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

ABSTRACT

Five non-systemic fungicides: 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: 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%. The least efficacious fungicide was copper oxychloride with only 29.81% mycelial growth inhibition. Among the systemic fungicides evaluated at concentrations of 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% followed by difenconozole (71.24%). Myclobutanil 10WP was least efficacious among the systemic fungicides and resulted in only 52.76% of mycelial growth inhibition. 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 the most effective which resulted in disease incidence and intensity of 32.25 and 20.04%, respectively. Combination treatment comprising of seed treatment with *Bacillus subtilis* at concentration of 10^9spores ml^{-1} and foliar spray with mancozeb 63% WP+ carbendazim12%WP at the concentration of 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* at the concentration 10^9 spores ml^{-1}alone was least efficacious and resulted in highest disease incidence of 53.80% with disease intensity of 37.25%. It was, however superior than control where disease incidence and intensity was 80.00% and 51.34 %, respectively.

Key words: *Ascochyta, Bio-agents, Chickpea, Disease incidence, Fungicides, Seed Treatment*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop belongs to family *Fabaceae* grown world-wide and is ranked third after beans and dry peas (Shifa *et al*, 2018). India is the largest producer, consumer and importer of the chickpea in world, contributing 71 % in area and 71.95% in production (Samriti, 2020). Chickpea contributes nearly 48 to 52% of total pulse production in India (Anonymous, 2018). The total area under its cultivation in India is about 105.61 lakh hectares with annual production of 112.29 lakh tonnes and an average productivity of 1063 kgha^{-1} (FAO, 2019). The land placed under production of chickpea in Jammu and Kashmir during the year 2012-13 was 2000 hectare with average production of 1000 tonnes ha^{-1} and yield of 542 kg ha^{-1}(Anonymous, 2016).

Chickpea crop is affected by several diseases, pests and poor management practices resulting in heavy yield losses. Amongst them, diseases play important role in reducing yield potential of chickpea (Nene, 1982). About 50 diseases have been reported to affect chickpea with huge economic importance. These include wilt [*Fusarium oxysporum* f. sp. *ciceri* (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). Lesions can occur on leaves, leaflets, stems, pods and seeds. Symptoms begin to appear after 4-6 days after the spores infect the plant. Conidia are exuded from fruiting body called pycnidia in a sticky spore mass and are spread to the nearby plants during rain-splashes (Reddy and Singh, 1990). 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 causes necrotic spots which develop into conidia at the centre. These conidia work as inoculum for secondary infection during rains (Shtienberg, 2010). Ascochyta blight is mainly controlled with fungicides and bio-agents (Salman, 2021).

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 compare and evaluate new fungicides along with some compatible bio-agents for controlling fungal diseases such as ascochyta blight. Use of fungicides create environmental pollution, but bio-agents are environment friendly. Hence, the present study was carried out to evaluate non-systemic and systemic fungicides and some bio-control agents against the ascochyta blight of chickpea.

Material and methods

Five non-systemic fungicides: chlorothalonil, carbendazim (12%)+ mancozeb (63%), captan 50WP, dodine 65WP and copper oxychloride 50WP and four systemic fungicides: difenoconozole 25EC, carbendazim 50WP, hexaconozole 5EC and mycobutanil 10WP were evaluated against aschochyta blight of chickpea using poisoned food technique (Carpenter,1942) where potato dextrose agar was medium. The non-systemic fungicides were evaluated at different concentrations of 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. On sterilized petri plates, 25 ml of PDA medium was aseptically poured. After cooling, 5mm 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 the 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 described 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: 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 season of 2018-19 at Dryland Agriculture Station (DARS), SKUAST-Kashmir, Rangreth. Field was equally divided into beds with plot size of 2m×1m, with each plot having 50 stands of chickpea plants. Three replications for each treatment were maintained in completely randomized blocks designs. In first method (seed treatment), the chickpea seeds of susceptible variety (Local channa) 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. The third method involving sowing, susceptible chickpea seeds and the crop was sprayed with fungicides and bio-agents after 15 days of seed emergence. Disease incidence and intensity for each treatment were recorded after 30 days after seed emergence.

The bio-control agent and the fungicide which proved most effective in *in-vitro* were further evaluated under field conditions for their efficacy in controlling the disease. Further integrated management trials were conducted during Rabi season 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 50 stands of chickpea plants. Three replications for each treatment were maintained in completely randomized blocks designs. The chickpea seeds of susceptible variety (Local channa) were sown after being dressed with different combination of bio-agent and fungicides as seed treatment. The foliar sprays were done 15 days after seed emergence. The plots where only water was sprayed served as check. Observations on disease incidence and intensity were recorded 30 days after seed 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%. The least efficious fungicide among tested fungicide was copper oxychloride with 29.81% mycelial growth inhibition (Table 1), while among the systemic fungicides (Table 2), carbendazim proved the most effective in causing the mycelial growth inhibition of 90.23% followed by difenoconozole with 71.24% mycelial growth inhibition. The least effective fungicide was myclobutanil with 52.76% 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% disease incidence and disease intensity. The least efficacious fungicide was copper oxychloride with 60. 25% and 40.32% disease incidence and intensity which is still superior over check with disease incidence and intensity of 83.33% and 71.11%, respectively. The foliar spray also showed better results than soil application method, where disease incidence and disease intensity was 20.25% and 6.24% when sprayed with carbendazim (12%) + mancozeb (63%) followed by carbendazim with 25.00% and 7.15% 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%, respectively, followed by *Pseudomonas fluorescens* with 42.00% and 25.34% 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%, respectively (Table 3).

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 (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% and the mean disease intensity ranged from 4.82% to 51.34%. Lowest disease incidence of 7.80% was recorded in treatment combination of seed treatment with *Bacillus subtilis* applied at concentration of 10^9 spores $ml^{-1}$and foliar spray with carbendazim (12%) + mancozeb (63%) applied at concentration of 2.5%. Highest disease incidence of 80.00% was observed in check plots. Seed treatment with *Bacillus subtilis* applied at 10^9 spores ml^{-1} and foliar spray with carbendazim 50WP applied at concentration of 2.5% resulted in the disease incidence and disease intensity of 17.37% and 8.25% followed by foliar spray with carbendazim applied at concentration of 0.5% with disease incidence and disease intensity of 18.75% and 9.23%, respectively. Among the different seed treatments, least disease incidence and intensity of 36.50% and 20.25% was observed when the seeds were treated with mancozeb 63% WP+ carbendazim12%WP applied at the concentration of 2g/kg of seed.

Under field conditions, during Rabi 2020-2021 integrated disease management option that involved seed dressing with *Bacillus subtilis* applied at the concentration of 10^9 spores ml^{-1} and foliar spray with mancozeb (63%WP) + carbendazim (12%WP) applied at the concentration of 2.5% at 15 days after seed 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 bio-agents which can effectively inhibit the test fungus in laboratory conditions are supposed to be effective against the same fungal pathogen under 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 crop. Although with the increase in concentrations, few fungicides completely inhibited the growth of *Ascochyta rabiei* (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 this approach of integrating fungicides with bio-agents eliminates the effect of *Ascochyta rabiei* through double action. The fungicides eliminates the seed and soil borne inoculums, while the bio-agents takes care of soil-borne inoculums and increases crop productivity by improving nutrient status and soil health (Bretag *et al*.,2006; Warkentin *et al.*, 1996 and 2000).

Conclusions and recommendations

It is important to integrate all available methods into a management package for *Ascochyta rabiei* under chickpea production. The use of seed free of contaminating pathogens will reduce the probability of transmitting seed-borne disease to the seedlings. 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. Inclusion of fungicides and bio-agents in the integrated management of *Ascochyta rabiei* under chickpea production in Kashmir.

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

S No.	Treatment	% 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	% 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

Table 3: 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	36.50 (37.16)	20.25 (26.74)

	seed		
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* @10^9spores 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* @10^9spores 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 (25.65)	9.23 (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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