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SUPPRESSION OF ROOT ROT DISEASE OF CHICKPEA BY THE ENDOPHYTIC *PENICILLIUM* SPECIES UNDER SOIL AMENDMENT WITH OILSEED CAKES

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ABSTRACT

Root rot diseases are a serious global concern since they decrease the yield of crop plants. In the present study, the biocontrol potential of endophytic *Penicillium* species was assessed under soil amendment with oilseed cakes in the screen house. Fourteen isolates of endophytic *Penicillium* species were applied individually or in soil amended with neem cake (*Azadirachta indica*) and mustard cake (*Brassica juncea*). Significant reduction in *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, and *Macrophomina phaseolina* infections was found in chickpea roots with enhancement in plant growth. Efficacy of *Penicillium* spp. was found improved in amended soil compared to unamended soils.

KEY WORDS: Organic Amendment, Endophytic *Penicillium*, Root rot fungi, Chickpea, Host growth.

INTRODUCTION

Root rot pathogens affect crops globally and have a wide range of hosts (Gonzalez *et al.,* 2011; Nzungize *et al.,* 2011). Among root rot pathogens, *Rhizoctonia* species affects several crops such as bean (Ajayi *et al.,* 2013), apple (Marimuthu *et al.,* 2013), tobacco, blueberry (Kamfwa *et al.,* 2013), tomato (Hegde *et al.,* 2012), pea (Díaz-Arias *et al.,* 2011), and canola (Akhtar and Azam, 2014). *Macrophomina phaseolina* is another root rot fungi that cause dry root rot of chickpea in India (Kou and Wang, 2010). Overall, root rots are a complex disease. For instance, black root rot in strawberries is a complex disease caused by *Pythium*, *Fusarium*, and *Rhizoctonia* species (Young, 1996). The use of biocontrol agents is an eco-friendly approach compared to other management approaches against root rot fungi. Biocontrol agents are antagonists of microbes having the potential to suppress pathogens directly or produce resistance against pathogens in their host plants (Cook and Baker, 1983, Eilenberg *et al.,* 2001).

Endophytes are microbes. They are either bacteria or fungi, which live inside plants without causing any apparent symptoms to their host and are best known for their biocontrol potential (Wilson, 1995). *Penicillium species* have been isolated as endophytes of multiple plant species (Nicoletti *et al.*, 2014). Endophytic *Penicillium* species have been reported to provide growth enhancement to plant through protection against biotic stress (Waqas *et al.,* 2015; Hassan, 2017) and reduce the chances of pathogens attack by producing bioactive/antagonist compounds that protect their host.

The organic amendment is used to enhance crop health and yield (Shafique *et al.,* 2016). Several researchers have reported negative effects of organic amendment on pests (Sultana *et al.,* 2011; 2018; Rahman *et al.,* 2016). Many researchers reported the efficacy of neem cake against phytopathogens (Rahman *et al.,* 2016; Shafique *et al.,* 2016). Jangir *et al*. (2020) reported the combined effect of *Trichoderma harzianum* with mustard cake, which significantly reduced the *Fusarium* infection and enhanced the growing tomato plant.

The present study offers the individual potential of endophytic *Penicillium* species and with the combination of organic amendments like neem (*Azadirachta indica*) cake and mustard (*Brassica juncea*) cake against root rotting fungi on the growth of chickpea. It was previously isolated and morphologically identified (Urooj *et al.,* 2018).

MATERIALS AND METHODS

Effect of endophytic *Penicillium* with neem cake to inhibit the root diseases and chickpea growth

The experiments were performed in earthen pots (15cm diam.) in the screen house, where neem (Azadirachta indica) cake (Neemex powder) was mixed with sandy loam soil (pH 8.0) at 1% w/w and transferred one kg to each pot. The soil was naturally infested with root infecting fungi. 3-10% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (Wilhelm, 1955), *Macrophomina phaseolina* (2-8 sclerotia g-1 of soil) as determined by wet

sieving and dilution plating (Sheikh and Ghaffar, 1975), and 3000 cfu g-1 of the soil of a mixed population of *Fusarium oxysporum* and *F. solani* as determined by soil dilution technique (Nash and Snyder, 1962). The pots were irrigated for seven days to allow the complete decomposition of organic matter. Six chickpea seeds (*Cicer arietinum*) were sown per pot, and aqueous suspension (25mL) of *Penicillium* species ($8x10^7$ cfu/mL), grown in Potato Dextrose Broth, was drenched onto each pot. After seed germination, four seedlings per pot were kept, and excess was removed. In another set, *Penicillium* was inoculated in an un-amended soil for comparison. The plants grown in un-inoculated soil are served as control, while carbendazim (25mL of 200ppm) are served as a positive control. The observations were measurement after 45 days. The plants were uprooted to assess the efficacy of soil amendment with neem cake and *Penicillium*. The roots were washed thoroughly with sterilized water. Root rot fungi were isolated described by Mansoor et al. (2007). Fungi, which were emerged from root pieces, were identified to calculate the infection percentages. The data of the plant growth were recorded. Another similar experiment was conducted in which mustard cake was used as an organic amendment.

RESULTS

Effect of endophytic *Penicillium* with neem cake in suppressing the root diseases and growth of chickpea

The plants grown in pots received endophytic *Penicillium* isolates, *P .purpurogenum* (EPSML3), and *P. thomii* in natural soil and amended soil with neem cake. *P. decumbens*, *P. nigricans*, *P. purpurogenum* (EPSML3) *P. purpurogenum* (EPEHS7), *P. javanicum*, and *P. purpurogenum* (EPAER14) showed no infection of *F. oxysporum.* In an un-amended soil, *F. solani* was found significantly reduced except isolate *P. asperum*. Whereas in amended soil infection of *F. solani* was not significant. In unamended soil, *M. phaseolina* was significantly reduced. Combine effect of isolates *P. purpurogenum* (EPSML3), *P. purpurogenum* (EPEHS7), *P. purpurogenum* (EPAER14) and neem cake showed significant result on *M. phaseolina* infection. Application of *P. rugulosum*, *P. decumbens*, *P. restrictum*, *P. duclauxi*, *P. asperum*, and *P. thomii* showed no infection of *R. solani* in natural soil. Amended soil with neem cake has not infected *R. solani*. (Table 1)

The greater height of the plant was produced by *P. decumbens*, *P. nigricans*, *P. rugulosum*, and *P. duclauxi* when applied in natural soil. Effect of *P. restrictum* and *P. citrinum* with neem cake showed the highest plant height. Untreated control of amended soil showed the highest value of fresh shoot weight and fresh root weight related to other approaches. Fresh shoot weight in natural soil showed a significant result in all treatments except *P. thomii*. *P. decumbens* and *P. duclauxi* alone showed the highest root length and fresh root weight. In amended soil, *P. purpurogenum* (EPAER14) showed significant root length. (Table 2)

Table 1. Effect of endophytic *Penicillium* and neem cake on the infection of *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, and *Macrophomina phaseolina* on chickpea roots in a greenhouse experiment.

				Infection%					
Treatments	Code #	*F. oxysporum*		*F. solani*		*M. phaseolina*		*R. solani*	
		NS	AS	NS	AS	NS	AS	NS	AS
Control	…	37.5	0	50	12.5	43.7	37.5	25	0
Carbendazim	…	0	0	25	25	31.2	37.5	12.5	0
P. decumbens	EPAIR6	18.7	0	12.5	31.2	37.5	68.7	0	0
P. nigricans	EPSLR4	12.5	0	31.2	43.7	37.5	56.2	37.5	0
P. regulosum	EPAAR5	6.2	6.2	18.7	43.7	37.5	50	0	0
P. citrinum	EPSMR1	31.2	18.7	18.7	31.2	37.5	50	18.7	0
P. lilacinum	EPSMS2	6.2	6.2	43.7	12.5	6.2	62.5	25	0
P. purpurogenum	EPSML3	0	0	37.5	25	6.2	31.2	6.2	0
P. duclauxi	EPASS9	18.7	37.5	12.5	25	37.5	50	0	0
P. lividum	EPMCL12	6.2	6.2	43.7	12.5	6.2	62.5	25	0
P. purpurogenum	EPEHS7	18.7	0	25	37.5	12.5	31.2	6.2	0
P. restrictum	EPCTS8	37.5	37.5	25	25	12.5	50	0	0
P. thomii	EPAER11	0	18.7	43.7	18.7	6.2	25	0	0
P. purpurogenum	EPAER14	18.7	0	25	37.5	12.5	31.2	6.2	0
P. javanicum	EPSLR13	31.2	0	18.7	43	31.2	56.2	37.5	0
P. asperum	EPHAL10	12.5	6.2	50	12.5	12.5	81.2	0	0
	$LSD_{0.05}$	Treatment =4.90[1]	Pathogen =2.45[2]	Soil Type =1.73[3]					

1 Difference higher than LSD values among means in the column is significant at $p<0.05$

2 Difference greater than LSD values among means in a row are significant at $p<0.05$

3. Mean values in the NS and AS column showing difference higher than LSD value are significantly different at $p<0.05$; NS= Natural Soil, AS=Amended Soil.

Table 2. Effect of endophytic *Penicillium* and neem cake on the growth of chickpea in a greenhouse experiment.

Treatments	Code #	Shoot Length (cm)		Shoot Weight (g)		Root Length (cm)		Root weight (g)	
		NS	AS	NS	AS	NS	AS	NS	AS
Control	…	23.69	22.25	2.74	8.37	2.74	9.75	2.11	3.03
Carbendazim	…	23.9	29.75	3.2	8.21	21.87	15.37	3.76	2.35
P. decumbens	EPAIR6	29.25	29.11	3.76	3.88	30.37	12.93	5.22	1.16
P. nigricans	EPSLR4	29.3	33.57	3.39	6.61	23.31	13.91	3.76	1.2
P. regulosum	EPAAR5	29.28	33.15	3.32	6.33	22.96	9	3.87	1.17
P. citrinum	EPSMR1	26.7	33.84	3.13	6.68	23.97	9.75	3.94	0.98
P. lilacinum	EPSMS2	27.68	28.01	3.1	6.98	21.55	11.32	3.5	1.09
P. purpurogenum	EPSML3	25.87	33.32	3.075	7.38	26.7	13.7	4.32	1.41
P. duclauxi	EPASS9	29.25	29.11	3.76	3.88	30.37	12.93	5.22	1.16
P. lividum	EPMCL12	27.68	28.01	3.1	6.98	21.55	11.32	3.5	1.09
P. purpurogenum	EPEHS7	26.98	30.77	3.26	5.06	22.02	15.65	4.13	1.39
P. restrictum	EPCTS8	26.67	33.84	3.205	6.68	27.35	9.75	3.51	0.98
P. thomii	EPAER11	23.9	30	2.96	7.99	24.16	10.62	4.27	1.25
P. purpurogenum	EPAER14	26.98	30.77	3.26	5.06	22.02	15.65	4.13	1.39
P. javanicum	EPSLR13	26.18	33.57	3.41	6.61	25.87	13.91	4.38	1.2
P. asperum	EPHAL10	28.56	28.91	3.44	7.63	19.21	13.52	3.06	1.3
	$LSD_{0.05}$	4.7[1]	4.93[1]	0.94[1]	3.33[1]	7.32[1]	5.45[1]	1.61[1]	1.107[1]

1 Difference higher than LSD values among means in the column is significant at $p<0.05$
NS= Natural Soil, AS=Amended Soil.

Table 3. Effect of endophytic *Penicillium* and mustard cake on the infection of *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, and *Macrophomina phaseolina* on chickpea roots in a greenhouse experiment.

Treatments	Code#	Infection %							
		F. oxysporum		*F. solani*		*M. phaseolina*		*R. solani*	
		NS	AS	NS	AS	NS	AS	NS	AS
Control	…	37.5	12.5	50	31.2	43.7	0	25	18.7
Carbendazim	…	0	12.5	25	43.7	31.2	6.2	12.5	12.5
P. decumbens	EPAIR6	18.7	0	12.5	6.2	37.5	0	0	0
P. nigricans	EPSLR4	12.5	0	31.2	43.7	37.5	18.7	37.5	43.7
P. regulosum	EPAAR5	6.2	0	18.7	31.2	37.5	18.7	0	25
P. citrinum	EPSMR1	31.2	0	18.7	62.5	37.5	18.7	18.7	31.2
P. lilacinum	EPSMS2	6.2	6.2	43.7	50	6.2	25	25	12.5
P. purpurogenum	EPSML3	0	0	37.5	6.	6.2	0	6.2	12.5
P. duclauxi	EPASS9	18.7	0	12.5	62.5	37.5	6.2	0	31.2
P. lividum	EPMCL12	6.2	6.2	43.7	100	6.2	25	25	31.2
P. purpurogenum	EPEHS7	18.7	0	25	18.7	12.5	0	6.2	12.5
P. restrictum	EPCTS8	37.5	6.2	25	12.5	12.5	12.5	0	6.2
P. thomii	EPAER11	0	6.2	43.7	12.5	6.2	6.2	0	6.2
P .purpurogenum	EPAER14	18.7	0	25	18.7	12.5	12.5	6.2	12.5
P. javanicum	EPSLR13	31.2	0	18.7	31.2	31.	18.7	37.5	43.7
P. asperum	EPHAL10	12.5	0	50	18.7	12.5	0	0	0
	$LSD_{0.05}$	Treatment=4.46[1]	Pathogen=2.23[2]	Soil Type=1.58[3]					

1 Difference higher than LSD values among means in the column is significant at $p<0.05$
2 Difference greater than LSD values among means in a row are significant at $p<0.05$
3. Mean values in the NS and AS column showing difference higher than LSD value are significantly different at $p<0.05$
NS= Natural Soil, AS=Amended Soil.

Table 4. Effect of endophytic *Penicillium* and mustard cake on the growth of chickpea in a greenhouse experiment.

Treatments	Code#	Shoot Length (cm)		Shoot Weight (g)		Root Length (cm)		Root weight (g)	
		NS	AS	NS	AS	NS	AS	NS	AS
Control	…	23.69	21.88	2.74	4.06	2.74	6.92	2.11	5.8
Carbendazim	…	23.9	21.34	3.2	4.2	21.87	9.37	3.76	4.99
P. decumbens	EPAIR6	29.25	15.25	3.76	2.88	30.37	7.5	5.22	5.3
P. nigricans	EPSLR4	29.3	19.55	3.39	4.76	23.31	7.58	3.76	13.7
P. regulosum	EPAAR5	29.28	19.07	3.32	6.33	22.96	8.75	3.87	12.38
P. citrinum	EPSMR1	26.7	19.16	3.13	5.56	23.97	7.56	3.94	11.72
P. lilacinum	EPSMS2	27.68	19.29	3.1	4.17	21.55	9.46	3.5	3.83
P. purpurogenum	EPSML3	25.87	12	3.075	2.41	26.7	6.5	4.32	5.32
P. duclauxi	EPASS9	29.25	19.2	3.76	5.61	30.37	11.15	5.22	8.19
P. lividum	EPMCL12	27.68	19.29	3.1	4.17	21.55	9.46	3.5	3.83
P. purpurogenum	EPEHS7	26.98	17.87	3.26	5.5	22.02	9.25	4.13	7.34
P. restrictum	EPCTS8	26.67	18.5	3.205	3.15	27.35	4.5	3.51	0.99
P. thomii	EPAER11	23.9	23.05	2.96	6.26	24.16	9	4.27	9.31
P. purpurogenum	EPAER14	26.98	17.87	3.26	5.5	22.02	9.25	4.13	7.39
P. javanicum	EPSLR13	26.18	23.05	3.41	6.26	25.87	9	4.38	9.31
P. asperum	EPHAL10	28.56	16.62	3.44	5.82	19.21	9.25	3.06	8.34
	$LSD_{0.05}$	4.7[1]	6.13[1]	0.94[1]	3.01[1]	7.32[1]	2.92[1]	1.61[1]	6.15[1]

1 Difference higher than LSD values among means in the column is significant at $p < 0.05$
2 Difference greater than LSD values among means in a row are significant at $p < 0.05$
NS=Natural Soil AS=Amended Soil.

Effect of endophytic *Penicillium* with mustard cake in suppressing the root diseases and growth of chickpea

Root rot fungi infection was less in amended soil compared to un-amended soil. No infection of *F. oxysporum* was found in *P. purpurogenum* (EPSML3) and *P. thomii* in un-amended soil. *P. citrinum*, *P. purpurogenum* (EPSML3), *P. nigricans*, *P. rugulosum*, *P. decumbens*, *P. purpurogenum* (EPEHS7), *P. duclauxi*, *P. javanicum*, and *P. purpurogenum* (EPAER14) with mustard cake amendment showed complete suppression of *F. oxysporum*. *P. decumbens* and *P. purpurogenum* (EPSML3) in amended soil showed lesser infection of *F. solani*, while *P. lividum* showed 100% infection of *F. solani* in amended soil. Infection of *M. phaseolina* in unamended soil was significant, whereas, in amended soil, untreated control showed no infection of *M. phaseolina*. Treatment of *P. thomii* and *P. purpurogenum* (EPAER14) in mustard cake amended soil showed lower infection of *R. solani* and *P. citrinum*, *P. nigricans*, *P. rugulosum*, *P. duclauxi*, *P. javanicum*, and *P. lividum* showed non-significant results. (Table 3)

Natural soil showed greater plant height compared to mustard cake amended soil. *P. nigricans* showed greater plant length compared to other treatments. In amended soil, plant height was statistically not significant. (Table 4).

DISCUSSION

Combining the application of endophytic fungi with the organic amendment is a biological approach for root rot diseases in plants. In this study, endophytic *Penicillium* species with neem and mustard cake showed a significant result compared to their application alone. However, the efficacy of biocontrol agents with oilseed cakes is less explored against phytopathogens (Thilgavathi *et al.,* 2007; Karthiba *et al.,* 2011). In the present study, the efficacy of endophytic *Penicillium* with neem cake against root rot fungi is conformity with our previous report (Urooj *et al.,* 2018). The study evaluated the potential of 14 isolates of endophytic *Penicillium* species with neem and mustard cake on the growth of chickpea *in vivo* and amended soil, with neem cake *P. decumbens*, *P. nigricans*, *P. purpurogenum* (EPSML3), *P. purpurogenum* (EPEHS7), *P. javanicum* and *P. purpurogenum* (EPAER14) showed no infection of *F. oxysporum*. The combined effect of isolates *P. purpurogenum* (EPSML3), *P. purpurogenum* (EPEHS7), *P. purpurogenum* (EPAER14), and neem cake showed significant results on *M. phaseolina* infection. Amended soil with neem cake has not infected *R. solani*. Few researchers have reported the biocontrol potential of oilseed cake against phytopathogens that support our outcomes (Arora *et al.,* 2017; Meng *et al.,* 2015; Monika *et al.,* 2020). Effect of *P. restrictum* and *P. citrinum* with neem cake showed greater plant height.

P. citrinum, *P. purpurogenum* (EPSML3), *P. nigricans*, *P. rugulosum*, *P. decumbens*, *P. purpurogenum* (EPEHS7), *P. duclauxi*, *P. javanicum* and *P. purpurogenum* (EPAER14) with mustard cake amendment showed

complete suppression of *F. oxysporum*. Tiyagi (1995) reported that castor, mustard, neem, and Duan, cakes were significantly efficient against many plant infections resulting from pathogenic fungi, viz., *Macrophomina phaseolina*, *Rhizoctonia solani*, *Phyllosticta phaseolina,* and *F. oxysporum* f. sp. *ciceri*, in mungbean and chickpea, which is an agreement to the outcomes of the current study.

Singh (2015) reported the biocontrol potential of *T. harzianum* in combination with de-oiled seed cake (Jatropha, neem, and karanja and mahua) that is in close agreement with our current findings. Multi-fold improvement in growth parameters was measured after treatment with oilseed cakes such as fresh shoot and root weight, plant height including root and shoot lengths, and root length. In earlier studies that are in close agreement with outcomes of the current study (Rizvi *et al.,* 2012; Tiyagi *et al.,* 1995; Jat *et al.,* 2017).

REFERENCES

Ajayi O.O., T.C. Paulitz, K. Campbell, K.M. Murphy, and S.E. Ulrich (2013). Identification of resistance to *Rhizoctonia* root rot in mutant and wild barley (*Hordeum vulgare* subsp. *spontaneum*). *Phytopath,* 103: S2.3.

Akhtar M.S. and T. Azam. (2014). Effects of PGPR and antagonistic fungi on the growth, enzyme activity, and *Fusarium* root-rot of a pea. *Arch. Phytopathol,* 47(2): 138-148.

Arora K., S. Sharma, S.B.N. Krishna, J.K. Adam and A. Kumar (2017). Non-edible Oil cakes as a novel substrate for DPA production and augmenting biocontrol activity of *Paecilomyces variotii*. *Front. Microbiol,* 8:1-12.

Cook R.J., and K.F. Baker. (1983). The nature and practice of biological control of plant pathogens. St. Paul, MN: *American Phytopathological Society* 539 pp.

Díaz-Arias M.M., G.P. Munkvold G.P. and L.F. Leandro (2011). First report of *Fusarium proliferatum* causing root rot on soybean (*Glycine max*) in the United States. *Plant Dis,* 95(10): 1316.

Eilenberg J., A. Hajek, and C. Lomer (2001). Suggestions for unifying the terminology in biological control. *BioControl,* 46(4): 387-400.

Gonzalez M., M. Pujol, J.P Metraux, V. Gonzalez-Garcia, and M.D. Bolton (2011). Tobacco leaf spot and root rot caused by *Rhizoctonia solani* Kühn. *Mol. Plant Pathol,* 12(3): 209-216.

Hassan S.E.D. (2017). Plant growth-promoting activities for bacterial and fungal endophytes isolated from the medicinal plant of *Teucrium polium* L. *J. Adv. Res. 8*(6): 687-695.

Hegde N., D. Baer, P. Asija, K. Shetty, J.B. Rasmussen, and R.S. Goswami (2012). Evaluating seed treatments for their ability to control *Fusarium* root rot in legumes. *Phytopathol,* 102(7): 52-52.

Jangir M., S. Sharma and S. Sharma (2020). Synergistic effect of oilseed cake and biocontrol agent in the suppression of *Fusarium* wilt in *Solanum lycopersicum*. *Braz. J. Microbiol, 51*(4): 1929-1939.

Jat M.K., R.R. Ahir, S. Choudhary and G.L. Kakaraliya (2017). Management of coriander wilt (*Fusarium oxysporum*) through cultural practices as organic amendments and date of sowing. *J. Pharmacogn. Phytochem*, 6:1499–1501

Kamfwa K., M. Mwala, P. Okori, P. Gibson, and C. Mukankusi (2013). Identification of QTL for *Fusarium* root rot resistance in common bean. *J. Crop Improve,* 27(4): 406-418.

Karthiba L., K. Saveetha, S. Suresh, T. Raguchander, D. Saravanakumar and R. Samiyappan (2011). PGPR and entomopathogenic fungus bioformulation for the synchronous management of leaf folder pest and sheath blight disease of rice. *Pest Manag. Sci,* 66(5):555–564.

Kou Y. and S. Wang. (2010). Broad-spectrum and durability: understanding of quantitative disease resistance. *Curr. Opin. Plant Biol,* 13(2): 181-185.

Mansoor F., V. Sultana and S. Ehtesham ul-Haque (2007). Enhancement of biocontrol potential of *Pseudomonas aeruginosa* and *Paecilomyces lilaceous* against root rot of mungbean by a medicinal plant *Launaea nudicaulis* L. *Pak. J. Bot.,* 39(6): 2113-2119.

Marimuthu S., V. Ramamoorthy, R. Samiyappan and P. Subbian (2013). Intercropping system with combined application of *Azospirillum* and *Pseudomonas fluorescens* reduces root rot incidence caused by *Rhizoctonia bataticola* and increases seed cotton yield. *J. Phytopathol.,* 161(6): 405-411.

Meng X., J. Li, F. Bi, L. Zhu and Z. Ma (2015). Antifungal activities of crude extractum from *Camellia semiserrata* Chi (Nanshancha) seed cake against *Colletotrichum musae*, *Colletotrichum gloeosporioides*, and *Penicillium italicum in vitro* and *in vivo* fruit test. *Plant Pathol. J.,* 31(4): 414-420.

Nash S.M., and W.C. Snyder (1962). Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathol.* 52: 567-572.

Nicoletti R., A. Fiorentino and M. Scognamiglio. (2014). Endophytism of *Penicillium* species in woody plants. *Open Mycol. J., 8:*1 -26.

Nzungize J., P. Gepts, R. Buruchara, A. Male, P. Ragama, J.P Busogoro and J.P. Baudoin (2011). Introgression of *Pythium* root rot resistance gene into Rwandan susceptible common bean cultivars. *Afr. J. Plant Sci*, 5(3): 193-200.

Rahman A., V. Sultana, J. Ara and S. Ehtesham-ul-Haque (2016). Induction of systemic resistance in cotton by the neem cake and *Pseudomonas aeruginosa* under salinity stress and *Macrophomina phaseolina* infection. *Pak. J. Bot*. 48: 1681-1689.

Rizvi R., I. Mahmood, S.A. Tiyagi and Z. Khan (2012). The conjoint effect of oil-seed cakes and *Pseudomonas fluorescens* on the growth of chickpea about the management of plant-parasitic nematodes. *Braz. Arch. Biol. Technol.*, 55(6):801–808

Shafique, H.A., V. Sultana, S. Ehteshamul-Haque and M. Athar (2016). Management of soil-borne diseases of organic vegetables. *J. Plant Prot. Res, 56* (3):221-230.

Sheikh A.H., and A. Ghaffar. (1975). A population study of sclerotia of *Macrophomina phaseolina* in cotton fields. *P. J. Bot*,7: 13- 17.

Singh, R.., A. Kumar, and A. Tomer (2015). De-oiled cakes of neem, Jatropha, mahua, and karanja: a new substrate for mass multiplication of *T. harzianum*. *J. Plant Pathol. Microbiol, 6*(7): 288–292

Sultana. V., G.N. Baloch, J. Ara and S. Ehtesham-ul-Haque (2011). Effect of soil amendment with seeds of *Vernonia anthelmintica* on soil-borne diseases and growth of okra. *Phytopath*. 101: S173.

Sultana, V., S. Tariq, K. Hira, A. Tariq, J. Ara, R.M. Tariq and S. Ehteshamul-Haque (2018). Seaweed bio-fertilizer for the management of root rotting fungi and root-knot nematodes affecting the cotton crop. *Pak. J. Bot*. 50: 2409-2412.

Thilagavathi, R., D. Saravanakumar, N. Ragupathy and R. Samiyappan (2007). Integration of biocontrol agents for the management of dry root rot (*Macrophomina phaseolina*) disease in green gram. *Phytopathol Mediterr.* 46: 157–167

Tiyagi, S.A. and M. M. Alam (1995). Efficacy of oil-seed cakes against plant-parasitic nematodes and soil-inhabiting fungi on mungbean and chickpea. *Bioresour. Technol.*, 51(2-3):233–239

Urooj, F., H. Farhat, S.A. Ali, M. Ahmed, V. Sultana, Z.I. Shams, J. Ara and S. Ehteshamul-Haque (2018). Role of endophytic *Penicillium* species in suppressing the root rotting fungi of sunflower. *Pak. J. Bot*., 50: 1621-1628.

Waqas, M., A.L. Khan, M. Hamayun, R. Shahzad R., S.M. Kang, J.G. Kim and I.J. Lee (2015). Endophytic fungi promote plant growth and mitigate the adverse effects of stem rot: an example of *Penicillium citrinum* and *Aspergillus terreus*. *J. Plant Interact.* 10(1): 280-287.

Wilhelm, S. (1955). Longevity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopathol,* 45: 180-181.

Wilson, D. (1995). Endophyte: the evolution of a term, and clarification of its use and definition. *Oikos,* 73(2): 274–276.

Wilson, D. (1995). Fungal endophytes which invade insect galls: insect pathogens, benign saprophytes, or fungal inquilines?. *Oecologia* 103(2): 255-260.

Young, N.D. (1996). QTL mapping and quantitative disease resistance in plants. *Annul. Rev. Phytopathol.,* 34: 479-501.