In vitro evaluation of fungicides, bioagents and botanicals against *Erysiphe polygoni* DC in black gram

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Abstract:

Powdery mildew caused by *Erysiphe polygoni* DC is one of the major constraints in the production of black gram. In order to find out the effective fungicides, botanicals and bioagents against *Erysiphe polygoni* experiment was carried out under *in vitro* evaluation of fungicides, botanicals and bioagents was carried out with respect to inhibition of conidial germination of *E. polygoni.* Azoxystrobin 250% SC at 0.1 per cent concentration (94.16%) inhibited maximum conidial and it was followed by hexaconazole 5% EC (90.51%). Among botanicals maximum of 79.93 per cent conidial inhibition observed with Azadirachtin (1500 ppm) followed by NSKE (76.64%).Among bioagents @ 6 g/L concentration *Bacillus subtilis* (75.47%) inhibited maximum conidial germination followed by *Pseudomonas flurescens* (72.19%).

Key words: *Erysiphe polygoni, In vitro,* powdery mildew and black gram

Introduction:

Powdery mildew has long been known as important disease of plants in all parts of world. Linnaeus (1767) established a genus *Erysiphe*. De Condolle (1802) described many species of the genus. Although chemical control by fungicides may have negative environmental effects and limitations but fungicides still constitute the predominate part of the control measures used against powdery mildew. Use of chemicals has become more popular in recent times because of their quick results, especially in absence of resistant varieties. Now a day, use of plant extracts and bioagents in managing the disease has gaining importance because of their eco-friendly nature and low cost. *In vitro* screening of the fungicides, botanicals and bioagents provide information confirming their efficacy against specific pathogen and therefore, serve as a guide for field testing.

Material and Methods

***In vitro* evaluation of fungicides-**Totally twelve fungicides were tested against *E. polygoni.* And their details are given below.

Sl.No.	Common name	Trade name	Concentration
1	Azoxystrobin	Amistar 250% SC	0.1%
2	Carbendazim	Bavistin 50% WP	0.1%
3	Difenconazole	Score 25% EC	0.05%
4	Dinocap	Kerathane 48% EC	0.2%
5	Hexaconazole	Contaf 5% EC	0.1%
6	Myclobutanil	Systhene 10% WP	0.1%
7	Penconazole	Topas 10% EC	0.1%
8	Propiconazole	Tilt 25% EC	0.1%
9	Triadimefon	Bayleton 25% EC	0.1%
10	Tridemorph	Calixin 80% EC	0.1%
11	Trifloxystrobin	Flint 50% WG	0.1%
12	Wettable sulphur	Sulfex 80% WP	0.3%
13	Control	-	-

***In vitro* evaluation of botanicals**

The present investigation was aimed to study the anti-fungal activity of botanicals. Nine botanicals were tested 5% concentrations. Details of the botanicals, which were tested against *E. polygoni* are given below.

Sl. No.	Botanicals	Scientific name	Concentration
1	Neem Seed Kernel Extract	*Azadirachta indica*	5%
2	Azadirachtin (1500 ppm, 1:10 dilution)	-	
3	Turmeric leaf extract	*Curcuma longa*	
4	*Ipomoea* leaf extract	*Ipomea carnea* sp. *fistulosa*	
5	Ballari jali leaf extract	*Prosopis juliflora*	
6	Papaya leaf extract	*Carica papaya*	
7	Lantana leaf extract	*Lantana camara*	
8	Adusoge leaf extract	*Adathoda vasica*	
9	Tridax leaf extract	*Tridax procumbens*	

Preparation of plant leaf extracts

Ten grams leaves of corresponding 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 5 per cent concentration. The experiment was conducted in one factor completely randomised design (CRD).

***In vitro* evaluation of bioagents:**

Four bioagents were tested at 0.4% and 0.6% concentrations. Details of the bioagents, which were tested against *E. polygoni* are given below.

Sl. No.	Bioagents	Concentration
T_1	*Bacillus subtilis*	0.4%
T_2	*Bacillus subtilis*	0.6%
T_3	*Pseudomonas fluorescens*	0.4%
T_4	*Pseudomonas fluorescens*	0.6%
T_5	*Trichoderma harzianum*	0.4%
T_6	*Trichoderma harzianum*	0.6%
T_7	*T. viride*	0.4%
T_8	*T. viride*	0.6%
T_9	Control	-

Various fungicides, botanicals and bioagents were evaluated under *in vitro* condition by spore germination technique against *E. polygoni*. Required concentrations were prepared by dissolving known quantity of fungicides, botanicals and bioagents in sterile distilled water separately under aseptic conditions. The conidial suspension was prepared separately in sterile distilled water. A drop of a spore suspension was mixed with one drop of Fungicides, Botanicals and Bioagents solution in a cavity slide to achieve the required concentration. In each treatment three replications were maintained. Slides were then incubated at a room temperature (25±1°C) for 24 hours. The observation on the spore germination was recorded 24 hours after incubation under microscope at 40X magnification. A control

with only sterile water was maintained. Percent conidial germination was calculated by the following formula.

$$\text{Per cent germination (PG)} = \frac{A}{B} \times 100$$

Where,

PG - Per cent germination

A - Number of conidia germinated

B - Number of conidia observed

The Per cent inhibition was calculated by the following formula given by Vincent (1927).

$$\text{Per cent inhibition of spore germination} = \frac{C - T}{C} \times 100$$

Where,

C- Germination of conidia in control

T- Germination of conidia in treatment

Results and Discussion:

Although chemical control by fungicides may have negative environmental effects and limitations but fungicides still constitute the predominate part of the control measures used against powdery mildew. Use of chemicals has become more popular in recent times because of their quick results, especially in absence of resistant varieties. Now a day, use of plant extracts and bioagents in managing the disease has gaining importance because of their eco-friendly nature and low cost.*In vitro* screening of the fungicides, botanicals and bioagents provide information confirming their efficacy against specific pathogen and therefore, serve as a guide for field testing.

***In vitro* evaluation of fungicides against *Erysiphe polygoni* DC**

In the present study*, in vitro* evaluation of 12 fungicides was carried out with respect to inhibition of conidial germination of *E*. *polygoni* DC. All fungicides tested were statistically significant in inhibiting the conidial germination(Table 1). Azoxystrobin at 0.1 per cent concentration gave maximum per cent inhibition of conidial germination (94.16%) followed by hexaconazole at 0.1% (90.51%), myclobutanil 10% WP (89.78%), and Trifloxystrobin 50% EC (86.86%). The effectiveness of fungicides

against powdery mildew pathogens was reported by several researchers. Dinesh (2009) and Divyajyothi (2012).

Investigation under *in vitro* studies carbendazim has showed less per cent inhibition of conidial germination of 68.98 per cent with the concentration of 0.1 per cent. Hence, the present study results do not agree with efficacy of carbendazim against powdery mildew reported by several workers (Singh *et al.*, 1994 and Bhardwaj (1992)). Investigation also revealed that the tridemorph at 0.1 per cent was less effective in reducing the powdery mildew and it is supported by the work of Steve *et al.* (1990) who reported the loss of efficacy of sterol biosynthesis inhibiting fungicides and increase in tolerance of the pathogen against the chemicals. Among the non-systemic fungicides sulphur at 0.3% gave relatively high conidial germination inhibition followed by dinocap at 0.2%. Similar results were observed by Patel *et al.* (1992) while working with powdery mildew of mustard.

***In vitro* evaluation of botanicals**

Generally synthetic fungicides are used against phytopathogenic fungi. But continuous use of chemical fungicides in management of plant diseases has become a major threat to mankind which often imposes various undesirable side effects. Hence, in recent years there has been increased awareness on toxic hazards of chemicals to crops, consumer and environment due to residual phytotoxity and pollution effects. So screening of plant products for their effective antifungal activity against the pathogen is essential to minimize the use of fungicide.

The present investigation was carried out to evaluate the nine different plant species for the possible presence of fungitoxic substance against *E.polygoni* DC under *in vitro* condition. The results revealed that the effect of plant leaf extracts on conidial germination was significant. Azadirachtin 1500ppm (79.93%) was most effective in inhibiting conidial germination followed by NSKE (76.64%); adusoge (68.25%), lanata leaf extract (70.07%) and turmeric leaf extract (67.88%) (Table 2). Least inhibition was recorded with tridax (39.41%) followed by papaya leaf extract (43.06%) and ballari jali (53.28%). The plant leaf extract at 5 per cent was significantly superior. The findings of the present study are in confirmation with Venkatrao (1997), Gangwar *et al.* 2000 and Dinesh *et al.* (2009).

The fungicidal spectrum of neem (*Azadirachta indica*) has already been investigated by Singh and Pande (1966) and reviewed in detail by Parveen and Alam (1993). Antifungal properties of *Azadirachta indica* were also established by Mukherjee (1976). The chemical basis of this antifungal activity has been attributed to the presence of oil in the plants parts of *Azadirachta indica* (Singh and Dwivedi, 1990).

***In vitro* evaluation of bioagents**

The concept of organic farming and eco-friendly management encouraged the plant protection specialists to go for the use of bioagents for the management of pest and diseases. Recently use of fertilizers and chemicals has been discouraged to avoid the pollution of air, water and soil. Bioagents which were previously known for their antimicrobial nature were evaluated against *E. polygoni* DC following spore germination technique. Levente (2003) reported that there are approximately 40 fungal species that have so far been reported as natural antagonists of powdery mildews or have been tested as their potential biocontrol agents and known for different mode of action.

The present investigation was carried out to evaluate the four bioagents with different concentration against *E.polygoni* DC under *in vitro* condition. The results revealed that the effect of various bioagents on conidial germination was statistically significant. *Bacillus subtillis* @ 6 g/L was most effective (75.47%) in inhibiting conidial germination followed by *Pseudomonas flouraescens* @ 6 g/L (72.19%) (Table 3). Least inhibition was recorded with *Trichoderma viride* @ 4 g/L (65.40%). *Bacillus subtilis* @ 4 g/L concentration was equally good with 68.69% per cent inhibition and it was on par with *Trichoderma harzianum* @ 6 g/L concentration. Similar observations were made by Sudha and Lakshmanan (2007), Romero *et al.* (2007) and Gaber and Aly (2011) while working with chilli, cucurbit and sqush powdery mildew respectively.

Romero *et al.* (2007) studied *in vitro* bio efficacy of *Bacillus subtilis* strains, and found that strains such as, UMAF6614, UMAF6619, UMAF6639, and UMAF8561, having ability to suppress the conidial germination on melon in detached leaf assay.

Conclusion:

In vitro evaluation of fungicides revealed that maximum inhibition of conidial germination was observed with azoxystrobin 250% SC @ 0.1% (94.16%) followed by hexaconazole 25% EC at 0.1% (90.51%). The least per cent inhibition was recorded in dinocap 48% EC (64.60%) at 0.2 per cent, wettable sulphur 80% WP (66.79%) and carbendazim 50% WP (68.98%).

Out of nine botanicals tested *in vitro* against *E. polygoni* DC, the effect of neem based products on conidial germination was significantly superior over control. Azadirachtin 1500 ppm showed maximum conidial inhibition of 79.93 per cent followed by NSKE (76.64%), adusoge (68.25%) and lantana leaf extract (70.07%).

In vitro evaluation of four bioagents with different concentrations revealed that all the bioagents were significantly superior over the control. Maximum conidial inhibition was recorded with *Bacillus subtilis* (75.47%) at 6 g/L followed by *Pseudomonas fluorescens* (72.19%) @ 6 g/L.

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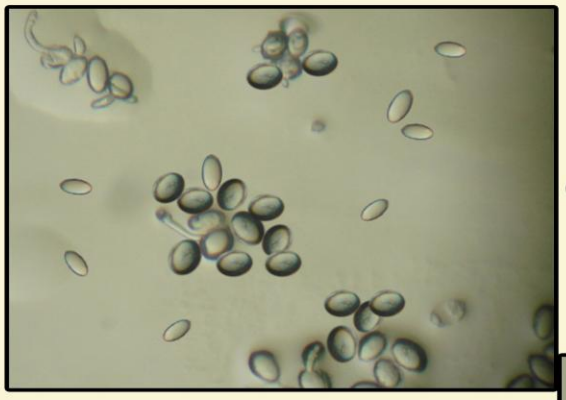
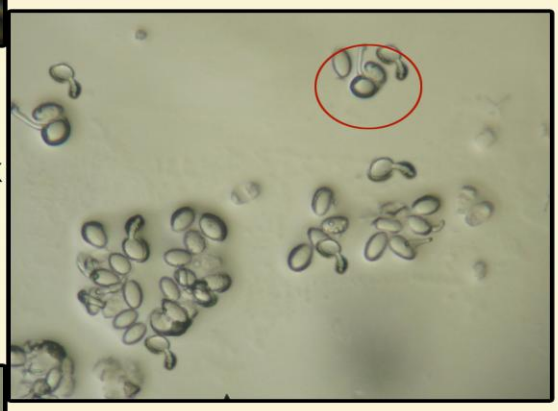
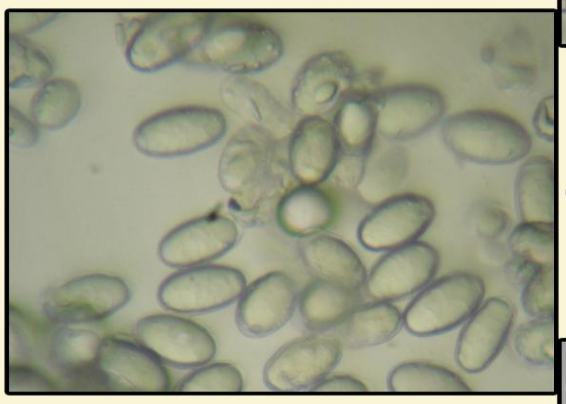


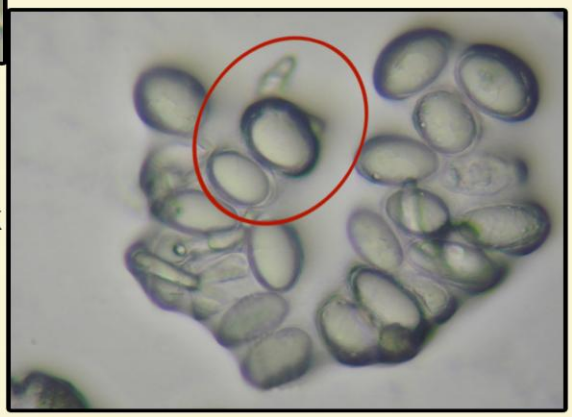
Conidia at 100X



Germinated conidia at 100X

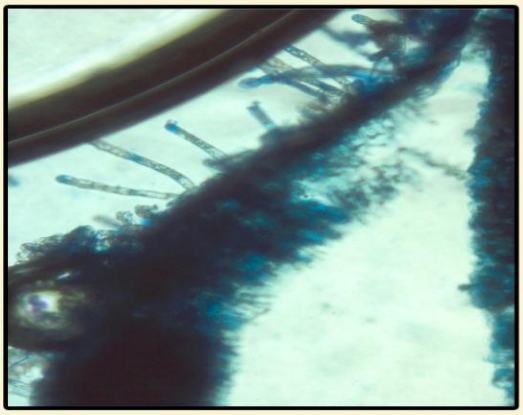


Conidia at 400X



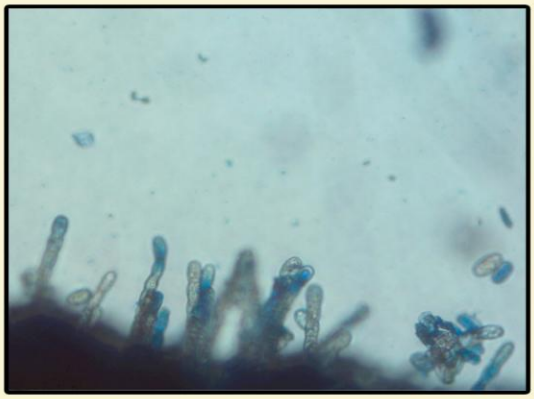
Germinated conidia at 400X

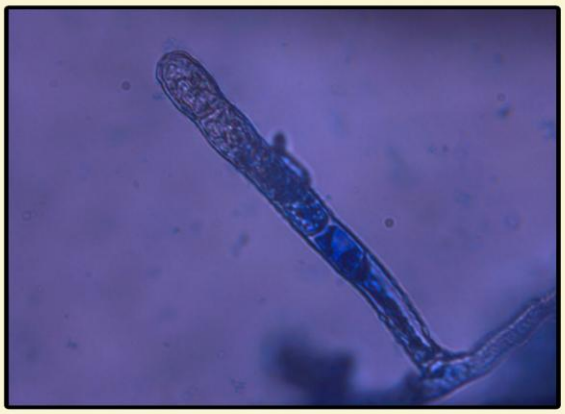
Plate 1: Microphotograph showing conidia and germinated conidia of *E. polygoni*



Conidia and conidiophores at 100X

Conidia and conidiophores at 100X





Conidia and conidiophores at 400X

Conidia and conidiophores at 400X

Plate 2: Microphotograph showing conidia and conidiophores of *E. polygoni*

Table 1. *In vitro* evaluation of fungicides against *Erysiphe polygoni* DC

Fungicides	Concentrations (%)	Germination (%)	Inhibition (%)
Azoxystrobin 250% SC	0.1	5.33 (13.35)*	94.16 (76.55)
Carbendazim 50% WP	0.1	28.33 (32.17)	68.98 (56.95)
Difenconazole 25% EC	0.05	22.67 (28.44)	75.19 (60.15)
Dinocap 48% EC	0.2	32.33 (34.67)	64.60 (53.45)
Hexaconazole 5% EC	0.1	8.67 (17.12)	90.51 (72.56)
Myclobutanil 10% WP	0.1	9.33 (17.79)	89.78 (71.86)
Penconazole 10% EC	0.1	17.67 (24.87)	80.66 (64.35)
Propiconazole 25% EC	0.1	14.33 (22.26)	84.31 (67.15)
Triadimefon 25% WP	0.1	16.33 (30.45)	82.12 (65.43)
Tridemorph 50%EC	0.1	23.33 (28.90)	74.82 (60.30)
Trifloxystrobin 50% WG	0.1	12.33 (20.55)	86.86 (69.23)
Wettable sulphur 80% WP	0.3	30.33 (33.43)	66.79 (55.19)
Control	-	91.33 (72.96)	-
S.Em.±		0.49	0.43
C.D at 1%		1.94	1.70

* Values in parenthesis are arc sine transformed

Table 2. *In vitro* evaluation of botanicals @ 5%against *Erysiphe polygoni* DC

Botanicals	Germination (%)	Inhibition (%)
Adusoge leaf extract	28.67 (32.39)*	68.25 (55.73)
Azadirachtin (1500 ppm, 1:10dilution)	18.23 (25.36)	79.93 (63.42)
Ballari jali leaf extract	42.67 (40.80)	53.28 (46.91)
Ipomoea leaf extract	37.33 (37.68)	59.12 (50.28)
Lantana leaf extract	27.33 (31.54)	70.07 (56.86)
Neem Seed Kernel Extract	21.83 (27.52)	76.64 (61.13)
Papaya leaf extract	52.00 (46.17)	43.06 (41.01)
Tridax leaf extract	55.33 (48.09)	39.41 (38.95)
Turmeric leaf extract	29.33 (32.81)	67.88 (55.51)
Control	91.33 (72.96)	-
S.Em.±	0.52	0.41
C.D at 1%	2..09	1.65

* Values in parenthesis are arc sine transformed

Table 3. *In vitro* evaluation of bioagents against *Erysiphe polygoni* DC

Bioagents	Concentrations (g/L)	Germination (%)	Inhibition (%)
Bacillus subtilis	4	28.67 (32.39)*	68.69 (55.97)
Bacillus subtilis	6	22.33 (28.22)	75.47 (60.32)
Pseudomonas fluorescens	4	30.33 (33.43)	66.94 (54.90)
Pseudomonas fluorescens	6	25.33 (30.24)	72.19 (58.74)
Trichoderma harzianum	4	27.33 (31.53)	67.47 (55.23)
Trichoderma harzianum	6	23.67 (29.12)	68.19 (55.70)
Trichoderma viride	4	31.67 (34.26)	65.40 (53.97)
Trichoderma viride	6	29.67 (33.02)	66.74 (54.78)
Control	-	91.33 (72.96)	-
S.Em.±		0.39	0.13
C.D at 1 %		1.57	0.43

* Values in parenthesis are arc sine transformed