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Integrated disease management of *Rhizoctonia bataticola* causing dry root rot of chickpê

PA Gaikwad, DN Dhutraj, CV Ambadkar and KD Navgire

Abstract
Integrated disease management of *Rhizoctonia bataticola* causing dry root of chickpea (*Cicer arietinum* L.) crop were studied during 2018-19 at VNMKV, Parbhani. In present studies, Carboxin 37.5% + Thiram 37.5% WP seed treatment @ 3g / kg seed + *T. asperellum* @ 10g / kg seed + Neem seed cake @ 50g / kg soil, followed by Carbendazim 50WP seed treatment @ 1g / kg seed + *T. asperellum* @ 10g / kg seed + FYM @ 50g / kg soil and Carbendazim 50% WP (ST) @ 1 g + *T. asperellum* (ST) @ 10 g + NSC (SA) @ 50 g were found most efficient with significantly highest reduction in pre-emergence seed rot, post-emergence seedling mortality and total mortality, over untreated control

Keywords: *R.bataticola*, chickpea, disease management, dry root rot

Introduction
Chickpea is an important *Rabi* crop sown in September – November. The production of chickpea is largely constrained by *Fusarium* wilt (*Fusarium oxysporum* f. sp.*cicer is*); however, recent reports indicated that dry root rot (DRR) is emerging as a potential threat to chickpea production (Ghosh *et al*., 2013, Pande *et al*., 2010 and Sharma *et al*., 2010) [7, 10]. The dry root rot is caused by *Rhizoctonia bataticola* (Taub.) Butler. (Synonym: *Macrophomina phaseolina* (Maubl.) Ashby.) and it is an important component of the disease complex that causes root rots and seedling blight in many grain legumes when they are weakened by other stress factors (Hwang *et. al*. 2003) [5]. It can affect chickpea production, causes considerable yield losses that vary from 5 to 50 per cent and may cause 100 per cent losses in susceptible cultivars under favourable condition (Pande *et al*., 2012) [8]. Considering the economic importance of disease, present investigation was planned to study the integrated disease management of *Rhizoctonia bataticola* causing dry root rot of chickpea.

Material and Methods
Isolation of *Rhizoctonia bataticola*
Naturally diseased chickpea plants showing typical symptoms of dry root rot in standing chickpea crop fields, collected during survey of Marathwada region was brought to the laboratory, washed thoroughly with sterile distilled water, blot dried, cut with sharp sterilized blade into small bits (5mm) and subjected to tissue isolation (Tuite, 1969) [12] on PDA. These bits were sterilized with 0.1 per cent aqueous solution of Mercuric chloride ($HgCl_2$) for two minutes, washed by giving three successive changes with sterile distilled water in glass Petri plates to remove traces of mercuric chloride and blot dried. These were inoculated separately (location-wise) and ascetically on autoclaved and cooled PDA medium in sterilized glass Petri plates under aseptic conditions of Laminar-air-flow cabinet (make: ACS, Bangalore) and were inoculated in BOD incubator (make: MAC, Delhi) at 28 ± 2^0C temperature. Within 2-3 days of incubation, blackish mycelia mat was developed and within next 7-8 days, microsclerotia were initiated in the plates. Applying hyphal tip and/or single spore/sclerotial isolation technique, the test pathogen was isolated aseptically on PDA medium, sub-cultured and the pure cultures of the test isolates obtained were maintained separately on PDA slant test tubes in refrigerator for further studies. After a week of incubation, the pure culture developed was again transferred aseptically by hyphal tip technique on PDA slant test tubes and incubated at 28 ± 2° C.

Pathogenicity test
Pathogenicity of *R. bataticola* test isolates was attempted by employing sick soil method. For the purpose, autoclaved and cooled potting mixture of soil: sand: FYM (2:1:1) was filled into black coloured nursery pot/polybags (20 x 30 cm), disinfected with 5 per cent copper sulphate solution.

The test isolatesmultiplied on sand: maize medium was inoculated @ 50g/kg potting mixture separately in these bags, mixed thoroughly in top 5-6 cm layer, watered lightly and maintained in screen house for two weeks, so as to proliferate the test pathogen and make the potting mixture sick with *R. bataticola.*

Surface sterilized (0.1% $HgCl_2$) healthy seeds of susceptible chickpea Cv. JG-62 were sown (10 seeds / bag) in these bags, watered lightly and maintained in the screen house. Three bags per test isolate were sown and maintained. The observations on seedling mortality were recorded at two weeks after sowing and based on per cent seedling mortality, pathogenic / non-pathogenic potential of the test isolates was determined.

The test pathogen isolates were re-isolated aseptically on PDA plates, from artificially dry root rot diseased chickpea seedlings (pathogenicity test), compared their cultural and morphological characteristics with the original culture of *R. bataticola* isolates isolated from naturally dry root rot diseased chickpea plants to fulfill Koch's postulates.

Identification of the pathogen

On the basis of symptoms expressed (both on naturally and artificially diseased) on chickpea plants, pathogenicity test, cultural and morphological characteristics and microscopic characteristics, the test pathogen was identified and further confirmed by comparing the description of *R. bataticola* given by Barnett and Hunter (1972) [3].

Integrated disease management (polybag culture)

Those fungicides, bioagents and organic amendments found effective against *R. bataticola* during present *In vitro* and pot / polybag culture studies were selected and used (alone and in-combination) for integrated management of dry root rot of chickpea (polybag culture), by applying 'sick soil' method, as detailed described under pathogenicity test heading.

The test fungicides and talc based formulations of the test bioagents were applied (alone and in-combination) as pre-sowing seed treatment to the healthy seeds of chickpea JG-62 and sown (10 seeds / bag) in the polybags (20 x 30 cm.) containing *R. bataticola* (Rb-6 isolate) sick soil / potting mixture. The powdered test oil cakes were applied (@ 50 g/kg soil or potting mixture) in these polybags, mixed thoroughly, watered lightly and kept in screen house. After 72 hrs, these bags were seeded with surface sterilized healthy seeds (10 seeds / bag) of chickpea JG-62. For each treatment, two bags / replications were maintained. The polybags sown (10 seeds / bag) with surface sterilized healthy seed of chickpea JG-62, containing *R. bataticola* (Rb-6 isolate) were maintained as untreated control.

Experimental details:

Design: CRD
Replications: Three
Treatments: Twelve

Treatment details

Tr. No.	Treatments	Dosages (g/kg seed or potting mixture)	
		Seed	Soil
T_1	Carboxin 37.5% + Thiram 37.5% (75% WP) (ST)	3.0 g	-
T_2	Carbendazim 50% WP (ST)	1.0 g	-
T_3	Carbendazim 12% + Mancozeb 63% (75% WP) (ST)	2.5 g	
T_4	*T. asperellum* ($5x10^7$ cfu/g carrier) (ST)	10 g	-
T_5	*T. harzianum* ($5x10^7$ cfu/g carrier) (ST)	10 g	
T_6	*T. hamatum* ($5x10^7$ cfu/g carrier) (ST)	10 g	-
T_7	$T_4 + T_5$ (ST)	10 g + 10 g	-
T_8	$T_2 + T_4$ (ST)	1.0 g + 10 g	
T_9	$T_2 + T_4$ (ST) + FYM	1.0 g + 10 g	50 g
T_{10}	$T_2 + T_4$ (ST) + NSC (SA)	1.0 g + 10 g	50 g
T_{11}	$T_1 + T_4$ (ST) + NSC (SA)	3.0 g + 10 g	50 g
T_{12}	Control (untreated)	Sick soil	

ST: Seed Treatment, SA: Soil Application

Observations on pre-emergence seed rot (PRESR) and post-emergence seedling mortality (POESM) were recorded, respectively at 7-8 days and 15 and 30 days after sowing and total mortality was computed. Per cent PRESR, POESM and total mortality were calculated by applying the formulae as detailed below.

$$\text{PRESR (\%)} = \frac{\text{No. of Seeds un-germinated}}{\text{Total no. of Seeds sown}} \times 100$$

$$\text{POESM (\%)} = \frac{\text{No. of Seedlings died}}{\text{Total no. of Seedlings}} \times 100$$

Total mortality (%) = PRESR + POESM

Further, per cent reduction in total mortality with the treatments, over untreated control (sick soil alone) was calculated by formula as detailed below.

$$\text{\% Disease control} = \frac{C - T}{C} \times 100$$

Where,
C = Total mortality in untreated control
T = Total mortality in treatment

Results and Discussion

Isolation of the pathogen

Applying tissue isolation technique, the test fungus was isolated successfully from naturally dry root rot diseased roots and stems of chickpea plants, on autoclaved and cooled Potato Dextrose Agar plates. After 2-3 days of incubation, black

mycelial mat on PDA plates was developed and after 7-8 days of incubation, microsclerotia were developed and identified as *R. bataticola* based on morphological and cultural characters using the descriptions given by C.M.I. (1970). The culture of these isolate were purified by hyphal tip / single spore isolation technique and their pure culture on PDA slant tube was maintained separately and mass multiplication of the test isolate was done by using Sand: Maize and PDB medium.

Pathogenicity test

Pathogenicity of the *R. bataticola* test isolate was proved by applying sick soil method in polybag / pot culture, under screen house conditions, by sowing the seeds of susceptible chickpea cultivar JG-62. The test isolate induced the symptoms such as pre-emergence seed rot, post-emergence seedling mortality, yellowing and drooping of the leaves, microsclerotia production on stems and roots, rotting and shredding of roots and stems etc. Further, this test also revealed constant association of *R bataticola* with dry root rot disease of chickpea.

Reisolation of artificially dry root rot diseased chikcpea plants specimens also consistently yielded *R. bataticola* typical colony growth on PDA plates. Also, their cultural and morphological characteristics were exactly identical with *R. bataticola* pure cultures of the isolates obtained from naturally dry root rot diseased chickpea plants specimens. Thus, by applying Koch's postulates, pathogenicity of *R. bataticola* test isolates was conclusively proved.

Identification of the pathogen

Based on typical symptomatology of naturally / artificially dry root rot diseased chickpea plants, morpho-cultural characteristics, microscopic observations and pathogenicity test, the test pathogen was identified as *R. bataticola*, the cause of dry root rot of chickpea as described by Butler (1918) [4] and Sneh *et. al.*, (1991) [11].

Integrated disease management strategies

Integrated management of chickpea dry root rot, caused by *R. bataticola* (Pot/Polybag culture)

Those fungicides, bio-control agents and organic amendments found most effective during the various *in vitro* studies were integrated (alone and in combination) so as to manage chickpea dry root rot disease (*R. bataticola*), by applying sick soil method and by sowing susceptible Chickpea Cv. JG-62, pot / polybag culture, under screen house conditions. The results obtained on pre-emergence, post-emergence and average mortality are represented in the Table 1, PLATE I and Fig.1.

Per cent incidence of mortality (PRESR, POESM and Average)

With various treatments integrated, pre-emergence seed rot (PRESR), post-emergence seedling mortality (POESM) and average mortality incidence were observed to be in the range of 6.67 to 40.00, 9.03 to 43.24 and 7.85 to 41.62 per cent, respectively, as against 83.33, 100.00 and 91.67 per cent in control (untreated). However, the best effective treatment found was T_{11} (Carboxin 37.5% + Thiram 37.5% WP (ST) @ 3.0 g + *T. asperellum* (ST) @ 10 g + NSC (SA) @ 50 g), with significantly minimum PRESR (6.67%), POESM (9.03%) and average mortality (7.85%) This was followed by Carbendazim 50% WP (ST) @ 1 g + *T. asperellum* (ST) @ 10 g + FYM (SA) @ 50 g (10.00, 15.10 and 12.55 per cent), Carbendazim 50% WP (ST) @ 1 g + *T. asperellum* (ST) @ 10 g + NSC (SA) @ 50 g (13.33, 20.05 and 16.69 per cent) and *T. asperellum* (ST) @ 10 g + *T. harzianum* (ST) @ 10 g (16.67, 19.03 and 17.85 per cent). Whereas in rest of the treatments PRESR observed in the range 18.33 to 40.00 per cent, POESM in the range of 21.06 to 43.24 per cent and average mortality was found in the range of 19.70 to 41.62 per cent as against comparatively maximum PRESR (83.33%), POESM (100.00%) and average mortality (91.67%) in control (untreated).

Reduction in mortality (PRESR, POESM and Average)

Results (Table 1, PLATE I and Fig. 1) showed that all the treatments employed resulted in the significant reductions in per-emergence seed rot (PRESR), post-emergence seedling mortality (POESM) and total mortality, which were found in the range of 55.56 to 92.13 per cent, 66.67 to 90.97 per cent and 58.02 to 91.55 per cent, respectively, over control (untreated).

However, the treatment T_{11} (Carboxin 37.5% + Thiram 37.5% WP (ST) @ 3.0 g + *T. asperellum* (ST) @ 10 g + NSC (SA) @ 50 g), was found to be best effective with significantly highest reduction in PRESR (92.13%), POESM (90.97%) and average mortality (91.55%). This was followed by Carbendazim 50% WP (ST) @ 1 g + *T. asperellum* (ST) @ 10 g + FYM (SA) @ 50 g (87.96, 84.90 and 86.43 per cent), Carbendazim 50% WP (ST) @ 1 g + *T. asperellum* (ST) @ 10 g + NSC (SA) @ 50 g (84.26, 79.95 and 82.10 per cent) and *T. asperellum* (ST) @ 10 g + *T. harzianum* (ST) @ 10 g (79.86, 80.97 and 80.42 per cent). However in the rest of the treatments PRESR recorded in the range of 55.56 to 78.01 per cent, POESM reduction was in the range of 60.48 to 78.94 per cent and average mortality reduction was found to be in the range of 58.02 to 78.47 per cent, over control (untreated).

Results of this present study revealed that the fungicides (Carbendazim, Carboxin + Thiram, Carbendazim + Mancozeb), biocontrol agents (*T. asperellum* and *T. harzianum*) and organic amendment (Neem seed cake and FYM), applied as seed treatment and soil application (alone and in combination) were found to be effective against *R. bataticola*. Among the treatments taken, Carboxin37.5% + Thiram37.5% WP seed treatment @ 3g / kg seed + *T. asperellum* @ 10g / kg seed + Neem seed cake @ 50g / kg soil, followed by Carbendazim50WP seed treatment @ 1g / kg seed + *T. asperellum* @ 10g / kg seed + FYM @ 50g / kg soil and Carbendazim 50% WP (ST) @ 1 g + *T. asperellum* (ST) @ 10 g + NSC (SA) @ 50 g were found to be most effective with significantly highest reduction in the pre-emergence seed rot, post-emergence seedling mortality and total mortality, over control (untreated).

The reason for a good control of dry root rot by fungal antagonists in integration with combi fungisides & organic amendments in this study may be due to the combi product of fungicide which performed dual action to control the plant disease *i.e.* systemic and contact. These dual action which was totally inhibit the fungal growth. Whereas *Trichoderma* is a saprophytic fungus that grows on dead organic matter and cell wall of pathogenic fungi. It secretes a range of extracellular compounds, which inhibit pathogens through antibiosis. *Trichoderma* was found effective to control the disease. Neem cake which contains azadirachtin, salannin, nimbin, azadiradione as the major component might be responsible to reduce the disease. It acts as a biofertilizer and helps in providing the required nutrients to plants. Neem seed cake performs the dual function, acts as a soil enricher, reduces the population of soil pest and bacteria, provides macro nutrients

essential for all plant growth and these plant nutrients inhibits the growth of soil borne fungi (Pawar *et al*., 2018) [9].

Thus, these promising fungicides, bioagents and Neem seed cake could be used for integrated disease management of chickpea dry root rot disease, caused due to *R. bataticola.* These results similar with the findings of Moradia & Khandar (2011) [6], Vishwanathan *et al*., (2015) [14], Veena *et al*., (2016) [13] & Agale R.C. (2018) [1]. The present finding supports the results of Deshmukh *et al*., (2016).

Plate I: Integrated disease management against chickpea dry root rot JG- 62 (Polybag culture)

Table 1: Integrated efficacy of fungicides, bioagents, and organic amendments against *R. bataticola*, causing dry root rot of Chickpea (Polybag culture)

Tr. No.	Treatments	Dosages (g/kg seed or potting mixture)	Incidence (%) *		Av. Mor. (%)	Red. (%) over control		Av. Red. (%)
			Presr	Poesm		Presr	Poesm	
T_1	Carboxin 37.5% + Thiram 37.5% (Vitavax power 75% WP)	ST @ 3.0 g	28.33	32.14	30.23	65.97	**67.87**	66.92
			(32.16)	(34.53)	(33.35)	(54.31)	(55.47)	(54.89)
T_2	Carbendazim 50% WP	ST @1.0 g	23.33	26.04	24.69	71.99	73.96	72.97
			(28.88)	(30.68)	(29.79)	(58.05)	(59.32)	(58.67)
T_3	Carbendazim 12% + Mancozeb 63% (SAAF 75% WP)	ST @ 2.5 g	36.67	39.52	38.09	55.56	60.48	58.02
			(37.27)	(38.95)	(38.11)	(48.79)	(51.05)	(49.62)
T_4	*T. asperellum* ($5x10^7$ cfu/g carrier)	ST @ 10 g	30.00	32.61	31.31	63.89	67.39	65.64
			(35.26)	(37.45)	(34.02)	(53.06)	(55.18)	(54.11)
T_5	*T. harzianum* ($5x10^7$ cfu/g carrier)	ST @ 10 g	18.33	21.06	19.7	78.01	78.94	78.47
			(25.35)	(27.32)	(26.35)	(62.03)	(62.68)	(62.35)
T_6	*T. hamatum*($5x10^7$ cfu/g carrier)	ST @ 10 g	33.33	36.98	35.16	60.19	63.02	61.6
			(33.21)	(34.82)	(36.37)	(50.88)	(52.55)	(51.7)
T_7	$T_4 + T_5$	ST @ 10 g	16.67	19.03	17.85	79.86	80.97	80.42
			(24.09)	(25.86)	(24.99)	(63.33)	(64.14)	(63.74)
T_8	$T_2 + T_4$	ST @ 1.0 g + 10 g	40.00	43.24	41.62	51.85	56.76	54.31
			(39.23)	(41.11)	(40.18)	(46.06)	(48.89)	(47.47)
T_9	$T_2 + T_4$+ FYM	ST @ 1.0 g + 10 g + SA @ 50 g	10.00	15.1	12.55	87.96	84.9	86.43
			(18.43)	(22.87)	(20.75)	(69.7)	(67.13)	(468.38)
T_{10}	$T_2 + T_4$ + NSC	ST @ 1 g + 10 g + SA @ 50 g	13.33	20.05	16.69	84.26	79.95	82.1
			(21.42)	(26.6)	(24.11)	(66.63)	(63.4)	(69.47)
T_{11}	$T_1 + T_4$ + NSC	ST @ 3.0 g + 10 g + SA @ 50 g	6.67	9.03	7.85	92.13	90.97	91.55
			(14.96)	(17.48)	(16.27)	(73.71)	(72.51)	(73.1)
T_{12}	Control	--	83.33	100	91.67	0.00	0.00	0.00
			(65.91)	(90)	0.00	0.00	0.00	0.00
	SE ±	--	2.58	0.35		2.76	0.35	
	CD (P=0.05)		7.53	1.01		8.07	1.01	

*-Mean of three replications, Av.: Average, Mor.: Concentration, Incr.: Increase Red.: Reduction, PRESR: Pre emergence seed rot, POESM: Post Emergence Seedling Mortality, ST: Seed Treatment, SA: Soil Application, NSC: Neem Seed Cake, Figures in parentheses are arcsine transformed values

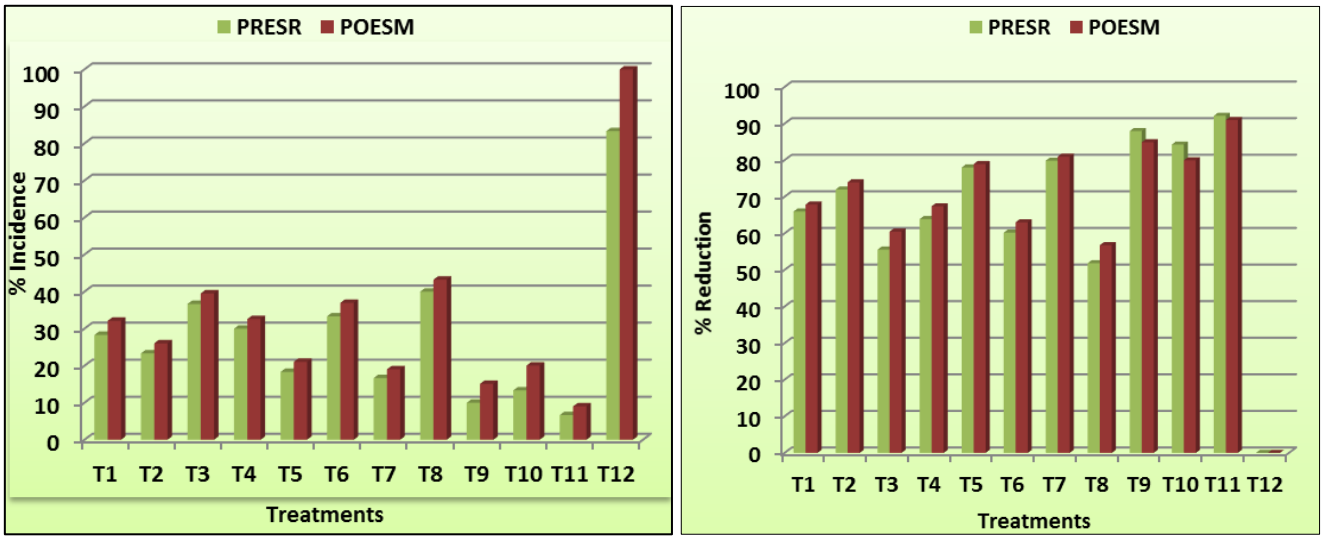

Fig 1: Integrated efficacy of fungicides, bioagents, and organic amendments against *R. bataticola*, causing dry root rot of Chickpea

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