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SOYBEAN ROOT-ROT MANAGEMENT USING SOME INDUCER RESISTANCE COMPOUNDS

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ABSTRACT: ***Fusarium solani, Rhizoctonia solani and Macrophomina phaseolina were the dominant associated fungi with soybean root-rotted seedlings collected from Giza, El-Menia, Minoufiya and Sharkia governorates. Pathogenicity test experiments cleared that F. solani and R. solani were more virulent to soybean cultivar Giza 21, while M. phaseolina was less virulent. Dipping soybean seeds of Giza 21 and Giza 35 cultivars for 30 second before one hour of planting, in six different inducers significantly reduced seedling damping-off and root-rot. Oxalic acid showed the best effect while neem oil was the least effective one, under greenhouse conditions. Oxalic and Salicylic acid treatments increased the survived plants, as it reached 97.4% under field conditions. Application of the inducers significantly improved both growth and yield parameters of both tested soybean cultivars. Peroxidase, polyphenol oxidase and chitinase activities were significantly higher, than control, in response to the application of different inducers and the fungicide Rizolex-T to Giza 21 soybean seeds planted in artificially infested soil with R. solani. Generally, Rizolex-T, Oxalic and Salicylic acid were the most efficient treatments, while Neem oil showed the lowest effect.***

Key words: ***Root-rot, soybean, Rhizoctonia solani, Fusarium solani, Macrophomina phaseolina, chemical inducers, Rizolex-T and defence related enzymes.***

INTRODUCTION

Soybean [*Glycine max* (L.) Merr] is considered as one of the main oil crops all over the world. It occupies special importance because it contains 20% oil and 40% protein of dry seed weight (Ahmed, 2001).

Fungi belonging to various genera were isolated from infected plants. *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina* were the main root-rot pathogens of soybean plants as they recorded the highest isolation frequency (Killebrew *et al.,* 1988; Naito and Itoh, 1999 and Umasingh and Thapliyal, 1999).

Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of the induced resistance; in both SAR and ISR, plant defences are preconditioned by prior infection or treatment that results in resistance or tolerance against subsequent challenge by a pathogen. Great achievements have been made over the past 20 year in understanding the physiological and biochemical basis of SAR and ISR. Much of this knowledge is due to the identification of a number of chemical and biological elicitors; some of which are commercially available for use in conventional agriculture (Vallad and Goodman, 2004). Several investigators studied the effectiveness of these chemical inducers on root-rot disease (Segarra *et al.,* 2006). The induced systemic resistance (ISR) sensitizes the plant to respond rapidly after treatment. These responses include phytoallexin accumulation, phenol, lignifications and activation of peroxidase, poly-phenoloxidase and chitinase, enzymes. The aim of this study was to test the efficiency of some resistance inducers to control root-rot of soybean.

MATERIALS AND METHODS

1. Isolation and identification of the causal organisms:

Diseased samples of soybean seedlings were collected from four different governorates in Egypt, i.e. Giza, El-Menia, Minoufiya and Sharkiya.

2. Preparing of pathogen inoculum:

Infected parts were cut into small pieces, washed thoroughly with running tap water to remove any adhering soil particles. These pieces were surface sterilized by immersing in 5% sodium hypochlorite solution for 2 min, followed by 70% ethanol for 2 min, then washed several times in sterilized water then dried within sterilized filter papers. Four surface sterilized pieces were aseptically transferred onto potato dextrose agar medium (PDA) containing 40 ppm streptomycin sulphate to avoid any bacterial contamination. Plates were incubated at 25°C for 3 – 7 days.

Fungi were purified and individually grown on sand-barley medium (25 g clean sand, 75 g barley and enough water to cover the mixture). Flasks contained sterilized medium were inoculated with each particular fungus and incubated at 25°C for two weeks. Flasks vigourously shaked daily to enhance the homegenous fungal growth. Potted soil was watered daily for a week to enhance fungi growth. Fungi were identified at the Department of Mycology, Plant Pathology Institute, Agricultural Research Center (ARC), Giza, Egypt.

3. Effect of the chemical inducers on disease incidence (under green-house conditions):

This experiment was carried out in sterilized pots (30 cm diam.) containing sterilized clay soil in the greenhouse. Both pots and soil were sterilized by 5% formalin solution and left to dry for 2 weeks before planting. Soil was individually infested with *Fusarium solani,* *Rhizoctonia solani* and/or *Macrophomina phaseolina* (3% of soil weight). Soybean seeds (cvs. Giza 21 and Giza 35 were just dipped for 30 second in solutions of six chemical inducers i.e. Salicylic acid (SA) at the concentration of 2.5, 5.0 and 8.0 Mm. Oxalic acid (OA) (2.5, 500 and 800 Mm), potassium phosphate (20, 30 and 50 µm), Ethephon (200, 400 and 500 ppm), Neem oil 200, 400 and 600 ppm and the fungicide Rizolex-T (3 g / kg seeds). Treated seeds were left in open air for 2 hours before sowing. Seeds dipped in sterilized water served as a control treatment.

Five seeds were sown per pot and three replicates were used for each treatment. Percentage of pre- and post-emergence damping-off, root-rot and healthy survival plants were recorded.

4. Effect of chemical inducers on disease incidence and plant growth (under field conditions):

This experiment was conducted in naturally infested soil at Sers El-Layian Agricultural Research Station during 2008 and 2009 growing seasons. The experimental layout was a completely randomized plots each one consisting of 3 rows (3.5 m long and 60 cm in width).

The recommended agricultural practices were done.

Seed treatment:

Soybean seeds cvs. Giza 21 and Giza 35 were individually treated with the highest concentration of each tested chemical inducers and the fungicide Rizolex-T (3 g per kg seeds) as mentioned in the greenhouse experiment. Three replicates were used for each treatment. Percentages of pre- and post-emergence damping-off, plant height, healthy survival and root rotted plants were recorded. The effect of chemical inducers on yield parameters i.e. number of pods / plant and weight of 100 seeds were also estimated.

5. Effect of some chemical inducers on enzymes activities of soybean grown in soil infested with *Rhizoctonia solani*:

Soybean cv. Giza 21seedlings of 15 days-old plants grown in soil infested with *R. solani*, both treated with the highest concentration of the tested chemical inducers or the untreated control, were used for assay of 3 defence related enzymes. The activity of Peroxidas, Polyphenol oxidase and Chitinase enzymes were respectively determined according to Snell and Snell (1953), Allam and Hollis (1972) and Tuzun *et al.* (1989).

Statistical analysis:

All obtained data were statistically analysed according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

1. The causal fungi of soybean root-rot:

Identification of the isolated fungi clear that *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina* were the dominant pathogens of soybean root-rotted plants collected from Giza, El-Menia, Minoufiya and Sharkia governorates. These results are in agreement with those obtained by the Department of Mycology, Plant Pathology Institute, Agricultural Research Center (ARC), Giza, Egypt.

2. Pathogenicity tests:

Results present in Table (1) indicate that all obtained fungal isolates were pathogenic to Giza 21 soybean cultivar. However *Fusarium solani* isolates were more pathogenic than the other ones. The most aggressive isolate was that obtained from El-Menia governorate which caused 40% damping-off.

***Fusarium solani* also was reported as the causal organism of soybean root-rot and damping-off by Orellana *et al.* (1976), Hassanein (1978), Jasnic and Vidic (1986), Abou-Zeid *et al.* (1987), Montoya (1991), Naito and Itoh (1999), Ali *et al.* (2009) and El-Hai *et al.* (2010).**

Table (1): Pathogencity tests of the isolated fungi on Giza 21 soybean cultivar.

Fungui	Locality	Pre-emergence damping-off infection (%)	Post-emergence damping-off infection (%)	Survived plants (%)
Rhizoctonia solani	Giza	8.0	3.3	88.7
	El-Menia	20.0	16.7	63.3
	Minoufiya	5.0	3.2	91.8
	Sharkia	7.3	10.0	82.7
Fusarium solani	Giza	9.0	13.3	77.7
	El-Menia	16.7	23.3	60.0
	Minoufiya	6.7	10.0	83.3
	Sharkia	7.7	9.0	83.3
Macrophomina phaseolina	Giza	5.0	3.3	91.7
	El-Menia	7.3	6.7	86.0
	Minoufiya	9.0	12.0	79.0
	Sharkia	6.7	10.0	83.3
Control		0	0	100
L.S.D at 5%		1.4	1.5	2.5

Rhizoctonia solani **occupied the second rank as the causal organism of root-rot and damping-off of soybean where dead plants ranged from 8.2% (El-Menia isolate) to 36.7% (Minoufiya isolate). This pathogen was also recorded by Killebrew *et al.* (1988), Naito and Itoh (1999) and Umasingh and Thapliyal (1999) as soybean root-rot and damping-off causal organism. *Macrophomina phaseolina* was weak pathogen to the cv. Giza 21 soybean seedlings (up to 30 days). This is of logic where this pathogen attacks old plants under drought conditions (Arafa, 1994; Lichangsong *et al.,* 1997 and Umasingh and Thapliyal, 1999).**

3. Effect of chemical inducers on root-rot disease incidence:

Under greenhouse and artificial soil infestation conditions, results present in Table (2) indicate just dipping of soybean seeds for 30 seconds in solutions of six chemical inducers, before planting, significantly reduced seedling damping-off, root-rot disease severity and increased survived plants of the cvs. Giza 21 and Giza 35.

The higher concentration is better effective and vice versa. Oxalic acid showed the best results of both tested cultivars. On the other hand, Neem oil applications were less effective than the tested inducers, while Rizolex (3 g / kg seeds) gave nearly similar results of the inducers.

Under natural infested soil field, Rizolex-T showed the best action against root-rot pathogens of Giza 21 soybean cultivar (Tables 3 & 4). It resulted the average of 97.4% survival plants and this was 95.3% when Oxalic acid was applied. As for Giza 35 cultivar, Oxalic and Salicylic acid gave the best results (97.0% survivals). The untreated control plants of both Giza 21 and Giza 35 soybean cultivars, respectively resulted 91.3 and 89.4% survived plants which showed 55.63 and 60.17% root-rot disease severity, in the same respect.

4. Effect of chemical inducers on soybean plant growth and yield parameters:

Under natural infested soil field, the tested inducers, Neem oil and Rizolex-T increased plant height, abundant branches and yield of Giza 21 and Giza 35 soybean cultivars (Tables 5 & 6). Significant results of plant height compared with control, were achieved when Oxalic acid, Salicylic acid, Potassium phosphate and Rizolex-T were individually applied. However, Salicylic acid was the longly used compound which significantly increased the number of formed branches of both cultivars at 2008 and 2009 growing seasons. On the other hand, all tested compounds significantly increased yield production. These results were noticed when Salicylic acid was applied to any of tested soybean cultivars. In general, Ethephon and Neem oil showed the least efficacy.

5. Effect of chemical inducers on some enzymes activity:

Under greenhouse conditions with *R. solani*, soybean plants cv. Giza 21 (one month old) those grown from seeds treated with different inducers were picked up and analysed for some enzymes activity. Results present in Table (7) clear that Rizolex-T, Salicylic and Oxalic acid caused the highest activities of peroxidase, polyphenol oxidase and chitinase. These respectively were 188, 131 and 267% when Rizolex-T was applied in comparison with control. These percentages were 183, 126 and 245% over control when soybean seeds were treated with Salicylic acid and they were 174, 117 and 240% due to the application of Oxalic acid. However, minimum enzymes activities were recorded when Neem oil was applied to Giza 21 cv. soybean seeds before planting. The above mentioned enzymes activity recorded 137, 110 and 156% over the untreated control. Increasing the

Table 2

Table 3, 4

Table 5 , 6

Table (7): Activity of peroxidase, polyphenol oxidase and chitinase as affected by chemical inducers, neem oil and rizolex-T.

Treatment	Concen.	Peroxidase activity / min.	% control	Polyphenol-oxidase activity/min.	% control	Chitinase activity / min.	% control
Salicylic acid	8 mM	4.00	183	2.15	126	6.13	245
Oxalic acid	8 mM	3.80	174	2.00	117	6.00	240
Potassium phosphate (K_2HPO_4)	4%	3.20	146	1.90	111	4.18	167
Ethephone	500 ppm	3.30	1.51	1.98	116	4.66	186
Neem oil	200 ppm	3.00	137	1.87	110	3.90	156
Rizolex-T	3 g/kg seed	4.10	188	2.23	131	6.69	267
Control	-	2.18	-	1.70	-	2.50	-

enzyme activities indicate the high potential of plant physiology which improves the acquired resistance to the disease. These were also reported by Vallad and Goodman (2004) and Segarra *et al.* (2006).

On the other hand, the noticeable increases of chitinase could be the main reason of the pathogen chitin analysis and disintegration which consequently decreased the infection.

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مكافحة مرض عفن جذور نباتات فول الصويا باستخدام بعض المستحثات والمبيد الفطرى ريزولكس–ت

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الملخص العربى :

وجد أن الفطريات (فيوزاريوم سولانى ، رايزوكتونيا سولانى و ماكروفومينا فاسيولينا) هى الأكثر تكراراً عند العزل من نباتات فول الصويا المصابة بمرض عفن الجذور جمعت من محافظات المنوفية، الشرقية، الجيزة والمنيا. أظهرت تجارب إثبات القدرة المرضية أن الفطرين فيوزاريوم سولانى و رايزوكتونيا سولانى هما الأكثر إحداثاً للمرض، فى حين كان الفطر ماكروفومينا فاسيولينا أقل ضراوة. أدى معاملة بذور صنفى فول الصويا جيزة ٢١، جيزة ٣٥ بالمستحثات المختبرة أو المبيد الفطرى ريزولكس–ت كتغطية فيلمية للبذرة بالنقع لمدة ٣٠ ثانية قبل الزراعة بساعة إلى خفض معنوى فى موت البادرات وأعراض عفن الجذور والزيادة المعنوية فى النباتات القائمة. وكان المبيد الفطرى ريزولكس–ت هو الأفضل تأثيراً تحت ظروف الصوبة والعدوى الصناعية بالفطر رايزوكتونيا سولانى، ويليه فى ذلك حمض الأوكساليك ثم حمض السالسليك فى حين كان زيت النيم هو الأقل تأثيراً فى ذلك. كما أظهرت المعاملات – تحت ظروف العدوى الطبيعية بالحقل – زيادة معنوية فى نمو النبات وإنتاج المحصول بنفس كفاءة المركبات المستخدمة فى الصوبة. إزداد نشاط إنزيمى الأكسدة (بيروكسيديز، بوليفينول أوكسيديز) وكذلك إنزيم شيتينيز كاستجابة للمعاملات المختلفة فى النباتات عمر شهر المنزرعة فى تربة سبق عدواها صناعياً بالفطر رايزوكتونيا سولانى، وكان أفضلها فى ذلك المبيد الفطرى ريزولكس–ت، حمض الأكساليك وحمض السالسيلك.

Table (2): Effect of chemical inducers on damping-off and root-rot disease severity of soybean Giza 21 and Giza 35 cultivars under greenhouse and artificial soil infestation conditions.

Inducer	Conc.	Giza 21				Giza 35			
		Pre-emergence damping-off infection (%)	Post-emergence damping-off infection (%)	Disease severity (%)	Survived plant (%)	Pre-emergence damping-off infection (%)	Post-emergence damping-off infection (%)	Disease severity (%)	Survived plant (%)
Salicylic acid	2.5 mM	4.0	1.3	27.0	94.7	7.0	2.0	44.1	91.0
	5.0 mM	2.6	1.0	22.5	96.4	4.0	1.3	27.5	94.7
	7.5 mM	1.0	0.3	10.2	98.7	1.6	0.3	22.5	98.1
Oxalic acid	100 ppm	3.3	1.3	10.2	95.4	5.0	2.3	22.5	92.7
	150 ppm	2.0	1.0	2.7	97.0	4.0	1.3	10.2	94.7
	200 ppm	0.3	0	1.8	99.7	2.0	0	2.7	98.0
Potassium phosphate (K_2HPO_4)	5 mM	4.7	2.3	27.0	93.0	5.0	1.6	27.0	93.4
	10 mM	2.6	1.7	22.5	95.7	4.0	1.3	22.5	94.7
	15 mM	1.3	1.7	22.5	97.0	2.0	1.0	20.0	97.0
Ethephon	200 ppm	4.0	2.3	50.4	93.7	6.3	1.6	60.0	92.1
	400 ppm	3.3	2.0	27.0	94.7	6.0	1.3	55.3	92.7
	600 ppm	2.3	1.7	22.5	96.0	6.0	1.7	50.0	92.3
Neem oil	100 ppm	5.3	1.7	50.0	93.0	5.6	2.3	66.7	92.1
	150 ppm	4.0	1.0	44.1	95.0	4.3	2.0	66.7	93.7
	200 ppm	3.3	1.3	44.1	95.4	2.6	1.3	50.0	96.1
Rizolex-T	3 g/kg seeds	0.6	0.0	0.9	99.4	3.0	0.0	0.9	97.0
Control		5.3	2.0	66.7	92.7	0.3	2.0	100.0	91.7
L.S.D at 5%		1.50	0.90	0.81	1.33	1.60	0.90	0.99	1.50

Table (3): Effect of chemical inducers on damping-off of soybean cultivars under natural infested soil field during season 2008.

Treatment	Giza 21				Giza 35			
	Pre-emergence damping-off infection (%)	Post-emergence damping-off infection (%)	Disease severity (%)	Survived plant (%)	Pre-emergence damping-off infection (%)	Post-emergence damping-off infection (%)	Disease severity (%)	Survived plant (%)
Salicylic acid	2.7	3.3	22.5	94.0	1.7	1.3	24.18	97.00
Oxalic acid	2.0	2.7	28.11	95.3	1.3	1.7	30.11	97.00
Potassium phosphate (K_2HPO_4)	3.3	3.3	36.00	93.4	2.0	2.3	38.17	95.7
Ethephon	4.0	2.0	40.16	94.0	2.3	3.7	45.00	94.0
Neem oil	5.7	4.0	35.27	90.3	3.0	4.7	58.16	92.3
Rizolex-T	1.3	1.3	16.16	97.4	3.0	3.0	20.11	94.0
Control	4.7	4.0	55.63	91.3	4.3	6.3	60.17	89.4
L.S.D at 5%	1.3	1.5	0.9	1.0	1.3	1.2	1.0	1.5

Table (4): Effect of chemical inducers on damping-off of soybean cultivars under natural infested soil field during season 2009.

Treatment	Giza 21				Giza 35			
	Pre-emergence damping-off infection (%)	Post-emergence damping-off infection (%)	Disease severity (%)	Survived plant (%)	Pre-emergence damping-off infection (%)	Post-emergence damping-off infection (%)	Disease severity (%)	Survived plant (%)
Salicylic acid	2.3	3.0	25.3	94.7	2.0	2.0	30.3	96.0
Oxalic acid	2.3	2.7	36.7	95.0	1.3	2.0	43.7	96.7
Potassium phosphate (K_2HPO_4)	3.7	3.3	38.16	93.0	4.7	2.7	45.18	92.6
Ethephon	5.7	4.7	45.3	89.6	5.0	5.0	50.6	90.0
Neem oil	6.7	2.1	50.0	91.2	5.3	3.7	55.16	91.0
Rizolex-T	2.3	1.3	20.6	96.4	0.3	2.3	30.7	97.4
Control	6.3	3.0	60.17	90.7	5.3	6.3	70.7	88.4
L.S.D at 5%	1.2	1.4	0.8	1.5	2.4	1.5	0.1	1.6

Table (5): Effect of chemical inducers on yield characteristics of Giza 21 and Giza 35 soybean cultivars under field conditions during season 2008.

Treatment	Giza 21				Giza 35			
	Plant height (cm)	No. of branches	Yield of 5 plant (g)	100 seeds weight (g)	Plant height (cm)	No. of branches	Yield of 5 plant (g)	100 seeds weight (g)
Salicylic acid	87.07	2.7	265.4	70.00	98.8	3.3	290.37	84.3
Oxalic acid	87.30	3.7	295.4	71.70	96.7	4.0	294.00	90.0
Potassium phosphate (K_2HPO_4)	85.17	2.3	218.6	50.00	95.3	2.7	250.40	80.0
Ethephon	78.20	2.7	194.7	36.70	92.5	2.3	228.77	73.3
Neem oil	76.77	2.3	181.7	45.17	86.7	2.3	210.20	65.0
Rizolex-T	92.00	2.7	265.2	75.00	100.8	3.0	306.83	95.0
Control	70.27	2.0	120.1	40.00	84.0	2.7	195.17	58.3
L.S.D at 5%	9.90	0.93	20.46	7.80	7.97	1.08	19.2	8.90

Table (6): Effect of some inducers on yield characteristics of Giza 21 and Giza 35 soybean cultivars under field conditions during season 2009.

Treatment	Giza 21				Giza 35			
	Plant height (cm)	No. of branches	Yield of 5 plant (g)	100 seeds weight (g)	Plant height (cm)	No. of branches	Yield of 5 plant(g)	100 seeds weight (g)
Salicylic acid	88.3	3.3	68.3	375.10	95.6	2.7	80.5	286.8
Oxalic acid	83.3	5.0	75.2	300.60	98.0	4.3	88.6	318.3
Potassium phosphate (K_2HPO_4)	88.3	3.0	50.3	213.60	90.0	2.3	78.5	241.9
Ethephon	84.7	2.3	41.5	193.27	91.7	3.0	68.9	230.4
Neem oil	73.3	2.0	50.6	155.87	84.3	2.7	63.6	215.4
Rizolex-T	95.0	4.0	80.3	315.00	100.0	4.0	90.3	323.3
Control	71.7	2.0	43.7	106.70	81.7	2.3	55.0	200.2
L.S.D at 5%	9.6	1.3	9.9	12.06	6.17	1.08	9.4	29.6