight. No obvious proportional trend could be observed between concentrations of the amino acids and growth parameters. Although highest and lowest concentration levels of L-glutamic acid gave significant increase in shoot and root weights, negligible or little increase were obtained in such parameters by the modest concentration. Also, lowest concentration level of L-proline markedly increased shoot and root weights more than other concentration levels did. Likely, the other amino acids had more or less effects on growth parameters of soybean, regardless, to its concentration levels.

DISCUSSION

Evidently, most of the tested amino acids had affected the development and reproduction of the root-knot nematode *M. incognita*. Infecting soyben Such effect may be endogenously processed inducing a variety of resistance to the susceptible host against the invading pathogen. Also, amino acids may improve the vigour of the host plant enabling it to compensate the damage caused by the pathogen or it may behave as a tolerant host. Significant reductions in root gall numbers in roots of the treated plants were obtained by most tested amino acids. Reduction in root galling in different host plants due to application of certain amino acids has, also, been reported (Overman & Woltz, 1962, Krishna Prasad & Setty, 1974; Reddy *et al.* ,1975; Osman & Viglierchio, 1981 ; AL-Sayed & Thomason,1988 ;Oka *et al.*, 1999 and Vaitheeswaran *et al.*,2003). Likewise, such amino acids succeeded in decreasing counts of the nematode inside the infected roots. Oka *et al.*, (1999) speculated that roots of plants treated with amino acids became less attractive to nematode and harder to be invaded.

Table 1: Effect of some amino acids on development and reproduction of *M. incognita infecting* soybean.

Treatments	Conc.* (M)	No. of Galls	Nematode counts					
			In soil	In root			final population	Build up (Pf/Pi)
				D. stages	Eggmasses	Eggs/EM.		
L-Alanine	0.01	213 bcd	5353 ab	1033 ab	125 a	202 fg	6511 a	6.5 a
	0.10	193 cde	1385 fg	920 bcd	35 ij	152 hij	2340 fgh	2.3 fgh
	0.25	136 fg	934 g	644 fgh	35 ij	96 l	1613 h	1.6 h
DL-aspartic	0.01	228 bc	3127 cde	669 efgh	95 bc	154 hij	3891 cde	3.9 cde
	0.10	165 ef	5265 ab	517 h	90 bcd	279 cd	5872 ab	5.9 ab
	0.25	106 g	5757 a	328 i	75 def	359 b	6160 a	6.2 a
L- Cysteine	0.01	229 bc	3564 c	941 bc	50 ghi	262 de	4555 c	4.6 c
	0.10	197 cde	1548 fg	830 cde	40 ij	119 jkl	2418 fgh	2.4 fgh
	0.25	138 fg	1018 g	763 defg	25 j	107 kl	1806 gh	1.8 gh
L-Glutamic	0.01	183 de	2099 efg	530 h	60 fgh	130 ijkl	2689 fgh	2.7 fgh
	0.10	165 ef	1260 fg	596 gh	45 hij	88 l	1901 fgh	1.9 fgh
	0.25	182 de	2054 efg	771 def	80 cdef	114 jkl	2905 efg	2.9 efg
L-Hydroxyproline	0.01	132 fg	957 g	627 fgh	50 ghi	39 m	1634 h	1.6 h
	0.10	138 fg	2107 efg	563 h	40 ij	192 gh	2710 efgh	2.7 fgh
	0.25	186 de	3598 c	552 h	80 cdef	193 gh	4230 cd	4.2 cd
L-Phenylalanine	0.01	181 de	4276 bc	528 h	105 ab	192 gh	4909 bc	4.9 bc
	0.10	235 b	3480 cd	652 fgh	100 bc	143 ijk	4232 cd	4.2 cd
	0.25	186 de	2400 def	635 fgh	85 cde	126 ijkl	3120 def	3.1 def
L- Proline	0.01	270 a	5530 a	921 bcd	110 ab	469 a	6561 a	6.6 a
	0.10	206 bcd	5229 ab	781 cdef	95 bcd	311 c	6105 a	6.1 a
	0.25	179 de	3504 cd	621 fgh	70 efg	236 ef	4195 cd	4.2 cd
Check	-	286 a	5530 a	1150 a	100 bc	165 ghi	6780 a	6.8 a

* Each amino acid was applied at the rate of its molecular weight(in gram) dissolved in one litre of water.

Means followed by the same letter(s) within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

Table 2: Effect of amino acids on growth parameters of soybean infected with *M. incognita*.

Treatments	Conc. (M)*	Shoot				Root			
		Sh. Weight	% Increase	Sh. Length	% Increase	R. weight	% Increase	R. length	% Increase
L-Alanin	0.01	5.0 cdef	22	28.6 ab	28	4.8 bcd	102	34.2 bc	57
	0.1	4.9 cdef	20	25.4 bcd	13	4.4 bcdef	87	21.8 e	0
	0.25	5.1 cdef	23	27.2 abc	21	5.0 bc	113	36.2 ab	66
D_Laspartic	0.01	6.0 bcd	47	25.4 bcd	13	2.9 hijk	23	29.8 bcde	37
	0.1	5.3 cdef	30	23.2 cd	4	3.3 fghijk	40	28 cde	28
	0.25	5.6 bcdef	38	24.6 bcd	10	2.5 jk	8	28 cde	28
L- Cysteine	0.01	5.4 bcdef	32	29 ab	29	4.4 bcdef	88	23.8 e	9
	0.1	4.9 cdef	20	28.6 ab	28	3.6 defghij	53	22.2 e	2
	0.25	4.5 def	10	28.2 ab	26	2.8 ijk	18	22.2 e	2
L-Glutamic	0.01	4.3 ef	5	24.4 bcd	9	3.1 ghijk	30	25.2 de	16
	0.1	6.8 ab	65	26.2 abcd	17	4.7 bcde	99	29.8 bcde	37
	0.25	7.8 a	91	27.2 abc	21	6.3 a	165	29.8 bcde	37
L-Hydroxyproline	0.01	4.5 def	10	24.6 bcd	10	4.0 cdefgh	70	32.8 bcd	50
	0.1	4.7 def	15	25.6 bcd	14	4.0 cdefghi	69	29 bcde	33
	0.25	4.5 def	10	26.2 abcd	17	3.2 ghijk	36	42 a	93
L-Phenylalanine	0.01	6.4 bc	55	25 bcd	12	3.6 efghij	51	27 cde	24
	0.1	4.5 def	10	25.4 bcd	13	3.2 ghijk	36	23.4 e	7
	0.25	4.4 def	8	26.2 abcd	17	3.6 defghij	53	27.2 cde	25
L- Proline	0.01	6.7 ab	63	28.8 ab	29	5.5 ab	131	36.6 ab	68
	0.1	5.5 bcdef	35	30.4 a	36	4 cdefgh	71	27.2 cde	25
	0.25	5.0 cdef	22	30.4 a	36	4.2 cdefg	76	34 bc	56
Check		4.1 f	-	22.4 d	-	2.4 k	-	21.8 e	-

* Each amino acid was applied at the rate of its molecular weight(in gram) dissolved in one litre of water.

Means followed by the same letter(s) within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.

Production of eggmasses and eggs /eggmass was adversely affected by the tested amino acids, which in turn reflected on the nematode final population and its build–up. Thus, it seems that such amino acids affect development, reproduction and fecundity of the nematode. Many assumptions had been postulated to explain the bio-activity of amino acids when applied to plants. The most acceptable interpretation was this assumed by Prasad and Webster (1967) , Evans and Trudgill (1971) and Sharma and Tiagi (1984). They referred that those acids may act as anti- metabolites which block essential cycles in either plants or in nematodes; and they could interfere with the supply of nutrients to nematodes, delaying its maturation and build-up. Activities of enzymes in the host plants may also be generated to induce a sort of resistance by producing and/or increasing some beneficial substances.

REFERENCES

Al-Sayed, A.A. and Thomason, D.J. (1988). *Meloidogyne incognita* and tomato response to thiamine, ascorbic acid, L-arginine and L-glutamic acid. J. Nematol., 20 (3) : 451 - 456.

Evans, K. and Trudgill, D.L. (1971). Effect of amino acids on the reproduction of *Heterodera rostochiensis.* Nematologica, 17 : 495 – 500.

Giebel, J. and Krunz, J. (1975). Role of amino acids in plant tissue response to *Heterodera rostochiensis.* II- Effect of proline and hydroxyproline. Nematol. Mediter., 3: 49 – 53.

Kesba, H.H.H. (1999). Ecological and pathological studies on some plant parasitic nematodes infecting grape, *Vitis vinifera.* M.Sc. Thesis, Fac. Agric., Cairo Univ., 114 pp.

Kesba, H.H.H. (2003). Integrated nematode management on grapes grown in sandiness soil. Ph. D. Thesis, Fac. Agric., Cairo Univ., 189pp.

Krishna-Prasad, K.S. and Setty, K.G.H. (1974). The effect of amino acids as foliar spray on root-knot nematode (*Meloidogyne incognita*) and tomato. Indian J. Nematol., 4 : 88 – 90.

Oka, Y. ; Cohen,Y. and Spiegel,Y. (1999). Local and systemic induced resistance to the root-knot nematode in tomato by DL-beta-amino-n-butyric acid. Phytopath., 89 (12): 1138-1143.

Oka.Y. and Cohen, Y. (2001). Induced resistance to cyst and root-knot nematodes in cereals by DL-beta-amino-n-butyric acid. Euro. J. Pl. Path., 107(2): 219-227.

Osman, A.A. and Viglierchio, D.R. (1981). Foliar spray effects of selected amino acids on sunflower infected with *Meloidogyne incognita.* J. Nematol.,13 (3) : 417 - 419.

Overman, A.J. and Woltz, S.S. (1962). Effects of amino acid antimetabolites upon nematodes and tomato. FL. Agric. Exp. Stations J. Series No. 1524 : 166 – 170.

Prasad, S.K. and Webster, J.M. (1967). The effect of amino acid antimetabolites on four nematode species and their host plants. Nematologica, 13: 318 – 320.

Reddy, P.P.; Govindu, H.C., and Setty, K.G.H. (1975). Studies on the effect of amino acids on the root-knot nematode, *Meloidogyne incognita* infecting tomato. Indian J. Nematol., 5: 36-41.

Sharma, R.K., and Tiagi, B. (1984). Studies on the effect of some amino acids and amino acid metabolite foliar sprays on the root-knot nematode infected pea plants. Indian J. Nematol. , 14: 61-62.

Vaitheeswaran, M. ; Ibrahim, S. M.; Selvapriya, P. and Kumar, J. V. (2003). Chemotherapeutic action of alanine as soil drench against root-knot nematode, *Meloidogyne incognita* on *Vigna unguiculata*. Indian J. Nematol. 33: 2, 136-142.

التأثير المثبط لبعض الأحماض الأمينية ضد نيماتودا تعقد الجـذور (ميلـودوجين انكوجنيتا) في فول الصويا

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تم تقييم الأحماض الأمينيه : الأنين ، اسبارتيك ، سيستين ، جلوتاميـك ، هيدروكسـي برولين ، فينيل الأنين ، برولين لمعرفة كفاءتها في مكافحة نيماتودا تعقـد الجـذور (ميلـودوجين انكوجنيتا) وتحسين نمو نباتات فول الصويا المصابة بها.

وأوضحت معاملات الرش على المجموع الخضري للنباتات بثلاث تركيزات مـن تلـك الأحماض الامينية كفاءتها في التأثير على نمو وتكاثر النيماتودا. وكان هناك تفاوت واضـح بـين الأحماض الامينية وبعضها وكذلك بين التركيزات. وبصفه عامة فان تعداد النيماتودا النهائي وكذلك معدل تكاثرها قد انخفض في معظم المعاملات عند مقارنتها بمعاملة المقارنة ، كذلك كان هنـاك تحسن ملحوظ في نمو النباتات المصابة عند رشها بالأحماض الامينية . ولم يلاحظ تـأثير سـلبي على النمو إلا في حالات محدودة. وبذلك فأنة يمكن استنتاج أن الأحماض الامينية المستخدمة يكون لها تأثير على النيماتودا إما بطريقة مباشرة أو غير مباشرة عن طريق تحسين وسائل المقاومة في النباتات المصابة. وخاصة أحماض ألانين و سيسـتين و جلوتاميـك و هيدروكسـي بـرولين بالتركيزات المناسبة.