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Identification of Resistant Genotypes and Integrated Management of Dry Root Rot of Chickpea

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ABSTARCT

Background: Dry root rot is an economically important soil borne disease of chickpea in India. The pathogen, *Rhizoctonia bataticola,* is a soil borne fungus resulting in significant losses in yield. Therefore, the present investigation was aimed to identify the management strategy of disease through identification new sources of resistance and integrated management of disease.

Methods: *R. bataticola* was isolated and purified by using hyphal tip technique and molecular detection was done by using ITS primers. One hundred chickpea germplasm entries were screened under in both field and advanced phenotyping in glass house. The field trials on integrated management of the disease were conducted with different treatments using randomized block design.

Result: Fourteen resistant and five moderately resistant genotypes were identified under artificial epiphytotic conditions. Advanced screening of these 19 genotypes under phenotyping technique yielded four resistant and five moderately resistant genotypes. Among nine treatments, seed treatment with mancozeb 50% + carbendazim 25% WP @ 3.5 g/kg followed by soil drenching of mancozeb 50% + carbendazim 25% WP @ 3 g/l water to infected and surrounding plants was found highly effective by recording least disease incidence with highest seed yield, test weight and benefit cost ratio.

Key words: Dry root rot, Integrated disease management, Resistant genotypes.

INTRODUCTION

Dry root rot caused by *Rhizoctonia bataticola* is an important disease of chickpea causing significant yield losses. The farmers are facing the problem of disease in all states that grow the crop and it is particularly more in Karnataka. The recent reports indicated that dry root rot is an emerging as a potential threat to chickpea productivity and production (Ghosh *et al*., 2013).

The dependence on chemical control or any other single control method is not advisable to manage dry root rot. Although, growing of resistant cultivars for the management of disease is a cost effective, sustainable and ideally fit into integrated disease management, identification of sources of resistance against soil borne diseases is not easy and requires artificial epiphytotic conditions or sick plots as the pathogen, is a soil and seed borne and survives in the form of sclerotia for many years.

Since the disease is a major hindrance to chickpea production Karnataka, it was felt necessary to identify the sources of resistance and develop eco-friendly integrated disease management strategy to manage the emerging and destructive dry root rot of chickpea. Hence, the present studies aimed at identification of sources for resistance and integrated management of emerging dry root rot of disease of chickpea.

MATERIALS AND METHODS

Isolation and purification of *R. bataticola*

Chickpea plants showing typical dry root rot symptoms were transferred to sterilized potato dextrose agar (PDA) medium in Petri dishes and incubated at 25±2°C to obtain mycelial growth. The pathogen was purified by hyphal tip technique.

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Molecular detection of *R. bataticola*

The pathogen was detected molecularly using ITS-1 (5′ CCTGTGCACCTGTGAGACAG-3′) and ITS-4 (5′-TGTCC AAGTCAATGGACTAT-3′) as reverse primer. For this, standard protocols were used for the isolation of DNA according to Liu *et al*. (2000) and amplified products was sent for sequencing and identification of species to Eurofins, Bangalore.

Identification of dry root rot resistant sources of chickpea

Preliminary disease screening

One hundred germplasm lines chickpea germplasm entries obtained from Indian Institute for Pulses Research, Kanpur) were screened in ‘sick plot’ at Agricultural Research Station, Kalaburgi, Karnataka during *rabi*-2019 and *rabi*-2020.

The crop was raised as per the recommended package of practices except the plant protection measures against dry root rot and observations were recorded. Disease incidence (%) was calculated using the following formula and genotypes were categorized (Khan *et al*., 2013) for their reaction (Table 1).

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased seedlings}}{\text{Total no. of seedlings}} \times 100$$

Advanced screening of promising entries by phenotyping technique

Nineteen promising (Resistant and moderately resistant) germplasm lines (Table 2) which were resistant/moderately resistant in preliminary disease screening were further subjected for advanced screening using phenotyping technique during *rabi*-2021 (Bera *et al*., 2016). For this, nineteen germplasm lines were sown in block with 2 meter width bed with spacing of 0.5 meter between beds with spacing of 30 × 10 cm.

Integrated management of the disease

Field experiments were conducted in randomized block design (RBD) during *rabi*-2019-20 and *rabi*-2020-21 at Agricultural Research Station, Kalaburgi, Karnataka by following recommended agronomical practices with highly susceptible variety Annigeri-1 with eight treatment combinations of bio-agents replicated thrice with plot size of 5 × 3 m^2 in sick pot along with untreated control plot. The observations on per cent disease, seed yield and test weight were recorded and benefit cost (B:C) ratio was also calculated as per standard methodologies.

RESULTS AND DISCUSSION

Isolation and identification of *R. bataticola*

The pathogen produced black, brown to grey coloured mycelium and the young hyphae were thin, hyaline, septate and typical right angle branching of the mycelium and constriction of the branch near the point of origin. The sclerotia formed were black, smooth, varying from spherical through oblong to irregular shapes (Fig 1 and 2). The results are in line with Sharma *et al.* (2012) with respect to isolation and characters of *R. bataticola.*

Molecular detection of pathogen

Both ITS-1 and ITS-4 primers produced amplified product size of 500-650 bp and nucleotide sequencing was done for ITS region of 18S rRNA. The BLAST data results revealed that the *R. bataticola* matched with the reference strain and confirmed as *Rhizoctonia bataticola* and accession number (HQ649832.1) was obtained by depositing the sequence in NCBI GeneBank, Maryland, USA.

Preliminary disease screening

Fourteen were resistant (0-10 per cent disease incidence) and five moderately resistant (10.1-20 per cent) out of 100 germplasm lines screened against the disease (Table 1,3 and Fig 3). The present findings are well supported by Saifulla *et al*. (2011) who screened chickpea entries for dry root rot pathogen under field condition and reported that 20 entries as resistant and 36 entries moderately resistant out of 196 genotypes screened in sick plot. Further, Nagamma and

Table 1: Reaction of chickpea germplasm lines against dry root rot in sick plot during *rabi,* 2019 and *rabi,* 2020.

Germplasm line	Disease incidence (%)				Reaction
	Rabi, 2019	*Rabi,* 2020	Mean	Maximum	
EC219999	100	89.00	94.50	100	HS
EC220078	47.00	42.95	44.97	47.00	S
EC267240	100	100	100	100	HS
EC267484	100	100	100	100	HS
EC267504	23.83	15.06	19.44	23.83	MS
EC441805	90.00	100	95	100	HS
EC489870	88.00	96.11	92.05	96.11	HS
EC489870	100	100	100	100	HS
EC489907	100	100	100	100	HS
EC528345	64.67	100	82.33	100	HS
EC555002	87.5	100	93.75	100	HS
IC555217	06.50	02.20	4.35	6.50	R
IC83344	77.00	79.00	78.00	79.00	HS
IC83370	49.18	53.15	51.16	53.15	HS
IC83409	38.00	45.41	41.70	45.41	S
IC83526	08.20	6.00	7.10	8.20	R
IC83538	10.00	10.00	10.00	10.00	R
IC83603	14.81	15.24	15.02	15.24	MR
IC83775	32.50	50.00	41.25	50.0	S
IC83797	87.0	100	93.50	100	HS

Table 1: Continue...

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IC83798	3.00	0.0	1.50	3.00	R
IC83803	43.33	39.08	41.20	43.33	S
IC83816	43.12	48.10	45.61	48.10	S
IC83965	100	100	100	100	HS
IC83996	100	100	100	100	HS
IC83997	48.84	89.00	68.92	89.00	HS
IC84008	100	100	100	100	HS
IC84011	18.70	17.5	18.10	18.70	MR
IC95135	05.60	2.60	4.10	5.60	R
IC116287	46.98	81.00	63.99	81.00	HS
IC116304	49.22	65.60	57.41	65.60	HS
IC116325	79.00	100	89.50	100	HS
IC116343	100	100	100	100	HS
EC498765	81.40	87.53	84.46	87.53	HS
EC498767	34.40	40.96	37.68	40.96	S
EC498773	31.66	34.12	32.89	34.12	S
EC498779	100	100	100	100	HS
EC498780	100	100	100	100	HS
EC498812	100	100	100	100	HS
EC498818	10.90	14.90	12.90	14.90	MR
EC498823	100	100	100	100	HS
EC498825	24.85	29.71	27.28	29.71	MS
EC498827	8.70	3.80	6.25	8.70	R
IC83358	4.25	1.45	2.85	4.25	R
IC83359	100	100	100	100	HS
IC83361	90.56	100	95.28	100	HS
IC83362	88.00	100	94.00	100	HS
IC83363	100	100	100	100	HS
IC83353	100	100	100	100	HS
IC83354	95.0	100	97.50	100	HS
IC83355	100	100	100	100	HS
IC83356	28.41	30.00	29.20	30.00	MS
IC83357	5.50	1.30	3.40	5.50	R
IC83487	9.34	9.15	9.24	9.34	R
IC83489	100	100	100	100	HS
IC83490	89.00	100	94.50	100	HS
IC83493	90.00	100	95.00	100	HS
IC83494	100	100	100	100	HS
IC83497	100	100	100	100	HS
IC83500	02.50	1.25	1.87	2.50	R
IC83501	06.70	3.57	5.13	6.70	R
IC83502	100	100	100	100	HS
IC83504	76.00	100	88.00	100	HS
IC83506	6.00	6.78	6.39	6.78	R
IC83508	78.00	90.00	84.00	90.00	HS
IC83509	49.21	80.20	64.70	80.20	HS
IC83510	100	100	100	100	HS
IC83511	100	100	100	100	HS
IC83512	100	100	100	100	HS
IC83511	100	100	100	100	HS
IC83512	100	100	100	100	HS
IC83513	98.20	100	99.10	100	HS

Table 1: Continue...

Table 1: Continue...

IC83514	07.80	5.45	6.62	7.80	R
IC83515	100	100	100	100	HS
IC83516	100	100	100	100	HS
IC83517	100	100	100	100	HS
IC83722	34.35	34.35	34.35	34.35	S
IC83723	36.56	40.73	38.64	40.73	S
IC83725	100	100	100	100	HS
IC83728	99.0	100	99.50	100	HS
IC83729	100	100	100	100	HS
IC83730	100	100	100	100	HS
IC83731	100	81.20	90.60	100	HS
IC83732	15.40	19.83	17.61	19.83	MR
IC83733	100	100	100	100	HS
IC83734	100	100	100	100	HS
IC83735	49.19	41.16	45.17	49.19	S
IC83736	9.50	7.45	8.47	9.50	R
IC83739	75.00	82.00	78.50	82.00	HS
IC83740	86.00	92.00	89.00	92.00	HS
IC83741	100	100	100	100	HS
IC83742	100	100	100	100	HS
IC83742	16.00	16.45	16.22	16.45	MR
IC83743	32.70	32.26	32.47	32.27	S
IC83744	100	100	100	100	HS
IC83745	100	100	100	100	HS
IC83746	90.00	100	95	100	HS
IC83747	93.50	100	96.75	100	HS
IC83748	99.00	90.13	94.56	99.00	HS
IC83749	44.05	44.21	44.11	44.21	S
IC83750	100	70.40	85.20	100	HS
IC83751	100	100	100	100	HS
Susceptible Check (A-1)	100	100	100	100	HS

HS- Highly susceptible; S- Susceptible; MS- Moderately susceptible; MR- Moderately resistance; R- Resistance.

Table 2: Reaction of promising chickpea germplasm lines against dry root rot using phynotyping technique during *rabi,* 2021.

Germplasm lines	Disease incidence (%)	Reaction
IC83736	16.00	MR
IC 83526	17.5	MR
IC 83538	36.09	S
IC83798	2.8	R
IC95135	4.9	R
EC498827	29.00	MS
IC83358	15.40	MR
IC83357	26.00	MS
IC83487	38.11	S
IC83500	2.87	R
IC83501	16.00	MR
IC83506	5.6	R
IC83514	18.33	MR
IC 83736	29.00	MS
IC83603	25.00	MS
IC84011	33.06	S
EC498818	21.50	MS

Saifulla (2012) out sixty four Kabuli genotypes against dry root rot of chickpea, six were resistant and nine showed moderately resistant reaction.

Advanced disease screening of promising lines by phenotyping technique

Four germplasm lines *viz*., IC83798, IC95135, IC83506 and IC83500 showed resistant reaction and five germplasm lines *viz*., IC83736, IC83526, IC83358, IC83501 and IC83514 were moderately resistant (Table 2 and Fig 4). Similarly, Pande *et al*. (2004) tested 47 lines of chickpea against the dry root rot in advanced screening and reported that only ICC 14395 and ICCV 2 were resistant and the remaining 22 lines moderately resistant against dry root rot of chickpea. Similarly, Jayalakshmi *et al*. (2008) also tested 12 genotypes against dry root rot disease and found only four genotypes as resistant.

Integrated management of dry root rot of chickpea

The results on pooled data (Table 4 and Fig 5) indicated that the treatment combination, seed treatment with mancozeb 50% + carbendazim 25% WP @ 3.5 g/kg followed

Fig 1: Cultural characters of *R. bataticola.*

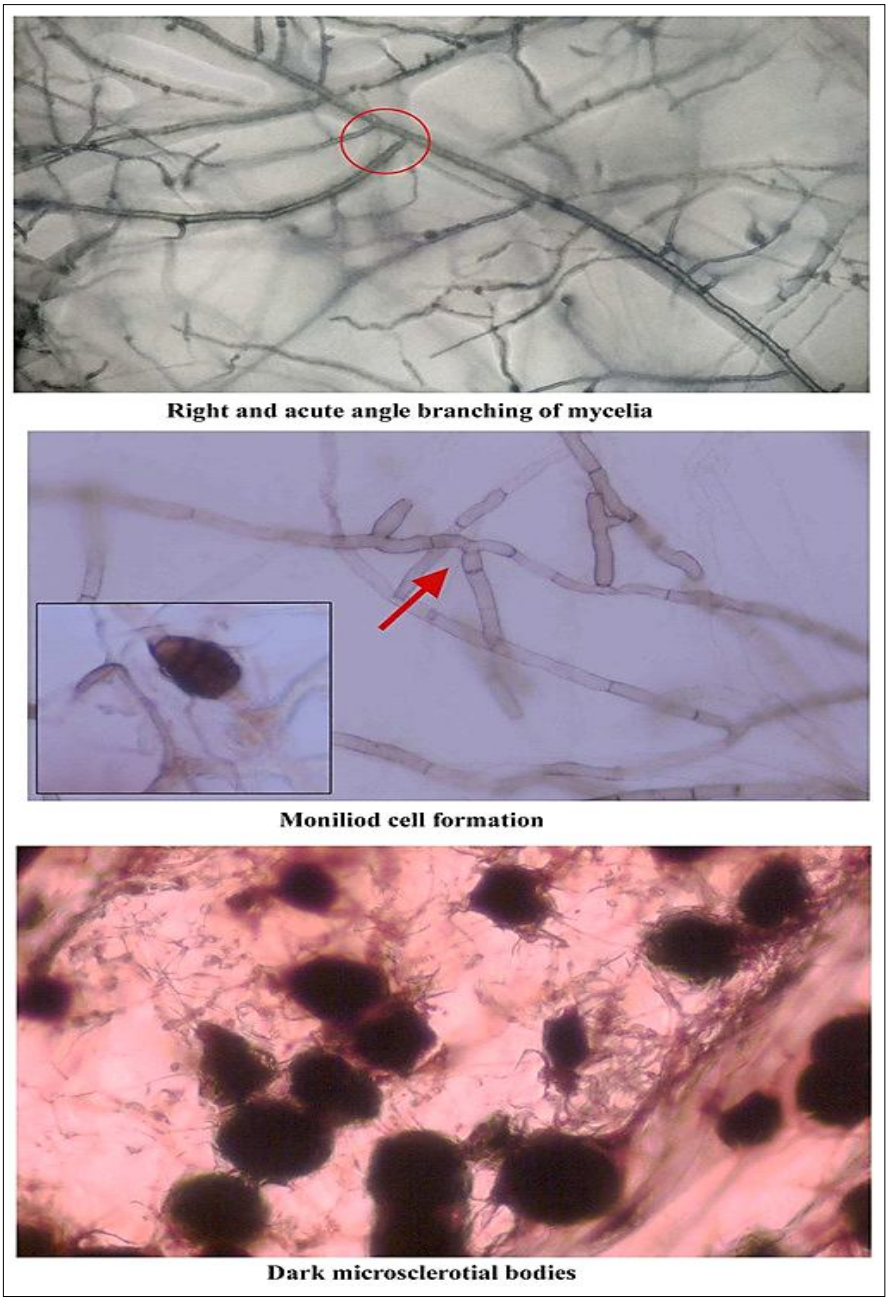

Fig 2: Morphological characters of *R. bataticola.*

Fig 3: Screening of chickpea germplasm entries against dry root rot of chickpea under field conditions.

Fig 4: Advanced disease screening of promising lines by phenotyping technique.

Table3: Categorization of chickpea germplasm entries against dry root rot based on their disease reaction.

Scale	Disease reaction	Name germplasm lines	Number of lines
0-10% mortality	Resistance (R)	IC83736, IC 83526, IC 83538, IC83798, IC95135, EC498827, IC83358, IC83357, IC83487, IC83500, IC83501, IC83506 IC83514 and IC 555217	14 5
10.1-20% mortality	Moderately resistance (MR)	IC83603, IC84011, EC498818, IC83732 and IC83742	
20.1-30% mortality	Moderately susceptible (MS)	EC 267504, EC498825 and IC83356	3
30.1-50% mortality	Susceptible (S)	EC 220078, IC 83409, IC 83775, IC83803, IC83816, EC498767, EC498773, IC83722, IC83723, IC83735, IC83743 and IC83749	12
> 50% mortality	Highly susceptible (HS)	EC219999, EC267240, EC267484, EC441805, EC489870, EC489870, EC489907, EC555002, IC83344, IC83797, IC83965, IC83996, IC84008, IC116325, IC116343, EC498765, EC498779, EC498780, EC498812, EC498823, IC83359, IC83361, IC83362, IC83363, IC83353, IC83354, IC83355, IC83489, IC83490, IC83493, IC83494, IC83497, IC83502, IC83504, IC83508, IC83510, IC8351, IC83512, IC83513,IC83515, IC83516, IC83517, IC83725, IC83728, IC83729, IC83730, IC83731, IC83733, IC83734, IC83739, IC83740, IC83750, IC83741, IC83742, IC83751, IC83744, IC83745, IC83746, IC83747, IC83748, EC 528345, IC83370, IC83997, IC116287, IC116304 and IC83509	66

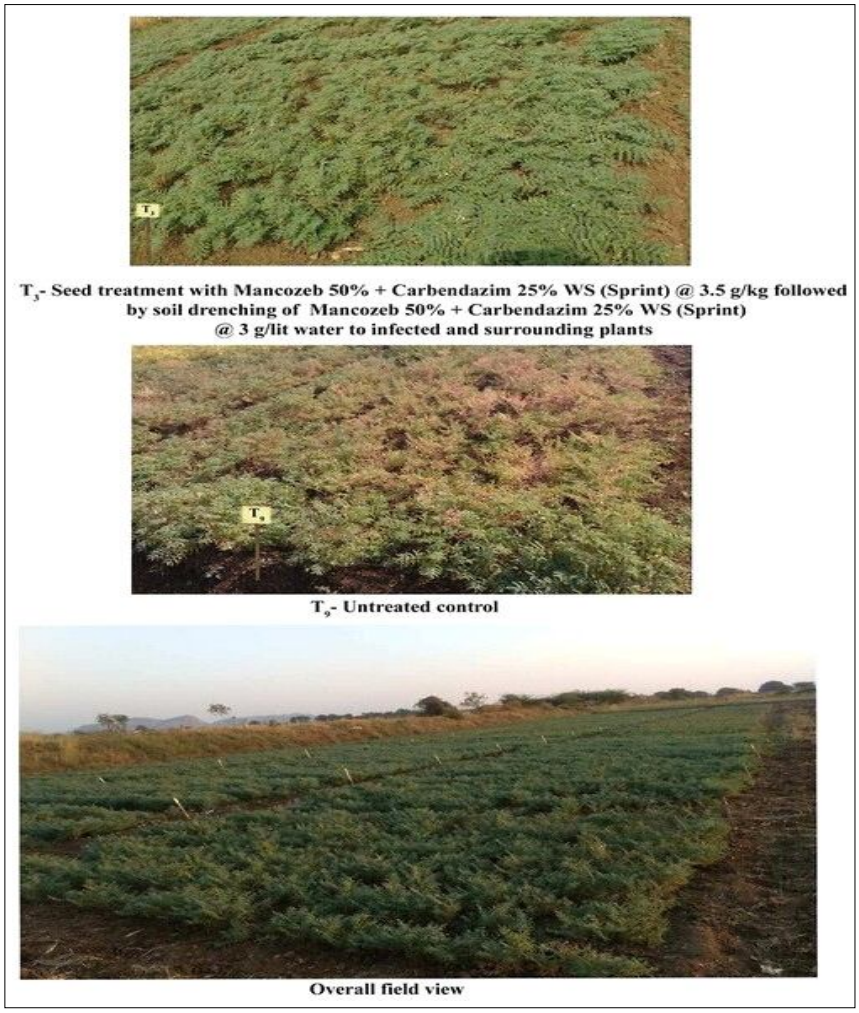

Fig 5: Integrated disease management of dry root rot.

Table 4: Integrated management of dry root rot of chickpea during *Rabi*, 2019 and *Rabi,* 2020.

Treatment	Disease incidence (%)			Seed yield (q/ha)			BC ratio
	Rabi, 2019	*Rabi*, 2020	Pooled mean	*Rabi*, 2019	*Rabi*, 2020	Pooled mean	
Seed treatment with *T. harzianum* @ 5 g/kg seed followed by soil application of enriched *T. harzianum* @ 2.5 kg/250 kg of FYM during sowing	21.90 (27.26)	23.55 (29.03)	22.27	8.20	7.72	7.96	2.22
Seed treatment with *P. fluorescens* @ 5 g/kg seed followed by soil application of enriched *T. harzianum* @ 2.5 kg/250 kg of FYM during sowing	24.27 (29.51)	25.00 (30.0)	24.63	8.30	7.80	8.05	2.25
Seed treatment with mancozeb 50% + carbendazim 25% WP @ 3.5 g/kg followed by soil drenching of mancozeb 50% + carbendazim 25% WP @ 3 g/lit water to infected and surrounding plants	7.70 (16.11)	8.25 (16.69)	7.97	13.10	11.42	12.26	3.40
Seed treatment with *T. harzianum* @ 5 g/kg seed followed by soil drenching of mancozeb 50% + carbendazim 25% WP @ 3 g/lit water to infected and surrounding plants	22.59 (28.38)	20.59 (26.99)	21.59	9.62	8.88	9.25	2.01
Seed treatment with *P. fluorescens* @ 5 g/kg seed followed by soil drenching of mancozeb 50% + carbendazim 25% WP @ 3 g/lit water to infected and surrounding plants	26.00 (30.66)	25.15 (26.67)	25.55	8.10	6.30	7.20	2.39
Seed treatment with *T. harzianum* @ 5 g/kg seed + *P. fluorescens* @ 5 g/kg seed followed by soil application of enriched *T. harzianum* + *P. fluorescens* @ 2.5 kg/250 kg of FYM during sowing	22.33 (28.20)	20.47 (26.90)	21.40	8.97	11.15	10.06	2.81
Seed treatment with *T. harzianum* @ 5 g/kg seed + *P. fluorescens* @ 5 g/kg seed followed by soil drenching of mancozeb 50% + carbendazim 25% WS (Sprint) @ 3 g/lit water to infected and surrounding plants	9.57 (18.04)	10.30 (18.72)	9.93	10.90	8.50	11.20	3.02
Seed treatment with mancozeb 50% + carbendazim 25% WP @ 3.5 g/kg	22.10 (28.04)	24.12 (29.41)	23.11	7.52	8.00	7.76	2.17
Untreated control	33.45 (34.11)	36.25 (33.38)	34.85	5.11	6.37	5.74	1.60
S.Em. ±	3.12	3.08	3.10	0.56	0.39	0.47	
CD at 5%	9.06	8.14	9.02	1.32	1.54	1.42	

by soil drenching of mancozeb 50% + carbendazim 25% WP @ 3 g/lit water to infected and surrounding plants was highly effective and significantly superior in recording least disease incidence when compared to other treatments. The treatment recorded lowest pooled mean disease incidence of 7.97% with highest pooled mean yield (11.42 q/ha) and B:C ratio (1:3.40). Khan *et al.* (2012) tested eight fungicides against dry root rot fungus, among them mancozeb, carbendazim, copper-oxy- chloride and benomyl completely inhibited the growth of the fungus compared to control. Further, in fungicidal trails on management of dry root rot of chickpea caused by *R. bataticola,* carbendazim (0.2 per cent) used as seed treatment, soil drenching and seed treatment plus soil drenching recorded lowest disease incidence of 15.6 per cent highest grain yield of 192 q/ha respectively (Vijay Mohan *et al*., 2006).

CONCLUSION

Preliminary screening of 100 germplasm lines resulted in the identification of fourteen resistant and five moderately resistant germplasm lines. Advanced screening of 19 genotypes under phenotyping technique yielded only four genotypes resistant and five moderately resistant genotypes. The integrated disease management, seed treatment with mancozeb 50% + carbendazim 25% WS @ 3.5 g/kg followed by soil drenching of mancozeb 50% + carbendazim 25% WS @ 3 g/l water to infected and surrounding plants was highly effective by recording least disease incidence of dry root rot with highest seed yield, test weight and highest benefit cost ratio.

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Conflict of interest: None.

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