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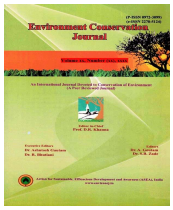

Resistance screening and *in-vitro* efficacy of fungicides for the management of dry root rot of chickpea caused by *Rhizoctonia bataticola*

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ABSTRACT

Dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler is an emerging threat for chickpea production. It is among one of the chief and common soil borne diseases of chickpea. The present investigation was conducted firstly to identify the resistant source for dry root rot in chickpea and secondly to evaluate the efficacy of different fungicides in inhibiting the growth of *R. bataticola* under *in vitro* conditions. Screening of a set of 50 chickpea entries resulted in identification of three entries namely ICCV 191317, ICCV 191306, and Ujjain 21 as moderately resistant to dry root rot of chickpea. No entry could be identified as completely resistant for dry root rot in chickpea. Further, among the different fungicides tested, pyraclostrobin alone and in combination of Thiophanate methyl completely checked the growth of *R. bataticola* at 100 ppm concentration under *in vitro* conditions. However, another combination product of fungicides namely carboxin + thiram and carbendazim + mancozeb also showed complete inhibition in growth of test pathogen at higher concentration of fungicides i.e. at 300 ppm concentration.The identified moderately resistant genotypes could be a useful resource for development of resistant varieties in chickpea for dry root rot using molecular breeding approaches.

Introduction

Pulses are critical for providing affordable protein to the world's rising human population. In comparison to cereal crops, pulses have fallen behind in terms of genetic development. Nonetheless, in recent years, significant progress has been achieved in utilizing current genomic techniques and breeding approaches that support pulse genetic improvement (Kumar et al., 2021).

Chickpea (*Cicer arietinum* L.) is the most frequently farmed pulse, accounting for 75 % of India's total pulse production (Ali *et al*., 2020). Chickpea seeds have a protein content of 29%, a carbohydrate content of 59 %, a fibre content of 3%, an oil content of 5%, and an ash content of 4%. The therapeutic benefits of malic acid and oxalic acid from leaves are well established (Singh *et al.*, 2020). After the common bean, it is the world's second most important food legume. It is a high-protein crop that also improves soil fertility through biological nitrogen fixation (Zia-Ul-Haq *et al.*, 2007).

Chickpeas are infected by 172 different pathogens, including fungi, bacteria, viruses, and nematodes. Dry root rot caused by *Rhizoctonia bataticola*, wilt

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caused by *Fusarium oxysporum ciceri*, and collor rot caused by *Sclerotium rolfsii* are major soil-borne diseases that inflict serious damage to chickpeas in favourable conditions (Ravichandran *et al*., 2014).

Dry root rot is one of the soil-borne diseases that can cause 10-35 percent yield losses in chickpea production (Pal, 1998). Chickpea dry root rot caused by the nectrotropic fungus *R. bataticola* is becoming a severe danger to global chickpea agriculture (Pandey and Sharma, 2010). It is most severe in Madhya Pradesh's chickpea-growing regions. *R. bataticola* is a polyphagous soil-borne disease that has infected over 500 plant species around the world, resulting in massive economic losses. Despite the fact that the fungus is both seed and soil borne (Dhingra and Sinclair, 1994), soil borne inoculum is more essential in infecting and spreading disease. The fungus is propagated by irrigation water, agricultural practises, and equipment.The stages of pod setting and late flowering are typically when the plant is most vulnerable to dry root rot disease. Plants that have been infected look to have entirely dried out. The most typical symptoms of disease are the destruction of lateral roots and widespread rotting. Yellowing of the leaves is a common indicator of root rot, and these leaves could fall off in two to three days. Within a week, the plant may wilt. In the advanced stages of disease, sclerotial bodies can be seen distributed on the damaged tissues (Singh and Srivastava, 1998).

Looking to the enormous losses imposed by this pathogen, there is a dire need for the control of this pathogen. Although different chemicals and biocontrol agents (Kumar *et al*., 2009; Srivastava *et al*., 2009) have so far been utilized for control of different plant diseases including dry root rot of chickpea but so far limited success have been achieved. Further evaluation of fungicides will certainly open up new avenues for control of this pathogen. Simultaneously, huge genomic resources are now available in different pulses including chickpea (Hiremath *et al*., 2011, 2012; Gujaria *et al*., 2011) which can easily assist in genetic dissection of region harbouring resistance for dry root rot of chickpea. However, to accomplish this trait mapping, identification of donar lines for dry root resistance is a pre-requisite which can be utilized in molecular breeding programmes to incorporate resistance for DRR in elite chickpea lines (Chamarthi *et al*., 2011). Apart from this, use of host plant resistance is not only one of the most feasible eco-friendly approaches for dry root rot management in chickpea which will not only provide immediate solution but also can contribute to identification of source of resistance,likely to be used in molecular breeding programme. Looking to the economic importance of dry root rot of chickpea, the present investigation, was conducted firstly to screen different fungicides against *R. bataticola in-vitro* and secondly to identify resistance source for DRR in chickpea.

Material and Methods

In-vitro evaluation of fungicides against *R. bataticola*

The experiment was conducted *in-vitro* to know inhibitory effect of different fungicides viz. Carboxin+Thiram, Azoxystrobin+Difenoconazole, Thiophanatemethyl+pyraclostrobin, Carbendazim+ Mancozeb, Difenoconazole, Thiophanate methyl and Pyrochlostrobin alone at 100 and 300 ppm using poisoned food technique (Nene and Thapliyal, 1973). Potato dextrose agar (PDA) media was amended with 100 and 300 ppm of the appropriate fungicide, then placed separately in a Petri plate and allowed to solidify. The study used *R. bataticola* cultures that were seven days old and actively developing. Without using fungicides, a fungal disc (5mm diameter) was placed in the middle of the PDA Petri plate and proper control was maintained. The plates were incubated at room temperature (28±2ºC) for seven days. The diameters of colonies measured and the per cent inhibition of growth estimated on the seventh day.

$PI = C – T/C \times 100$

Where

PI = Per cent inhibition

C = Radial growth of pathogen in control plates

T = Radial growth of test pathogen in treatment plates

Resistance screening

A collection of 50 chickpea varieties and advanced breeding lines were employed to screen for resistance using an *in vitro* blotter paper approach (Nene *et al*., 1981). A 5 mm disc of pure culture of a seven-day-old, vigorously developing *R.*

bataticola was transferred to 250 ml flasks with 100 ml Himedia potato dextrose broth for each flask (PDB). The mycelial mats were taken from two such flasks after 7 days of incubation at 25°C, and were added to 100 ml sterilised distilled water in a beaker after proper crushing for 1-2 minutes in the blender. Seeds of various chickpea lines were surface sterilised and sown on plastic trays with sterilised soil + sand (1:1) mixture. Each genotype's ten-day-old seedlings were uprooted in such a way that the root system was not disrupted. These seedlings' root systems were thoroughly cleansed in flowing water before being rinsed in sterilised distilled water. All genotypes (test lines) had their roots immersed in the inoculum kept in a beaker for about 30 seconds, and the excess inoculum was removed by contacting the roots to the beaker's edge. Each test line was given ten seedlings, which were stored separately on two blotter papers (size 45 cm x 25 cm with one fold). The blotter paper was sufficiently saturated with water, and the seedlings were held in such a way that just the cotyledons and roots were covered, leaving the green tops of the seedlings exposed. A check JG 12 seedling was inoculated and kept with each batch of seedlings. The folded blotter papers were stacked in a batch of ten papers in a tray, one on top of the other. These trays were kept in the incubator for 8 days at 35°C. On alternate days, artificial light was provided for 12 hours and the blotter papers were suitably moistened. The seedlings were assessed for dry root rot after 10 days using the scale mentioned in table 1.

Results and Discussion

In vitro evaluation of fungicides against *R. bataticola*

At two distinct concentrations, 100 and 300 ppm, a set of seven fungicides were tested for their fungicidal activity on *R. bataticola* radial growth. When compared to the control, all of the fungicides were observed to suppress the growth of test pathogen to varied degrees. Pyraclostrobin and Thiophanate methyl + Pyraclostrobin were shown to be the most effective and significantly superior to all other fungicides, inhibiting 100% mycelial growth of *R. bataticola* at 100 and 300 ppm, respectively. Further, Carboxin + Thiram and Carbendazim + Mancozeb also showed complete inhibition in growth of test pathogen at higher concentration of fungicides i.e. at 300 ppm concentration. However, at 100 ppm concentration Carboxin + Thiram and Carbendazim + Mancozeb exhibited 87.21 % and 84.24% inhibition. As mentioned in table 2, more inhibitory effect of pyraclostrobin is exhibited then thiophanate methyl because of complete inhibition of test pathogen. In earlier reports also similar findings have been reported by Ravichandran and Hegde, 2017 where carbendazim + Mancozeb, carboxin + thiram were reported as best fungicides against *R. bataticola* with 100 per cent inhibition of *R. bataticola*. The findings of present investigation are in agreement of their findings.

Screening of chickpea lines for identification of resistant source

In total, a set of 50 entries of chickpea consisting of released varieties, advanced breeding lines of different crosses, local checks were evaluated for resistance against dry root rot of chickpea. It was observed that after 10 days of incubation period, no entry showed complete resistance against dry root rot. However, a set of three entries namely ICCV 191317, ICCV 191306, Ujjain 21 exhibited 10.1-20% dry root rot incidence and grouped under the category of moderately resistant entries. A set of 11 entries namely JAKI 9218, JG 226, ICCV-D 201215, JG 12xJG 16-1, ICCV191312, ICCV191305, ICCV191303, JG 14, JG 11, JG 2018-52, C20264 exhibited 20.1-30% dry root rot incidenceand grouped under the category of tolerant. However, 36 entries exhibited 30.1 to 40% dry root rot and grouped under the category of susceptible entries. None of the entry could be grouped under the category of highly susceptible (Table 3).

Under *in vitro* circumstances, Pandey *et al*. (2004) investigated twenty-nine chickpea germplasm accessions, ten cultivars, and eight advanced breeding lines for resistance to dry root rot. Dry root rot resistance was found in one germplasm accession (ICC 14395), a cultivar (ICCV 2), and an advanced breeding line. The other 22 lines were moderately resistant, 19 susceptible, and two highly susceptible lines (BG 212 and ICC 12267) were utilized as controls. Gupta *et al*. (2012) also identified BG 212 as a vulnerable cultivar with 100% mortality, which corroborated the findings of this study. Jagre *et al.* (2018) tested 98 chickpea entries *in vitro* and identified 5 to be disease

Table 1: Rating scale for scoring of dry root rot of chickpea

Rating	Category	Symptoms of DRR	DRR percentage
1	Resistant	No infection on roots	0.0-10.0
2-3	Moderately resistant	On roots very few small lesions	10.1-20.0
4-5	Tolerant	Lesions on roots clear but small, new roots free from infection	20.1-30.0
6-7	Susceptible	Many lesions on roots,Usually new roots free from lesions	30.1-40.0
8-9	Highly susceptible	Roots are infected and completely discoloured	40.1 and above

Table 2: *In vitro* evaluation of fungicides against *R. bataticola* at 100 and 300 ppm

Treatment No.	Treatment details	Average radial growth (mm)	Per cent inhibition	Average radial growth (mm)	Per cent inhibition
		100 ppm		300 ppm	
T_1	Carboxin + Thiram	11.16	87.21	0.00	100.00
T_2	Azoxystrobin + Difenoconazole	10.50	88.44	11.83	86.70
T_3	Thiophanate methyl + pyraclostrobin	0.00	100.00	0.00	100.00
T_4	Carbendazim + Mancozeb	14.00	84.24	0.00	100.00
T_5	Difenoconazole	42.16	52.15	26.00	70.78
T_6	Thiophanate methyl	14.50	83.25	14.83	83.41
T_7	Pyraclostrobin	0.00	100.00	0.00	100.00
T_8	Control	90.00	-	90.00	-
	CD (5%)	2.37		0.83	
	SE(m)±	0.80		0.28	

Table 3: Response of chickpea entries to dry root rot under *in vitro* conditions

SN	Genotypes	Dry Root rot incidence(%)	Number of entries	Reaction of Genotypes
1	No genotypes	0.0-10	0	Resistant
2	ICCV 191317, ICCV 191306, Ujjain 21	10.1-20	3	Moderately Resistant
3	JAKI 9218, JG 226, ICCV-D 201215, JG 12xJG 16-1, ICCV191312, ICCV191305, ICCV191303, JG 14, JG 11, JG 2018-52, C20264	20.1-30	11	Tolerant
4	ICCV191313, Local-check, ICCV191315, ICCV191309, ICCV191316, ICCV191304, Kak2, ICCV191310, ICCV191308, ICCV191307, ICCV191311, ICCV191301, ICCV191318, JG 12xICC06301, JG 12xICC4958, JG 12 x ICC251741, JG 26xICC251741, JG 14xJG 24, JG2017-47, JG2018-53, ICVT-Desi local check, IVTC20247, ICCV-202117, YELLOW TOP, IVT-C-20257, ICCV201212, ICCV201218, ICVTD201214, IVT-MHC-20467, IVT late C-20281, JG 12xICC4959, JG 26xICC251742, JG23 x ICC251742, JG 24, JG 36, JG 12	30.1-40	36	Susceptible
5	No germplasm	40.1 and above	0	Highly susceptible

resistant, with a disease incidence of less than 10%. 42 genotypes, on the other hand, were found to be moderately resistant. The disease's prevalence ranged from 10% to 20%. They are supported by the current investigation's findings. The identified lines in present investigations can not only be directly used under DRR prevalent areas but can also be deployed further in development of mapping populations, identification of QTL for dry root resistance in chickpea which can be utilized in crop improvement programmes.

Conclusion

Looking to the economic losses of dry root rot of chickpea, the findings of present investigation identified two fungicides namely Pyraclostrobin and Thiophanate methyl + Pyraclostrobin which were found completely inhibitory to *R. bataticola* even at 100 ppm concentration. Further, three entries namely ICCV 191317, ICCV 191306, Ujjain 21 grouped under the category of moderately resistant and can not only be utilized in dry root rot prone areas but also can significantly contribute in genetic dissection and development of improved varieties for dry root rot resistance in chickpea using genomic tools and molecular breeding platforms in future.

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Conflict of interest

The authors declare that they have no conflict of interest.

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