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Effect of fungicides, botanicals and bioagents against purple blotch of onion caused by *Alternaria porri*

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ABSTRACT

A study was conducted in the of Department Plant Pathology, College of Agriculture, Latur, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India, during 2011 to control *Alternaria porri* causing Alternaria blight of onion with fungicides, botanical and bio-agents. Among nine treatments, six fungicides (@ 100, 200, 250 and 500 ppm concentrations), one plant extract and two bioagents (@ 500 ppm) were evaluated *in vitro in vivo* and were found effective against *A. porri* and recorded significant inhibition of the test pathogen over untreated control. However, *in vitro* result revealed that in hexaconozole cent per cent (100.00 %) inhibition was observed, followed by difenoconazole (83.91 %), mancozeb (63.58%), *P. florescence* (58.94 %) and *T. viride* (54.45%). The minimum per cent inhibition was observed in chlorothalonil (31.40 %) followed by plant extract NSKE (43.92 %), copper oxychloride (46.87 %) and carbandazim (47.11 %). *In vivo* results revealed that hexaconozole (0.1%) was found most effective and recorded significantly least mean disease incidence (6.03 %) and intensity (13.33 %) with corresponding significantly increased bulb yield (438.00 q/ha) followed by mancozeb (@ 0.2%) and copper oxychloride (0.25%) which recorded significantly mean disease incidence of 6.83 and 8.53 per cent and intensity, 15.00 and 20.00 per cent, respectively and gave correspondingly bulb yield, respectively of 375.00 and 429.00 q/ha. The botanical tested, *A. indica* (@ 5%) was found antifungal against *A. porri* and recorded significantly disease incidence (7.96 %) and intensity (27.00 %), and gave the bulb yield (290.00 q/ha). Both fungal and bacterial antagonists tested were found not so effective to reduce incidence and intensity, attempt increased the bulb yield over unsprayed control.

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INTRODUCTION

Onion (*Allium cepa*) a bulbous, biennial herb, is one of the most important vegetable crops grown thought world and in India. Onion is supposed to have its origin in the Middle East Asian Countries and introduced in India from Palestine. It belongs to family *Alliaceae*, and genus *Allium* with about 300 species.

In India area under onion is 579.9 thousand hectare production 7158.4 million tones and productivity 12357 kg/ha and in the state of Maharashtra area, production, and

productivity of onion are 109.0 thousand hectare, 1112.0 million tones, 10202 kg/ha, respectively (Anonymous, 2008).

Onion crop is attacked by 66 diseases, of which 10 bacterial, 38 fungal, 6 nematodes, three viral, one phytoplasmal, one phanerogamic plant parasite and seven miscellaneous diseases and disorder. The major bacterial diseases are: Bacterial flower stalk and leaf necrosis (*Pantoea agglomerans*) fungal diseases are: Purple blotch (*Alternaria porri*) and Stemphylium leaf blight (*Stemphylium vesicarium*), viral diseases are: Yellow dwarf (yellow dwarf virus) and nematode diseases are: Stem and bulb nematode (*Ditylenchus dipsaci*) and root knot nematode (*Meloidogyne incognita*). Among these diseases, the purple blotch (*A. porri*) is one of the major constrains in onion cultivation. The pathogen is polyphagus infecting crop like onion, garlic, shallot and other *Allium* crops. High relative humidity (80 to 90%) and optimum temperature (24±2°C) are needed for further development of purple blotch disease symptoms causing considerable yield losses and is seed borne pathogen causing up to 20-60 per cent loss in bulb yield and extent of loss depends on time of infection and stage of crop growth. Shahanaz *et al.* (2007) reported losses of about 50 to 100 per cent with relative occurrence of *A. porri.*

Typical symptoms of the disease appears on foliage and on foliage sheath small white sunken spots develop on the leaves which enlarge, become zonate and under moist conditions, turn purple and are also prominent on the inflorescence and stalks. Infection can cause a semi watery rot on necks of bulbs that turn yellow red in colour. Infected bulb tissues eventually become papery.

MATERIAL AND METHODS

Isolation and identification :

Naturally infested onion leaves showing typical symptoms of Purple blotch of onion were collected from the fields, brought to the laboratory and washed thoroughly with distilled water. These leaves were then blot dried and cut with sharp sterilized blade into small bits (5 mm), keeping half-healthy and half-diseased portion intact. These pieces were surfaces sterilized with 0.1 per cent aqueous solution of mercuric chloride ($HgCl_2$) for two minutes and then washed by giving three changes with sterile distilled water to remove the traces of mercuric chloride and blot dried. The surface sterilized diseased leaf bits were then inoculated on the solidified and cooled PDA (Potato dextrose agar) medium in Petri plates under aseptic conditions. Inoculated plates were then incubated in BOD incubator at 24 ± 2°C temperature. After three to four days of incubation, the well-developed mycelial growth, free from any contaminant was obtained. Following hyphal-tip technique, the fungus was transformed/ subcultured aseptically on the PDA slants in culture tubes. Through frequent sub-culturing, the fungus was purified and pure culture was maintained on agar slants in culture tubes and stored in refrigerator for further studies.

Pathogenicity test :

Seeds of onion cv. N-53 susceptible to purple blotch were surface sterilized with 0.1per cent $HgCl_2$ and sown @ 10 seeds/ pot in the earthen pots (25 cm dia.) filled with steam sterilized potting mixture of soil: sand: FYM (2:1:1). Five healthy growing onion seedlings per pot were maintained, watered regularly and kept in the green house for further development. The test pathogen (*A. porri)* was mass multiplied on the basal culture medium PDA in Petri dishes. Spore-cum mycelial suspension of the test pathogen was prepared from 7-8 days old culture in plates by flooding with 5-10 ml sterile distilled water. The resultant spore-cum-mycelial suspension was filtered through double-layered muslin cloth and filtrate obtained was suitably diluted with sterile distilled water to get inoculum's concentration of 5×10^4 spores/ml. Forty five days old seedlings of onion cv. N-53 already grown in earthen pots were artificially inoculated by spraying with atomizer the spore-cum-mycelial suspension (5×10^4 spores/ml) of the test pathogen. Onion seedlings in earthen pots sprayed with sterile water (without inoculums) were maintained as uninoculated suitable control. Pots (both inoculated and uninoculated) were incubated in the screen house, where high humidity (80-90%) and optimum temperature (24±2°C) were maintained for further development of purple blotch symptoms.

Reisolation :

The pathogen was reisolated on basal medium (PDA) from the culture medium artificially inoculated onion seedling showing typical symptoms of purple blotch. Growth of the fungus obtained was transferred on Potato dextrose agar slants and compared with original pure culture of the test fungus obtained from naturally infected leaf of onion.

Identification of pathogen :

Pure culture of the fungus obtained was inoculated aseptically on autoclaved PDA in Petri plates and plates were incubated at 24±1°C for a week. On the basis of pathogenicity test, cultural characteristics and microscopy, the test pathogen was identified *A. porri.*

***In vitro* evaluation of fungicides :**

Efficacy of six fungicides *viz.*, Mancozeb 75, Copper oxychloride 50 WP, Chlorothalonil 75 WP, Difenoconazole 25 EC, Hexaconozole 5 EC and Carbendazim 50 WP, were evaluated *in vitro* against purple blotch applying poisoned food technique (Nene and Thapliyal,1993). The requisite quantity of each fungicide based on active ingredient was calculated and mixed thoroughly with autoclaved and cooled

(40°C) Potato dextrose agar medium (PDA) in conical flasks to obtain desired concentrations of plain PDA medium without fungicide which served as control. Fungicide amended PDA medium was then poured aseptically in Petri plates (90 mm dia.). After solidification of the medium, all the plates were inoculated aseptically with 5 mm culture disc of the test fungus obtained from a week old actively growing pure culture of *A. porri.* The disc was placed on PDA in inverted position in the centre of the Petri plate and plates were incubated at 24 ± 2°C. Each treatment was replicated thrice. When medium in the untreated control plates was fully covered with mycelial growth of the test fungus, radial mycelial growth was measured in all the treatment plates. Per cent inhibition of mycelial growth in treated plates was calculated by applying the formula given by Vincent (1927) :

$$\text{Per cent Inhibition (I)} = \frac{C - T}{C} \times 100$$

where,

C = Growth (mm) of test fungus in untreated control plates

T = Growth (mm) of test fungus in treated plates.

***In vitro* evaluation of botanical (plant extract) :**

Plant extract of one botanical NSKE was evaluated against *A. porri.* Plant extract was prepared by grinding with mixture-cum grinder in 100 ml distilled water and filtered through double layered muslin cloth. The filtrates obtained were further filtered through Whatman No. I filter paper using funnel and volumetric flasks (100 ml cap.). The final clear extracts filtrates obtained formed the standard plant extracts of 100 per cent concentration, which were evaluated in *vitro* against *A. porri* applying poisoned food technique.

An appropriate quantity of the plant extract (100 %) was mixed thoroughly with PDA medium in conical flasks (100 ml cap.) to obtain desired concentrations and autoclaved at 15 lbs/inch^2 pressure for 15 to 20 minutes. Sterilized and cooled PDA amended with plant extract was then poured (15 to 20 ml/plate) into sterile glass Petri plates (90 mm dia.) and allowed the medium to solidify at room temperature. Each plant extract and its respective concentrations were replicated thrice. The plates containing PDA without any plant extract were maintained as untreated control. Upon solidification of PDA, all the plates were aseptically inoculated by placing in the centre a 5 mm mycelial disc obtained from one week old actively growing pure culture of *A. porri*. Plates containing plain PDA and inoculated with mycelial disc of test fungus served as untreated control. All these plates were then incubated at 24±2°C temperature for a week or until the untreated control plates were fully covered with mycelial growth of the test fungus.

Observations on radial mycelial growth/colony diameter of the test fungus were recorded treatment wise at 24 hours interval and continued till mycelial growth of the test fungus was fully covered in the untreated control plates. Per cent inhibition of mycelial growth over untreated control was calculated by applying the formula given by Vincent (1927) as detailed under.

***In vitro* efficacy of bioagents :**

Two fungal antagonists *viz.*, *Trichoderma viride* and one bacterial antagonist *Pseudomonas fluorescens* were evaluated *in vitro* against *A. porri,* applying dual culture technique (Dennis and Webster, 1971). Seven days old cultures of the bioagents and test fungus (*A. porri*) grown on agar media were used for the study. Discs (5 mm dia.) of PDA along with culture growth of the test fungus and bioagents were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and bioagents were placed at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates under aseptic conditions and plates were incubated at 24±2°C. Plates inoculated with culture disc of test fungus were maintained as untreated control. Observations on linear mycelial growth of test fungus and bioagents were recorded at an interval of 24 hours and continued until untreated control plate was fully covered with mycelial growth of the test fungus. Per cent inhibition of the test fungus over untreated control was calculated by applying the formula given by Arora and Upddhyay (1978).

***In vivo* evaluation of fungicides, botanical and bioagents field evaluation :**

The field experiment was conducted on the research farm of the Department of Plant Pathology during *Kharif,* 2011 to evaluate the efficacy of fungicides, botanical and bioagents, which were found most fungistatic/fungicidal against *A. porri* during *in vitro* studies.

The seeds susceptible to purple blotch of onion cv. N-53, were sown (15/06/2011) on the raised beds. Before sowing, the beds were soil drenched with Copper oxychloride (@ 0.3%) to prevent the infection by other soil borne organisms. Seed of N-53 were sown on beds and watered regularly twice a day till the full growth at transplanting. Fourty five days old seedlings of onion cv. N-53 were transplanted (04/08/ 2011) in the experimental field of the Department of Plant Pathology. The crop was raised as per recommended package of practices and protective irrigation was given as and when required.

Three sprayings of all the treatments were undertaken at an interval of 15 days, starting first spraying at 60 days after transplanting of the crop *i.e.* when first symptoms of disease appeared. One plot/replication was maintained as unsprayed control without receiving any fungicide, botanical and bioagents. Observations on disease incidence and severity were recorded before each spray treatment and lastly 15 days

after last spraying. Observations on disease incidence were recorded by counting treatment wise the number of plants affected with purple blotch disease, and perc ent disease incidence was calculated by applying the formula :

$$\text{Incidence (\%)} = \frac{\text{No. of plants showing disease symptoms}}{\text{Total no. of plants/plot}} \times 100$$

Ten plants per treatment per replication were selected randomly and tagged, and five leaves/plant were selected for recording purple blotch intensity. The purple blotch disease intensity was recorded applying 0-5 point disease rating scale (Mayee and Datar, 1986).

Based on numerical ratings/scale observed, per cent disease intensity (PDI) was worked out applying the formula given by Mc-Kinney (1923) :

$$\text{PDI)} = \frac{\text{Summation of numerical ratings}}{\text{No. of leaves / plants observed} \times \text{maximum rating}} \times 100$$

Further, per cent disease control (PDC) was worked out by applying the formula :

$$\text{PDC} = \frac{\text{PDI in control plate} - \text{PDI in treatment plot}}{\text{PDi in control plate}} \times 100$$

Score/Grade	Description
0	Healthy (No disease).
1	Traces (< 5% leaf area affected).
2	Light (6-10% leaf area affected)
3	Moderate (11-25% leaf area affected).
4	High (26-50% leaf area affected).
5	Very high (> 50% leaf area affected).

Disease rating scale :

At harvest, fresh onion bulb yield per treatment plot was recorded and finally yield data presented on hectare basis.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads :

***In vitro* evaluation of fungicides, botanical and bioagents :**

Per cent inhibition :

Results revealed that all the treatments tested (fungicides @ 200, 250, 100, 500, 100 and 100, botanical and bioagents @ 500 ppm, respectively significantly inhibited mycelial growth of the test fungus over untreated control (90.00 %). Further, it was found that percentage mycelial inhibition of the test pathogen increased with the increase in concentration of the fungicides tested.

Cent per cent (100.00 %) inhibition was observed in plate with Hexaconazole followed by Difenoconazole (83.91%), which was followed by Mancozeb (63.58%), bacterial and fungal antagonist, *P. fluroscense* (58.94 %) and *T .viride* (54.45%*)* at 100, 500, 200 and bioagents 500 ppm concentrations. Minimum per cent inhibition was observed with fungicide with chlorothalonil (31.40%), and this was followed by plant extract NSKE (43.92%), Copper oxychloride (46.87%), Carbendazim (47.11%) at 100, 500, 250 and 100 ppm concentrations as compared to untreated control.

Results of the present study are in conformity with those reported earlier by several workers. Efficacy neem (*A. indica*) was agreed with the observations of several workers (Pal *et al.*, 2008), fungal bioagents, *viz., T. harzianum, P. fluorescens, T. viride,* and *T. hamatum* (Kumar *et al.*, 2006; Verma *et al.*, 2007 and Kharbhari *et al.*, 2008) againt *A. porri*.

***In vivo* evaluation of fungicides, botanicals and bioagents**
Disease incidence (%) :

Results of (Table 2) indicated that all the treatments significantly influenced the purple blotch disease incidence in onion cv. N-53. The purple blotch disease incidence (%) was recorded after 1st, 2nd and 3rd spray treatments which ranged from 18.12 (hexaconozole) to 43.90 (*T. viride*), against 48.00 to 50.00 per cent incidence in unsprayed control.

The mean purple blotch disease incidence (%) recorded

Table 1 : In *vitro* effect of fungicides at different concentrations on radial mycelial growth and inhibition of *A. porri*.

Treatments	Concentrations (ppm)	Mycelial growth (mm)*	Per cent inhibition
Mancozeb	200	32.77	63.58 (52.89)**
Copper oxychloride	250	47.81	46.87 (43.22)
Chlorothalonil	100	61.74	31.40 (34.08)
Difenoconazole	500	14.48	83.91 (66.340
Hexaconozole	100	00.00	99.99 (88.19)
Carbendazim	100	47.60	47.11 (43.34)
NSKE	500	50.47	43.92 (41.50)
T. viride	500	40.99	54.45 (47.52)
P. fluroscense	500	36.95	58.94 (50.13)
Control	--	90.00	00.00
S.E. ±	--	--	2.68
C.D. (P=0.05)	--	--	7.89

* Average of three replication, ** Figures in parenthesis are angular transformed values

with all the treatments ranged from 6.03 (hexaconozole) to 16.2 (control) per cent. Among the fungicides tested, hexaconozole recorded significantly least mean disease incidence (6.03%). This was followed by fungicides mancozeb (6.83%), copper oxychloride (8.53%), bioagent, *P. fluorescense* (9.33%), fungicides difenocozole (10.13%), carbendazim (10.83%), chlorothalonil (11.56%) and bioagent *T. viride* (14.63%). Of the botanicals tested, NSKE (*A. indica*) recorded significantly least mean disease incidence (7.96%). Biocontrol agents tested were also found equally effective as that of the fungicides and botanical tested.

Results (Table 2) obtained on the per cent reduction in purple blotch incidence over untreated control revealed that fungicides, hexaconozole percentage over control caused highest reduction (62.77%) in the disease incidence. This was followed by mancozeb (57.83%), plant extract NSKE (50.86%), copper oxychloride (47.34%), *P. fluorescense* (42.40%), difenoconazole (37.46%), carbendazim (33.14%), chlorothalonil (28.64%), *T. viride* (9.69%), chlorothaonil and *T. viride* were found least effective which recorded minimum reduction of 28.64, 9.69. per cent, respectively in disease incidence (%).

Hexaconozole recorded significantly highest reduction (62.77%) in the disease incidence. The second and third best fungicides found effective in reducing the disease incidence were mancozeb (57.83%) and copper oxychloride (47.34%). The fungicide chlorothalonil was found least effective and recorded minimum disease reduction (28.64%).

Result revealed that hexaconozole (@ 0.1%), mancozeb (@ 0.2%), followed by plant extrat NSKE (@ 0.5%) and copper oxychloride (@ 0.25%) were found most effective in reducing the purple blotch incidence in onion cv. N-53.

Disease intensity :

Results (Table 2), revealed that all the treatments significantly influenced the purple blotch disease intensity and effectively reduced the same over unsprayed control in onion cv. N-53. The percentage disease intensity recorded ranged from 13.33 to 58.66 per cent.

Among the fungicides tested, hexaconozole recorded significantly least mean disease intensity (13.33%), which was followed by fungicdes mancozeb (15.00%), difenocozole (17.66%), copper oxychloride (20.00%), plant extract NSKE (27.00%), chlorothalonil (27.66%) and *P. fluorescense* (28.00%) and carbendazim (29.66%) and *T. viride* (31.33%). Of the botanicals tested, NSKE (*A. indica*) recorded significantly least mean disease intensity (27.00%), the bioagents tested, were found comparatively least effective than the fungicides and some what equally effective as that of botanicals tested.

Bulb yield (q/ha) :

Results of Table 2, obtained in respect of efficacy of the fungicides, botanical and bioagents tested in reducing incidence and intensity of purple blotch in onion cv. N-53 and their ultimate effect on bulb yield, indicated that all the treatments significantly reduced the disease incidence and intensity over unsprayed control and thereby increased the bulb yield. However, fungicides followed by bioagents and botanical were found most effective in reducing the disease and increasing the bulb yield.

Results of Table 2 revealed that all the fungicides, botanical and bioagents evaluated were found effective in reducing the purple blotch disease incidence and intensity and there by gave significantly increased bulb yield over unsprayed control in onion cultivar N-53.

Among the fungicides tested, hexaconozole (0.1%), gave significantly highest bulb yield (438.95 q/ha) and recorded highest increase of 51.01 per cent in bulb yield over unsprayed control (yield, 215.00q/ha), with significantly least mean

Table 2: Effect of fungicides, botanical and bioagents on per cent purple blotch incidence (PI) in onion Cv. N-53

Treatments	Con. (%)	Mean PI (%)	Mean PI reduction (%) over control	Mean PDI	Mean PDI reduction (%) over control	Fresh bulb yield (%)
Mancozeb	0.20	6.83* 15.12)	57.83	15.00 (22.79)	74.42 (54.42)**	375.42
Copper oxychloride	0.25	8.53 (16.95)	47.34	20.00 (26.51)	65.90 (46.87)	428.66
Chlorothalonil	0.10	11.56 (19.91)	28.64	27.66 (31.76)	52.84 (36.49)	369.25
Difenconazole	0.05	10.13 (18.53)	37.46	17.66 (24.88)	69.89 (50.24)	371.45
Hexaconazole	0.10	6.03 (14.18)	62.77	13.33 (21.39)	77.27 (57.22)	438.95
Carbandazim	0.10	10.83 (19.19)	33.14	29.66 (33.02)	49.43 (33.97)	324.00
NSKE (A. indica)	0.50	7.96 (16.32)	50.86	27.00 (31.31)	53.97 (37.39)	290.00
T. viride	0.50	14.63 (22.16)	9.69	31.33 (34.02)	46.59 (31.97)	348.35
P. fluorescenes	0.50	9.33 (17.76)	42.40	28.00 (31.95)	52.26 (36.11)	312.47
Control (water spray)	-	16.2 (23.73)	00.00	58.66 (50.01)	00.00	215.00
S.E. ±	--	1.50	--	1.70	--	21.00
C.D. (P=0.05)	--	4.46	--	5.10	--	63.69

*Average of three replication, **Figures in parenthesis are angular transformed values, Con. – Concentration, PI – Per cent incidence, PDI – Per cent disease intensity

purple blotch incidence (6.03%) and intensity (13.33%). The second best fungicide found was mancozeb (0.2%). which recorded bulb yield (375.42 q/ha) with increase of 42.73 per cent in bulb yield; with mean disease incidence (6.83%) and intensity (15.00%). difenocozole and bioagent *T.viride* were recorded bulb yield of 371.11, 348.35 q/ha, respectively. All the botanical and bioagents tested, significantly reduced the purple blotch mean incidence and intensity and recorded significantly higher bulb yield over unsprayed control. NSKE recorded (290 q/ha) bulb yeild and increase of 25.86 per cent over control with disease incidence and intensity, respectively 7.96 and 27.00 per cent.

Thus, all the fungicides, botanical and bioagents evaluated under field conditions against purple blotch of onion were found most effective in reducing the purple blotch disease incidence and intensity in onion cv. N-53 and there by increased the bulb yield over unsprayed control.

Mancozeb 75 WP (@ 0.2%) was found effective against Stemphylium blight (*S. vesicarium*), purple blotch (*A. porri*) of onion and *Alternaria* spp. causing blights in other crops, as earlier reported by several workers (Deshmukh *et al.*, 2007; Pandey *et al.*, 2008; Ilhe *et al.*, 2008 and Pal *et al.*, 2008).

Carbendazim, copper oxychloride, difenoconazole, chlorothalonil and hexaconozole have been reported, in the order of their merit as effective in the management of the Stemphylium blight and Alternaria blight of onion, potato, mustard and other crops by Gorawar and Hegde (2005) and Kumari *et al.* (2006).

Botanicals/plant extracts *viz.*, neem, mehandi and ginger were also reported effective in the control of Stemphylium blight of onion, garlic and blights caused by *Alternaria* spp. in other crops (Kumari *et al.*, 2006 and Sharma *et al.*, 2007).

Antagonists/bioagents *(T. harzianum*, *T. viride* and *P. fluorescens)* have been founded effective in the management of Stemphylium blight of onion and garlic caused by *Alternaria* spp. similar to the present study as reported by (Shahanaz *et al.*, 2007).

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