International Journal of Chemical Studies

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Integrated management strategies in chilli powdery mildew

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Abstract
Powdery mildew of chilli caused by *Leveillula taurica* is one of the most serious diseases of chilli. To assess the efficacy of fungicides, bioagents and botanicals against chilli powdery mildew *in vitro* evaluation was carried out. Among the ten fungicides evaluated, the maximum inhibition of conidial germination was recorded in azoxystrobin (90.38%) and least inhibition of conidial germination was observed in carbendazim (71.47 %) at 0.15 per cent concentration. Among the four bioagents evaluated *Bacillus subtilis* (50.48 %) was found effective and nimbicidin (65.05 %) at 2.5 was recorded maximum conidial germination inhibition among the botanicals. Based on the results of *in vitro* evaluation, the effective fungicide, botanical and bioagent were selected for field trial to check their efficacy. Among the eight treatments three sprays of azoxystrobin (0.1%) was found effective by showing less per cent disease index of 6.86 with yield of 13.25 q/ha and cost benefit ratio of 1: 3.33 followed by treatment with two sprays of azoxystrobin (0.1%) and one spray of Nimbicidin (2.5%) with per cent disease index of 13.89 and yield of 12.54 q/ha and cost benefit ratio of 1:3.26. Whereas, three sprays of *Bacillus subtilis* (1%) was found to be least effective by showing high per cent disease index of 34.58 with yield of 8.78 q/ha and cost benefit ratio of 1:2.52.

Keywords: Azoxystrobin, *Bacillus subtilis*, Chilli, *Leveillula taurica*, Nimbicidin powdery mildew

Introduction

Chilli (*Capsicum annuum* L.) is an important vegetable cum spice crop grown in both tropical and subtropical regions of the world.Chilli is a rich source of vitamin A, vitamin C and E, potassium and folic acid too. It is valued for its pungency (imparted by an alkaloid, capsaicin) and the red pigments (capsanthin, capsorubin and capxanthin). India is the major producer, consumer and exporter of chilli in the world. In India, the area under chilli cultivation during 2015 was 1.81 lakh ha and the production was 1.9 mt and productivity of 10.1Mt/ha. It is the second most important spice in the Indian export market. (Anon., 2016) [6].

Powdery mildew caused by *Leveillula taurica* is a major constraint in chilli production causing yield loss of 42.82 per cent due to severe defoliation and reduction in size and number of fruits per plant (Ashtaputre, 2014) [7]. Therefore present investigation was carried out by using integrated approach.

Materials and Methods

For this initially *in vitro* evaluation was conducted at Department of Plant Pathology, College of Agriculture Dharwad during 2017-18. Effectiveness of fungicides, bio agents and botanicals were evaluated under *in vitro* condition against *L.taurica*. Eight systemic fungicides (Azoxystrobin, carbendazim, difenconazole, hexaconazole, myclobutanil, propiconazole, penconazole and tebuconazole) and two combi products (Metiram55 % + Pyraclostrobin 5 % and Tebuconazole 50 % + Trifloxystrobin 25 %) were evaluated at two different concentrations 0.10% and 0.15% by spore germination inhibition method.

Required concentrations were prepared by dissolving known quantity of fungicides in sterile distilled water containing 2 per cent sucrose separately under aseptic conditions. Two drops of fungicide solution was taken on cleaned cavity slide to which powdery mildew spores were suspended by camel hair brush and sterile water kept as control. In each treatment three replications were maintained. Slides were then incubated at temperature (22 ± 1^0 C) for 24 hr. The observation on the spore germination was recorded 24 hr after incubation under

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microscope at 40X magnification. Conidial germination (%) calculated by the following formula.

$$\text{Germination (\%)} = \frac{A}{B} \times 100$$

Where,
G – Conidial germination (%)
A – Number of conidia germinated
B – Total number of conidia observed
The inhibition of conidial germination (%) was calculated by the following formula given by Vincent (1947) [12].

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

Where,
C –Germination of conidia in control (%)
T –Germination of conidia in treatment (%)
The bio efficacy of four bio agents (*Bacillus subtilis, Psuedomonas fluorescens, Trichoderma harzianum* and *Lecanicillium lecanii*) were evaluated at two different concentrations at 1% and 1.5% concentrations. Similarly, the bio efficacy of seven botanicals (*Parthenium hysterophorus, Annona reticulata, Allium sativum, Bougaainvillea glabra, Allium cepa,Sorghum bicolor* and Nimbicidin) were carried out at two different concentrations (2.5% and 5%).10 g leaves of plant material were rinsed in water and cut into small pieces and macerated using pestle and mortar with 50 ml of water. The contents were filtered through a clean double layered muslin cloth. Then, the volume was made up to 100 ml to get ten per cent concentration. Further, it was diluted with distilled water to get 2.5 and 5 per cent concentration. These extracts were centrifuged for 5 min at 3000 rpm to get a clear plant extract this supernatant extract was used for evaluation.

The plant extract solutions were prepared in 2 per cent sucrose. Two drops of plant extracts were taken on cleaned cavity slide to which powdery mildew spores were suspended by camel hair brush and sterile water kept as control. Slides were incubated at temperature $(22 \pm 1^0 \text{ C})$ for 24 hr.

The field trial was conducted during *kharif* 2017 at Main Agricultural Research Station at UAS, Dharwad. Based on the results of *in vitro* evaluation, the most effective fungicide, bioagent and botanicals were selected for the field efficacy trial. The experiment was laid out in Randomized Block Design (RBD) with three replications and eight treatments with control. The seeds of Byadgi dabbi variety were sown in small beds and the nursery was raised. The seedlings of 35 days old were transplanted to the main field by following spacing of 60×60 cm and with plot size of 4.2 m². The recommended package of practice was followed for the trial.
The first spray was done immediately after the appearance of disease and successive two sprays at 12 days intervals. The severity of powdery mildew was scored at 7 days interval after each spray. The disease severity of powdery mildew was recorded on 10 plants and 10 leaves on lower, middle and upper leaves by using 0-9 disease rating scale (Mayee and Datar, 1986) [10] and expressed as per cent disease index (PDI) (Wheeler, 1969) [13]. Yield in each treatment was calculated separately and the data were analysed statistically.

$$\text{PDI} = \frac{\text{Sum of individual disease ratings}}{\text{Total No. of leaves observed} \times \text{Maximum disease rating}} \times 100$$

Statistical analysis
All the data related to disease severity and yield was statistically analyzed. Calculations were made after applying the test of significance of the means (Panse and Sukhtame, 1978) [11].

Results and Discussion
The results revealed that, among ten fungicides irrespective of concentrations of fungicides tested, the treatment involving azoxystrobin was recorded maximum conidial germination inhibition (90.16 %) followed by myclobutanil (87.82 %) and least inhibition was noticed in carbendazim (69.99 %) (Table 2).The findings are in accordance with Marthand (2016) [9].
Irrespective of concentrations of bioagents tested, *Bacillus subtilis* was recorded maximum conidial germination inhibition (50.23%) followed by *Psuedomonas fluorescens* (47.36 %) and least inhibition was noticed in *Lecanicillium lecanii* (31.92 %).Several workers like Dinesh *et al.* (2015) [8], have reported the effectiveness of *Psuedomonas fluorescens* against *L. taurica*. But in the present investigation under *in vitro* studies, *Bacillus subtilis* (1.5 %) was found effective (Table 3). The reduction in conidial germination *Bacillus subtilis* due to may be attributed to the production of lipopeptides, antibiotics and enzymes and systemic induced resistance.
Among the evaluated botanicals, maximum inhibition of conidial germination was observed in Nimbicidin (59.73 % and 65.05 %) at 2.5 and 5 per cent concentrations. Whereas, among the different plant extracts maximum conidial germination inhibition was observed in leaf extract of *Sorghum bicolor* (51.78 %) followed by bulb extract of *Allium cepa* L. (46.81 %) at 5 per cent concentrations and least inhibition was observed in leaf extract of *Parthenium hysterophorus* (34.33 %) at 5 per cent concentration. At 2.5 per cent maximum inhibition was noticed in *Sorghum bicolor* (45.96 %) followed by *Allium cepa*L. (41.90 %) and least inhibition was observed in *Parthenium hysterophorus* (26.16 %) (Table 4).

Table 1: Powdery mildew of chilli disease rating scale (0-9)

Score	Description
0	No symptom of powdery mildew.
1	Small scattered powdery mildew specks covering 1 % or less leaf area.
3	Small powdery lesions covering 1-10 % of leaf area.
5	Powdery lesions enlarged covering 11-25 % of leaf area.
7	Powdery lesions coalesce to form big patches covering 26-50 % of leaf area.
9	Big powdery patches covering 51 % or more of leaf area and defoliation occurs.

Based on results of *in vitro* evaluation the effective and economically viable fungicide (Azoxystrobin), bio agent (*B. subtilis*) and botanical (Nimbicidine) were used in formulating the spray schedule. The results revealed that, all treatments reduced the disease significantly compared to unsprayed check. Least per cent disease index 6.86 was observed in T₃ (azoxystrobin @ 0.1 %) and is significantly superior over all treatments followed by T₅ (azoxystrobin @ 0.1 % + Nimbicidin @ 2.5 % + azoxystrobin @ 0.1 %) recording 13.89 per cent disease index. The next best treatments were T₄ (azoxystrobin @ 0.1 % + *Bacillus subtilis* @ 1 % + azoxystrobin @ 0.1 %) and and maximum per cent disease index of 65.34 was recorded in untreated control.

Maximum yield of 13.25 q/ha was obtained from T_3 (azoxystrobin @ 0.1 %) followed by T_5 (azoxystrobin @ 0.1 % - Nimbicidin @ 2.5 %- azoxystrobin @ 0.1 %) with yield of 12.54 q/ha. Least yield of 8.78 q/ha was recorded in T_1 (*Bacillus subtilis* @ 1 %) among the treatments. Whereas, yield of 6.71 q/ha was recorded in untreated control.

Among the different treatments T_3 (azoxystrobin @ 0.1 %) has showed higher cost benefit ratio (1:3.33) followed by T_5 (azoxystrobin @ 0.1 % - Nimbicidin @ 2.5 % - azoxystrobin @ 0.1 %) (1:3.26). Least cost benefit ratio (1:2.52) was obtained from T_1 (*Bacillus subtilis* @ 1 %). Use of chemicals to control the disease is an age old practice in plant protection in the absence of resistant varieties. Use of newer chemicals has become more popular in recent years because of their quick results. Now a days, people are more concentrating on their health and are becoming fonder of organic products so in view this, integrated management of the disease using plant extracts and bioagents along with chemicals was undertaken.

T_3 (azoxystrobin @ 0.1 %) was found effective by showing less per cent disease index of 6.86 with yield of 13.25 q/ha and cost benefit ratio of 1: 3.33 followed by T_5 (azoxystrobin @ 0.1 % + Nimbicidin @ 2.5 % + azoxystrobin @ 0.1 %) with per cent disease index of 13.89 and yield of 12.54 q/ha and cost benefit ratio of 1:3.26. Whereas, T_1 (*B. subtilis* @ 1%) was found to be least effective by showing high per cent disease index of 34.58 with yield of 8.78q/ha and cost benefit ratio of 1:2.52. Finding was in accordance with Ahiladevi and Prakasam (2014) [3].

The strobilurin fungicides, azoxystrobin (Amistar) have recently been labelled for the management of powdery mildew on chilli, but only preliminary reports are available on the efficacy of these fungicides against the severe form of the disease (Alexander and Waldenmaier, 2002) [5-2]. The strobilurin fungicides represent important class of chemicals for the management of a broad range of fungal diseases in agricultural production systems (Ajithkumar *et al.*, 2014) [4-1].

Table 2: *In vitro* evaluation of fungicides against chilli powdery mildew

Fungicide	Conidial germination inhibition (%)		Mean
	Concentrations (%)		
	0.1	0.15	
Azoxystrobin (Amistar)	89.94 (70.48) *	90.38 (71.90)	90.16 (71.19)
Carbendazim (Bavistin)	68.51 (55.86)	71.47 (57.71)	69.99 (56.78)
Difenconazole (Score)	77.29 (61.54)	78.62 (62.46)	77.95 (62.00)
Hexaconazole (Contaf)	85.88 (67.93)	87.50 (69.30)	86.69 (68.61)
Myclobutanil (Systhane)	86.92 (68.80)	88.73 (70.38)	87.82 (69.59)
Propiconazole (Tilt)	78.12 (62.11)	80.07 (63.49)	79.09 (62.80)
Penconazole (Topaz)	76.96 (61.31)	78.43 (62.33)	77.69 (61.82)
Tebuconazole (Folicur)	83.59 (66.10)	81.76 (64.72)	82.67 (65.41)
Metiram + pyraclostrobin (Cabrio Top)	85.58 (67.68)	86.41 (68.37)	85.99 (68.02)
Tebuconazole + Trifloxystrobin (Nativo)	84.25 (66.62)	85.41 (67.54)	84.83 (66.18)
	S.Em. ±	C.D. at 1 %	
Fungicides (F)	0.34	1.31	
Concentration (C)	0.18	0.68	
F ×C	0.49	1.86	

*Angular transformed values

Table 3: *In vitro* evaluation of bioagents against chilli powdery mildew

Bio agents	Conidial germination inhibition (%)		Mean
	Concentration (%)		
	1	1.5	
Bacillus subtilis	49.98 (44.19) *	50.48 (45.35)	50.23 (44.77)
Psuedomonas luorescens	46.13 (42.78)	48.59 (44.40)	47.36 (43.59)
Trichoderma harzianum	33.70 (35.49)	35.53 (36.59)	34.61 (36.04)
Lecanicilliumlecanii	29.81 (33.09)	34.04 (35.69)	31.92 (34.39)
	S. Em. ±	C.D at 1 %	
Bioagent (B)	0.15	0.59	
Concentration (C)	0.13	0.51	
B × C	0.22	0.83	

*Angular transformed values

Table 4: *In vitro* evaluation of botanicals against chilli powdery mildew

Botanicals	Conidial germination inhibition (%)		Mean
	Concentration (%)		
	2.5	5	
Annona reticulate (Custard apple)	31.69 (34.24) *	36.85 (37.36)	34.27 (35.80)
Allium sativum L. (Garlic bulb extract)	39.5 (38.92)	43.35 (41.16)	41.42 (40.04)
Allium cepa L. (Onion bulb extract)	41.90 (40.32)	46.81 (43.15)	44.35 (41.73)
Bougainvillea glabra (Paper flower leaf extract)	32.62 (34.82)	39.06 (38.66)	35.84 (36.74)
Parthenium hysterophorus (Parthenium grass leaf extract)	26.16 (30.74)	34.33 (35.85)	30.25 (33.29)
Sorghum bicolor(Sorghum leaf extract)	45.96(42.66)	51.78 (46.02)	48.87 (44.34)
Nimbicidin (0.25 & 0.5 %)	59.73 (50.59)	65.05 (53.73)	62.39(52.16)
	S. Em. ±		C.D. @ 1 %
Botanicals (B)	0.18		0.71
Concentrations (C)	0.12		0.46
B ×C	0.27		0.96

*Angular transformed values

Table 5: Integrated management of chilli powdery mildew

Treatments	PDI	Per cent disease reduction over control	Yield of dry chilli (q/ha)	Per cent increase in yield over control	Cost Benefit ratio
T$_1$: *Bacillus subtilis* (B) – B- B	34.58 (36.00) *	47.07	8.78	30.84	1:2.52
T$_2$:Nimbicidine (Ni) - Ni -Ni	27.95 (31.90)	57.22	9.31	38.74	1:2.60
T$_3$:Azoxystrobin (A) – A- A	6.86 (15.17)	89.50	13.25	97.46	1:3.33
T$_4$: A- B –A	16.10 (23.64)	75.35	11.05	64.67	1:2.90
T$_5$: A-Ni – A	13.89 (21.37)	78.74	12.54	86.88	1:3.26
T$_6$: Ni - A – B	23.13 (28.73)	64.60	9.98	48.73	1:2.71
T$_7$: Wettable sulphur (WS) -WS- WS	18.39 (23.08)	71.85	10.32	53.80	1:3.25
T$_8$:Water spray (C) - C - C	65.34 (53.91)	-	6.71	-	1:2.23
S. Em. ±	2.01		0.41		
C.D. at 5 %	6.10		1.24		

*Angular transformed values

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