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Seed Treatment With Chitosan and Ethanol-Extracted *Propolis* for Suppression Bean Root Rot Disease Under Greenhouse Conditions

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Abstract

Root rot disease caused by *Fusarium solani*, *Sclerotium rolfsii* and *Rhizoctonia solani* is a serious and persistent disease problem of bean plants during growing season. The effects of ethanol-extracted propolis (EEP) and chitosan as seed treatments on bean root rot disease under greenhouse conditions was evaluated. Complete inhibition of linear growth was obtained with chitosan at 8.0 g / L concentration. The heights increase of inhibition zone area was obtained with EEP at 15.0 %. Greenhouse experiments revealed that all tested concentrations of chitosan and/or EEP significantly reduced the bean root rot disease caused by *R. solani* and *S. rolfsii*. The highest reduction in root rot disease incidence was obtained with chitosan at 8.0 g /L and EEP at 15 % which showed highly significantly reduction in both root rot disease incidence and disease severity. Other treatments showed moderate effect.

1. Introduction

Bean plants (*Phaseolus vulgarus* L.) is one of the most important leguminous crops in Egypt. Root rot disease caused by *Fusarium solani Sclerotium rolfsii* and *Rhizoctonia solani* is a serious and persistent disease problem of bean plants during growing season [1-3].

Controlling this disease mainly depends on fungicidal treatments. Therefore, there are needed to alternative fungicidal treatments for controlling plant diseases[4-7]. Among the possible usable materials are extracts of propolis, as they have a high potential to possess antimicrobial, antifungal, antioxidant, antiviral and antiprotozoal activity[8]. Propolis is a naturally occurring brownish-green resinous product that honeybees collect from different plant exudates. It possesses many biological properties, including antibacterial, antiviral, and antifungal, and has been used for pharmacological applications [9-11].

Although its antimicrobial activity against human pathogenic fungi, bacteria and viruses has been demonstrated, [12,13]. In vitro and in vivo few studies have been conducted against plant pathogenic microorganisms [14-16]. The application of ethanol-extracted propolis (EEP) inhibited the growth of *P. digitatum* in vitro[17,18] and limited the growth of *B. cinerea* on strawberry [19]. Chitosan (Ch) deacetylated chitin, is currently obtained from the outer shell of crustaceans such as crabs, krills and shrimps. Chitosan exhibits a variety of antimicrobial activities [20-22], which depend on the type of chitosan (native or modified), its degree of polymerization, the host, the chemical and/or nutrient composition of the substrates, and environmental conditions. In some studies, oligomeric chitosans have been reported to exhibit a better antifungal activity than larger units[20]. In others, the antimicrobial activity increased with the increasing chitosan molecular weight , and seems to be faster on fungi and algae than on bacteria [23].

The purpose of the present study is to evaluate the effects of ethanol-extracted propolis (EEP) and chitosan as seed treatments on bean root rot disease incidence under greenhouse conditions.

2. Material and Methods

2.1.Source of pathogenic fungi and bean seeds

Pathogenic fungal isolates , *i.e. Rhizoctonia solani* and *Sclerotium rolfsii* as the causal agents of bean root rot disease were kindly obtained from Plant Pathology Dept., National Research Centre, Giza, Egypt.

Meanwhile, Bean seeds cv. Giza 3 were obtained from Vegetable Crops Research Dept., Agricultural Research Centre, Giza, Egypt.

2.2.Testing of the different concentrations of ethanol extracted propolis (EEP) on inhibition zone (mm) of R. solani and S. rolfsii growth

Ethanol extracted propolis (EEP) at four concentrations *i.e* . 0.0 , 5.0, 10.0 and 15.0 % were tested to study their effect on inhibition zone of *R. solani* and *S. rolfsii* growth . Sterilized filter paper disks were used as carrier material for testing EEP . Disks (10- mm diameter) were dipped in tested concentrations of EEP , Ethanol or sterilized water then air dried and transferred to Petri plates containing PDA medium inoculated with mycelial suspension (10^6 cfu / mL) of *R. solani* and *S. rolfsii* . Inoculated plates were incubated at 25°C for 5 days and diameter of inhibition zone (mm) was measured.

2.3.Testing of chitosan solutions on the linear growth of pathogenic fungi in vitro

The inhibitory effect of chitosan solutions at five concentrations, i.e. 0, 2, 4 , 6 and 8 g /L. against linear growth of bean root rot fungi was evaluated . Chitosan solutions were added to conical flasks containing sterilized PDA medium before its solidifying to obtain the previous concentrations and rotated gently then disbanded into sterilized Petri-plates (9 cm diameter). Plates were individually inoculated at the centre with equal disks (6- mm diameter) taken from 10 days old cultures of each R. solani and S. rolfsii then incubated at 25±2°C. Linear growth of tested fungi was measured, when the control plates reached full growth and the average growth diameter was calculated. Each treatment was represented by 5 plates as replicates.

2.4. *Greenhouse experiments*

2.4.1. Effect of different concentrations of chitosan and ethanol -extracted propolis (EEP)on bean root rot disease under greenhouse conditions

2.4.2.Preparation of fungal inocula

Inocula of *R. solani* and/*or S. rolfsii* were prepared by culturing each fungus on 50.0 mL potato dextrose broth (PDB) medium in 250 mL Erlenmeyer flasks for 15 days at 25° C. and inocula of *R. solani* and *S. rolfsii* were prepared from the growing upper solid layers which washed and air-dried with sterilized filter paper layers. The air-dry mycelium was blended in distilled water to obtain inocula pieces of 1-2 mm in diameter. Soil infestation was carried out at rate of 2.0 g dry mycelium / kg soil [24].

2.4.3. Soil infestation

Sandy -loamy soil was autoclaved at 120°C for 60 min. Plastic pots (30 cm diameter, 5.0 kg soil) containing sterilized sandy -loamy soil were artificially infested individually with the inoculum of each fungus as mentioned above . Eight pots were used as replicates for each treatment. Disinfected rootstock bean seeds cv. Giza 3 , were sown individually at the rate of 8 seeds / pot.

2.4.4. Seed Treatment with chitosan and ethanol extracted propolis (EEP)

chitosan solutions at five concentrations, *i.e.* 0, 2, 4 , 6 and 8 g /L. In addition to ethanol extracted propolis (EEP) at four concentrations *i.e* . 0.0 , 5.0, 10.0 and 15.0 % were tested as seed treatments to study their effect on bean root rot caused by *R. solani* and *S. rolfsii*. Seeds were primed with different prepared solutions of Chitosan and ethanol extracted propolis.

2.4.5. Assessment of Bean root rot disease

Root rot disease incidence was expersed as percentages of pre-and post emergence stages as follow : Pre-emergence stage : Percent of diseased plants was recorded after 15 days of sowing. Post – emergence stage : Percent of diseased plants was recorded after 40 days of sowing ,meanwhile disease severity was determined according[25] and modified as follow:- 0= Healthy roots, 1= 25 % or less, 2= 26 to 50, 3= 51 to 75, 4= 76 to 100 % infected roots.

2.4.6. Statistical analysis

Tukey test for multiple comparisons among means was utilized [26].

3. Results and discussion

3.1. Effect of different concentrations of ethanol extracted propolis (EEP) on inhibition zone (mm) of R. solani and S. rolfsii growth

Ethanol extracted propolis (EEP) at four concentrations *i.e* . 0.0 , 5.0, 10.0 and 15.0 % were tested to study their effect on inhibition zone of *R. solani* and *S. rolfsii* growth . Results in Table (1) indicate that

the height increase in inhibition zone area was obtained with EEP at 15.0 % which recorded 3.2 and 3.0 mm for. *R. solani* and *S. rolfsii respectively*. While EEP at 10.0 % recorded 2.1 and 1.9 mm as inhibition zone area for *R. solani* and *S. rolfsii respectively*. EEP at 5.0 % was less effective.
Propolis is a naturally occurring brownish-green resinous product that honeybees collect from different plant exudates. It possesses many biological properties, including antibacterial, antiviral, and antifungal, and has been used for pharmacological applications [9-11].

Table 1: Effect of different concentrations of EEP on inhibition zone of bean root rot fungi

Ethanol extracted propolis (%)	Zone of inhibition growth (mm)	
	R. solani	*S. rolfsii*
5.0	1.5 c	1.3 c
10.0	2.1 b	1.9 b
15.0	3.2 a	3.0 a
Ethanol	0.0 d	0.0 d
Water	0.0 d	0.0 d

Figures with the same litter are not significantly different (P =0.05)

3.2. Effect of chitosan solutions on the linear growth of R. solani and S. rolfsii in vitro
The inhibitory effect of chitosan solutions at five concentrations, *i.e.* 0, 2, 4 , 6 and 8 g /L. against linear growth of bean root rot fungi was evaluated . Results in Table (2) reveal that All tested concentrations significantly reduced the linear growth of *R. solani* and *S. rolfsii*. Complete inhibition in linear growth was obtained with chitosan at concentration of 8.0 g / L. The highest reduction was achieved with chitosan at 6.0 g /L. which reduced the linear growth by 88.7 and 87.2 % for *R. solani* and *S. rolfsii respectively.* Meanwhile, chitosan at 4.0 g/ L showed moderate effect.

Table 2: Linear growth of bean root rot fungi as affected with different concentrations of chitosan.

Treatment	Conc.	*R. solani*		*S. rolfsii*	
		Linear growth (mm)	Reduction %	Linear growth (mm)	Reduction %
Chitosan (g/ L)	2.0	48.4 b	46.2	52.0 b	42.2
	4.0	21.4 c	76.2	25.0 c	72.2
	6.0	10.2 d	88.7	11.5 d	87.2
	8.0	0.0 e	100.0	0.0 e	1.00
Control	0.0	90.0 a	0.0	90.0 a	0.0

Figures with the same litter are not significantly different (P =0.05)

3.3. Greenhouse experiments
3.3.1. Effect of different concentrations of chitosan and ethanol -extracted propolis (EEP) on bean root rot disease under greenhouse conditions
Chitosan solutions at five concentrations, *i.e.* 0, 2, 4 , 6 and 8 g /L. In addition to ethanol extracted propolis (EEP) at four concentrations *i.e* . 0.0 , 5.0, 10.0 and 15.0 % were tested as seed treatments to study their effect on bean root rot disease caused by *R. solani* and *S. rolfsii*.

3.3.2. Effect on bean root rot disease incidence caused by Rhizoctonia solani
Results in Table (3) reveal that all tested concentrations of chitosan and EEP significantly reduced the bean root rot disease caused by *R. solani.* The highest reduction in disease incidence was obtained with chitosan at 8.0 g /L and EEP at 15 % which reduced the disease incidence by 78.7 & 75.9 and 75.2 & 74.1 % for pre and post emergence respectively. Followed by chitosan at 6.0 g /L and EEP at 10 % which reduced the disease incidence more than 75.1 and 51.7 % for pre and post emergence respectively. Other concentrations were less effective.
The application of 5% and 10% concentrations of EPP extended the storage life of Fremont mandarins, as compared to untreated control fruits[27]. Treatment with EEP was also effective in preventing fungal decay in cherries stored for 4 weeks, but adversely affected sensory quality and stem color[28]. Moreover, [29] reported that the 8% concentration of EEP controlled powdery mildew disease severity by 31.33 and 43.68% for cucumber and soybean cops, respectively. The application of the concentrations 24 hours before and 24

hours after pathogen inoculation showed less severity, but this difference was not significant for either crop. The EEP induced increasing phytoalexin levels in soybean cotyledons as the applied EEP concentration increased. Ethanol extracts of propolis could have potential for the control of powdery mildew, principally in cucumbers, through the preventive application of an 8% concentration .The application of ethanol-extracted propolis (EEP) inhibited *P. digitatum* growth in vitro[17,18] and limited the growth of *B. cinerea* on strawberry [19].

Table 3: Bean root rot disease incidence caused by *Rhizoctonia solani* as affected different concentrations of chitosan and ethanol -extracted propolis (EEP) under greenhouse conditions.

Treatment		Root rot caused by *Rhizoctonia solani* %			
		Pre-emergence		Post-emergence	
		Disease incidence	Reduction %	Disease incidence	Reduction %
Chitosan (g/ L)	2.0	38.5 c	31.7	41.0 b	29.3
	4.0	32.0 dc	43.3	30.0 d	48.3
	6.0	21.0 d	62.8	22.0 e	62.1
	8.0	12.0 e	78.7	14.2 f	75.9
EEP (%)	1.0	50.4 b	11.3	44.0 b	24.1
	5.0	36.2 c	35.8	36.0 c	37.9
	10.0	24.2 d	57.1	28.0 d	51.7
	15.0	14.0 e	75.2	15.0 f	74.1
Control	0.0	56.4 a	0.0	58.0 a	0.0

Figures with the same litter are not significantly different (P =0.05)

3.3.3.Effect on bean root rot disease incidence caused by Sclerotium rolfsii

Results in Table (4) reveal that all tested concentrations of chitosan and EEP significantly reduced the bean root rot disease caused by *S. rolfssi* . The highest reduction in disease incidence was obtained with chitosan at 8.0 g /L and EEP at 15 % which reduced the disease incidence by 74.4 & 73.3 and 69.1 & 68.3 % for pre and post emergence respectively. Followed by chitosan at 6.0 g /L which reduced the disease incidence by 60.0 and 55.0 % for pre and post emergence respectively. Other concentrations were less effective.

3.3.4.Effect on bean root rot disease severity after 40 days of sowing

Results in Table (5) indicate that all tested concentrations of chitosan and EEP significantly reduced the disease severity. The highest reduction in disease incidence was obtained with chitosan at 8.0 g /L and EEP at 15 % which reduced the disease severity more than 71.4 % for each by *R. solani* and *S. rolfsii* . Followed by chitosan at 6.0 g /L and EEP at 10 % which reduced the disease severity by 57.1 and 71.4 % for *R. solani* and *S. rolfsii* respectively. Other treatments showed moderate effect. Chitosan exhibits a variety of antimicrobial activities [20,21,22] . The mechanism by which chitosan affects the growth of several pathogenic fungi has not been fully elucidated, but several hypotheses have been postulated , first: its polycationic nature, it is believed that chitosan interferes with negatively charged residues of macromolecules exposed on the fungal cell surface. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents (30). Second the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of mRNA and protein synthesis(31), third the chelating of metals, spore elements and essential nutrients[32]. Forth : the interaction of chitosan with fungal DNA and RNA [33]. Five : Malformation of fungal mycelial . Chitosan is not only effective in inhibition the growth of the pathogen fungi, but also induces marked morphological changes, structural alterations and molecular disorganization of fungal cells [34,35]. Moreover, [30] reported that, chitosan caused morphological changes such as large vesicles or empty cells devoid of cytoplasm in the mycelium of *B. cinerea*. Furthermore, (36) revealed that by microscopic observation of fungi treated with chitosan, it can affect the morphology of the hyphae. Moreover, [37] reported that all tested concentrations of chitosan significantly reduced the wilt disease incidence of watermelon plants. The highest reduction in disease incidence was obtained with soil treatment with chitosan at concentrations of 6 and 8 g / kg soil which reduced the percentage of diseases plants with all tested cultivars. In addition to reduced the population of pathogenic fungus in soil and root rhizosphere. Chitosan treatment caused the highest increase in enzyme activities which increased the peroxidase, chitinase and β-1,3 - glucanase.

Table 4: Bean root rot disease incidence caused by *Sclerotium rolfsii* as affected different concentrations of chitosan and ethanol -extracted propolis (EEP) under greenhouse conditions.

Treatment		Root rot caused by *Sclerotium rolfsii* %			
		Pre-emergence		Post-emergence	
		Disease incidence	Reduction %	Disease incidence	Reduction %
Chitosan (g/ L)	2.0	44.0 b	20.0	47.0 b	21.7
	4.0	31.0 c	43.6	38.0 c	36.7
	6.0	22.0 d	60.0	27.0 d	55.0
	8.0	14.0 e	74.5	16.0 e	73.3
EEP (%)	1.0	51.0 a	7.3	53.0 b	11.7
	5.0	38.0 b	30.9	37.0 c	38.3
	10.0	28.0 c	49.1	31.0 c	48.3
	15.0	17.0 e	69.1	19.0 e	68.3
Control	0.0	55.0 a	0.0	60.0 a	0.0

Figures with the same litter are not significantly different (P =0.05)

Table 5: Bean root rot disease severity after 40 days of sowing as affected different concentrations of chitosan and ethanol -extracted propolis (EEP) under greenhouse conditions.

Treatment		*Rhizoctonia solani*		*Sclerotium rolfsii*	
		Disease severity	Reduction %	Disease severity	Reduction %
Chitosan (g/ L)	2.0	0.4 bc	42.6	0.5 b	28.6
	4.0	0.4 bc	42.6	0.2de	71.4
	6.0	0.3 cd	57.1	0.2de	71.4
	8.0	0.2 de	71.4	0.1ef	85.7
EEP (%)	1.0	0.5 b	28.6	0.4 bc	42.6
	5.0	0.5 b	28.6	0.3 cd	57.1
	10.0	0.3 cd	57.1	0.2 de	71.4
	15.0	0.2 de	71.4	0.2 de	71.4
Control	0.0	0.7 a	0.0	0.7 a	0.0

Figures with the same litter are not significantly different (P=0.05)

Conclusions

The present study shows that complete inhibition in linear growth was obtained with chitosan at concentration of 8.0 g / L. Under greenhouse experiments revealed that all tested concentrations of chitosan and EEP significantly reduced the bean root rot disease caused by *R. solani* and *S. rolfsii.* The highest reduction in disease incidence was obtained with chitosan at 8.0 g /L and EEP at 15 % which reduced the disease incidence and disease severity.

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References

1. R.M. Harveson, J. Smit, W.W. Stroup, *Plant Disease* 89 (2005) 279-184.
2. K.Wen, P. Seguin, M.S. Arnaud, S. Hare, *Phytopathology* 95 (2005) 345-353.
3. N.S. El-Mougy, F. Abd-El-Kareem, M. M. Abdel-Kader, Y.O. Fotouh, *Plant Pathology & Quarantine* 41 (2013) 42-52.
4. F. Abd-El-Kareem, W. M. Haggag, *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 5 (2014) 941-945.
5. F. Abd-El-Kareem, W. M. Haggag*, Pvt. Ltd. New Delhi, India* (2015) 126-138.
6. M. A. El-Naggar, H. Abouleid, F. Abdel-Kareem, H. M. El-Deeb, I. E. Elshahawy, *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 7 (2016) 1338-1348.

7. A. Bakeer, R. S. R. El-Mohamedy, N. M. Saied, F. Abd-El-Kareem, *British Biotechnology Journal* 13 (2016) 1-10.
8. C. S. Pereira, R. J. Guimaraes, E. A. Pozza, A. A. Silva, *da. Revista Ceres*. 55 (2008) 369-376.
9. K. Bosio, C. Avanzini, A. D'Avolio, O. Ozino, D. Savoia, *Lett Appl Microbiol*. 31 (2000)174-177.
10. N. Sahinle, A. Gul, *III. Ulusal Zootekni Kongresi, Ankara* (2002) 113-118.
11. N. Sahinler, O. Kaftanoglu, *Natural Prod Res*. 19 (2005) 183-188.
12. G.A. Burdock*, Food Chem Toxicol.* 36 (1998)347-363.
13. A. Kujumgiev, T. Tsvetkoca, Y. Serkedjieva, V. Bankova, R. Christov, S. Popov , *J Ethnopharmacol*. 64 (1999) 235-240.
14. F.G. Fahny, M.O.M. Omar, *Assiut J Agric Sci*. 20 (1989) 265-275.
15. M.N. Abd al-Fattah, A.Fahmy, M. Zeinab, M. Moursy, A. Maysa, *6th Nat Conf of Pest & Dis of Veg and Fruits. Ismailia, Egypt* (1995) 118-128.
16. E. N. Quiroga, D. A. Sampietro, J. R. Soberon, M. A. Sgariglia, M. A.Vattuone, *J. Microbiol*. 101 (2006) 103-110.
17. E. M. Soylu, A. E. Ozdemir, E. Ertürk, N. Sahinler *Proceedings of the First European Conference ofApidology 'EurBee Udine, Italy* (2004)160.
18. E. M. Soylu, A. E. Ozdemir, E. Ertürk, N. Sahinler, S. Soylu, *Asian J Chem.* 20 (2008) 4823-4830.
19. A.LaTorre, G.Imbroglini, M.Guccione, *First Observation Agriculture*. 6 (1990) 169-177.
20. M.T.Badawy, M.T. Rabea, T.M.Rogge, C.V.Stevens, G.Smagghe, *Polymer Bull.* 54 (2003) 279–289.
21. R.S.R. El-Mohamedy, M.M.Abdel-Kader, F.Abd-El-Kareem, N.S.El-Mougy, *Journal of Agricultural Technology.* 9 (2013) 1521-1533.
22. W.M.Haggag, H.Abouziena, F.Abd-El-Kreem, S. El Habbasha, *Journal of Chemical and Pharmaceutical Research*. 7(2015) 882-889.
23. S.N. Kulikov, S.N.Chirkov, S. Lopatin, V.P. Varlamov, *Prik Biokhim Mikrobiol.*42 (2006) 224–228.
24. F.A.A. Al-Mahareeq *M. Sc., Thesis, Fac. Graduate Studies, An- Najah National Univ., Nablus, Palestine* (2005) 93 pp.
25. R. Buruchara, C. Estevez de Jensen, G. Godoy, G. Abawi, J. Pasche, M. Lobo Junior, C. Mukankusi, *International Conference July* 20-23, 2015, *Protea Hotel, Kruger Gate Skukuza, South Africa* (2015) 22-42.
26. J. Neler, W.Wassermann, M. H. Kutner, 2nd *Irwin Inc. Homewood Illionois*. (1985) 117-155.
27. A.E. Ozdemir, E. Ertürk, N. Sahinler, M. Kaplankıran, A. Gül, *III. Bahçe rünlerinde Muhafaza ve Pazarlama Sempozyumu, Antakya-Hatay* (2005) 204-211.
28. E. E. Candır, O A. E. Zdemir, E. M. Soylu, N. Sahinler, A. Gul, *Asian J Chem*. 21 (2009) 2659-2666.
29. C. Guginski-Piva, I. D. Santos, A.W.Wagner, D. W. Heck, M. F. Flores, K. Pazolini, *IDESIA (Chile).* 33 (2015) 39-47.
30. M. El Hassni, A. El Hadrami, F. Daayf, M. Cherif, I. El Hadrami, *Phytopathol Mediterr.* 43 (2004) 195-204.
31. N.I. Vasyukova, G.I. Chalenko, N.G. Gerasimova, E.A. Perekhod, O.L. Ozeretskovskaya, A.I. Albulov, *Appl Biochem Microbiol*. 36 (2005) 372-376.
32. M.T. Rabea, T.M. Rogge, C.V. Stevens, G. Smagghe, *Pest Manag Sci*. 61(2005) 951-960.
33. J. Palma-Guerrero, I.C. Huang, H.B. Jansson, J. Salinas, L.V. Lopez, N.D. Read, *J. Appl Microbiol*. 104 (2008) 541-553.
34. A. El- Ghaouth, J. Arul, A. Asselin, N. Benhamou, *Mycol Res.* 96 (2002) 769-779.
35. E. Ait Barka, P. Eullaffroy, G. Vernet, *Plant Cell Rep*. 22 (2004) 608-614.
36. S.B. Banos, A.N. Hernandez-Lauzardo, M.G.Velazquez-del Valle, M. Hernandez-lopez, E. Ait Barka, E. Bosquez-Molina, C.L. Wilson, *Crop Protection*, 25 (2006) 108-118.
37- N.M. Saied, *Ph.D. Thesis Fac. Agric. Banha Univ.* (2015) 250.

(2018) ; http://www.jmaterenvironsci.com