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EFFECT OF SOME PLANT EXTRACTS ON CONTROL OF RHIZOCTONIA DISEASES IN CHICKPEA (*Cicer arietinum* L.) PLANTS

El-Barougy, Ebtehag [1] and Manal Eid[2]

1- Department of Legume Diseases, Plant Pathology Research Institute, Agricultural Research Institute, Egypt

2- Agricultural Botany, Faculty of Agriculture, Suez Canal University 41522, Ismailia, Egypt

ABSTRACT

Chickpea (*Cicer arietinum* L.) is subject to stand injury and yield loss due to several diseases. *Rizhoctonia solani* found to be pathogenic to Chickpea causing damping-off and root-rot. Chemicals are effective in controlling these diseases but, these chemicals are expensive and not friendly to the environment. There are great efforts to reduce environmental pollution by reducing the dependence on agrochemicals to control plant diseases. Plant extracts and resistant chickpea varieties are used between many other means to control some diseases. Some plant extracts such as Rosemary(*Rosemerinus officinalis*) leaves and Colocynth (*Citrullus colocynthis*) fruits inhibited mycelial growth of *Rhizoctonia solani* on PDA medium. The results from this study demonstrated that both plant extracts were effective against *Rhizoctonia.solani* growth. In pots, all tested plant extracts significantly decreased damping-off, and increased healthy plants with corresponding increases of plant height and seed yield weight**.** Reducing sugar decreased whereas, the level of phenols increased in infected plants. In addition to performance of pretreated plants are similar to uninfected control plants, it might be due extracts of both Rosemary and Colocynths which have a role in plants react to pathogen attack by activating an elaborate defense mechanisms. However, the immediate impact of this research is promising in that it provides a safer alternative than synthetic fungicides. Especially, Rosemary extract is often used in food preparations for human consumption; thus there should be a major concern over toxicity.

Keywords; Chickpea cultivar, *Rhizoctonia.solani*, natural products, Rosemary leaves, Colocynth fruits.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) belongs to the leguminous family which has been extensively cultivated for centuries. This ancient crop probably originated in Turkey over 7000 years ago and spread to the Middle East and South Asia and North Africa where it became an important crop (Poplelka *et al*, 2004). Chickpea productivity, however remained virtually stagnant over recent decades because of its intolerance of the insect pests *Helicoverpa* amigera and because of its susceptibility to diseases such root-rot caused by *R solani*.

R solani is a prevalent fungal pathogen in cultivated prairie soils and is an important factor affecting seedling establishment of chickpea. Seed treatment, soil application and foliar spray with systemic fungicides and antibiotics have given effective control of the disease (Kazempour, 2004). However, these treatments are expensive for the small farmer and therefore

the chemicals are not always applied the six or seven times as recommended. In addition, there are environmental concerns regarding the use of chemicals and fears of exacerbating the development of resistance. Methods other than synthehetic chemical use are being investigated as alternative solutions. Developing genetic resistance against this disease is considered the most effective and reliable method for control. However, this method is usually slow in developing varieties for farmer use. Until resistant varieties can be developed and distributed to the farmer, the uses of natural products are a more practical alternative (Baraka *et al.* 2006). Plant extract of Rosemary and Colocynthis have been found to be antibacterial and antifungal (Caroles and Harrison, 2000). The fungicidal activity of Rosemary essential oil was effective in inhibiting the fungal growth of *Fusarium* spp. (Bartynska and Budzikur, Ramza, 2001). Widmer & Laurent (2006) reported that Rosemary and lavender leaf extracts could provide an economically safe methods for reduction damage caused by *Phytophthora* black pod disease on cacao. Zian (2005) found that some plant extracts (Rosemary leaves, Colocynth fruits and others) were effective in inhibiting the fungal growth of *R.solani, Macrophomina phaseolina, Fusarium oxysporum* isolated from lupine diseased plant. Also, he found that Rosemary and Colocynth showed to be the most effective in controlling damping-off, root-rot and wilt fungi.

This paper describes the impact of Rosemary and Colocynth plant extracts on controlling damping-off, and root rot diseases in Chickpea plants. It is also discusses if these natural products were associated with secondary plant metabolites to express resistant to pathogen infection.

MATERIALS AND METHODS

***Rhizoctonia solani* inoculum:**

Isolation of *R. solani* from chickpea:

R.solani was originally isolated on PDA from damped and rotted chickpea plants grown at Ismailia Agric. Res. Station . Purification of the isolated fungus was carried out using hyphal tip techniques and identified according to Barnett and Hunter (1986).

Pathogenicity tests :

Pathogenicity test of the fungus isolated from rotted root (*R.solani*) was tested in infested soil at Ismailia Agric . Res. Station , A.R.E. . Five varieties of chickpea i.e. Giza 1, Giza 2 Giza 195 Giza 531and Giza 88 were used in this experiment.

Preparation of fungal inoculum and soil infestation:

Sterilized sorghum medium (250 g sorghum grains/1000 ml bottle and enough sterilized water to cover the sorghum) was used for preparation of fungal inoculum. The medium was autoclaved then inoculated with the desired fungus and incubated at 25 °C for 15 days. Pots (30 cm in diameter) filled with un-sterilized soil were infested with the fungal inoculum at rate of 3 % (W/W) of soil weight. The infested soil was watered and mixed thoroughly for one week to insure even distribution of the inoculum. Chickpea

seeds were sown at the rate of 10 seeds / pot (30 cm in diameter) A set of four replicates was used for each treatment. Four pots containing non-infested soil were used as control. Percentages of damping off, infested survival and healthy survival plants were calculated 30 and 90 days after planting respectively**.** However, plant height and seed weight were recorded four months after planting.

Disease severity of root rot and any discoloration of tissue were recorded according to the scale proposed by Turner and Van Alfan (1983). 0= none 1= <2% 2= 3-20% 3= 21-50% 4= 51-90% 5= 91-100%.

Effect of some plant extracts on control of Rhizoctonia diseases (damping-of and root-rot) in chickpea plant:

1- *In-vitro* experiments:

Plant extracts :

Laboratory and pot experiments were conducted to study the effect of Rosemary and Colocynth plant extract on *R. solani* isolated from damped and rotted chickpea plants.

Method of extraction :

Extracts of Rosemary leaves (*Rosmarinus officinalis*) , Colocynth fruits (*Citrullus colocynthis*) , Leucaena seeds (*Leucaena leucocephala*) and Alfalfa roots (*Medicago sativa*) were used in this study. The plant portions were completely air – dried under laboratory condition, then grounded to fine powders in a grinder and kept for further tests. A weight of 250 g of each dried sample was soaked with ethyl alcohol (95%) in one liter flask for 48 hours, based on methods described by Meisner *et. al*.(1970) and Freedman *et. al*. (1979) .The flask was shaked for 30 min. by shaker and then filtered . The extract was evaporated under reduced pressure at temperature not exceeding 50°C; this yielded the ethyl alcohol extract as crude gum obtained weighted and re-dissolved in 70 % ethyl alcohol (one gram extract /5 ml ethyl alcohol) to give solution of 20% concentration.

Effect of Rosemary and Colocynth extracts on linear growth of the pathogenic fungus :

In this experiment ,250 ml flasks each contained 100 ml of PDA were prepared , a set of three flasks containing a mixture of plant extract solution of 2% , 5% and 10 % concentrates before plating the medium and one flask of PDA untreated to be a control . The previous method was used with each of the prepared extracts .The tested fungus *R.solani* was inoculated onto the prepared plant extract media in 9 cm diameter Petri-dishes, using 5 mm discs of active growing culture as inoculum . Incubation was carried out at 25°c, and the growth was allowed to continue for complete growth in the control dishes. The effect of plant extracts on the linear growth of the tested fungus was measured by determining two dimensions and the mean was calculated. The effect was recorded photographically.

II- Pots experiment :

Effect of Rosemary and Colocynth plant extracts on seedling survival of chickpea grown in infested *Rhizoctonia solani* soil :

Chickpea Seeds (Giza 2) were soaked in the solution of each extract for 8 h. at the rate of 10 %, 5 % and 2 % concentrations . The soaked seeds were left to dry in the air for 2 hours before sowing in the potted infested soil with the pathogenic fungus. This experiment was conducted in 30 cm diameter clay pots. Seeds were sown at the rate of 5 seeds / pot .A set of four replicates was used for each treatment.

There were 8 treatments as follows:

1-Infested soil+ Rosemary leaves extract (soaked seeds at 10 %).
2- Infested soil+ Rosemary leaves extract (soaked seeds at 5 %) .
3- Infested soil+ Rosemary leaves extract (soaked seeds at 2 %) .
4- Infested soil+ Colocynth fruits extract (soaked seeds at 10 %).
5- Infested soil+ Colocynth fruits extract (soaked seeds at 5 %) .
6- Infested soil+ Colocynth fruits extract (soaked seeds at 2 %) .
7- Infested soil, soaked seeds in water (Control 1).
8-Non-infested soil, soaked seeds in water (Control 2).

Five pots were used per treatment, five soaked seeds representing each extract were sown in each pot.

Cultural practices were done as usual. Percentage of plant survival was recorded 2 months after seeding and plants were harvested at 3 months old. Plant growth parameters (plant height, seeds weight) were recorded. However, samples were taken for chemical analysis 35 days after sowing.

Chemical analysis:

Chickpea leaves are collected from seedlings for all treatments with both plant extracts to determine reducing sugar and phenol contents. Reducing sugar content was measured according to Nelson's method (Moore 1974), while phenol content was determined by a modified Folin-Ciocalteu method (William *et. al*., 1965)

RESULTS

Isolation and identification of the causal pathogen and pathogencity:

Isolation trails from rooted plants collected from different localities of Ismailia Governorate yielded a fungus which was identified as *R solani* in seedling stage, infested plants may be seen with damping off symptoms. In adult stage, dark-cankers form near the base of the stem. This fungus also causes root rot then the plant rots and wilts.

Cultivars reaction:

Disease symptoms attributed to *R. solani* were observed on plants grown in soil artificially infested with the tested fungus in pot experiment. Symptoms were almost similar to those noticed under the field conditions; the fungus produces damping off and root rot symptoms of the plant development.

Data presented in Table (1) revealed that all the tested chickpea cultivars were susceptible to infection with *R. solani* at different degrees. Giza- 2 was the highly susceptible cultivar as it recorded the highest percentage of damping off (50%) followed by Giza-88 (33.3%), Giza-195 (26.7%), Giza-531 (23.3%) and Giza- 1(13.2%). Significant differences were observed between cultivars. For invested survival, it is obvious that Giza-2 recorded the highest percentage reached 33.4% followed by Giza-351 ,Giza-1, Giza-195 and Giza-88 which showed 33.3%.,33.2%, 30% and 26.7% , respectively.

Concerning healthy plants percentage, Giza-2 was the most susceptible cultivar to *R solani* (26.7%) followed by Giza-88 (40%) and both of Giza531 and Giza 195 (45% and 55% respectively).. Disease severity was also recorded in Table (1) the lowest degree was obtained from Giza 531cultivar (2.7) but the highest one (4.7) was observed on Giza-2

Table (1): Susceptibility of chickpea cultivars to *Rhizoctonia* solani

Cultivar	*R solani*			
	Damping off	Survived plants		Diseases severity scorr **
		Infested	healthy	
Giza531	23.3	33.3	43.4	2.7
Giza-195	26.7	30	43.3	4.3
Giza-1	13.2	33.2	56.6	4
Giza-2	50	33.4	26.6	4.7
Giza-88	33.3	26.7	40	4.3
L.S.D	13.34		11.4	

**** the scale proposed by Turner and Van Alfan(1983)**

Effect of some plant extracts on the control of Rhizoctonia diseases in chickpea plants:

1- Laboratory experiment

Effect of plant extracts on linear growth of *R. solani* :

Two plant extracts namely rosemary leaves and colocynth fruit with three concentrations (i.e. 2 , 5 and 10 % of plant extract solution) were added to PDA medium to study their effect on the growth of the pathogenic fungus

Data presented in Table (2) indicate that rosemary extract at all concentrations gave the highest effect on linear growth of the pathogenic fungus , as it showed at 2% , 5% and 10% concentrations , the lowest values of linear growth were (47, 35 and 11 mm respectively) comparing with colocynth(72 , 60 and 15 mm) .

Table (2): Effect of plant extracts on linear growth of *R. solani* :

Treatments	Linear growth of *R.solani* (mm)		
Concentrations	2 %	5 %	10 %
Rosemary(leaves)	47	35	11
Colocynth(fruit)	72	60	15
Control	90	90	90

Data showed also that the increasing of concentration for both of the two plant extracts decreased the growth of the tested fungus, this mean that the concentration 10 % has the highest effect for all plant extracts.

2-Pots experiment:

Effect of three concentrations of either rosemary or colocynthis extracts on damping –off and survived chickpea seedlings grown in infested soil with *R.solanii*:

Plant extracts (Rosemary) leaves and (Colocynthis) fruits with three concentrations, i.e. 2, 5 and 10 % of plant extract solution) reduced significantly *R. solani* infection Table (3). Percentage of healthy chickpea survival plants increased using plant extracts over the control (Infested soil).On the other hand; differences in the effect of plant extract concentration on the tested fungal pathogen were noted.

Data in Table(3) indicate that soil infested with *R solani* used as a control show the lowest percentage of healthy survived plants (8%) compared with the highest survival percentage (92%) recorded from non-infested soil (control-2). Significant differences were realized between control-1 (infested soil) and other treatments, the most effective treatments were obtained from rosemary and colocynthis plants at 5% concentration, all of them recorded 88 % of healthy plants followed by 84%, 80% recorded from Rosemary and colocynthis at 2% concentration respectively ,while 72% and 64% obtained from Colocynthis and rosemary at 10% concentration respectively.

Table (3): Effect of three concentrations of rosemary and colocynthis extracts on damping –off and survival of chickpea seedlings grown in infested soil with *R. solani* .

Plant extracts	Concentrations	Damping -off	Survived plants		Disease severity scale
			Infested	Healthy	
Rosemary	2%	12	4	84	1.4
	5%	8	4	88	1.2
	10%	16	20	64	1.6
Colocynthis	2%	12	8	80	1.1
	5%	12	0	88	1
	10%	20	8	72	1.1
Infested soil (Control1 **)**	-	48	44	8	3.8
Non-infested soil (Control 2)	-	8	0	92	-
L.S.D.	-			20.3	0.6

Data presented in Table (4) reveal that the infection with *R.solani* was reflected in plant height and seed yield of chickpea plants. The highest plant high (36.4 cm) was obtained from colocynthis at 5% Concentration followed by 35.12, 34.9, 33.18, 32.5 and 30.9 cm) which recorded from treatment of Rsemany at 5%, colocynthis at% 10 , colocynthis at% 2, Rsemary at 10% and Rsemary at 2% concentration respectively) but sill significantly less than recorded in control2(35.9cm). Meanwhile, control recorded the lowest plant height (27.2cm) as compared with other treatments. The same trend was noticed with seed yield. The most effective was colocynthis treatment at 5% (12.7) compared with 3.5 g/5 plants which obtained from control1 (infested soil).

Table (4): Effect of three concentrations of either rosemary or colocynthis extracts on some plant parameters (shoot height and seed weight).

Plant extracts	Concentrations	Plant height (cm.)	Seed yield weight g / 5 plants
Rosemary	2%	30.9 ab	8.6 b
	5%	35.12 a	10.9 ab
	10%	32.5 a	9.6 ab
Colocynthis	2%	33.18 a	10.06 ab
	5%	36.4 a	12.7 ab
	10%	34.9 a	10.8 ab
Infested soil (Control1 **)**	-	27.2 b	3.5 c
Non-infested soil (Control 2)	-	35.9 a	13.1 a

Figures in the same column followed by the same letters are not significantly different (p> 0.05) based on Duncan's multiple range test.

Data in Table (5) revealed that the highest total phenol content (8.86 /100gD.W) was produced in leaf tissue of plant, seeded in infected soil with *R.solani* (control-1) comparing with the lowest amount (2.29//100gD.W) recorded from leaf tissue of chickpea, seeded in non-infested soil (control- 2) Leaf tissue of plant pre-treated with Colocynthis at 10 % concentration before seeded in infested soil with *R.solani* produced 2.4//100g D.W followed by 2.93, 2.98 at 5% and 2.% concentration ,while 3.68 ,4.39 //100gD.W were obtained at 2% and 5% concentrations of Rosemary leaves extract

Table (5): Effect of three concentrations of either rosemary or colocynthis extracts on reducing sugar, total phenols.

Plant extracts	Concentrations%	Sugar reduction%	total Phenols%
Rosemary	2%	8.3	3.68
	5%	7.4	4.39
	10%	12.9	9.5
Colocynthis	2%	6.6	2.98
	5%	4.5	2.93
	10%	4.12	2.4
Infested soil (Control1 **)**	-	4.05	8.86
Non-infested soil (Control 2)	-	11.4	2.29

Data also showed that the highest amount of reducing sugar (11.4/100gD.W) was present in leaf tissue of chickpea seeds ,planted in non-infested soil, comparing with the lowest amount (4.05/ 100gD.W) recorded from leaf tissue of chickpea, seeded in infested soil. Three concentrations of both plant extracts caused a decrease in reducing sugar in leaf tissue of chickpea, seeded in infested soil.

DISCUSSION

Isolation trails from rotted chickpea plants yielded *R solani* conforming to other reports (Hassanein *et. al.,* 1996; Abou-Zeid *et.al.,* 1997; Demirci *et.al.,* 1998; and Pande *et.al.,* 2006).They reported that *R.solani* is one of the most important pathogens of chickpea. Pathogenicity test was conducted and led to symptoms which were almost similar to those noticed under field conditions. The present investigation demonstrated that the isolated fungus from naturally infested field could reduce seedling emergence and healthy plants and could directly affect yield and yield components.

The results from this study demonstrated that both plant extract displayed inhibition towards *R solani* to chickpea seedlings to some degree.

Under the condition preparing an extract concerning a concentration of 5 % was effective in inhibiting the pathogen although at 2 % concentration also reduced infection. In this study, the rosemary extract proved to be the most effective in inhibiting *R.solani*. This is probably an effect of the responsible compound within the extract. Results of this study are similar to those reported by Zian (2005) he found that rosemary extract inhibited the linear growth of *R.Solani* on PDA in Petri dishes .The same trend was noticed with those mentioned by Timothy and Laurent, 2006 ,they reported that Rosemary extract was found to be effective in reducing germination of *Phytophthora capsici*, *. Phytophthora megakarya*, and. *Phytophthora palmivora* zoospores when supplemented to agar plates at different dilutions.

Plant extracts (Rosemary, Colocynthis) decreased significantly damping-off as well as infested survived plants caused by *R. solani* and increased Plant height and seed yield over the control , these findings are in agreement with those recorded by(Baraka *et al*.,2006) who mentioned that extracts of Rosemary leaves and Colocynth fruits decreased disease incidence caused by *R solani* in Lupine plants .The mechanism by which plant extract affections fungal growth may be attributed to the presence of some effective substances in their chemical structure such as rosmic acid, rosmanol, rosmarinic acid and oleoresin as in Rosemary leaves or albuminoids colocynth in, colocynth fruits extract. James, Duke, 1983; Oleeszok *et al.*, 1990; Omar and Aly, 1996 and Moreno *et al.,* 2006 found that Rosmarinic acid and Carnosic acid may be the main bioactive antimicrobial compounds present in rosemary extracts.

The decrease in reducing sugar in infected plants is expected due to deficiency in photosynthetic pigments, the magnitude of which has been reported to be directly proportional to the rate of photosynthesis Ghosh, et al; 2003.Whereas, the rise in the level of phenols in infected plants may be due to their release from cell wall structures during their destruction (Mondavia *et al*, 1997). However, results of this study are similar to those recorded by (Ghosh,*et al* 2003) who found remarkable reduce in sugar content but phenol content increased significantly in the leaves of disease affected mulberry as compared to those in healthy mulberry. Conversely Shetty(1983)found few healthy leaves in infected plants may be to due to

higher concentrations of phonemics in these leaves as one of the internal factors inhibiting downy mildew infection.

According to Mondavia *et al*, (1997) and Colpas *et a*l (2003) phenol compounds are related to defense against pathogen and known to impart resistance to fungal disease. However, this hypothesis remains unproven because the chemical analysis was conducted long after resistance was expressed. The known resistance to biotic stress is often based on major genes and therefore one gene products. So the role of phenols would presumably come later (Bajaj *et al* 1983).

Consider the results obtained for infected plants, it may be concluded that due to change of reducing sugar and phenol in infected leaves, the metabolic process of infected plant is altered as compared to healthy and pretreated ones which leads to cause reduction of plant height and seed yield.

Performance of pretreated plants are similar to uninfected control plants, it might be due extracts of both Rosemary and Colocynthis have a role in plants react to pathogen attack by activating an elaborate defense mechanisms. Either, the defense mechanism is local or systemically. May be the development of lesions restricts pathogen growth .It could be the induction of a diverse group defense-related gene and several species of pathogenesis related proteins (Katz *et al* 1998.Cultured parsley cell are reported to provide a suitable system to induce resistance against both fungal and viral diseases Siegrist *et al* (1998).

In view of the fact that breeding chickpeas for resistance to soil –borne fungi inducing wilt and root diseases might be hampered by pathogenic variability in the pathogens, the complex nature of genetics of resistance in the host, and the non availability of suitable resistance screening techniques. Consequently, the impact of this research could be significant in terms of two future research areas in an intergretated control system of fungal diseases of chickpea. First, the concentration of rosmasrinic acid and albuminoids colocynth within chickpea tissue could be used as an indicator for breeding to biotic resistance.

The second area of research would be to investigate the possibility for transgenic. If a correlation between these natural products and biotic resistance was determined, then chickpea plants could be genetically modified to have this in plant at high enough concentration to control these diseases. Another approach would be to insert the genes responsible for rosmatic production into a microorganism, so that it will exogenously produced the chemical and organism could be used as biological control agent. This strategy would have several advantages over applying a plant extract if it becomes ethically acceptable. The cost of application would be reduced significantly since theoretically, the organisms would only need to be applied one time whereas, the plant extract would mostly likely need to be applied as often as chemical fungicides.

However, the immediate impact of this research is promising in that it provides a safer alternative than synthetic fungicides, especially, rosemary extract is often used in food preparation for human consumption; thus there should be a major concern over toxicity. Of-course, rosmarinic acid and

Carnosic acid are chemical and over time, some degree of resistance could be expeteced as is observed with synthetic fungicides Moreno *et al.,* (2006). Despite all of the work that still needs to be done, this study provides these natural plant extract which could give the local farmer a safe alternative for some immediate control until resistant varieties are developed and become commercially available.

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تأثير بعض مستخلصات النباتات فى مقاومة امراض موت البادرات و عفن الجذور المتسببة عن فطر الريزوكوتنيا فى نباتات الحمص

ابتهاج الباروجى[١] و منال عيد [٢]

١- قسم أمراض المحاصيل البقولية - معهد بحوث أمراض النباتات- مركز البحوث الزراعية- مصر

٢- قسم النبات الزراعى- كلية الزراعة بالإسماعيلية – جامعة قناة السويس- مصر

اجرى هذا البحث فى محطة البحوث الزراعية بالاسماعيلية عام ٢٠٠٦ لدراسة تأثير استخدام مستخلصات اوراق نباتات حصى اللبان و ثمار الحنظل على مقامة امراض موت البادرات و عفن الجذور المتسببة عن فطر الريزوكوتينيا سولانى فى نباتات الحمص و تاثير ذلك على نسبة الفينولات و السكريات المختزلة.

و قد اوضحت النتائج ان استخدام مستخلصات اوراق نباتات حصى اللبان و ثمار الحنظل ادى الى خفض معنوى فى نسبة موت البادرات و عفن الجذور فى النباتات النامية فى تربة محقونة مسبقا بفطر الريزوكوتينيا سولانى. كما لوحظ ان استخدام هذه المستخلصات ادت الى خفض نسبة الفينولات و زيادة نسبة السكريات المختزلة فى النباتات النامية فى التربة المحقونة بالفطر مقارنة بالنباتات النامية فى تربة غير معاملة بالفطر (الكنترول).