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ELICITING THE RESISTANCE IN CHICKPEA AGAINST RHIZOCTONIA ROOT- ROT DISEASE

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ABSTRACT

Four chemical substances *i.e*. salicylic acid, oxalic acid, disodium phosphate and ethephon were tested, as seed soaking treatments, to study their effect on reducing the severity of root-rot disease of chickpea plants caused by *Rhizocotnia solani*. In vitro studies showed that the tested chemicals significantly reduced the linear growth of the fungus .

The greenhouse experiments showed that salicylic acid, disodium phosphate and ethephon were effective chemicals in reducing the root-rot disease and significantly increases seedling survival,.A significant increase in the activity of chitinase enzyme was also recorded in plants grown from the treated seeds. Salicylic acid increases the activity of oxidative reductive enzymes (peroxidase and chitinase enzymes) in the plants tissues . In the field experiments salicylic acid proved to be the most effective chemical in reducing the root-rot disease as will as increases the yield components.

Keywords: Induce, peroxidase of resistance, chitinase, root-rot, *Rhizoctonia solani* , chickpea.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the earliest grain crops cultivated by man and has been found in middle eastern archaeological sites dated at 7500 – 6800 BC (Zohary and Hopf, 2000).

It is well established that seed rot, seedling damping-off, root-rot, wilt and collar rot are serious fungal diseases of chickpea. Induced resistance has also been implicated in the control of *Rhizoctonia Solani* on mung bean hypocotyls by the plant growth regulator ethephon (Arora and Bajaj, 1985). In Egypt, this disease complex is caused by different pathogens i.e., *Fusarium oxysporum* f.sp. *ciceri*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Macrophomin phaseolina* (Al-Awadi, 1994 and Omar *et al.* 1999).

Plant disease control in the 21st century faces considerable challenges. There are a continuing problem of pathogen adaptability due to fungicide resistance and breakdown in the effectiveness of host genetic resistance. Also there is a newer problem of slowing down the rate of delivery the new fungicides to market and increasing public concern related to the environmental effects of widespread fungicide uses. There is also a potential problem of climate change and its impact on pathogen spread. It is reported that treating of plants with various agents (e.g., virulent or avirulent pathogens, non-pathogens, cell wall fragments, plant extracts, or synthetic chemicals) lead to induction of resistance to subsequent pathogen attack, both locally and systemically **(**Vallad and Godman, 2004; Walters *et al*, 2005). A number of chemical and biological agents are commercially available as elicitors for use conventional agriculture (Vallad and Godman, 2004). The ultimate aim of this research is to elicit resistance against root-rot disease of chickpea caused by *R. Solani* by using a recommended chemicals by Gamil,1995 that may induce resistance against such pathogen.),

MATERIALS AND METHODS

1- Source of the pathogen:

A pathogenic isolate of *Rhizoctonia solani Sacc.* was selected from the gene bank of the Plant Pathol. Res. Inst. Agric. Res., Center . The isolate was tested to confirm its virulence.

2- Tested Chemicals

Four chemical compounds i.e., salicylic acid, oxalic acid, sodium phosphate and ethephon were used in this investigation while different concentrations of each were applied.

3- Effect of selected chemical on *Rhizoctonia solani* growth, *in vitro*

Salicylic acid, oxalic acid, sodium phosphate and ethephon at different concentrations were tested to study their effect on the mycelial linear growth of *Rhizoctonia solani, in vitro*. Four concentrations of salicylic acid, oxalic acid *i.e*., 1, 3, 5 and 10 Mm, disodium phosphate at 10, 20, 30 and 50 Mm, ethephon 100, 200, 300 and 500 ppm were prepared and added separatelly to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized petri plates (10 cm diameter). Plates were individually inoculated at the center with equal disks (5 mm) of 3 days old culture of *R. solani* and incubated at 25 ^{0}C for 5 days. The average of the fungal linear growth was than recorded.

4- Greenhouse experiment:

Greenhouse experiment was carried out to evaluate the effect of the four chemicals on reducing the incidence of chickpea root-rot. Sterilized pots (25 cm diameter) contaning autoclaved sandy-clay soil were inculated with *Rhizoctonia solani* inoculm previously prepared on grain media (Mazen, 2004). The inoculum was added to autoclaved soil at the rate 3% (w/w) and mixed thoroughly at the upper layer of the soil. The treatments were watered and left for one week to ensure the distribution of the inoculum. Three pots were used for each treatment and three left free of the inoculum to serve as controls.

Salicylic acid, oxalic acid, sodium phosphate and ethephon at the selected concentration were used for seed soaking. Seed samples (cv. Giza 2) were soaked in aqueous solutions of 10 Mm salicylic acid, 10 Mm oxalic acid, 50 Mm disodium phosphate and 500 ppm ethephon for 12 h (Ismail, 2004), before sowing in soil. Five seeds were sown in each pot while three replicates were presented one treatment.

Disease incidence was recorded as a percentage of pre- and post-emergence damping-off as well as healthy survival plants after 15 and 30 days from sowing, respectively. Plants were uprooted 45 days after sowing and the root-rot disease severity was recorded according to Soleman *et al.* (1988).

5- Field application:

The efficacy of the promising chemicals in controlling chickpea seed rot was assessed under field conditions. The experiment was carried out at Sers El-Lyain Research Station, during 2008/2009 and 2009/2010 growing seasons. The experimental plot was designed to be 3.6 m^2 (2 rows each of 3 m long and 60 cm width). Chickpea seeds (cv. Giza 2) were soaked in aqueous solutions of the four tested compounds as previously described in green house experiments. The rows were hollowing out at 10 cm spaced while 2 seeds was seeded in each hollow. When seedlings grow up to 15 height, one plant was left at each hill. All recommended agricultural practices were followed.

Disease incidences were recorded as pre- and post-emergence damping-off 15 and 30 days after sowing respectively. Root-rot incidence was recorded after 75 days of sowing.

Plant growth characters as plant height, number of branches, number of pods per plant, weight of one hundred seed's per plant as well as the average weight of seed / kg / plot, were recorded at harvest

6- Effect of resistance selected chemicals on chitinase activity in chickpea plants:

Fresh tissues of 15 days old chickpea seedlings were collected to study the activity of chitins enzyme in each treatment .Tuzun et al 1986 used this enzyme activity as a marker for the biochemical changes associated with the induced resistance.

Statistical analysis:

The obtained data were subjected to analysis of variance using the fisher L.S.D method. Means were separated by fisher's protected least significant differences (L.S.D) at $p < 0.05$ level (Gomez and Gomes,1984).

RESULTS

Effect of tested chemical on growth of *Rhizoctonia solani*, *in vitro:*

The effect of the tested chemicals on mycelial growth of *Rhizoctonia solani*, the cause of root-rot in chickpea are shown in Table (1). Data indicate that low concentrations of salicylic acid, oxalic acid, disodium phosphate and ethephon slightly reduced the linear growth of *Rhizoctonia solani*. The highest reduction in the linear growth was achieved when salicylic acid at 10 Mm was used to present 92.55% reduction as compared with untreated ones. Oxalic acid, and disodium phosphate at 10 and 50 mM or ethephon at 500 ppm showed a moderate effect on the mycelial growth of the fungus while other treatments had no significant effect.

Table (1): Effect of four selected chemicals on the linear growth of *Rhizoctonia solani*, grown on PDA medium.

Treatment	Concentration	Mycelia linear growth	
		Linear growth (cm)	% inhibition
S.A	1 Mm	6.00	33.33
	3 Mm	3.50	61.11
	5 Mm	2.00	77.77
	10 Mm	0	100.0
O.A	1 Mm	7.60	15.55
	3 Mm	4.50	50.00
	5 Mm	2.67	70.33
	10 Mm	0.67	92.55
Na_2HPO_4	10 Mm	6.30	30.00
	20 Mm	4.00	55.55
	30 Mm	2.00	77.77
	50 Mm	0	100.0
Ethephon	100 ppm	8.00	11.11
	200 ppm	5.00	44.44
	300 ppm	2.67	70.33
	500 ppm	1.00	88.88
Control		9.00	0.00

L.S.D at 0.01 for:
Treatment = 0.18 Concentration = 0.24 A × B = 0.53

Effect of the tested chemicals on root-rot incidence, under greenhouse conditions:

Data in Table (2) show significant effects of salicylic acid, oxalic acid, disodium phosphate and ethephon on reducing the percentages of pre- and post-emergence damping-off caused by *R. solani* compared to the control. However, salicylic acid was the most effective tested chemical on decreasing the percentage of pre-and post-emergence root-rot being 10.0 and 10.0% followed by oxalic acid being 10.00 – 16.67% respectively. On the other hand, disodium phosphate and ethephon showed less effect (16.67 – 13.33% and 16.67 – 13.33%). Salicylic acid enhanced the percentages of plant survival to be (80%) compared with the check treatment (23.33%).However all treatment significantly reduced the disease severity of root-rot symptoms caused by *R. Solani*.

Table (2): Effect of soaking chickpea seeds in the tested chemicals for controlling root-rot under greenhouse conditions.

Treatment	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survival plant (%)	Disease severity
S.A	10.00	10.00	80.00	19.53
O.A	10.00	16.67	73.33	30.00
Na_2HPO_4	16.67	13.33	70.00	23.20
Ethephon	16.67	13.33	70.00	21.00
Check	40.00	36.67	23.33	38.05
L.S.D. at 0.05	9.30	9.24	13.72	7.63

Activity of chitinase enzyme:

Data in Table (3) recorded that treatment of chickpea seeds with the different selected chemicals resulted in an increase in chitinase activity compared to the untreated check. Salicylic acid gave the highest increase(6.53 activity / min) while Na_2 HPO_4 presented the least (4.30 activity / min) compared to other treatments.

Table (3): Determination of chitinase activity (min/activity) in chickpea seedlings tissues grown from seeds previously soaked in the selected chemicals before planting in soil infested with *R. solani* under greenhouse conditions.

Treatment	Concentration	Activity of chitinase / min
S.A	10 Mm	6.53
O.A	10 Mm	5.70
Na_2HPO_4	50 Mm	4.30
Ethophon	500 ppm	5.20
Control	–	2.80

Efficiency of the tested chemicals on reducing the pre and post emergence damping off chickpea under field conditions:

Effect on Root-rot infection

Data in Table (4) clearly show the influence of the tested chemicals on root-rot disease of chickpea under field conditions during two successive seasons. The results revealed that the promising chemicals reduced the pre- and post-emergence damping-off compared to check (control). However, there were visual variations among these chemicals. Salicylic acid gave the best results in decreasing the percentage of pre- and post-emergence root-rot during the two seasons of (2008/2009 – 2009/2010). Disodium phosphate was the least effective chemical in controlling pre- and post-infection.

Table (4): Effect of soaking chickpea seeds in the solution of the tested chemicals on the incidence of root-rot during the two successive seasons under field condition.

Treatment	Season 2008/2009				Season 2009/2010			
	Preemergence damping off (%) *	Postemergence damping off (%)**	Survival (%) ***	Disease Severity ***	Preemergence damping off (%) *	Postemergence damping off (%)**	Survival (%) ***	Disease Severity ***
S.A	0.00	3.33	96.67	6.77	13.33	6.67	90.00	9.66
O.A	6.67	13.33	80.00	10.37	10.00	6.67	80.00	10.67
Na_2HPO_4	13.33	16.67	70.00	12.89	13.33	13.33	73.33	15.55
Ethephon	10.00	10.00	80.00	8.11	10.00	10.00	80.00	12.22
Cheak	23.33	20.00	56.67	15.92	26.67	23.33	50.00	22.22
L.S.D at 0.05	8.44	8.43	13.53	1.02	7.48	10.04	14.79	0.96

*** After 15 days ** After 30 days *** After 75 days**

Effects on yield component:

As for the effect of the tested chemicals on yield component of chickpea under field condition, the obtained data in Table (5) show a marked difference among all treatments and varieties on yield components as well as on root-rot diseases of chickpea including plant height, number of branches, number of pods, 100 seed weight and weight of seed/yield (kg/plot).

Table (5): Effect of soaking chickpea seeds in solutions of the tested chemicals on yield component/plant under field condition.

Treatment	Season 2008/2009					Season 2009/2010				
	Plant height after harvest	No. of branches	No. of Pods	100 seed (w/g)	Yield (kg/plot)	Plant height after harvest	No. of branches	No. of Pods	100 seed (w/g)	Yield (kg/plot)
S.A	97.67	3.67	52.33	31.67	1900	87.67	3.33	48.67	28.00	1850
O.A	90.00	3.00	47.67	28.67	1712	83.67	2.67	44.67	25.00	1670
Na_2HPO_4	84.33	2.00	41.33	26.00	1451	77.67	2.33	38.00	22.00	1393
Ethephon	89.33	2.33	45.00	30.00	1504	80.67	2.67	42.00	25.67	1473
Cheack	81.33	1.67	38.00	21.67	1387	75.00	1.67	34.00	18.67	1137
L.S.D at 0.5	2.12	0.84	2.13	2.20	21.00	2.80	1.03	1.56	1.11	19.73

DISCUSSION

Soil borne disease of chickpea including wilt and root-rot causes a considerable loss in its yield. *Rhizoctonia solani* caused the highest percentage of pre- and post-emergence root-rot (Al-Awadi, 1994 and Omar *et al.,* 1999). In this study induced resistances against root rot were shown when chemicals of antioxidant properties were applied as seed treatment these chemicals also decrease the fungal growth of *R. Solan in vitro*. The greenhouse results indicate that such chemicals reduce the percentage of pre- and post-emergence damping-off.This results are in agreement with the finding of (Kloepper *et al.,* 1992 and Gamil, 1995). In addition, seed soaking treatments in S.A, O.X, Na2HPa4 and ethephon significantly increased the vegetative growth and yield components of the plant.In the finding of Ragab,et al 2009. Chitinase enzymes were obviously higher in plants grown from treated seed compared to the untreated ones.Mona et al 2009 found also that the activities of peroxides, polyphenol oxidase and chitinase enzymes were obviously higher in plants grown from treated seed with these chemicals. Several investigators studied the effectiveness of these chemical on root-rot disease of (Segarra et al., 2006).While these results revealed significant effects of such elicitors on the control of root-rot disease and its severity. In general, the protection of chickpea plants against R.Solani may due to the role of such chemical in eliciting resistance in the plants.

On the other hand , SA, oxalic acid and ethephon were shown to be the more effective as a growth stimuli used in this investigatior .Under field conditions, SA, oxalic acid, ethephon and Na_2HPO_4 treatments significantly reduced pre- and post-emergence damping-off (Segarra *et al.,* 2006). In addition, seed soaking treatments in SA, oxalic acid, ethephon and Na_2HPO_4 significantly increased the vegetative growth parameters and yield components as well.

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الحث على مقاومة الحمص لمرض عفن الجذور الريزوكتونى

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أجريت هذه الدراسة تحت ظروف المختبر والصوبة والحقل بغرض حث نباتات الحمص على مقاومة مرض عفن الجذور المتسبب عن الاصابة بالفطر ريزوكتونيا سولانى، حيث استخدم كل من حمض سلسيلك ، حمض أوكساليك ، فوسفات الصوديوم والأثيفون لاختبار إمكانية استخدامهم في استحداث هذة المقاومة.

وكانت النتائج كالاتى:

1 - فى المختبر : أظهرت جميع المركبات المستخدمة انخفاض نمو الفطر *Rhizocotnia solani* مقارنة بالكنترول

2 – فى تجارب الصوبة : كان لكل من المركبات(حمض السلسيلك وحمض الأوكساليك وفوسفات الصوديوم والأثيفون) تأثير ايجابيا فى مقاومة مرض عفن الجذور حيث انخفضت شدة الاصابة بالمرض وسجل حمض السلسيلك أعلى تأثير فى مقاومة المرض وخفض نسبة الاصابة وبدراسة نشاط أنزيم البيروكسيدز فى أنسجة النباتات المعاملة سجلت معاملات حمض السلسيلك وحمض الأوكساليك وفوسفات الصوديوم والاثيفون أعلى نشاط أنزيمى فى المعاملات المختبرة بما فى ذلك النباتات الغير معاملة وكذلك أظهرت الدراسة أيضا زيادة كبيرة فى نشاط أنزيم الشيتينيز.

3 – فى الحقل : أدت المعاملة بالمواد المستحثة للمقاومة عند نقع البذور لمدة 12 ساعة قبل الزراعة الى انخفاض معدل الاصابة بالمرض وزيادة عدد القرون ووزن البذور ومعدل التفريع وزيادة وزن ال100 بذرة ووزن القطعة التجريبية حيث انعكس ذلك ايجابيا على المحصول .

قام بتحكيم البحث

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