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Evaluation of fungicides, plant extracts and bio-agents against dry root rot of chickpea (*Cicer arietinum*)

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ABSTRACT

Dry root rot (DRR) of chickpea caused by *Macrophomina phaseolina* is an important disease affecting chickpea production especially in tropical and sub-tropical ecologies of world. The present investigation was planned to evaluate the *in vitro* efficacy of various fungicides, plant extracts and bio-control agents against *Macrophomina phaseolina.* Results suggested that among fungicides, significantly maximum mean mycelium growth inhibition was recorded with Carbendazim (100%) followed by Carbendazim+Mancozeb (84.65%). Likewise, among phyto extracts, garlic extract was excellent with maximum mycelia growth inhibition (70.15 and 100 %) followed by neem extract (68.35 and 82.35%) at 5 and 10% concentration, respectively. Among bio-agents, *Trichoderma viride* showed significantly maximum mycelia growth inhibition (80.20%) followed by *Trichoderma harzianum* (65.10%). Therefore, from the findings of present study, it can be concluded that apart from use of fungicides (Carbendazim and Carbendazim+Mancozeb) phyto extracts such as extract of garlic and neem leaves and bio-control agent *Trichoderma viride* can also be used as an effective alternative for management of DRR in chickpea. Findings of our study may help in development of sustainable management strategies against DRR thus minimizing its yield consequences in chickpea. However, there is a need to further strengthen the investigations on this aspect based on thorough understanding of the biology of the pathogen and host×plant×environment interaction especially under field conditions.

Keywords: Bio- agents, Chickpea, Dry root rot, Fungicides*, Macrophomina phaseolina,* Plant extract

Chickpea (*Cicer arietinum* L.) is world's third most important legume after beans and peas (Sharma *et al.* 2015). It is a good source of proteins, fibers, folate, vitamins as well as minerals (Ferguson *et al.* 2010, Ghosh *et al.* 2013). Globally, India is the largest chickpea growing country with an annual production of 8.43 MT from 8.95 Mha with average productivity of 943 kg/ha (Anonymous 2018). In past, chickpea production was largely constrained by wilt and blight only but recent reports indicate that Dry root rot (DRR) is also emerging as a potential threat in most of the temperate and tropical chickpea growing regions causing around 10–25% crop loss to chickpea growers depending upon the level of infection and environment ((Janzen *et al.* 2006, Pande *et al.* 2010, Sharma *et al.* 2015).

DRR is a soil-inhabiting fungus capable of infection at any crop stage. In dry and warm regions, it most commonly infects the crop at post-reproductive stage (Sharma and Pande 2013). Sometimes seedling infection may also be seen. Infected plants at seedling stage show brown discoloration at the soil line extending up the stem that may turn dark brown to black (Sharma *et al.* 2015). Currently, chickpea growers manage DRR using some systemic fungicides as seed treatment and soil drench, and some contact fungicides as seed treatment (Prajapati *et al.* 2002). Fungicide seed treatments can provide only short-term protection. Imbalance and overuse of fungicides over the time has a variety of detrimental effects such as development of resistant strain of pathogens, negative impacts on soil health and ecosystems (Chen *et al.* 2001), and non-target toxicity linked to biodiversity loss (Geiger *et al.* 2010, Brahmanand and Pandey 2015). Therefore, evaluation and promotion of some alternate eco-friendly management options of DRR is increasingly sought. However, studies with special target to management of DRR in chickpea under rainfed ecologies are still lacking. Therefore, the present *in vitro* study was conducted to find out most effective fungicides, plant extracts and native biocontrol agents against DRR of chickpea.

MATERIALS AND METHODS

Experimental site and disease sample collection: All the experiments were conducted under laboratory under controlled environment (*in vitro*) during winter 2016–17 at SKN. College of Agriculture, Jobner, Jaipur, Rajasthan. The

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samples of infected chickpea plants with DRR were collected from the experimental field and brought to the laboratory and fungus was isolated and identified for further studies.

Efficacy of fungicides: Efficacy of six systemic and non-systemic fungicides, i.e. Thiophanate methyl (Topsin-M), Carbendazim (Bavistin), Carbendazim+Mancozeb (Saaf), Chlorothalonil (Kavach), Carboxin+Thiram (Vitavax power) and Trifloxystrobin+Tebuconazole (Nativo) alongwith their four different concentrations (50, 100, 250 and 500 ppm) were tested against *Macrophomina phaseolina* using poisoned food technique (Nene and Thapliyal 1993). A control was also maintained where medium was not supplemented with any fungicide. The liner growth of the test fungus was recorded and per cent growth inhibition was calculated as:

$$\text{Per cent mycelial growth inhibition} = \frac{C-T}{C} \times 100$$

where, C=Diameter of the colony in check (coverage of both diagonals); T = diameter of colony in treatment (average of both diagonals).

Efficacy of plant extract: Antimycotic properties of six natural phytoextracts, i.e Aak (*Calotropis procera)*, Datura (*Datura stramonium)*, Garlic (*Allium sativum)*, Neem (*Azadirachta indica)*, Kheep (*Leptadenia pyrotechnica)*, and Tumba (*Colocynthis citrullus*) on growth of *M. phaseolina* following poisoned food technique (Nene and Thapliyal, 1993). All the plant extracts were tested at two different concentrations (5 and 10%). A control was also maintained where medium was not supplemented with any plant extract. Colony diameters (two diagonals) were measured at 7th day of incubation and per cent growth inhibition was calculated.

Efficacy of bio-control agent against Macrophomina phaseolina: Efficacy of four bio-control agents i.e. *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtalis* and *Pseudomonas fluorescens* was tested using dual culture plate method (Dennis and Webstar 1971). The isolated bio-agents were obtained from Division of Plant Pathology, Rajasthan Agricultural Research Institute, Durgapura, Jaipur. For testing the efficacy of bio-agents, required quantity of each bio-control agent mixed with autoclaved PDA was poured into sterilized petriplates and allowed for solidification for 3 h. After solidification, plates were inoculated with 5 mm (diameter) mycelial bit taken from 7 days old culture of *M. phaseolina* and incubated at 25±1ºC in BOD incubator for 7 days. PDA petriplates inoculated with pathogen alone served as check and three replications for each treatment were maintained. Linear growth of pathogen as well as bio-control agent was measured 7 days after incubation and per cent growth inhibition was calculated.

RESULTS AND DISCUSSION

Efficacy of fungicides against Macrophomina phaseolina: Among fungicides, Carbendazim was found most effective which inhibited mean mycelial growth of *M. phaseolina* by 100% followed by Carbendazim+Mancozeb (93.20%) and Carboxin+Thiram (89.70%) (Table 1). Fungicides such as Thiophanate methyl (71.92%) and Chlorothalonil (78.35%) were found least effective in inhibiting mycelial growth. The higher inhibition with these systemic fungicides used against soil borne pathogens may be due to their quick spread in plant system through xylem vessels to the site of infection. These fungicides made a biochemical chain into plant system and remained present for longer period thus stopping further infection in the plant system (Singh *et al*. 2019). The results of our investigation of complete inhibition of mycelial growth of *M. phaseolina* with Carbendazim are in agreement with the earlier findings (Vijay *et al*. 2006, Kumari *et al*. 2012)

Fungitoxicity of plant extracts against Macrophomina phaseolina: Among plant extracts, Garlic extract was found

Table 1 Efficacy of different fungicides and plant extracts against *M. phaseolina* casing DRR of chickpea

Fungicides	Per cent mycelial growth inhibition					Plant extract	Per cent mycelial growth inhibition		
	Concentration (ppm)						Concentration (%)		
	50	100	250	500	Mean		5%	10%	Mean
Thiophanate methyl	62.10	67.15	72.35	86.20	71.92	Tumba	12.10	35.10	23.60
Carboxin +Thiram	81.05	83.10	94.65	100.00	89.70	Garlic	70.15	100.00	85.08
Carbendazim	100.00	100.00	100.00	100.00	100.00	Datura	52.15	70.10	61.13
Chlorothalonil	65.10	72.20	83.45	92.65	78.35	Neem	68.35	82.35	75.35
Trifloxystrobin+Tebuconazole	73.20	79.40	86.10	100.00	84.67	Kheep	35.45	50.15	42.80
Carbendazim+ Mancozeb	84.65	88.15	100.00	100.00	93.20	Aak	60.10	74.10	67.10
Control	0.00	0.00	0.00	0.00	0.00	Control	0.00	0.00	0.00
		SEm±		*CD (P=0.05)*				*SEm±*	*CD (P=0.05)*
Fungicide		1.34		3.71		Plant extracts		1.13	3.15
Concentration		1.50		4.15		Concentration		1.26	3.52
Fungicide × Concentration		3.00		8.30		Plant extracts × Concentration		2.53	7.03

Table 2 Efficacy of different bio-agents against *M. phaseolina* causing DRR of chickpea

Bio-agent	Percent mycelial growth inhibition
Trichoderma harzianum	65.10
Trichoderma viride	80.20
Bacillus subtilis	44.20
Pseudomonas fluorescens	40.20
Control	0.00
SEm±	0.63
CD (P=0.05)	1.93

most effective in inhibiting mean mycelial growth (70.15 and 100%) followed by Neem (68.35 and 82.35%), Aak (60.10 and 74.10 %) and Datura (52.15 and 70.10%) at 5 and 10% concentration, respectively (Table 1). Kheep (35.45 and 50.15%) and Tumba extract (12.10 and 35.10%) at 5 and 10% concentration, respectively, were found least effective in inhibiting mycelial growth. All the tested plant extracts showed significantly higher mycelial growth inhibition with 10% concentration as compared to 5% concentration. The higher inhibition with garlic extract against fungus may be due to presence of allicin protein reported to have antifungal activity. Allicin has thio di sulphide acid which reacts with host protein by free thiol group and shows antifungal action against soil borne pathogens (Khatik *et al.* 2005, Slusarenko *et al*. 2008). Likewise, azadirachtin and nimbin present in Neem extract make it a broad-spectrum antifungal agent thus help to reduce pathogen spread and increase systemic acquired resistance in plant system (Coventry and Allan 2001, Valled and Goodman 2004).

Efficacy of bio-agents against Macrophomina phaseolina: All the tested bio-agents were found antagonistic to *M. phaseolina* and showed significantly higher mycelial growth inhibition over each other (Table 2). *Trichoderma viride* was found most effective with maximum mycelial growth inhibition (80.20%) followed by *Trichoderma harzianum* (65.10%). Whereas, *Bacillus subtilis* (44.20%) and *Pseudomonas fluorescens* (40.20%), recorded lower mycelial growth inhibition and proved least effective compared to *Trichoderma viride* and *Trichoderma harzianum*. The higher effectiveness of *Trichoderma viride* against the pathogen might be due to the fact that build-up of this bio-agent is reported to induce systemic resistance into plants against pathogen thus reduced colonization and establishment of pathogen into plant system. Further, some of *Trichoderma spp*. provide heterologous proteins against phyto-pathogens and volatile and non-volatile substances and produce antimicrobial products like hydrolytic enzymes which altogether inhibit the overall growth of pathogens upon application (Abbas *et al.* 2017, Manandhar *et al*. 2019).

DRR of chickpea is a devastating disease leading to qualitative as well as quantitative losses especially in tropical and sub-tropical ecologies of the world. Results revealed that Carbendazim and Carbendazim+Mancozeb were most efficient fungicides to inhibit the mycelium growth of *M. phaseolina*. Likewise, some plant extracts such as Garlic and Neem extracts and native bio-control agents such as *Trichoderma viride* also proved highly effective in inhibiting fungal growth. Results of our study may help in development of sustainable management strategies against DRR of chickpea. However, there is a need of further investigations for thorough understanding of the biology of the pathogen considering host×plant×environment interactions especially under field conditions.

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