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IN VITRO MANAGEMENT OF PURPLE BLOTCH OF ONION (*ALTERNERIA PORRI)* BY NEW FUNGICIDES AND BIOAGENTS

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Abstract – The onion the" Queen of Kitchen "is one of the most important and oldest vegetables, and culinary commodities are grown in the world. They can be eaten raw, cooked, fried, dried or roasted. No Indian food is complete without onion. Purple blotch is one of the major foliar diseases having more than 90 % loss in yield. Ten different fungicides were tested *in vitro* at different concentration against the pathogen which was isolated from diseased onion leaf. Out of which, kavach (Chlolorothionil) at 0.5% concentration with 67.70% inhibition, Dithan M 45 @0.25% con. shows 77.35% inhibition of pathogen, biltox (Copper Oxy Chloride) at 0.1% concentration showing inhibition of pathogen as 86.47%. Recommended that Kavach and Dithan M-45 was use at higher concentration to manage the pathogen. In contrast to that the systemic fungicides like Score maximum inhibition were found @ 0.1% concentration as 91.52%. Nativo@0.1% concentration having inhibition as 98.52%.In Signet at 0.1% concentration as 99.11%. In opera at 0.1% concentration as 99.11% inhibition. Amister top at 0.1% concentration as 97.35% inhibition of pathogen. Tilt(Propiconazole 25Ec) fungicides, Where maximum inhibition were found at 0.1% concentration as 98.52%. *In vitro* study the use of bio agent shows that *T. harzanium* caused significantly maximum inhibition, 90.58 % against *A. porri* followed by *T. viride* (85.58%). The inhibitory effect of these bio agents was probably due to competition and/or antibiosis. While the bacterial bio agents like *Bacillus subtillus* (71.77%) and *Pseudomonas flurosence* (56.00%) found less effective against the pathogen.

INTRODUCTION

The onion plant (*Allium cepa*) also known as the bulb onion or common onion, is the most widely cultivated species of the genus *Allium*. It was first officially described by Carl Linnaeus in his 1753 work *Species Plantarum*. Besides being used as food articles; onion has a variety of medicinal effects. It is one of the most important winter vegetable crops of India. It is a nutritious vegetable and contains a good amount of Vitamin A and C, rich source of minerals (calcium, manganese and iron) and dietary fibers. The importance of these bulb crops in treating diversified ailments, viz .lowering blood sugar, cardiovascular problems, improving gastrointestinal health, fighting cholera, preventing urinary disorders, blood clot etc. is well recognized. The onion, according to National Onion Association (USA, 1918) the primary center of origin onion lies in Central Asia, Iran and somewhere in Pakistan (Vavilov, 1951). In India the crop was grown in kharif and rabi. Main crop is in rabi (50-60%), and kharif and late kharif contributes remaining about 20-25% each. Maharashtra, Karnataka, Gujarat, Bihar, Madhya Pradesh, Rajasthan, Andhra Pradesh and Tamil Nadu are the main onion growing states in India. In general, barring North Eastern states and Kerala, all other states grow onion. Country's 24% area and 27% production alone come from Maharashtra. The productivity in late kharif and rabi is around 25 tonnes per hectare, whereas in kharif season it is 8-10 tonnes per hectare.

Although India stood second in onion production after China at world level, India is far behind in productivity compared to many countries. The average productivity of onion in India in the past decade stood at only 13.78 t/ha, which is lower than world average of 18.75 t/ha (FAO Stat., 2013).

Table 1. Area and Production of onion in India(NHB,Database)

Year	2016-17	2017-18	2018-19	2019-20
Area	1306000 ha.	1285000 ha.	1220000 ha.	1293000 ha.
Production	22427000 tonne	.23265000 tonne	22810000 tonne	24454000 tonne
NHB, Database Indian Horticulture				
Productivity (2012-13)	17.92MS	24.09 MP	24.42 Guj.	

Table 2. Share of Different Indian states in onion production

Sr.No.	State name	Production/000 tonne	Share %
1.	Maharashtra	9099	37.20
2.	Madya Pradesh	3966	16.2
3.	Karnataka	2275	9.3
4.	Rajastan	1386	5.7
5.	Bihar	1312	5.4
6.	Gujarat	1256.80	5.1

NHB, Indian Horticulture Data base, 2019

Through the crop grown in khariff and rabbi season in India, their diversified farming having many constraints as abiotic and biotic stress, unavailability of true to type seeds, irrigation in summer and rabbi season, attack of pests like aphides, jassides, thrips, whitefly and nematodes. The most important factors responsible for lower yield are the diseases like purple blotch, downy mildew, stemphylium blight, basal rot and storage rots, pink rot etc., and non-availability of varieties resistant to biotic and abiotic stresses.

Among the foliar diseases, purple blotch is one of the most destructive diseases, commonly prevailing in almost all onion growing pockets of the India. The name purple blotch for this disease was proposed by Nolla (1927). He named the causal organism as *Alternaria allil* which was later amended to *Alternaria porri*. The yield loss of onion in India due to this disease under favorable conditions varies from 5.0-96.5 percent (Gupta *et al*., 1994). Older leaves tend to be more susceptible to purple blotch than younger leaves. Symptoms begin as water-soaked lesions that usually have a white center. Edges of lesions become brown to purple and the leaf turns yellow above and below the lesions. With time, dark brown to black concentric rings form throughout the lesions. On infected leaves small, sunken, oval to foot-ball shaped lesions were found. Concentric light and dark zones are also observed on the infected leaves. Brown lesions with reddish-purple margins resembling bull's-eye were also noticed. These are areas of sporulation and penetration of the fungus (Agale *et al*., 2015; Fahim 1966; Carrol and Carroll, 1971). The influence of environment on incidence of disease was studied by some workers from different part of countries and reported that high rainfall and high humidity favoured the disease development. *Alternaria porri* on onion occurred following a long period relative humidity > 90% or dew deposition and temperature ranges between 20-25 ^{o}C (Gupta and Pathak, 1986; Evert and Lacy, 1996). In our study we use different new and commercial ten fungicides against the pathogen at different concentration so that we fulfill the today's farmers need.

MATERIALS AND METHODS

Collection of diseased samples : Onion leaves infected with Alternaria purple blotch were collected from the infected leaf of the onion with typical spot symptoms. The infected leaves were collected from A.R.S., Niphad, Dist. Nasik and nearby onion fields in kharif 2020. Based on symptoms, microscopic examination of diseased samples association of the pathogen as *Alternaria porri* was recorded.

Glassware, plastic ware and other materials: Petri plates, glass petri dishes, conical flasks, test tubes, blotter paper and roll paper towel were used in the present studies.

Method adopted: Potato dextrose agar (PDA) medium was used for isolation and maintenance of cultures.

Disinfection /sterilization of laboratory materials: To detect the fungi on leaves, the plates were washed with cleaning powder under running water, dried and then disinfected with denatured spirit. However, glass plates were sterilized in hot air oven at 180^{0} C for 1 hr. before use.

Isolation of pathogen by tissue isolation method: Infected leaf samples were cut into small pieces with sterilized blade and disinfected with sodium hypochloride (0.2%) solution for two minute. Pieces were washed with three changes of sterilized

distilled water and bits after dried on sterilized filter paper and around flame of spirit lamp were placed on solidified PDA medium in plate. Each plate contained five bits. The plates were incubated at room temperature (28±2 °C). All these operations were carried out aseptically. The plates were examined regularly. Colonies were developed around the each bit were examined and sub cultured. Based on morphological characters and published literature the fungus was identified as *Alternaria porri*. The pure culture was transferred on PDA slants and maintained for further studies.

Purification and maintenance of fungal culture: Culture was purified by following hyphal tip method (Vincent, 1927) and culture obtained was maintained on potato dextrose agar (PDA) medium slants at room temperature by adopting subsequent subculturing at periodical, regular intervals. Seven days old culture was used for further studies.

Pathogenicity test by spray inoculation method

We identified the pathogen as young hyphae were hyaline, slender, radiating and septate. The white colonies turned purple to black color with advancing age of culture. The conidiophores arose singly or in groups and were pale brown, erect, simple, cylindrical, septate. Conidia were thick, solitary, straight or curved with the body of conidium ellipsoidal tapering to the beak and having 7 to 9 transverse septa and 1 to 3 longitudinal septa (Evert and Lacy, 1987), (Campbell, 1969). Pathogenocity was also studied as pathogen was incubated in PDA broth for 7 days at 28 +- 2 °C. Where the pathogen attained the CFU count of 1 *10 ^{8} were used for testing of pathogen city after spraying them on healthy plant after 10-15 days and found same symptoms as the diseased specimen shows.

***In vitro* preparation of different fungicides at different concentrations**

In, *in vitro* study commercial chemical fungicides are used as Score, Dithan M 45, Tilt, folicure, Kavch, Copper oxy chloride, Opera, Signet, Nativo, Amister top at different concentration as 0.1@, 0.2% 0.3% .0.4% and 0.5%. Sterile water was used to prepare the stock solution add them in sterile PDA medium at different concentration. They grow with the control without fungicides incubate it take observation at seven day. The experiment was conducted in randomized block design (RBD) with four replications in five treatments at Deptt. of Plant Pathology, Agricultural Research Station, Niphad, Dist. Nasik (M.S.). The inoculated Petri dishes were incubated at room temperature 28°C+-2°C in the laboratory. The poisoned food technique was adopted for in vitro testing of fungicides (Nene and Thapliyal, 1993). The colony diameters were measured after 7 days when the control plates were full of fungal growth. Percent inhibition of growth was calculated using the formula given by (Vincent 1947).

I = 100/ (C-T)*C

Where,

I = per cent inhibition of mycelium growth (%)

C = Growth of mycelium in control (cm.)

T = Growth of mycelium in treatment, i.e. control (cm)

***In vitro* preparation of different bio agents against the pathogen**

Biological control of plant pathogens through antagonistic microorganisms is potential, eco friendly and a suitable approach apart from being a promising alternative to the use of chemicals. With this back ground, in the present investigation efficacy of antagonist microorganisms in managing purple blotch under field conditions has been determined total four bio agent containing the two fungal bio-control agents, namely, *Trichoderma viride* and *Trichoderma harzianum* with two bacterial bio agent as *Psudomonas fliuroscenscs* and *Bacillus subtillus* were obtained from the NIPHM, Hydrabad, Telangana were evaluated against pathogen *A. porri* .

These bio-control agents were screened under *in vitro* conditions against *A. porri* for their antagonistic activity by using dual culture method as described subsequently. Culture discs (6mm) each of the fungal antagonist and the pathogen were taken from the margin of the actively growing cultures and transferred to PDA medium containing 90 mm Petri dishes on opposite sides, approximately at 1.0 cm from the wall of the plate. For bacteria the pathogen was placed at the center with 6 mm aliquotes and bacteria was striked 2.5 cm apart from it. A control check without the bio agent with the test pathogen only was kept for comparison. The petri dishes were subsequently incubated at 28 ±2°C till the control plate was completely covered by *A.porri*. Each treatment was replicated five times. Colony diameter of the test fungus as well as each antagonist up to the zone of inhibition was recorded

and the percent of growth inhibition of the test pathogen over the control was calculated according to the above formula given by Vincent (1947). All the statistical analyses done by using method (Panse and Sukhatme, 1985)

RESULTS

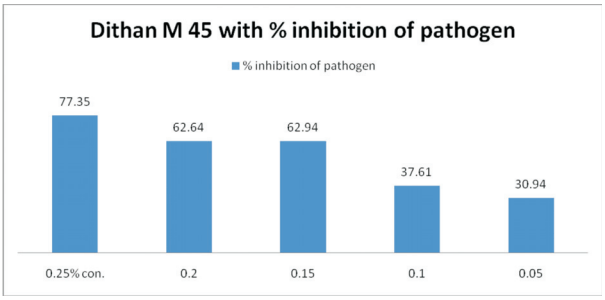
The Dithan M 45(Mancozeb) is the contact fungicide. where maximum inhibition were found significantly at 0.25% con.as 77.35%. While other concentration shows the result as 0.20% (62.64%), 0.15% (62.94%), 0.10%(37.61%) and minimum inhibition were found in 0.05% (30.94%) (Graph 1).

In vitro evaluation of Tilt (Propiconazole 25Ec) fungicides the systemic fungicide. Where maximum inhibition were found significantly at 0.1% concentration as 98.52%. Folicure (Tabuconazole 250 Ec) @0.1% concentration where maximum inhibition were found significantly at 0.1% concentration as 97.35%. While other concentration as 0.2% (99.70%), 0.3%(99.11%), 0.4%(99.11%) and 0.5%(100%). Amister top (Azoxystrobin 18.2%w/w+ Difenconazole 11.4%Sc) is the systemic fungicide.

Table 1. *In vitro* evaluation of Dithan M 45 at different concentration against the pathogen.

Concentrations % of Dithan M 45	% inhibition of pathogen	Control growth
0.25	77.35	8.5 cm
0.20	62.64	CD@ 5% =0.57
0.15	62.94	SEd =0.26
0.10	37.61	Significant
0.05	30.94	

In experiment % inhibition of pathogen* is figure with 5 replication mean in bio agents and 4 in different fungicides.
Growth of control plate without the fungicides was 8.5 cm.
All reaction was statistically significant.

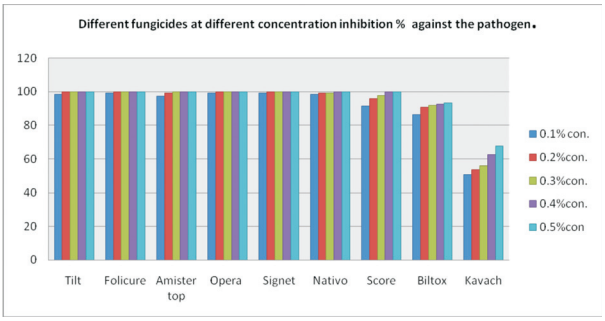


Graph 1. Dithan M 45 fungicides at different concentration reaction against the pathogen

Table 2. *In vitro* evaluation of different fungicides at different concentration against the pathogen.

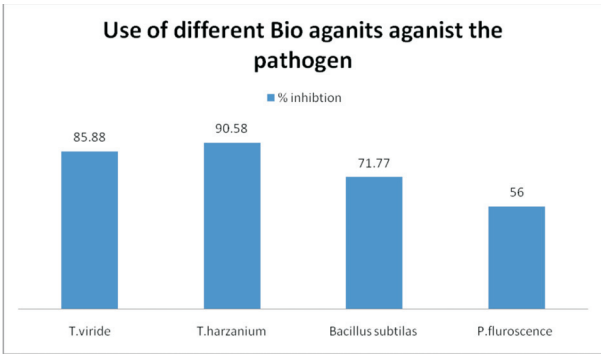
Sr No.	Name of the fungicides		Concentration %					Other Remarks
			0.1%	0.2%	0.3%	0.4%	0.5%	
1.	Tilt	% inhibition of pathogen*	98.52	99.70	100	100	100	CD@5%=0.034 SEd=0.015
2.	Folicure (Tabuconazole 250Ec)	-//-	99.11	99.70	100	100	100	CD@5%=0.035 SEd=0.015
3.	Amister top	-//-	97.35	99.11	99.70	99.81	100	CD@5%=0.034 SEd=0.015
4.	Opera	-//-	99.11	99.70	100	100	100	CD@5%=0.0345 SEd=0.016
5.	Signet	-//-	99.11	100	100	100	100	CD@5%=0.050 SEd=0.022
6.	Nativo	-//-	98.52	99.11	99.11	100	100	CD@5%=0.056 SEd=0.025
7.	Score	-//-	91.52	95.86	97.66	100	100	CD@5%=0.050 SEd=0.023
8.	Biltox	-//-	86.47	90.82	92.00	92.70	93.41	CD@5%=0.041 SEd=0.018
9.	Kavach	-//-	50.94	53.88	56.11	62.70	67.70	CD@5%=0.065 SEd=0.029

where maximum inhibition were found significantly at 0.1% concentration as 97.35%. In Opera shows maximum inhibition were found significantly at 0.1% concentration as 99.11%. In signet where maximum inhibition were found significantly at 0.1% concentration as 99.11%. Nativo@0.1% concentration having inhibition as 98.52%. In contrast to that the systemic fungicides like Score maximum inhibition were found significantly@ 0.1% concentration as 91.52%. While other concentration as 0.2% (95.88%), 0.3%(97.66%), 0.4% (100%) and 0.5%(100%). In biltox (Copper Oxy Chloride) the contact fungicides where different inhibition were found significantly at 0.1% concentration as 86.47%. While other concentration as 0.2% (90.82%), 0.3%(92.00%), 0.4%(92.70%) and 0.5% (93.41%). The Chlorothionil (kavach) where different inhibition were found significantly as at 0.1% concentration 50.94%. While other concentration as 0.2% (53.88%), 0.3% (56.11%), 0.4% (62.70%) and 0.5% (67.70%). Recommended that Biltox, Kavach and Dithan M-45 was use at higher concentration.Same tune was found in (Bhandeker *et al.*, 2019), (Deepti and Nidhi, 2015).



Graph 2. *In vitro* evaluation of Different fungicides at different concentration against the pathogen.

T. harzanium caused significantly maximum inhibition, that is, 90.58 % against *A. porri* followed by *T. viride* (85.58%) respectively. The inhibitory effect of these bio agents was probably due to competition and/or antibiosis. While other bacterial



Graph 3. *In vitro* % inhibition of pathogen by different bio agents.

bio agent like *Bacillus subtillus* (71.77%) and *Pseudomonas flurosence* (56.00%) found less effective against the pathogen.

DISCUSSION

All ten fungicides tested against the pathogen out of which, kavach (Cholorothionil) at 0.5% concentration with 67.70% inhibition, Dithan M 45 @0.25% con. shows 77.35% inhibition of pathogen, Biltox (Copper Oxy Chloride 50% WP) at 0.1% concentration showing inhibition of pathogen as 86.47%. While other concentration as 0.2% (90.82%), 0.3%(92.00%), 0.4%(92.70%) and 0.5% (93.41%). Recommended that Kavach and Dithan M-45 was use at higher concentration to manage the pathogen Same tune was found in (Rahman *et al.*, 1988, Chethana, 2000; Patel *et al.*, 2001; Pandey *et al.*, 2002) where Mancozeb @ 0.3% was highly effective followed by blitox and benlate and gradual reduction in fungal growth was found as the concentrations of the fungicides increased from 10 to 500 ppm.in different *alterneria* species management.

In contrast to that the systemic fungicides like Score (Difenconazole 25 % Ec) maximum inhibition were found @ 0.1% concentration as 91.52%. Nativo (Tabeconazole 50% + Trifloxystrobine 25%WG)@ 0.1% concentration having inhibition as 98.52%. In

Table 3. In vitro % inhibition of pathogen by different bio agents.

S/N	Bio agent	Mean radial Diameter cm.	Percent inhibition of mycelial growth (%)
1.	*T. viride*	1.2	85.88
2.	*T. harzianum*	0.9	90.58
3.	*Bacillus subtillus*	2.4	71.77
4.	*Pseudonomanas flurosence*	3.74	56.00
	Control plate	8.5 cm.	CD at 5% =1.35 significant result

Signet (Picoxystrobin 7.05% +Propiconazole 11.7Sc) at 0.1% concentration as 99.11%. In Folicure (Tabuconazole 250 Ec)@0.1% concentration where maximum inhibition were found significantly at 0.1% concentration as 97.35%. In opera (Pyraclostrobin 133g +Epoxiconazole 50g /l w/v) at 0.1% concentration as 99.11%inhibition. Amister top (Azoxystrobin 18.2%w/w+ Difenconazole 11.4%Sc) at 0.1% concentration as 97.35% inhibition of pathogen. Tilt(Propiconazole 25Ec) fungicides, (Table 02,Graph 02). Where maximum inhibition were found at 0.1% concentration as 98.52% (Dinesh Kumar *et al.,* 2020). Same report from (Roshan Kumar *et al.* 2020), where in the management of *A.altenata* of tomato at different fungicides in vitro and in field conditions found that tilt @300ppm, Nativo @500ppm, Amister @ 300ppm showing 100% inhibition of the pathogen.

Due to health risk and pollution hazards by use of chemical fungicides in plant disease control, it is considered appropriate to minimize their use. Use of antagonistic microorganisms in this context appeared logical and safe. Biological control of plant pathogens through antagonistic microorganisms is potential, eco friendly and a suitable approach apart from being a promising alternative to the use of chemicals.

We found that *T. harzanium* caused significantly maximum inhibition, that is, 90.58 % against *A. porri* followed by *T. viride* (85.58%) respectively. (Table 3, Graph 03). The inhibitory effect of these bio agents was probably due to competition and/or antibiosis. While other bacterial bio agent was found to be least effective. The bacterial bio agents like *Pseudomonas flurosence* (56.00%) and *Bacillus subtillus* (71.77%) found less effective against the pathogen. The antagonism of *T. viride, T. harzianum*, were also observed in the present studies in tune with the findings of various workers. Among the bioagents, *T. harzanium* was found to be more efficacious in inhibiting the mycelial growth.The best antagonistic fungi in reducing the linear growth of *A. porri* compared control followed by *Trichoderma viride* and other bacterial bio agents (Bhandekar *et al.,* 2019)

Similar result were recorded by (Chethana *et al.,* 2011; Yadav and Mishra, 2013; Mishra *et al.,* 2014), as they reported maximum mycelial inhibition 79.35%, 94.71% and 73.17% respectively of *A. porri* with *Trichoderma viride*.

Several workers have reported the effectiveness of *T. harziuanum* in control of diseases caused by *Alternaria* (Mathivanan *et al*., 2000; Patni *et al*., 2005; Sanjeet Kumar *et al*., 2005; Rao, 2006; Balai *et al*., 2017). The present investigation is in line with these reports.

CONCLUSION

Onion is one of the most important and oldest vegetables, culinary commodities grown in world. They can be eaten raw, cooked, fried, dried or roasted. No Indian food is complete without onion. Purple blotch is one of the major foliar diseases having more than 90 % loss in yield. In our study ten different fungicides were tested *in vitro* at different concentration against the pathogen which was isolated from diseased onion leaf. Out of which, kavach (Chlolorothionil) at 0.5% concentration with 67.70% inhibition, Dithan M 45 @0.25% con. shows 77.35% inhibition of pathogen, biltox (Copper Oxy Chloride)at 0.1% concentration showing inhibition of pathogen as 86.47%. Recommended that Kavach and Dithan M-45 was use at higher concentration to manage the pathogen. In contrast to that the systemic fungicides like Score maximum inhibition were found @ 0.1% concentration as 91.52%. Nativo@0.1%concentration having inhibition as 98.52%. In Signet at 0.1% concentration as 99.11%. In opera at 0.1% concentration as 99.11% inhibition. Amister top at 0.1% concentration as 97.35% inhibition of pathogen. Tilt (Propiconazole 25Ec) fungicides, Where maximum inhibition were found at 0.1% concentration as 98.52%. *In vitro* study the use of bio agent shows that *T. harzanium* caused significantly maximum inhibition, 90.58 % against *A. porri* followed by *T. viride* (85.58%). he inhibitory effect of these bio agents was probably due to competition and/or antibiosis. While the bacterial bio agents like *Bacillus subtillus* (71.77%) and *Pseudomonas flurosence* (56.00%) found less effective against the pathogen. Alternate use of bio fungicides and some systemic chemical fungicides plan the strategy for farmer to manage the disease in changing climatic scenario and condition of erratic environment with ecological bio safety.

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