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Management of collar rot of chickpea by *Pseudomonas fluorescens* and identification of sources of resistance

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ABSTRACT

An investigation was carried out at Department of Plant Pathology, JNKVV, Jabalpur to know the antagonistic potential of *Pseudomons fluorescens* against *Sclerotium rolfsii*. The result of the *In vitro* testing revealed that highest growth inhibition zone was obtained on King's B medium (82.2%) followed by *Pseudomonas* Agar (77.1%) and Potato Dextrose Agar medium (62.3%) after 9 days of incubation as compared to control. Combined application of soil and seed treatment of *P. fluorescens* was found best for increasing germination percentage i.e. 96.6%, 90%, 100% and 86.6% in JG 62, JG 63, JG 315 and JG 74 respectively as compared to seed and soil treatment alone. The combined application of soil and seed treatment of *P. fluorescens* effectively helped to increase plant growth, vigour index and to minimize disease incidence. Out of 190 chickpea entries comprising of 115 desi and 75 kabuli types evaluated for resistance against *S. rolfsii* under net house conditions, 18 entries of the desi ware found resistant, however, 12 entries were moderately resistant. In Kabuli type, 3 entries were found resistant, while 4 entries were graded as moderately resistant. In a field screening of 284 chickpea germplasm accessions against collar rot, 9 were found free from disease and 29 exhibited < 10 per cent mortality due to collar rot.

Key words: *Cicer arietinum* L., Chickpea, Collar rot, *Pseudomonas fluorescens* Resistance, Vigour index

Chickpea (*Cicer arietinum* L.) is one of the most important post rainy season pulse crops both in acreage and production next to wheat, cultivated in Madhya Pradesh. In India, it is cultivated in an area about 9.51 million ha with a production of 8.83 mt and 929 kg/ha productivity. Madhya Pradesh contributes an area 3.5 million ha, 4.7 mt production and 1350 kg/ha productivity during 2013-14 (www.mpkrishi.org). Collar rot caused by *Sclerotium rolfsii* Sacc. is becoming more serious disease. The pathogen caused significant reduction in plant population having wide host range. Biological control management is an ecology-conscious and environment friendly strategy to control the various root related diseases in chickpea. Several fungal (*Trichoderma sp.*) and bacterial (*Pseudomonas sp. and Bacillus sp.*) antagonists, have been successfully used as biocontrol agents in the control of seed and soil borne pathogens like *Sclerotium rolfsii, Rhizoctonia solani, Fusarium oxysporum and Sclerotinia sclerotiorum* in the various crops (Sharma *et al.*, 1999; Mukhopadhyay *et al.*, 1992; Raguchander *et al.*, 1997; Abrahm Mathew and Gupta, 1998). Plant growth promoting *Pseudomonas* and *Bacillus* species generally employ an array of mechanisms like antibiosis, site competition, HCN production, siderophore production, fluorescent pigments and/or antifungal volatiles (Weller 1988, Voisard *et al.*,1989, Gardener *et al.*, 2000, Pal *et al.*,2000, Validov *et al.*, 2005, Singh *et al.*, 2006) to antagonize pathogens. In the present studies efforts have been made to manage the disease by using *Pseudomonas fluorescens* as seed treatment and soil application and to find out possible sources of resistance against collar rot of chickpea.

MATERIAL AND METHODS

***Mass culturing of S. rolfsii in chickpea straw medium*:** The mass culture of the pathogen was done on chickpea straw, which was first washed with water, squeezes then sterilized in an autoclave by filling in polypropylene bags. The sterilized chickpea straw was inoculated with *S. rolfsii* and incubated at room temperature (25±2°C) for 15 days to obtain the profuse and dense growth of fungal mycelium and Sclerotia.

Testing of antagonistic potential of Pseudomonas fluorescens against S. rolfsii: An antagonistic activity (Jabalpur isolate) of *P. fluorescens* isolated from rhizosphere of chickpea field of Department of Plant Pathology, JNKVV Jabalpur (*in vitro*) and commercially available talk based formulation product of *P. fluorescens* from JNKVV Jabalpur (for *in vivo*) was studied against *S. rolfsii.*

***In vitro*:** *P. fluorescence* isolated from rhizosphere of chickpea field (Jabalpur isolate) was screened for their antagonistic activity by dual culture method on different media (PDA, King's B, *Pseudomonas* agar media). An amount of 20 ml media was poured in sterilized Petri plate, after solidification of media 4 days old pure culture of antagonist and the pathogen were inoculated separately at the same time. A five mm disc was taken from the margin of young vigoursly growing culture and placed in petri plate at the opposite point from each other. The plates were incubated in the BOD incubator at 27+2°C. Isolate of *Pseudomonas* spp. were tested for their effectiveness against the pathogen. Observation on the pathogen growth in the presence of *P.fluorescens* was recorded after 9 days of inoculation with the help of a scale. The mean of three replications was calculated and expressed in mm in each case.

$$I = \frac{C-T \times 100}{C}$$

Where, I = Percent inhibition; C = Growth of test pathogen in control plate; T = Growth of test pathogen in presence of *P. fluorescens*

In vivo : The experiment was conducted to find out an effective antagonistic treatment which enhance germination, shoot length, root length and minimize mortality of seedling. Trays were filled with inoculated soil. The soil was inoculated with test fungus *S. rolfsii* grown on chickpea straw medium. Seeds of chickpea variety JG 62 (early wilter), JG 63 (resistant), JG 315 (wilt resistant) and JG 74 (late wilter) were sown with different treatments *viz;* seed treatment, soil application and soil application + seed treatment with isolate of *Pseudomnas* sp. (commercial product of JNKVV). FYM was the best substrate for multiplication of *Pseudomonas fluorescens*. Ten seeds were sown in each tray. Untreated seeds sown in the inoculated soil with *S. rolfsii* and healthy soil served as control. Four replications were maintained. Observations on germination, pre and post emergence mortality were recorded.

Vigour index = Germination percentages × seedling length of final day count.

Types of treatment along with dosages:

Treatment	Types of treatment	Dosages
T_1	Seed treatment	10 g/kg seed
T_2	Soil treatment	50 g/kg soil
T_3	Seed + Soil treatment	10g/kg for seed and 50g/ kg for soil
T_4	Control *	-
T_5	Control **	-

Control* = Inoculated soil with *S. rolfsii* + no treatment, Control**= Healthy soil + no treatment

Screening of chickpea genotypes (Net house and Sick field): To locate the sources of resistance against collar rot, chickpea germplasm lines were procured from AICRP on chickpea and NBPGR. Germplasm lines were screened in the multiple disease sick field at Seed Breeding farm, JNKVV, Jabalpur. Two rows of test entries were sown in 4 m long row at 30 cm apart in multiple disease sick fields. Inoculum multiplied on chickpea straw were added in rows to maintain sickness. One row of susceptible check (JG 62) was alternated after every two row of test entries and replicated twice in randomized block design. Observation was recorded on emergence count and per cent seedling mortality and disease reaction was calculated by using the following formula as mentioned:

$$\text{Percent collar rot} = \frac{\text{Total infected plant}}{\text{Total emergence of plant}} \times 100$$

The percentage of mortality for each germplasm line was calculated and the level of resistance/susceptible was grouped as per disease rating scale.

Disease reaction

Rating	Category	Reaction type	Seedling mortality (%)
1	R	Resistant	0-10
2	MR	Moderately resistant	10.1-20
3	TR	Tolerant	20.1-40
4	S	Susceptible	40.1-60
5	HS	Highly susceptible	60 and above

RESULTS AND DISCUSSION

Testing of antagonistic potential of Pseudomonas fluorescens against Sclerotium rolfsii

In-vitro: The result of *in vitro* testing of *P. fluorescens* (Jabalpur isolate) on growth of *S. rolfsii*, presented in Table 1 showed that highest growth inhibition zone was obtained on King' B (81.2%) followed by *Pseudomonas* agar medium (77.1%) in comparison to potato dextrose agar (62.3%) after 9 days of incubation. Whereas in control plate the growth of *S. rolfsii* was found maximum i.e. 85.1 mm on PDA followed by King' B (48.0 mm) and *Pseudomonas* agar medium (45.0 mm).

Table 1: Antagonistic potential of *Pseudomonas fluorescens* (Jabalpur isolate) against collar rot on different media by dual culture technique.

Media	Radial growth of *S. rolfsii* (mm)* after 9 days		Per cent inhibition
	Control	Dual culture	
PDA	85.1	32.1	62.35
King' B	48.0	9.0	81.25
Pseudomonas agar medium	45.0	10.3	77.11

*Average of three replications

Effect of seed and soil treatment of Pseudomonas fluorescens on vigour index on four varieties of chickpea: The treatment with bio agent also affects the vigour index of plant. The vigour index is multiplication of germination percentages and seedling length of the final day count. Application of FYM colonized with *P. fluorescens* @ 10g/ kg of seed plus 50g/kg soil effectively help to increase the plant growth. The data presented in Table 2 revealed that the soil +seed treatment is found best in increasing vigour index (19831.9, 17631.0, 23570.0 and 19433.0 in JG 62, JG 63, JG 315 and JG 74 respectively) as well as enhanced germination (96.6%, 90%, 100% and 86.6% in JG 62, JG 63, JG 315 and JG 74 respectively) percentages as compared to seed and soil treatment alone. Hameeda *et al.* (2010) isolated bacteria from compost and macro fauna, seven of the 207 isolates showed antagonistic activity against *S. rolfsii* in pot culture. Two of the seven isolate were *Bacillus* sp. and rest belonged to *Pseudomonas* sp. Kaur and Sharma (2013) reported 35 isolates of rhizobacteria from 25 soil sample collected from healthy chickpea rhizosphere. Out of thirty five isolates, ten isolates of rhizobacteria were characterized as *Pseudomonas* sp. on the basis of morphological and growth promotion activities.

In vivo: The studies conducted to explore the use of *P. fluorescens* in improving germination percentage and

Table 2: Effect of seed and soil treatment with *Pseudomonas fluorescens* on vigour index of chickpea

Treatment	Germination (%)				Shoot length (cm)				Root length (cm)				Vigour index			
Varieties	JG 62	JG 63	JG 315	JG 74	JG 62	JG 63	JG 315	JG 74	JG 62	JG 63	JG 315	JG 74	JG 62	JG 63	JG 315	JG 74
T1	90.0	96.6	73.3	90.0	7.6	8.9	9.9	9.3	7.9	9.0	9.9	8.3	13988.7	17330.0	14545.6	15912.0
T2	100	96.6	96.6	96.6	9.9	10.3	11.9	10.9	6.9	8.8	9.8	7.7	16840.0	18624.4	21061.7	18141.4
T3	96.6	90.0	100	86.6	10.9	11.5	12.7	12.1	9.6	8.0	10.8	10.2	19831.9	17631.0	23570.0	19433.0
T4	90.0	96.3	93.3	86.6	6.8	7.1	7.5	6.9	7.0	7.6	6.2	7.0	12515.4	14212.9	12943.5	12123.1
T5	86.6	90.0	96.6	100	6.9	7.0	7.9	7.1	7.3	7.8	7.2	7.9	12321.4	13359.6	14682.2	15174.0
SE m(±)	0.04	0.03	0.02	0.01	0.003	0.003	0.002	0.001	0.038	0.003	0.002	0.001				
CD 5%	0.13	0.09	0.07	0.05	0.010	0.008	0.006	0.004	0.117	0.009	0.005	0.004				

reducing the mortality due to collar rot resulted that, combined application of seed treatment + soil application (T3) of commercial product of JNKVV, significantly increased the plant growth and decreased the per cent collar rot (13.6%, 5.5%, 7.5% and 8.1 % in JG 62,JG 63,JG 315 and JG 74 respectively) of chickpea as compared to soil treatment (20%, 5.6%, 10% and 13.3% in JG 62,JG 63,JG 315 and JG 74 respectively) and seed treatment individually (30.3%, 14.3%, 11.2% and 14.3% in JG 62,JG 63,JG 315 and JG 74 respectively) (Table 3). Among treatments application

Table 3: Testing of the antagonistic potential of *Pseudomonas fluorescens* against *Sclerotium rolfsii* using different treatment under Net house

Treatment	Percent mortality*			
Varieties	JG 62	JG 63	JG 315	JG 74
T1	30.3	14.3	11.2	14.3
T2	20 .0	5.6	10.0	13.3
T3	13.6	5.5	7.5	8.1
T4	97.2	81.5	45.8	74.7
T5	93.7	79.7	11.4	62.1
SE m (±)	2.868	2.061	0.903	2.224
CD 5%	8.72	6.27	2.74	6.76

*Average of four replications

of FYM colonized with *Pseudomonas fluorescens* @10 g/ kg of seed plus 50 g/kg soil effectively help to increased plant growth and minimum per cent disease incidence. Usharani *et al.* (2009) conducted pot experiment to evaluate different delivery system for *P. fluorescens* in the management of Fusarial wilt of tomato revealed that FYM enriched with *P. fluorescens* as seed and soil application was very effective in minimizing wilt incidence.

***Screening of chickpea entries against collar rot under Net house*:** Experiment was conducted to screen the chickpea entries (*Desi and kabuli*) against collar rot under artificially inoculated condition in Net house. It is evident that the pathogen caused both seed rot (0 to 55 %) and seedling mortality (6 to 100 %) up to 30 DAS. Of the 115 desi types screened in inoculated plastic tray, 11 entries (DC 2012-3, GNG 1581, GCP 105, GNG 2216, RSG 963, JG 38, H 10-05, Pant G186, H 10-41, JG 40, RVSSG 21) found free from disease, 7 entries showed <10 per cent mortality and may be graded as resistant, however 12 entries exhibited moderately resistant. Seventy five kabuli types were also screened to find out the resistant genotypes. The pathogen caused seed rot (0 to 80 %) and seedling mortality (16 to 100 %) up to 30 DAS. Of the 75 entries screened in inoculated plastic trays, 3 entries (Phule G 0627-3, GNG 2237and NBeG 176) was found free from disease and may be graded as resistant; however 4 entries exhibited moderately resistant reaction having the disease incidence up to 20 per cent, overall disease was found to occur in the range of 16 to 100 per cent (Table 4). Singh *et al.* (2012) reported 50 germplasm lines of chickpea against collar rot. The germplasm lines KG-1226, KG-8, B-321 and B-311 were found moderately resistant. Gupta and Babbar (2006) reported that among 423 Desi and kabuli chickpea genotypes screened in multiple diseased sick plot, 5 genotype of Desi and 4 of Kabuli type were found as resistant and 39 as moderately resistant.

Evaluation of chickpea germplasm accession against collar rot under field condition: To find out resistant sources against collar rot, chickpea germplasm accessions were also screened along with susceptible check JG 62 under disease sick field. Observations on per cent mortality due to disease were recorded up to 45 days after sowing (Table 5).

The disease was observed in the range of 0.0 to 100.0 per cent. The data exhibits that among 284 entries 33 entries were found resistant, out of them 9 entries (IC 305641, IC 83515, IC117779, IC117783, IC117784, IC117792, IC117800, IC487500, IC487394) were found free from disease, 24 entries showed <10 percent mortality, 82 entries exhibited 10.1 to

Table 4: Resistant chickpea genotypes (Desi and Kabuli) against collar rot identified in net house

Reaction		Genotypes	Total
Resistant (0 to 10.0%)	Desi	DC 2012-3, GNG 1581, GCP 105, GNG 2216, RSG 963, JG 38, H 10-05, Pant G186, H 10-41, JG 40, RVSSG 21,GJG 1001, IPC 2006-126, H 10-21, IPC 2008-69, H 10-22, JAKI 9218, IPC 2009-191	18
	Kabuli	Phule G 0627-3, GNG 2237, NBeG 176	3
Moderately resistant (10.1 to 20.0%)	Desi	H 10-57,Phule G 0405, RSG 963, IPC 2007-13, IPC 2010-62, RSG 931, CSJ 515, GAG 1107, GNG 2210, GNG 1115, JG 315, GL28295	12
	Kabuli	HK 2, BG 3026, BG 1003,GLK 10082	4

Table 5: Reaction of chickpea germplasm accession against collar rot under field condition

Reaction	Genotypes	Total
Resistant (0 to 10.0%)	IC 305641, IC 83515, IC 117779, IC 117783, IC 117784, IC 117792, IC 117800, IC 487500, IC 487394, IC 271762, IC 487469, IC 488127, IC 551962, IC 305523, IC 305297,IC 486528, IC 305299, IC 552343, IC 305510, IC305537, IC 486055,IC 248133, IC 248136, IC 837668, IC 83808, IC 305464, IC 485764,IC 271725, IC 117810, IC 117812, IC 209502, IC 209500, IC 117761.	33
Moderately resistant (10.1 to 20.0%)		82

20 percent mortality and regarded as moderately resistant (entries name are not given).

REFERENCES

Abrahm Mathew K and Gupta SK. 1998. Biological control of root rot of French bean caused by *Rhizoctonia solani*. Journal of Mycology and Plant Pathology 28:202-205.

Gardener BBM, Schroeder KL, Kalloger SK, Raaijmakers JM, Thomashow LS and Weller DM. 2000. Genotypic and phenotypic diversity of phlD-containing Pseudomonas strains isolated from the rhizosphere of wheat. Applied Environment Microbiology 66: 1939-1946.

Gupta O. 2001. Occurrence of sources of resistance in desi and kabuli chickpea genotypes against collar rot and yield attributing character, presented in International chickpea conference held at IGKVV, Raipur (Abst), p. 64.

Gupta O and Babbar A. 2006. Identification of desi and kabuli chickpea genotypes for multiple disease resistance against soil borne diseases. Indian Journal of Pulses Research 19:129-130.

Hameeda B, Harini G, Rupela OP, Rao JVDKK and Reddy G. 2010. Biological control of chickpea collar rot by co-inoculation of antagonistic bacteria and compatible rhizobia. Indian Journal of Microbiology 50: 419-424.

Mukhopadhyay AN, Shrestha SM and Mukherjee PK.1992. Biological seed treatment for control of soil borne plant pathogens. *FAO* Plant Protection Bulletin 40: 221-30.

Pal KK, Tilak KVBR, Saxena AK, Dey R and Singh CS. 2000. Antifungal characteristics of a *fluorescent Pseudomonas* strain involved in the biological control of *Rhizoctonia solani*. Microbiological Research 155: 233-242.

Raguchander T, Rajappan K and Samiappan R.1997. Evaluating methods of application of biocontrol agents in the control of Mungbean root rot. Indian Phytopathology 50:229-234.

Sharma SK, Verma BR and Sharma BK. 1999. Biocontrol of Sclerotinia sclerotiorum causing stem rot of chickpea. Indian Phytopathology 52:44-46.

Singh A, Verma R and Shanmugam V. 2006. Extracellular chitinases of fluorescent pseudomonads antifungal to *Fusarium oxysporum* f.sp. *dianthi* causing carnation wilt. Current Microbiology 52: 310-316.

Singh SP, Agarwal RK and Bhagawati R. 2012. Screening of chickpea germplasms, date of sowing and integrated management of collar rot caused by *Sclerotium rolfsii*. Annals of Plant Protection Sciences 20: 397-399.

Usharani S, Christopher DJ and Sujaritha A. 2009. Effect of delivery system of *Pseudomonas fluorescens* on the rhizosphere survival and management of fusarial wilt of tomato. Journal of Biological Control 23: 195-198.

Validov S, Mavrodi O, Fuente L, Boronin A, Weller D, Thomashow K and Mavrodi D. 2005. Antagonistic activity among 2,4-diacetylphloroglucinol producing fluorescent Psedomonads sp. FEMS Microbiology Letters, 242, 249.

Voisard C, Keel C, Haas D and Defago G. 1989. Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under genobiotic conditions. EMBO J. B., 351-358.

Weller DM. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology 26: 379-4.