Evaluation of fungicides and bio-agents against Ascochyta blight (*Ascochyta rabiei*) of chickpea under dryland conditions of Kashmir

ABSTRACT

Five non- systemic fungicides *viz.*, Chlorothalonil, carbendazim (12%)+mancozeb (63%), captan 50WP, dodine 65WP and copper oxychloride 50WP and four systemic fungicides *viz.*, difenoconozole 25EC, carbendazim 50WP, hexaconozole 5EC and mycobutanil 10WP were evaluated against Aschochyta blight of chickpea caused by *Ascochyta rabiei*. The *in-vitro* evaluation of non -systemic fungicides through poisoned food technique at five different concentrations *viz.*, 50, 100, 250, 500 and 1000 µg ml^{-1} fungicides showed that carbendazim(12%)+mancozeb(63%) proved most effective and resulted in highest mycelial growth inhibition (82.27%) of the pathogen followed by dodine with mycelial growth inhibition of 67.99 per cent. The least efficacious fungicide was copper oxychloride with only 29.81 per cent mycelia growth inhibition. Among the systemic fungicides evaluated at concentrations *viz.*, 25,50, 100, 200 and 500 µg ml^{-1},carbendazim proved to be the most effective and caused highest mycelial growth inhibition of 90.23 per cent followed by difenconozole with 71.24 per cent mycelial growth inhibition. Myclobutanil 10WP was least efficacious among the systemic fungicides and resulted in only 52.76 per cent mean inhibition of mycelial growth. Among the three methods used for evaluation of efficacy of fungicides and bio-agents, revealed that the seed treatment with carbendazim (12%) + mancozeb (63%) proved most effective with lowest disease incidence (15.0%) and disease intensity(4.02%). Among the biological control agents used, seeds treated with *Bacillus subtilis* proved most effective which resulted in disease incidence and intensity of 32.25 and 20.04 per cent, respectively. Combination treatment comprising of seed treatment with *Bacillus subtilis* @10^9 spores ml^{-1} and foliar spray with mancozeb 63% WP+ carbendazim12%WP @ 2.5% was most efficacious and resulted in lowest disease incidence (7.80 %) and disease intensity of (4.82%) while seed treatment with *Bacillus subtilis* @10^9 spores ml^{-1} alone was least efficacious and resulted in highest disease incidence of 53.80 per cent with disease intensity of 37.25 per cent. It was, however superior than control where disease incidence and intensity was 80.00 and 51.34 per cent, respectively.

KEYWORDS: Ascochyta, Bio-agents, Chickpea, Disease incidence, Fungicides, Seed Treatment

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop widely grown through world-wide and is third most important pulse crop. It belongs to family *Fabaceae* and after French beans (*Phaseolus vulgaris* L.) and dry peas (*Pisum sativum* L.). India is the largest producer, consumer and importer of the chickpea in world, contributing 71 per cent in area and 71.95 per cent in production. Chickpea contributes nearly 48 to 52 per cent of total pulse

production in India. The total area under its cultivation in India is about 10.56 million hectares with annual production of 11.23 million tonnes and an average productivity of 1063 kgha^{-1} (Anonymous, 2018).The production of chickpea in Jammu and Kashmir during the year 2012-13 was 2000 hac. with average production of 1000 tonnes ha^{-1} and yield of 542 kg ha^{-1}(Anonymous, 2016).Chickpea crop is affected by several pathogens, pests and poor management practices resulting in heavy yield losses. Amongst them, diseases play important role in reducing yield potential of chickpea. Nearly about 50 diseases have been reported to affect chickpea with huge economic importance. These include wilt [*Fusarium oxysporum* f. sp. *Cicero* (Padwick) Synd & Hans.], black root rot [*Fusarium solani*(Mart.)Sacc.], wet root rot (*Rhizoctonia solani*Kuhn.), dry root rot [*Rhizactonia bataticola*(Taub.) Butler], Aschochyta blight [*Aschochyta rabiei*(Pass.) Lab.], Pythium root and seed rot (*Pythium ultimum* Trow.) and collar rot (*Sclerotium rolfsii*Sacc.). Ascochyta blight (*Ascochyta rabiei*) is one of the most destructive diseases of chickpea and is wide spread in its distribution (Ahmad *et al.,* 2013). Symptoms begin to appear after 4-6 days after the spores infect the plant. Lesions can occur on leaves, leaflets, stems, pods and seeds. Conidia are exuded from fruiting body called pycnidia in a sticky spore mass and are spread to the nearby plants during rain-splashes. The pathogen survives on diseased crop debris and on diseased seeds in the form of pycnidia containing conidia. Primary infection takes place through conidia and cause necrotic spots which develop conidia at the centre. These conidia work as inoculum for secondary infection during rains (Shtienberg, 2010).Chickpea blight is mainly controlled through fungicides, therefore the use of fungicides and bio-agents are most effective and reliable methods of controlling the disease. Fungicides with novel chemistry are being introduced and evaluated before their application can be recommended to farmers. Therefore, *in-vitro* and *in-vivo* evaluation of these fungicides is of paramount importance. There is constant need to watch and evaluate new fungicides along with some compatible bio-agents for controlling this disease. Hence, the present studies were carried out to evaluate non-systemic and systemic fungicides and some bio-control agents against the disease and the results are presented herein.

Material and methods

Five non-systemic fungicides *viz.,*chlorothalonil, carbendazim (12%)+ mancozeb (63%), captan 50WP, dodine 65WP and copper oxychloride 50WP and four systemic fungicides *viz.*, difenoconozole 25EC, carbendazim 50WP, hexaconozole 5EC and mycobutanil 10WP were evaluated against aschochyta blight of chickpea caused by *Ascochyta rabiei* through poisoned

food technique (Carpenter,1942) using potato dextrose agar medium. The non-systemic fungicides were evaluated at 50, 100, 250, 500 and 1000µg $ml^{-1}$and systemic fungicides at 25, 50, 100, 200 and 500µg ml^{-1}. Different concentrations of fungicides were prepared by adding appropriate quantity of fungicides to sterilized molten potato dextrose agar(PDA) medium in conical flasks. Twenty-five millilitre of such PDA medium was aseptically poured in sterilized Petri plates. A five mm diameter mycelial disc of Ascochyta blight pathogen was aseptically placed in the centre of each Petri plate. Petri plates containing only PDA media amended with equal amount of sterilized water and inoculated with mycelial disc of such pathogen (5mm diameter) served as check. Each treatment was replicated four times and incubated at 28±2^{o}C for next 4 days. The comparative efficacy of each fungicide was calculated as per cent inhibition of mycelial growth of the test pathogen in each treatment as compared to check by the formula given by Vincent (1947):

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

Where

C = Radial mycelial growth (mm) in check

T = Radial mycelial growth in the treatment (mm)

The three methods *viz*., seed treatment, soil treatment and foliar application were used for evaluation of efficacy of fungicides and bio-agents against Ascochyta blight of chickpea in three different plots. Field trials were conducted during Rabi 2018-19 at Dryland Agriculture Station, SKUAST-Kashmir, Rangreth. Field was equally divided into beds with plot size of 2m ×1m, with each plot having 50 plants. Three replications for each treatment were maintained in completely randomized blocks. In first method (seed treatment), the chickpea seeds of susceptible variety were sown after treatment with fungicides and bio-agents. Similarly in the second method (soil treatment), soil was treated with fungicides and bio-agents at recommended concentrations and the seeds of susceptible variety was sown. Again in the third method, susceptible chickpea seeds were sown and the crop was sprayed after 15 days of emergence with fungicides and bio-agents. Observations on disease incidence and intensity was recorded after 30 days of crop emergence.

The bio-control agent and the fungicide which proved most effective in *in-vitro* were were further evaluated under field conditions for their efficacy in controlling the disease. Further integrated management trials were conducted during Rabi 2019-20 at Dryland Agriculture Station, SKUAST-K Rangreth. Field was equally divided into beds with plot size

of 2m ×1m with each plot having fifty plants. Three replications for each treatment were maintained in completely randomized blocks. The chickpea seeds of susceptible variety were sown after treatment with different combination of bio-agent and fungicides as seed treatment as well as foliar sprays. The foliar sprays were done after fifteen days of crop emergence. The plots where only water was sprayed served as check. Observations on disease incidence and disease intensity were recorded thirty days after crop emergence.

Results and Discussion

In *in-vitro* evaluation of non-systemic and curative fungicides revealed that carbendazim(12%)+mancozeb(63%) proved most effective in causing the highest mycelial growth inhibition(82.27%) of the pathogen followed by dodine with mycelial growth inhibition of 67.99 per cent. The least efficious fungicide among tested fungicide was copper oxychloride with 29.81 per cent mycelial growth inhibition, while among the systemic fungicides(Table1), carbendazim proved was most effective in causing the mycelial growth inhibition of 90.23 per cent followed by difenoconozole with 71.24 per cent mycelial growth inhibition. The least effective fungicide was myclobutanil with 52.76 per cent mycelia growth inhibition(Table 2).Further, the different methods of application of fungicides, seed treatment was found effective in controlling the disease followed by foliar spray . The data revealed that the seed treatment with carbendazim(12%) + mancozeb (63%) proved most effective with lowest disease incidence (15.0%) and disease intensity(4.02%) followed by carbendazim with 16.50 and 4.82 per cent disease incidence and disease intensity. The least efficious fungicide was copper oxychloride with 60. 25 and 40.32 per cent disease incidence and intensity which is still superior than check with disease incidence and intensity of 83.33 and 71.11 per cent, respectively,. The foliar spray also showed better results than soil application method, where disease incidence and disease intensity was 20.25 and 6.24 per cent when sprayed with carbendazim(12%) + mancozeb (63%) followed by carbendazim with 25.00 and 7.15 per cent disease incidence and intensity. Among the biological control agents used, seeds treated with *Bacillus subtilis* proved most effective and resulted in disease incidence and intensity of 32.25 and 20.04 per cent, respectively, followed by *Pseudomonas fluorescens* with 42.00 and 25.34 per cent disease incidence and intensity. Similarly, foliar spray with *Bacillus subtilis* also showed promising results as disease incidence and intensity was recorded 39.50 and 26.15 per cent, respectively (Table 3). Further the integrated disease management experiment conducted during Rabi 2020-21 to study the effect of some promising bio-agents and fungicides under field conditions as seed treatment and foliar spray against the ascochyta blight of chickpea at DARS, Rangreth revealed that carbendazim (12%)+mancozeb (63%) was most effective systemic fungicide (carbendazim 50 WP) and *Bacillus subtilis* was the most effective bio-agent for management of the disease. The results on disease incidence and intensity, presented in Table 4, revealed that all the seed treatments and foliar spray were superior over the check in controlling the disease incidence and intensity. In the experiment the mean disease incidence ranged from 7.80 to 80.00 per cent and the mean disease intensity ranged from 4.82 to 51.34 per cent. Lowest disease incidence of 7.80 per cent was recorded in treatment combination of seed treatment with *Bacillus*

subtilis@10^9 spores $ml^{-1}$and foliar spray with carbendazim (12%)+mancozeb (63%)WP @2.5per cent. Highest disease incidence of 80.00 per cent was observed in check plots. Seed treatment with *Bacillus subtilis* @10^9 spores ml^{-1}foliar spray with carbendazim 50WP @ 2.5% resulted in the disease incidence and disease intensity of 17.37 and 8.25 per cent followed by foliar spray with carbendazim @ 0.5 per cent with disease incidence and disease intensity of 18.75 and 9.23 respectively. Further it was observed that among different the seed treatments, least disease incidence and intensity of 36.50 and 20.25 percent was observed when the seeds were treated with mancozeb 63% WP+ carbendazim12%WP @ 2g/kg of seed .This is therefore concluded that under field conditions, during Rabi 2020-2021 the best disease management capsule was seed treatment with *Bacillus subtilis*@10^9spores $ml^{-1}$and foliar spray with mancozeb 63%WP + carbendazim12%WP @ 2.5 per cent,15 days of crop emergence proved effective in managing this disease. Our results are in conformity with those reported by Abdel Kader *et al* (1989); Ahmad *et al* (2008) Chongo *et al* (2003), Gurdip and Maninder (1990).The fungicides and bioagents which can effectively inhibit the test fungus in laboratory conditions are supposed to be effective against the same pathogen in field conditions. Generally, all the treatments and combinations check the activities of the inoculated fungus, *Ascochyta rabiei* and hence promote the healthy conditions of chickpea plant. Although with the increase in concentrations, few fungicides completely inhibited the growth of pathogen (Sabiya *et al* ., 2019). In the present investigation, *Bacillus subtilis* proved most effective in laboratory conditions and these findings were completely in agreement with many workers who found many isolates/strains of Bacillus isolated from the rhizosphere regions of host crop plants effective in managing the disease (Bais *et al*, 2004; Branda *et al* 2001; Chen *et al* 2012; Gong *et al* 2015; Stein,2005).In the interim, it is important to integrate all available methods into a management package to manage the diseases. The use of seed free of contaminating pathogens will reduce the probability of transmitting seed-borne disease to the seedlings. The present study concluded that the use of fungicide and biological agents could be two key measures for a rational integrated management of ascochyta blight of chickpea in sustainable cropping systems in Kashmir. In that approach, a fungicide possibly eliminates the seed and soil borne inoculum and a biocontrol agent takes care of the soil-borne inoculum and increases crop productivity by improving nutrients status and soil health (Bretag et,2006; Warkentin et al, 1996 and 2000). The present situation demands their incorporation in management schedule for better disease management. By utilizing this strategy using fungicides in combination with bio-control agents (BCAs) to synergistically suppress the Ascochyta blight of chickpea will not only improve the chickpea yields in Kashmir but will also be a contributing factor in agriculture sustainability through improvement of soil fertility.

Table 1 *In–vitro* evaluation of non-systemic fungicides against *Ascochyta rabiei* causing blight of chickpea during Rabi 2018-19

S No.	Treatment	Per cent inhibition of radial mycelial growth at concentration (µg ml^{-1})					
		50	100	250	500	1000	Mean

1.	Chlorothalonil	44.03 (41.03)	53.26 (46.87)	53.26 (46.87)	64.16 (53.23)	80.20 (63.62)	58.98
2	Carbendazim (12%)+mancozeb (63%)	61.66 (51.75)	75.33 (60.17)	81.43 (64.48)	92.96 (74.67)	100.00 (90.00)	82.27
3	Captan 50 WP	35.76 (36.76)	43.13 (41.05)	43.16 (41.06)	51.83 (46.04)	60.43 (51.02)	46.86
4	Dodine 65 WP	44.40 (41.77)	70.06 (56.84)	71.86 (57.98)	72.20 (58.20)	81.43 (64.48)	67.99
5	Copper oxychloride 50WP	25.90 (30.59)	28.33 (32.11)	27.06 (31.26)	29.56 (32.93)	38.20 (38.17)	29.81
	Mean	42.35	54.02	55.35	62.14	72.05	
CD (P=0.05) Non-systemic fungicide = (2.1) Concentration = (1.9) Fungicide × Concentration = (4.70)							

*Figures within parentheses are arc sign transformed value

Table 2 *In –vitro* evaluation of systemic fungicides against *Ascochyta rabiei* causing blight of chickpea during Rabi 2018-19

S No.	Treatment	Per cent inhibition of radial mycelial growth at concentration (µg ml^{-1})					
		25	50	100	200	500	Mean
1.	Difenoconozole 25 EC,	53.80 (41.17)	61.50 (51.64)	66.60 (54.69)	74.30 (59.53)	100.00 (90.00)	71.24
2	Carbendazim 50 WP	82.00 (64.89)	84.56 (66.86)	84.60 (66.89)	100.00 (90.00)	100.00 (90.00)	90.23
3	Hexaconozole 5EC	48.66 (44.23)	56.33 (48.63)	64.10 (53.19)	66.63 (54.71)	66.70 (54.74)	64.56
4	Mycobutanil 10 WP	43.53 (41.28)	51.23 (45.70)	51.20 (45.68)	56.33 (48.63)	61.50 (51.64)	52.76

	Mean	56.99	63.40	66.62	74.31	82.05	

CD (P=0.05)

Systemic Fungicide = (1.27)
Concentration = (1.25)
Fungicide × Concentration = (2.33)

*Figures within parentheses are arc sign transformed value

Table3 Evaluation of different fungicides and bio-control agents against Ascochyta blight of chickpea through seed treatment, soil treatment and foliar application during Rabi 2019-20

S No.	Treatment	Con. (%)	Seed treatment		Soil treatment		Foliar spray	
			Disease incidence (%)	Disease intensity (%)	Disease incidence (%)	Disease intensity (%)	Disease incidence (%)	Disease intensity (%)
1.	Chlorothalonil	0.30	35.50 (20.79)	10.75 (19.13)	49.25 (44.57)	17.37 (24.63)	43.25 (41.12)	13.24 (21.33)
2	Carbendazim (12%)+mancozeb (63%)	0.25	15.00 (22.78)	4.02 (11.56)	21.75 (27.79)	7.80 (16.21)	20.25 (26.74)	6.24 (14.46)
3.	Carbendazim 50 WP	0.05	16.50 (23.96)	4.82 (12.68)	28.75 (32.42)	9.23 (17.68)	25.00 (30.00)	7.15 (15.50)
4.	Difenoconozole 25EC	0.05	23.25 (28.82)	8.52 (16.97)	36.50 (37.16)	15.54 (23.21)	27.25 (46.26)	10.14 (18.56)
5.	Hexaconozole 5 EC	0.03	37.25 (37.61)	12.15 (12.15)	51.50 (45.85)	18.75 (25.65)	45.50 (42.41)	15.11 (22.87)
6.	Captan 50 WP	0.30	44.00 (41.55)	27.88 (31.87)	60.25 (50.91)	42.27 (40.55)	48.25 (43.99)	30.12 (33.28)
7.	Dodine 65 WP	0. 06	34.50 (35.97)	10.25 (18.67)	47.32 (25.60)	16.09 (23.64)	41.25 (39.96)	12.76 (20.92)
8.	Myclobutanil 10 WP	0.10	38.25 (22.48)	13.42 (21.48)	52.00 (46.14)	19.13 (25.93)	45.75 (42.56)	16.32 (23.82)
9.	Copper oxychloride	0.30	60.25 (50.91)	40.32 (39.41)	65.50 (54.02)	51.34 (45.76)	61.15 (51.44)	45.14 (42.21)
10.	*Trichoderma viridi*	0.2	44.25 (41.69)	31.13 (33.91)	52.15 (46.23)	37.11 (37.53)	47.25 (43.42)	33.42 (35.31)
11.	*Bacillus subtilis*	0.2	32.25 (34.60)	20.04 (26.59)	35.75 (36.72)	28.02 (31.96)	39.50 (38.93)	26.15 (30.75)

12.	*Pseudomonas fluorescens*	0.2	42.00 (40.39)	25.34 (34.42)	50.75 (45.42)	35.23 (36.40)	45.15 (42.21)	25.75 (30.49)
13.	Check (Untreated)		83.33 (65.90)	71.11 (57.48)_				

Table 4 *In vivo*/ field evaluation of the bio-agent and fungicide against the Ascochyta blight of Chickpea during 2020-21

S. No	Treatment	Mean disease Incidence (%)	Mean Disease Intensity (%)
T1	Seed treatment *Bacillus subtilis* @10^9 spores ml^{-1}	53.80 (41.17)	37.25 (37.61)
T2	Seed treatment with mancozeb 63% WP+ carbendazim12%WP @ 2g/kg of seed	36.50 (37.16)	20.25 (26.74)
T3	Seed treatment with carbendazim 50WP 1g/kg of seed	44.25 (41.69)	34.50 (35.97)
T4	Seed treatment with *Bacillus subtilis* @10^9 spores per ml and foliar spray with same @10^9	21.75 (27.79)	16.50 (23.96)
T5	Seed treatment with *Bacillus subtilis* @10^9 spores ml^{-1} and foliar spray with mancozeb 63%WP + carbendazim12%WP @ 2.5%	7.80 (16.21)	4.82 (11.56)
T6	Seed treatment with *Bacillus subtilis* @10^9 spores ml^{-1} foliar spray with carbendazim 50WP @ 2.5%	17.37 (24.63)	8.25 (16.97)
T7	Foliar with *Bacillus subtilis* @10^9 spores ml^{-1}	51.50 (45.85)	31.13 (33.91)
T8	Foliar spray with mancozeb 63% WP+ carbendazim12%WP @ 2.5%	20.25 (26.74)	13.42 (21.48)
T9	Foliar spray with carbendazim @ 0.5%	18.75	9.23

		(25.65)	(17.68)
T10	Control	80.00 (64.89)	51.34 (45.76)

CD ($p \leq 0.05$) 0.34 Drenching was done after 15 days of emergence

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