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In vitro evaluation of fungal and bacterial biocontrol agents against foliar fungal pathogens of Chickpea, *Cicer arietinum*. L

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Abstract
Chickpea (*Cicer arietinum* L.) is an important pulse crop grown and consumed all over the world, especially in the Afro-Asian countries. It is a good source of carbohydrates and protein. The reduction of chickpea production has remained virtually stagnant because, the crop suffers from many diseases such as Ascochyta blight, caused by *Ascochyta rabie* (Pass.). Therefore, effective management control is essential. Hence the present study was undertaken to evaluate the fungicides and biocontrol agents in a view to develop the effective management practices to mitigate the foliar fungal pathogens of chickpea. In this experiment an In *vitro* evaluation of fungicides, biocontrol agents on foliar fungal pathogens of chickpea and *In vivo* evaluation of fungal and bacterial biocontrol agents for antagonism against foliar fungal pathogens of chickpea was performed in the glasshouse condition. In glasshouse, a pot experiment was conducted in the Department of Agricultural Microbiology, UAS, GKVK, Bengaluru to evaluate antagonistic effect of selected fungal and bacterial biocontrol agents along with beneficial microorganisms against foliar fungal pathogens of chickpea. Among the fungicides Carbendazim (250ppm) was found effective against them in inhibiting growth of foliar fungal pathogens followed by mancozeb. The efficient target oriented bioagents to combat the foliar diseases of chickpea is in need of the hour to maintain soil sustainability and fertility under diverse agro climatic zones.

Keywords: chickpea (*Cicer arietinum* l.), ascochyta blight, carbendazim, mancozeb, hexaconozole, propiconozole, thiram, *Tricoderma harzianum*, *Tricoderma viridae*

1. Introduction
Chickpea (*Cicer arietinum.* L) Also known as Bengal gram is the only cultivated species of the genus *Cicer* which include 44 species. These crops have been named as "poor man's meat" and "rich men's vegetable". It is the high protein legume grown in India and is about 9.21 million hectares with production of 8.88 million ton with productivity of 995 Kg/ha [20]. Chickpea is a small bushy type annual plant characterized by unipinnate leaves attaining a height of 30- 45 cm. It is typically grown in semi-arid areas as winter/autumn (Rabi) crop. Its life span is 2-3 months and on maturity bears fruit which is called pod with 2-3 seeds per pod. The pods split vertically which is the characteristics of all pulses. All the chickpea cultivars are broadly grouped into 2 classes - "Desi" and "Kabuli". Chickpea after dehulling is valued for its nutrition, with a high protein content (12.3 - 31.5 %). Chickpea seed has 58.9 % carbohydrate, 3 % fiber, 5.2 % oil, 3 % ash, 0.2 % calcium, and 0.3 % phosphorus. Digestibility of protein varies from 76-78 % and its carbohydrate from 57-60 %. Among the legumes, chickpea is the most nutritive pulse extensively used as protein adjunct to starchy diet.
Chickpea (*Cicer arietinum* L.) is an important pulse crop grown and consumed all over the world, especially in the Afro-Asian countries. It is a good source of carbohydrates and protein. The protein quality of chickpea is considered to be better than other pulses [10]. There is a growing demand for chickpea due to its nutritional value.
Globally, chickpea is mostly consumed as a seed food in several different forms and preparations are determined by ethnic and regional factors [8, 12]. The reduction of chickpea production has however remained virtually stagnant over recent decades because the crop suffers from many diseases. The primary cause of low yields in chickpea is its susceptibility to a number of biotic and abiotic stresses. Among biotic stresses, *Ascochyta* blight, caused by *Ascochyta rabie* (Pass.), is a widespread foliar disease that causes extensive crop losses (up to 100 %) in most regions of the world where the crop is commonly grown [14].
Since new fungicides are scarce in the market and because of environmental problems, researchers are now emphasizing other alternatives, such as the genetic potential of plants, resistance against pathogens, and the use of biotic and abiotic agents to develop induced or acquired resistance [22]. Biological control is the best alternative especially against soil borne pathogens, compared to chemical controls. Biological control of pathogens, *i.e.*, the total or partial destruction of pathogen populations by other organisms, occurs routinely in nature [1]. Among the various antagonists used for the management of plant diseases, *Trichoderma* spp., plays a vital role [19]. Many studies have proved the potential of *Trichoderma* spp. as biological agents as antagonistic to several plant pathogens [7, 13, 19, 21] and many strategies to control this disease on chickpea have been investigated in the field [9, 17]. Hence the present study was undertaken to evaluate the fungicides and biocontrol agents in a view to develop the effective management practices to mitigate the foliar fungal pathogens of chickpea.

2. Materials and Methods
The experiment was conducted under green house during

The Rabi season, in the Department of Botany, Jnanabharathi Campus, Bangalore University, Bengaluru-56 and Department of Agricultural Microbiology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru-65. The *in vitro* studies include isolation and dual culture tests were conducted under aseptic condition in the laminar air flow cabinet, before working with the hood, the working surface was sterilized by swabbing with 70% alcohol.

2.1 *In vitro* evaluation of fungicides against some foliar fungal pathogens of chickpea (Cicer arietinum. L)

The efficacy of fungicides such as Carbendazim (Bavistin 50 WP), Mancozeb (Dithane M-45 75 WP), Hexaconozole (Contaf Plus), Propiconozole (Dilt 25% EC) and Thiram (Thiram 50 WP) were tested against selected foliar fungal pathogens of chickpea under *in vitro* condition. The fungicides were evaluated at 250, 500, 1000, 2000 and 3000 ppm respectively.

2.2 *In vitro* evaluation of biocontrol agents against some foliar fungal pathogens of chickpea (Cicer arietinum. L)

Biocontrol agents namely, *Tricoderma harzianum*, *Tricoderma viridae* obtained from Department of Microbiology, College of Agriculture GKVK, Bengaluru and bacterial bioagents like *Pseudomonas fluorescens* and *Bacillus subtilis* obtained from Al- ameen college, Bengaluru was evaluated for their efficacy against the infected pathogens under *in vitro* condition using dual culture technique.

2.3 Dual culture test

The antagonistic potential of the biocontrol agents against fungal pathogens were tested by dual culture method on potato dextrose agar (PDA) medium. The fungal bioagents like *Trichoderma harzianum* and *Trichoderma viridae.* Bacterial bioagents like *Bacillus subtilis* and *Pseudomonas fluorescens* were used for the study. The foliar fungal pathogens were inoculated simultaneously side by side at a distance of three cm from each other on a single Petri plate containing solidified PDA. Three replications were maintained for each bioagents. They were incubated for seven days. The diameter of the colony of both bioagents and the pathogens was calculated. The inhibition zone was measured from the edge of test fungal mycelium to the edge of bacterial colony after seven days of incubation and expressed as percent inhibition over control. The values obtained were statistically analyzed.

2.4 *In vivo* evaluation of fungal and bacterial biocontrol agents for antagonism against foliar fungal pathogens of chickpea (*Cicer arietinum*. L)

The isolates found efficient in *in vitro* studies were evaluated against foliar fungal pathogens of chickpea under glasshouse condition. Biocontrol agents and pathogens selected for *in vivo* studies were,

1. Fungal biocontrol Agents: Trichoderma harzianum and Trichoderma viridae
2. Bacterial biocontrol agents: *Bacillus subtilis and Pseudomonas fluorescens*
3. Test pathogens selected: Alternaria alternata and *Ascochyta rabiei*

2.5 Glasshouse evaluation:

In glasshouse, a pot experiment was conducted in the Department of Agricultural Microbiology, UAS, GKVK, Bengaluru to evaluate antagonistic effect of selected fungal and bacterial biocontrol agents along with beneficial microorganisms against foliar fungal pathogens of chickpea.

3. Results

3.1 *In vitro* evaluation of fungicides on growth inhibition of foliar fungal pathogens of chickpea (Cicer arietinum. L)

The efficiency of five fungicides such as Carbendazim, Hexaconozole, Propiconozole, Mancozeb and Thiram were evaluated at 250, 500, 1000, 2000 and 3000 ppm concentrations and percent inhibition of radial growth of the foliar fungal pathogens was highest in 250 ppm concentration of carbendazim 50% WP for all the fungal foliar pathogens of chickpea (*Cicer arietinum*. L) and among fungicides at 250 ppm concentration per cent growth inhibition of the foliar pathogens are presented in (Table-1).

The different fungicides evaluated against the foliar fungal pathogens significantly inhibited the growth of the *in vitro*. All the fungicides differed significantly with respect to inhibition. Among fungicide carbendazim 50 % WP was found to be superior in inhibiting the radial growth of the three fungal pathogens followed by mancozeb 75 % WP in *Fusarium oxysporum* (78.86 %), *Ascochyta rabie* (75.66 %) and *Alternaria alternata* (75.63 %). Whereas lowest inhibition of radial growth of fungal pathogens was recorded with the fungicide propiconozole 25 % EC in *Fusarium oxysporum* (59.33 %), *Ascochyta rabie* (57.66 %) and *Alternaria alternata* (54.66 %).

Table 1: *In vitro* evaluation of fungicides on growth inhibition of the foliar fungal pathogens of chickpea (*Cicer arietinum*. L)

Treatments	Percent inhibition at 250 ppm concentration		
	Fusarium oxysporum	***Ascochyta rabie***	***Alternaria alternata***
T_{1}- Control	0.00	0.00	0.00
T_{2}- Carbendazim 50% WP (Bavistin)	100.00	100.00	100.00
T_{3}- Hexaconozole 5% WP	60.93	59.97	57.66
T_{4}- Propiconozole 25% EC (Tilt 25 EC)	76.66	74.33	72.92
T_{5}- Mancozeb 75% WP (Indofil-M-45)	78.66	75.66	75.63
T_{6}- Thiram 50 WP	59.33	57.66	54.66
F Test	*p=0.506	*p=0.0.715	*p=1.240

* Significant at 5% level

3.2 *In vitro* evaluation of seed treatment with fungicides on percent germination on foliar fungal pathogens of chickpea (*Cicer arietinum*. L)

The percent germination on foliar fungal pathogens of chickpea treated with fungicides are presented in (Table-2). Significant differences were found among fungicides and *Fusarium oxysporum.* The highest percent germination of seeds was observed in carbendazim in all the three pathogens *i.e., Fusarium oxysporum* (71.24 %), *Ascochyta rabie* (68.25 %) and *Alternaria alternata* (65.25 %) followed by mancozeb treated seeds *viz., Fusarium oxysporum* (62.22 %), *Ascochyta rabie* (60.22 %) and *Alternaria alternata* (58.22 %). Lowest percent germination of *Fusarium oxysporum* (34.67 %), *Ascochyta rabie* (33.33%) and *Alternaria alternata* (29.33 %) was observed in the control which was significantly different from all the other treatments.

Table 2: *In vitro* seed treatment with fungicides on germination on foliar fungal pathogens of chickpea (*Cicer arietinum*. L)

Treatments	Percent germination		
	Fusarium oxysporum	***Ascochyta rabie***	***Alternaria alternata***
T_{1}- Control	34.67	33.33	29.33
T_{2}- Carbendazim 50% WP (Bavistin)	71.24	68.25	65.25
T_{3}- Mancozeb 75% WP (Indofil-M-45)	62.22	60.22	58.22
T_{4}- Propiconozole 25% EC (Tilt 25 EC)	49.43	47.42	46.09
T_{5}- Hexaconozole 5% WP	58.33	56.33	58.33
T_{6}- Thiram 50 WP	47.22	45.45	43.33
F Test	*p=0.303	*p=0.392	*p=0.680

* Significant at 5% level

3.3 *In vitro* evaluation of seed treatment with biocontrol agents on percent germination against foliar fungal pathogens of chickpea (*Cicer arietinum*. L)

The effect of microbial antagonists on the growth of three foliar pathogens of chickpea (*Cicer arietinum*. L) On percent germination were evaluated under laboratory condition and the data are presented in (Table -3). The results of the study indicated that all the antagonists significantly inhibited the growth of all the foliar fungal pathogens. The percent germination was ranged from 52.76% to 66.76%. Highest percent germination observed in the seeds treated with the bioagent *Tricoderma viridae* against all the three foliar fungal pathogens *i.e., Fusarium oxysporum* (66.76%), *Ascochyta rabie* (65.76%), *Alternaria alternata* (63.76%) and the least per cent germination was observed in the uninoculated control ranging from 42.32 % to 44.66 %.

Table 3: *In vitro* seed treatment with biocontrol agents on percent germination on foliar fungal pathogens of chickpea (*Cicer arietinum*. L)

Treatments	Foliar fungal isolates of percent germination		
	Fusarium oxysporum	***Ascochyta rabie***	***Alternaria alternata***
T_{1}- Control	44.66	43.32	42.32
T_{2}- *Trichoderma viridae (T.v)*	66.76	65.76	63.76
T_{3}- *Trichoderma harzianum (T.h)*	63.76	61.76	59.76
T_{4}- *Bacillus subtilis (B.s)*	58.32	56.32	54.32
T_{5}- *Pseudomonas fluorescens (P.f)*	55.47	54.32	52.76
F Test	*p=0.2270	*p=0.293	*p=0.507

Note: *T.v- Tricoderma viridae, T.h- Tricoderma harzianum, B.s- Bacillus subtilis, P.f- Pseudomonas florescens.* * Significant at 5% level.

3.4 *In vitro* evaluation of different biocontrol agents against foliar fungal pathogens of chickpea (*Cicer arietinum*. L) On percent inhibition

The effect of microbial antagonists on the growth of three foliar fungal pathogens were evaluated under laboratory condition with dual and triple inoculation culture technique and the observation on percent inhibition of foliar fungal pathogens against biocontrol agents are presented in (Table-4). Interactions of *Fusarium oxysporum*, *Ascochyta rabie* and *Alternaria alternata* with dual antagonists like *Th + B.s*, *Th + P.f*, *T.v + B.s* and *T.v + P.f* were studied and triple inoculation of antagonists with *Th + B.s + P.f* and *T.v + B.s + P.f* were studied under laboratory condition. The results of the study indicated that all the antagonists significantly inhibited the growth of fungal foliar pathogens of chickpea (*Cicer arietinum*. L).

Maximum inhibition of *Fusarium oxysporum* was observed in the treatment inoculated with triple antagonists *T.v + B.s + P.f* (84.52 %) which was on par with three treatment inoculated with dual antagonists of *T.v + B.s* (84.32 %) which are significantly different compared to all the other treatments. Whereas maximum inhibition of *Ascochyta rabie* was observed in the dual antagonists inoculated treatment with *T.v +P.f* (85.52 %) which was on par with the treatment inoculated with *T.v + B.s* (84.00 %) which are significantly different compared to all the other treatments and in case of fungal pathogen *Alternaria alternata* maximum inhibition was recorded in the treatment inoculated with *T.v + B.s* (82.97 %) which was on par with the treatment inoculated with *T.v + B.s + P.f* (82.33 %) which are significantly different compared to all other treatments.

Table 4: *In vitro* studies of biocontrol agents on percent inhibition on foliar fungal pathogens of chickpea (*Cicer arietinum*. L)

Treatments	Percent inhibition		
	Fusarium oxysporum	***Ascochyta rabie***	***Alternaria alternata***
T_{1}- control	0.00	0.00	0.00
T_{2}- *T.h*	75.65	72.65	69.65
T_{3}- *T.v*	83.32	80.52	78.33
T_{4}- *B.s*	76.65	72.65	69.66
T_{5}- *P.f*	68.52	66.66	61.33
T_{6}- *T.h + B.s*	78.65	76.42	75.66
T_{7}- *T.h + P.f*	76.52	80.66	73.33
T_{8}- *T.h + B.s + P.f*	82.32	79.96	78.97
T_{9}- *T.v + B.s*	84.32	84.00	82.97
T_{10}- *T.v + P.f*	83.97	85.52	79.52
T_{11}- *T.v + B.s + P.f*	84.52	83.33	82.33
F Test	*p=0.436	*p=0.835	*p=1.447

Note: *T.v- Tricoderma viridae, T.h- Tricoderma harzianum, B.s- Bacillus subtilis, P.f- Pseudomonas florescens.* * Significant at 5% level.

4. Discussion

Chickpea is one of the most important pulse crops of India. India has a distinct position covering about 76 percent of the total area and production of chickpea in the world. At national level, Karnataka stands sixth position in area and eighth in production. The inevitable importance of essential nutrients available in chickpea seeds is well recognized especially in the predominantly vegetarian population of India.

Several factors responsible for low production of chickpea has been recognized. High incidence of diseases namely *Fusarium* wilt, *Ascochyta* blight, root rot, *Alternaria alternata etc.*, chickpea wilt incited by *Fusarium oxysporum* f. sp. *ciceri* is catastropic disease resulting ultimately in the death of the plants which could cause severe loss to the farmers. The present investigations include symptomology, isolation, pathogenicity, cultural, nutritional, morphological, physiological, biochemical studies, *In vitro* and *In vivo* evaluation of different fungicides and bioagents.

All the fungicides differed significantly with respect to inhibition. Among the fungicides, carbendazim 50 % WP was found to be superior in inhibiting the radial growth of the three fungal pathogens followed by mancozeb 75 % WP in *Fusarium oxysporum* (78.86 %), *Ascochyta rabie* (75.66 %) and *Alternaria alternata* (75.63 %). Significant differences were found among fungicides and *Fusarium oxysporum.* High percent germination of seeds was observed with carbendazim in all the three pathogens i.e., *Fusarium oxysporum* (71.24 %), *Ascochyta rabie* (68.25 %) and *Alternaria alternata* (65.25 %) followed by mancozeb treated seeds *viz.*, *Fusarium oxysporum* (62.22 %), *Ascochyta rabie* (60.22%) and *Alternaria alternata* (58.22 %). Similar observations were made by others in the management of foliar fungal pathogens of muskmelon and chickpea was reported [2, 11].

Fungicides play an important role in checking the fungal growth. In the present study, *in vitro* evaluation of fungicides namely, carbendazim, mancozeb, zyneb, hexaconozole and propiconazole each at 250, 500, 1000, 2000 and 3000 ppm were evaluated against the pathogens. Among the fungicides evaluated carbendazim showed 100 per cent inhibition of mycelial growth at all concentrations, followed by mancozeb fungicide at 3000 ppm (86.7 %) and the least percent inhibition was noticed in zyneb at 250 ppm (37%). In general, carbendazim was the best, which gave considerable inhibition at all concentrations tested. These results are in agreement with the work of others [5] who reported that bavistin appeared to be the most toxic among seven fungicides against *Fusarium udum* and inhibited mycelia growth at 10 and 25 ppm. It was also reported among seven fungicides tested benlate performed best at 250 ppm followed by bavistin, thiram and vitavax at 2000 ppm and inhibited growth of *F. oxysporum* f. sp. *Cepae* [6].

Antagonists were evaluated *in vitro* to manage three selected fungal pathogens of chickpea. Two fungal antagonists namely, *Tricoderma harzianum* and *Tricoderma viridae* and two bacterial antagonists namely, *Bacillus subtilis* and *Pseudomonas fluorescens* were tested for their antagonistic effect against the pathogen by dual culture test. All isolates of antagonists successfully inhibited the growth of fungal pathogen. Further, the fungal antagonists inhibited the pathogen better than bacterial antagonists. Among fungal antagonists the maximum inhibition was observed in *T. viridae* (83.33%) followed by *T.harzianum* (75.66%). Among bacterial bioagents, the maximum inhibition was observed in *B. subtilis* (76.66 %), least inhibition was observed in *P. florescens* (68.51 %).

The inhibitory effect of these bioagents against the pathogen was probably due to competition, antibiosis and mycoparasitism. It was reported that significant suppression of chlamydospore germination of *F. oxysporum* f. sp *cucumaranium* by the use of *P. fluorescens* [18]. Antagonistic activity of *T. harzianum* and *T. viridae* against soil borne fungal pathogens [3] where *T. harzianum* and *T. viridae* were antagonists in vitro to *F. oxysporum* f. sp *lycopersici* and it was found that *T. harzianum* initially showed 2mm inhibition zone to *Fusarium* spp [15] and later it over grew the colony of the pathogen where the similar results were observed in present study.

5. Conclusion

Among the fungal biocontrol agents *Tricoderma viridae* inhibited the pathogens to maximum extent. *Bacillus subtilis* was found best effective among bacterial biocontrol agents tested against the pathogen. Among the fungicides Carbendazim (250ppm) was found effective against them in inhibiting growth of foliar fungal pathogens followed by mancozeb. In this view, advanced research tool for diagnostic detection of foliar fungal pathogens is necessary. In this regard, efficient target oriented bioagents to combat the foliar diseases of chickpea is in need of the hour to maintain soil sustainability and fertility under diverse agro climatic zones.

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