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Evaluation of different fungicides against chickpea dry root rot caused by *Macrophomina phaseolina* (Tassi) Goid

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Abstract
Chickpea (*Cicer arietinum* L.) is the second most important pulse crop after dry beans in tropical, subtropical and temperate regions of the world. Among the different soil borne diseases dry root rot caused by fungal pathogen *Macrophomina phaseolina* is the most important, devastating and challenging disease of chickpea. An experiment was conducted to test the efficacy of fungicides on radial growth of *M. phaseolina* which collected during *Rabi* 2021 from Sangareddy district of Telangana. In this study eight different fungicides were tested at 5 different concentrations under *in vitro* conditions for their inhibitory effect on test pathogen. Among these fungicides maximum inhibition (100%) on radial growth of *M. phaseolina* was observed with four fungicides *viz*. Propiconazole 25% EC, Tebuconazole 60 FS, Mancozeb 63% + Carbendazim 12% WP and Tebuconazole 50% +Trifloxystrobin 25% WG at all concentrations tested and minimum inhibition was observed with Copper oxychloride among all fungicides tested ranging from zero to 24.63 percent with radial growth of 9 cm to 6.78 cm at 1000ppm and 3000ppm concentrations respectively. Whereas remaining fungicides exhibited 81.48 to 91.86 percent inhibition at tested concentrations.

Keywords: Evaluation, fungicides, chickpea, *Macrophomina phaseolina*

Introduction
Chickpea (*Cicer arietinum* L.), is commonly called as gram, Bengal gram, or garbanzo bean and Shanaga in Telugu is the world's second most important pulse crop after dry beans in tropical, subtropical and temperate regions. India is one of the leading producer by producing 11.35 Mt of chickpea on 10.17 Mha of land with an average productivity of 1116 Kg ha^{-1} (Agricultural Statistics at a Glance, 2020) [1]. It is a self-pollinating, Rabi season legume. It is an annual diploid species (2n=16) and it is the only species cultivated under genus *Ciceri* (Atta and shah 2009) [3]. Chickpea has the ability to increase soil fertility by increasing the nitrogen through a symbiotic relationship with nitrogen-fixing bacteria. Besides this it will assist in breaking the disease cycle of numerous important cereal pathogens in addition to improving soil fertility.

Despite the high total production, yields of chickpea are low due to many biotic and abiotic constraints. Among the biotic constraints chickpea suffers from about 172 pathogens consisting of 67 fungi, 22 viruses, 3 bacteria, 80 nematodes and mycoplasma have been reported from all over the world, out of which 89 pathogens have been reported from India alone (Andrabi *et al*., 2008) [2]. Out of these dry root rot caused by *Macrophomina phaseolina*, (Synonym *Rhizoctonia bataticola* (Taub.) Butler), wilt caused by *Fusarium oxysporium* f.sp. *ciceris,* ascochyta blight caused by *Ascochyta rabiei* and collar rot caused by *Sclerotium rolfsii* Sacc. Has become major constraints in getting good yield.

Dry root rot is the most important, devastating and challenging disease which is caused by *Macrophomina phaseolina* (Tassi.) Goid. *M. phaseolina* is soil, seed and stubble inhabiting necrotrophic fungus infects chickpea plant most frequently at flowering and pod formation stage or seed development stage (Lakhran *et al*., 2018) [6]. Disease reaction of *M. phaseolina* on stem and leaves appear as dry and become brittle and straw coloured lesions. Yellowing and dropping of leaves within two to three days is prominent symptom of this disease. Plants infected by *Macrophomina* show root rot symptom at ground level by producing black lesions on the bark of the stem initially, when pulled out from the soil and examined sclerotial bodies may be visible on the affected tissues the basal stem and main root in the advanced stages (Singh and Srivastava, 1988) [11]. *M. phaseolina* survives in or on the seed and persists in the

soil as black sclerotia, which are generated in great numbers on infected host tissues and then disseminated in the soil during tillage activities. Dry root rot was found in all chickpea growing areas of the central and southern part of India and incidence ranged from 8.9 to 10.3 per cent (Ghosh *et al*., 2013) [5].

Keeping in view of the above losses an experiment was conducted to find out rapid and efficient management strategies against chickpea root rot disease, use of chemical fungicides choose as most popular and effective disease control strategy despite of its excessive use and development of resistance by pathogen. Additionally, some fungicides are eradicated and can help in getting rid of the pathogen that has already been established. In view of the above facts, the present study was carried out under *in vitro* to assess antifungal potential of different fungicides against chickpea dry root rot caused by *M. phaseolina*.

Materials and Methods

The study was carried out with the facilities available at the Department of Plant Pathology, Agricultural college, Jagtial.

Sample collection, isolation and purification

Roving survey was conducted during *Rabi* 2021-22 at in the farmer's fields of ten major chickpea growing districts of Telangana for Dry root rot caused by *M. phaseolina.* Chickpea plants showing typical dry root rot symptoms were collected in paper bags separately, labelled properly and brought to the laboratory for isolation of the pathogen. The infected portions of the plant were washed properly under running tap water to remove excess soil adhered to the root zone and dried on blotter paper before isolation to avoid contamination and then cut the diseased portion along with healthy portion into small pieces of 1-2 mm size with the help of sterilized blade and surface sterilized with 1% sodium hypochlorite solution for one minute and later washed in sterile distilled water for 3-4 times to remove traces of sodium hypochlorite. Then sterile pieces were transferred to sterile blotter paper to remove water adhered to cut pieces and placed in Petri plates containing sterilized potato dextrose agar (PDA) medium and incubated at 27±1^{0}Cfor 3-5 days. Pathogen was isolated from nine samples collected during survey. Further pure cultures were developed through hyphal tip method and maintained on PDA medium for further studies.

Pathogenicity test of *Macrophomina phaseolina* against chickpea plants

The nine isolates were screened for their pathogenicity on chickpea susceptible cultivar JG-11. A pot culture experiment was conducted to determine the virulence of the isolates.

Mass multiplication

The test pathogen/isolates are mass multiplied on sorghum grains. The sorghum grains were soaked in 2% sucrose water overnight and excess water was drained out from it, then boiled in fresh water for 30 minutes and drained. Grains were filled into 500 ml conical flasks @ 200 grams and autoclaved at 121^{0}C, 15 lbs pressure for 20 minutes. Then flasks were allowed to cool at room temperature and inoculated with 5mm mycelial disc of 5daysold culture of *M. phaseolina* isolate. They were incubated for 15 days at 27±1^{0}C. The flasks were manually shaken on alternate days for a few minutes to break clumps in order to get uniform colonization on grains.

Soil inoculation method

The appropriate inoculum multiplied on sorghum grains was thoroughly mixed in pots of (12×10 cm) diameter filled with 3 kg steam sterilized soil at the rate of 10 g kg^{-1} soil and mixed thoroughly and moistened with water. The pots were incubated for 7 days by covering with polythene sheets. Pots without pathogen inoculum were maintained as control (Saabale and Dubey, 2014) [10].

Five seeds of chickpea variety JG 11 were sown in pots. After germination, plants were observed regularly for the appearance of symptoms. The symptoms occurring on artificially inoculated plants were compared with that of naturally infested plant and the pathogen was reisolated from the artificially inoculated rotted chickpea plants. The fungus obtained was compared with the original isolate (first one) in all respects to prove the pathogenicity.

The dry root rot incidence was recorded at 15day intervals upto maturity of the crop plants. The experiment was conducted in three replications.

In vitro evaluation of fungicides against the radial growth of *Macrophomina phaseolina*

The effectiveness of eight different fungicides *viz.* Propiconazole 25% EC, Tebuconazole 60 FS, Azoxystrobin 11% +Tebuconazole 18.30% SC, Azoxystrobin 20% + Difenconazole 12.5% SC, Mancozeb 63% + Carbendazim 12% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Carboxin 37.5% + Thiram 37.5% WS, Copper oxy chloride were evaluated against *M. phaseolina* at five different concentrations that are 1000, 1500, 2000, 2500 and 3000 ppm by poison food technique described by Nene and Thapliyal (1993) [8] under *in vitro*. The required quantities of fungicides were weighed and mixed in the potato dextrose agar medium by thorough shaking to facilitate uniform mixing of the fungicide before pouring into Petri dishes so as to get the desired concentration of each fungicide separately. Twenty ml of amended medium was poured in 90 mm sterilized Petri dishes and allowed to solidify. Then mycelial discs of 5 mm diameter from actively growing 5day old culture of highly virulent isolate of *M. phaseolina* (based on pathogenicity) were inoculated at the centre of the Petri plate and then incubated at 27±1^{o}Cfor 7 days. Plates without fungicide served as control and inoculated with test pathogen. Three replications were maintained for each treatment. Per cent inhibition of mycelial growth was calculated using the following formula (Vincent, 1927) [12].

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

Where,
I = Percent inhibition of mycelial growth
C = Growth in control
T = Growth in treatment

Results and Discussion

Based on survey conducted in Telangana state *M. phaseolina* pathogen was isolated from the disease samples collected from nine villages belong to four districts out of 10 districts surveyed. Further pathogenicity test was conducted, based on results of pathogenicity test one highly virulent *M. phaseolina*

isolate was selected to test the efficacy of fungicides on radial growth under *in vitro* conditions.

Effect of different fungicides on growth inhibition of *M. phaseolina*

The results pertaining to the effect of eight different fungicides at five different concentrations on inhibition of radial growth of *M. phaseolina* were found significant and reported in Table. 1. Among the eight tested fungicides four fungicides *viz.*, Propiconazole 25% EC, Tebuconazole 60 FS, Mancozeb 63% + Carbendazim 12% WP and Tebuconazole 50% +Trifloxystrobin 25% WG were found highly effective at all concentrations by inhibiting the 100 per cent mycelial growth of *M. phaseolina*. Out of these tested fungicides in Petri plates amended with copper oxy chloride were shown least effect on radial growth of *M. phaseolina* at all tested concentrations in comparison to other fungicides used in this study ranging from 0%, 5.37%, 9.36%, 18.42% and 24.63% at concentrations 1000ppm, 1500ppm, 2000ppm, 2500ppm and 3000 ppm respectively. Whereas in case of remaining fungicides it was observed that per cent inhibition in radial growth of test pathogen increases with the increase in the concentration of the fungicide evaluated. At 1000 ppm concentration, significantly highest growth inhibition over control was observed in Azoxystrobin 11% + Tebuconazole 18.30% SC (81.67%) followed by Azoxystrobin 20% + Difenconazole 12.5% SC (81.62%). At 1500 ppm concentration Azoxystrobin 20% + Difenconazole 12.5% SC recorded significantly highest per cent inhibition (83.98%) followed by Azoxystrobin 11% + Tebuconazole 18.30% SC (83.61%). At 2000 ppm concentration both Azoxystrobin 20% + Difenconazole 12.5% SC and Carboxin 37.5% + Thiram 37.5% WS showed same per cent inhibition (84.91%). At 2500 ppm concentration the treatments T_4, T_5 and T_8 (Azoxystrobin 11% + Tebuconazole 18.3% SC, Azoxystrobin 20% + Difenconazole 12.5% SC and Carboxin 37.5% + Thiram 37.5% WS showed statistically similar inhibition on radial growth of *M. phaseolina* i.e., 89.82%, 88.80% and 89.35% respectively. Whereas these treatments T4, T5 and T8 at 3000 ppm concentration exhibited more than 91% growth (T_4-91.85%, T_5-91.02% and T_8-91.26%).

According to present investigation, our results are in complete agreement with Chaudhary *et al*., (2017), who reported that Carbendazim 12% + Mancozeb 63% WP at 1500ppm, 2000ppm and 2500ppm concentrations showed cent per cent inhibition of mycelial growth of *M. phaseolina* dry root rot pathogen isolated from soyabean. Whereas, efficacy of fungicide Copper oxy chloride at tested concentrations revealed less inhibition in compare to the findings of Lokesh *et al.*, (2020) [7] who reported nearly 50% of inhibition at high concentrations 2000 ppm and 2500ppm while evaluating the non-systemic fungicides against *M. phaseolina* under *in vitro*. Besides this our findings on efficacy of fungicide treatment Carbendazim 12%+Mancozeb 63% are support the findings of Zope *et al*., (2019). Recent reports of Lokesh *et al.*, (2020) [7] are shown similar trend with our results on percent growth inhibition of *M. phaseolina* over the control with increase in the concentration among ready mix fungicides *viz.*, Carbendazim 12%+Mancozeb 63% and Carboxin 37.5%+ Thiram 37.5% tested. Ranvijay *et al.*, (2020) [9] in laboratory conditions evaluated the inhibitory effect of fungicides Tebuconazole and Propiconazole at low concentrations and complete inhibition of colony growth of *M. phaseolina* was observed.

Table 1: Evaluation of different fungicides against radial growth of *M. phaseolina* under *in vitro*

Fungicide Treatment / Concentration	1000ppm		1500ppm		2000ppm		2500ppm		3000ppm	
	Radial growth (cm)	Percent growth inhibition	Radial growth (cm)	Percent growth inhibition	Radial growth (cm)	Percent growth inhibition	Radial growth (cm)	Percent growth inhibition	Radial growth (cm)	Percent growth inhibition
T_1 - Copper oxychloride	9.00	0.00 (0.00)	8.52	5.37 (13.33)	8.52	9.35 (17.79)	7.34	18.43 (25.38)	6.78	24.63 (29.66)
T_2 - Propiconazole 25% EC	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)
T_3 - Tebuconazole 25% FS	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)
T_4 - Azoxystrobin 11% + Tebuconazole 18.30% SC	1.65	81.67 (64.63)	1.48	83.61 (66.10)	1.44	83.98 (66.38)	0.92	89.82 (71.37)	0.73	91.85 (73.40)
T_5 - Azoxystrobin 20% + Difenconazole 12.5% SC	1.65	81.62 (64.59)	1.44	83.98 (66.38)	1.36	84.91 (67.11)	1.01	88.80 (70.43)	0.81	91.02 (72.55)
T_6 - Mancozeb 63% + Carbendazim 12% WP	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)
T_7 - Tebuconazole 50% + Trifloxystrobin 25% WG	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)
T_8 - Carboxin 37.5% + Thiram 37.5% WS	1.67	81.48 (64.49)	1.57	82.59 (65.32)	1.36	84.91 (67.11)	0.96	89.35 (70.93)	0.79	91.26 (72.79)
T_9 – Control	9.00	0.00	9.00	0.00	9.00	0.00	9.00	0.00	9.00	0.00
C.D.	0.02	0.17 (0.12)	0.08	0.86 (1.08)	0.06	0.63 (0.58)	0.15	1.69 (1.37)	0.29	3.25 (2.35)
SEm±	0.01	0.06 (0.40)	0.03	0.28 (0.36)	0.02	0.29 (0.19)	0.05	0.56 (0.45)	0.10	1.07 (0.78)
SEd	0.01	0.08 (0.06)	0.04	0.40 (0.51)	0.03	0.30 (0.27)	0.07	0.79 (0.64)	0.14	1.52 (1.10)
C.V.	0.49	0.12 (0.10)	2.72	0.60 (0.86)	2.09	0.43 (0.46)	6.82	1.13 (1.05)	14.70	2.13 (1.77)

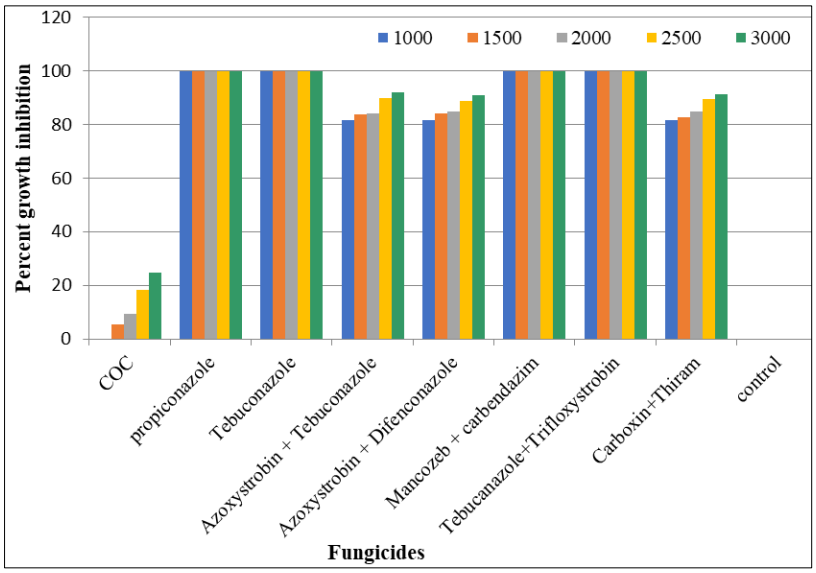

Fig 1: Percent disease incidence of fungicides at five different concentrations

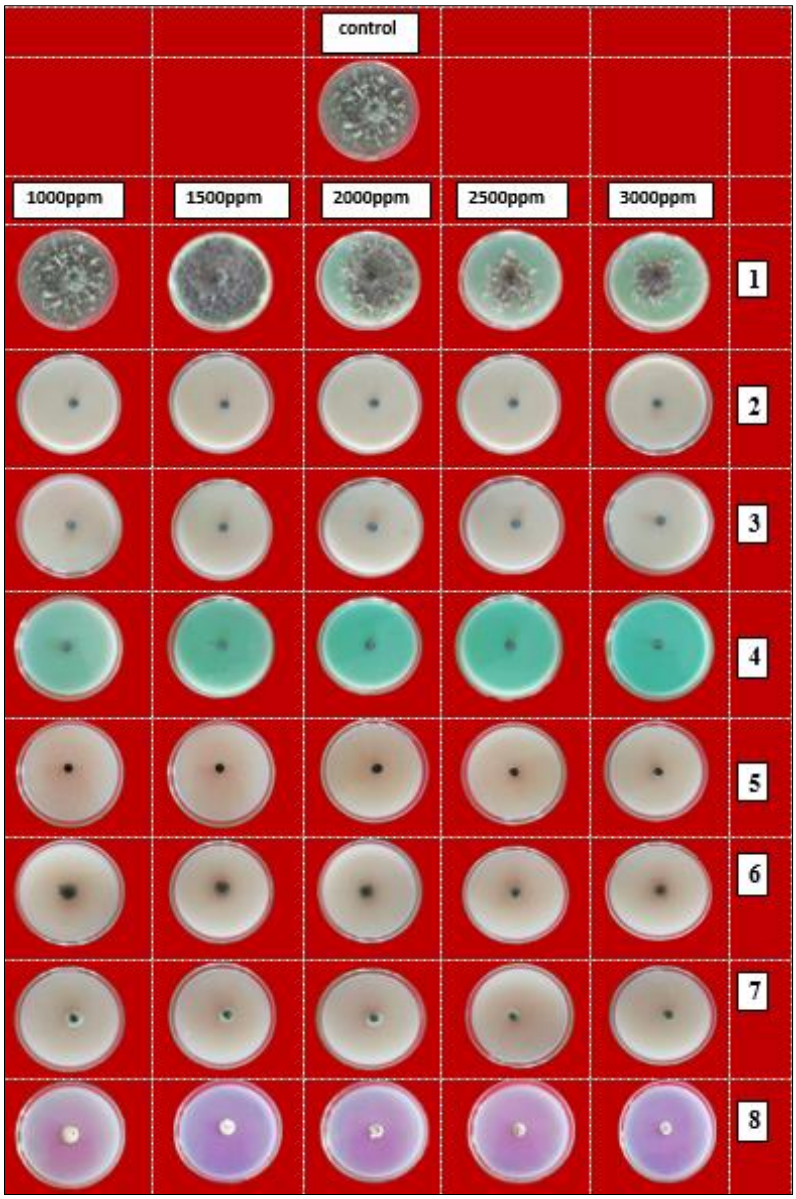

Fig 2: 1. Copper oxychloride, 2. Tebuconazole 25%, 3. Propiconazole25% 4. Mancozeb 63% + Carbendazim 12% WP5. Tebuconazole 50% +Trifloxystrobin 25% WG6.Azoxystrobin 20%+Difenconazole12.5% SC7. Azoxystrobin 11% +Tebuconazole 18.30% SC8. Carboxin 37.5% + Thiram 37.5% WS

Conclusion

In this study efficacy of fungicides showed greatly varied results on inhibition of radial growth of *M. phaseolina*. Among the eight fungicides tested systemic fungicides Propiconazole 25% EC and Tebuconazole 60 FS, in case of ready-mix fungicides Mancozeb 63% + Carbendazim 12% WP and Tebuconazole 50% + Trifloxystrobin 25% WG showed cent per cent mycelial growth inhibition of pathogen significantly at all concentrations including at low concentration of 1000ppm. However least per cent growth inhibition was observed with Copper oxychloride among all fungicides tested.

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