EFFECT OF SOME ANTIOXIDANTS ON THE RESISTANCE OF COTTON PLANTS AGAINST THE INFECTION WITH ROOT-KNOT NEMATODE *(MELOIDOGYNE JAVANICA)*

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Abstract

Role of some compounds and essential oils that work as antioxidants i.e. ascorbic acid, acetylsalicylic acid, sodium benzoate, salicylic acid, cinnamic acid, ginger, fennel, black pepper, basil and cumin oils for inducing resistance in cotton plants against the infection with root-knot nematode, *Meloidogyne javanica* were evaluated here. The effectiveness of ascorbic acid and ginger oil were superior with seed soaking or foliar spraying treatments followed by salicylic acid and fennel oil. The lowest effects were attained with sodium benzoate and cumin oil treatments. All the tested compounds reduced the root galling, number of females, egg masses and the final nematode population in potted soil, but enhanced plant growth as well as peroxidase activity, in comparison to the control. All tested antioxidants were more effective when used as seed soaking than foliar spraying. The highest reduction of nematode population (70.1 %) was attained with ascorbic acid in the seed soaking application.

INTRODUCTION

Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition. Typically this means that the antioxidant molecule becomes a free radical in the process of neutralizing a free radical molecule to a non-free-radical molecule. But the antioxidant molecule will usually be a much less reactive free radical than the free radical neutralized (http://www. benbest. com/nutrceut/Antioxidants.html)

Antioxidants (free radical scavengers) are potent compounds for delaying the loss of all membrane integrity and ethylene production (Elad, 1992). Removal of oxidants or reactive oxygen species (ROS) is important, as their accumulations are toxic to cells and impair resistance of plants against pests. The presence of hydrogen donors including antioxidants is essential for the activity of peroxidase that catalyzes the oxidation of these compounds using hydrogen peroxide (Mahadevan and Sridhar, 1982). Anti-oxidation of essential oils was tested by Ao *et al.* (2006) who found that most of the tested essential oils presented good antioxidant activity.

This present study aims to throw light on the role of some compounds and essential oils that work as antioxidants i.e. ascorbic acid, acetylsalicylic acid, sodium benzoate, salicylic acid, cinnamic acid, ginger oil, fennel oil, black pepper oil, basil oil

and cumin oil for inducing resistance in cotton plants against the infection with root–knot nematode, *Meloidogyne javanica*

MATERIALS AND METHODS

Solutions of the tested antioxidant compounds i.e. ascorbic acid, acetylsalicylic acid, sodium benzoate, salicylic acid, cinnamic acid, ginger oil, fennel oil, black pepper oil, basil oil and cumin oil, were prepared at the concentration of 500 p.p.m and applied as follows :-

1) Seed soaking

Cotton seeds were soaked in the prepared solutions of these compounds for 24 hours before planting in 10 cm clay pots filled with steamed loamy sand soil. Soaked seeds in free chemicals water served as control treatment. This group of pots was inoculated after one month from germination with 1000 freshly hatched larvae of *Meloidogyne javanica* per pot

2) Foliar spray

Other group of 20 days old seedlings, untreated before, were given 5 foliar sprays with 15 ml. per spray of the tested compounds at the concentration of 500 p.p.m. solution on alternate days using an atomizer.

Cotton seedlings were thinned in all treatments at fifteen days old to one plant per pot. Each treatment was replicated four times. Pots maintained at outdoor temperature in a completely randomized design. At the day following the last treatment in the spray procedure all plants were inoculated with 1000 freshly hatched larvae per plant. Untreated and Un inoculated pots served as control. At the end of the experiment after 5 months from planting, roots were washed and both shoots and roots were weighed. Nematode galls were counted, separated and stained with acid fuchsine in lactophenol for not more than 24 hours. Stained galls were rinsed in water, macerated and *Meloidogyne* females and egg masses were counted. Second stage larvae were extracted from soil by the combination of Baermann trays with elutriation and sieving, counted and recorded. Samples of plant tissues were taken for the estimation of peroxidase activity with guaiacol as an enzyme substrate using a modified procedure of Maehly and Chance (1954). The reaction mixture consisted of 3 ml phosphate buffer (0.1 M, pH 5.8) containing guaiacol (40 mM) and 200 µl of crude enzyme extract. The reaction was started by adding 50 µl H_2O_2 solution (250 H_2O_2/ 10ml distilled water).The activity was expressed as increase in absorbance at 470 nm/ mg protein / min.)

RESULTS AND DISCUSSION

Effects of two methods of applications (seed soaking and foliar spray) of five antioxidant chemical compounds and five essential oils on the response of cotton c.v. Giza 80 to the infection with *Meloidogyne javanica* are presented in Table (1). All treatments significantly reduced the final number of nematode galls, females, egg masses and juveniles when compared to these untreated and inoculated plants. These compounds varied in their effectiveness in reducing nematode infection or reproduction, and enhancing plant growth. The tested antioxidants were more potent when used in seed soaking than when used as foliar spray. The most potent compound was ascorbic acid followed by ginger oil, salicylic acid, fennel oil, black pepper oil, cinnamic acid, acetylsalicylic acid, basil oil, sodium benzoate and cumin oil in a descending order, recording with the seed soak application, 70.1, 70.0, 64.8, 64.2, 61.7, 61.6, 36.1, 31.1, 24.9 and 16.3 reduction % in the final nematode population in soil, respectively. Superiority of ascorbic acid in this experiment agrees with Al-Sayed (1989) who concluded that ascorbic acid was more effective in reducing nematode infection. Ascorbic acid can not only neutralize hydroxyl, alkoxyl and peroxyl radicals by hydrogen donation but also neutralize the radical form of other antioxidants such as glutathione and tocopherol. (http://www. benbest. com /nutrceut /Antioxidants.html). Data in Table (1) indicate that peroxidase activity increased in treated plants than other untreated and the activity in root extracts was higher than other in foliage extracts. Also the application methods used here were differ in increasing peroxidase activity, whereas the seed soaking with the tested antioxidants exhibit higher increase in peroxidase activity than foliar spraying method. The activity of peroxidase enzyme was the highest with ascorbic acid and ginger oil with the two application methods, although, this activity was stimulated slightly by sodium benzoate and cumin oil. The highest increase in peroxidase activity (282.2%) was attained by ascorbic acid in cotton root extract with the seed soaking application. The increase in peroxidase activity concomitant the reduction in the final nematode number and the enhancing of the plant growth, revealing the increase of resistance in the treated plants, and that enforce Ganguly and Dasgupta (1979) result that peroxidase activity significantly decreased in the *Meloidogyne incognita*- susceptible plants, and increased in the resistant plants. These results obtained here interpret the report of Melillo *et al.* (1983) that ascorbic acid induced resistance in hosts against plant parasitic nematodes. On the other hand it was obvious from the obtained data that peroxidase activity was high with untreated inoculated plants than other in untreated uninoculated plants and this agree with Zacheo *et al.* (1982) who concluded that peroxidase activity, significantly increased in resistant plants only after nematode inoculation.

Table 1. Effect of some antioxidants on the response of cotton c.v. Giza 80 to the infection with root-knot nematode (*Meloidogyne javanica)*

. Treatment Conc. (500 p.p.m.)	Galls/plant		Females/ Plant		Egg masses/ plant		Juveniles / pot		Reduction % in juveniles number		Fresh shoot wt.(g)		Fresh root wt. (g)		Peroxidase Unit/mg protein/min.			
															Activity in Foliage extract Root extract		Increase %	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Ascorbic acid	34*	39	43	49	29	33	2613	3135	70.1	64.1	42.0	36.8	15.0	13.3	0.851 2.721	0.759 2.366	194.5 282.2	162.6 165.4
Salicylic acid	75	87	96	99	66	74	3072	3624	64.8	58.5	37.5	33.5	12.0	10.8	0.795 1.988	0.691 1.743	175.1 179.2	139.1 144.8
Cinnamic acid	120	139	140	161	105	116	3355	4059	61.6	53.5	33.0	29.0	10.5	9.5	0.450 1.802	0.394 1.594	55.7 153.1	36.33 123.8
Acetylsalicylic acid	142	161	166	189	122	136	5580	6640	36.1	23.9	31.5	28.0	10.5	9.3	0.432 1.877	0.387 1.618	49.5 163.6	33.9 127.2
Sodium benzoate	330	381	379	450	310	347	6552	7862	24.9	10.0	27.0	24.2	9.0	8.4	0.421 1.672	0.362 1.466	45.7 134.8	25.25 105.8
Ginger oil	41	47	52	60	36	40	2620	3146	70.0	63.9	41.5	36.7	14.5	13.2	0.608 2.367	0.533 2.095	110.4 232.4	84.42 194.2
Fennel oil	80	92	103	117	68	76	3124	3717	64.2	57.4	36.0	32.1	12.0	11.0	0.576 2.245	0.514 1.987	99.3 215.3	77.85 179.1
Black pepper oil	134	154	156	177	116	129	3340	4024	61.7	53.9	31.5	27.7	11.0	10.2	0.448 1.867	0.403 1.666	55.0 162.2	41.55 133.9
Basil oil	156	178	184	211	135	149	6018	6980	31.1	20.1	30.0	26.7	10.5	9.6	0.416 1.656	0.368 1.440	43.9 132.6	27.33 102.2
Cumin oil	368	415	381	481	307	340	7325	8204	16.3	6.1	28.0	26.2	10.0	9.1	0.348 1.519	0.307 1.325	20.4 113.3	6.22 86.1
Untreated inoculated	489		516		438		8734		-	-	25.0		6.5		0.320 1.245	0.296 1.092	10.7 74.8	2.42 53.4
Untreated uninoculated	-		-		-		-		-	-	40.0		11.5		0.289 0.712			
L.S.D. 0.05	27.0	30.2	31.3	34.7	26.8	30.7	153.7	178.2			2.5	2.08	1.7	1.5				
L.S.D. 0.01	41.8	46.8	48.5	54.2	42.5	47.5	187.2	213.4			3.6	3.0	2.3	2.1				

A=Seed soaking B=Foliar spray All observation recorded are mean of four replicates

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تأثير بعض مضادات الأكسدة على مقاومة نباتات القطن للإصابة بنيماتودا تعقد الجذور (*Meloidogyne javanica*)

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تم تقييم بعض المركبات والزيوت الطيارة التى تعمل كمضادات للأكسدة مثل حمض الاسكوربيك ،حمض الأسيتيل سالسليمك، بنزوات الصوديوم، حمض السالسليك، حمض السينامك، زيت الزنجبيل، زيت الشمر ،زيت الفلفل الأسود،زيت الريحان وزيت الكمون فى تحفيز مقاومة نباتات القطن صنف جيزة ٨٠ ضد الإصابة بنيماتودا تعقد الجذور (***Meloidogyne javanica***). ولقد أوضحت النتائج تفوق حمض الاسكوربيك وزيت الزنجبيل عند معاملتهما نقعاً للبذور أو رشاً على المجموع الخضرى وجاء فى المرتبة الثانية حمض السالسليك وزيت الشمر أما بنزوات الصوديوم وزيت الكمون فكانا الأقل تأثيراً.

أظهرت النتائج أيضاً أن جميع المركبات والزيوت المستخدمة تفوقت على معاملة المقارنة فى خفض تعداد العقد النيماتودية وعدد الإناث وكتل البيض والتعداد النهائي للنيماتودا وأن النباتات المعاملة بهذه المركبات زادت فى النمو وفى نشاط إنزيم البيروكسيداز عن النباتات الغير معاملة، كما بينت النتائج أن طريقة المعاملة بنقع البذور أعطت نتائج أفضل فى خفض تعداد نيماتودا تعقد الجذور وتحفيز المقاومة فى نباتات القطن عن طريقة المعاملة بالرش على المجموع الخضرى وأن أعلى خفض لتعداد النيماتودا (٧٠,١%) قد تحقق باستخدام حمض الأسكوربيك ٥٠٠ جزء فى المليون بطريقة نقع البذور .