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Full Length Research Paper

In vitro evaluation of plant extracts, bio-agents and fungicides against Purple blotch and *Stemphylium* blight of onion

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Eight plant extracts, bio agents and fungicides were evaluated in *in vitro* conditions against purple blotch and *Stemphylium* blight of onion caused by *Alternaria porri* and *Stemphylium vesicarium*. Among the plant extracts, clove extracts of *Allium sativum* at 10% resulted in maximum inhibition of growth (58.05 and 57.31%) of *A. porri* and *S. vesicarium,* respectively followed by *Aloe vera* at 10% (53.5 and 47.15%). Among the bioagents, *Trichoderma viride* was effective in inhibition of growth (53.17 and 56.15%). Out of eight fungicides evaluated, Mancozeb at 0.2% completely inhibited the growth of both the pathogens. Azoxystrobin (0.1%), propiconazole (0.1%) and antracal (0.2%) were the other effective fungicides.

Key words: Onion, *Allium cepa*, *Alternaria porri*, S*temphylium vesicarium*, management.

INTRODUCTION

Onion (*Allium cepa* L.) is the most important *Allium* species cultivated worldwide, and it is used as vegetable, salad and spice in the daily diet by large population. Onion is attacked by many diseases, which vary from region to region, season to season and variety to variety. Diseases can affect it at production, harvesting, processing and marketing, which lower the quality, reduce the yield, and thereby increase the cost of production, and also, the export potential. Purple blotch and *Stemphylium* blight are the most important diseases in Northern India, which causes considerable losses in seed crops as well as bulb crops. It can cause severe damage, especially to the onion seed crop and losses of about 80 to 85% on the crop by affecting leaves and seed stalk (Tomaz and Lima, 1988). These diseases are currently managed by routine application of several fungicides. Most of the new generation pesticides are systemic in their mode of action leads to certain level of toxicity in the plant system and thus resulting health hazards. Further, it disturbs complete microbial diversity of whole ecosystem. All these factors have led to new dimension in research for biological control and integrated disease management. Hence, an attempt was made to evaluate different plant extracts, bioagents and fungicides against the pathogen to manage the *Alternaria porri* and *Stemphylium vesicarium* of onion.

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MATERIALS AND METHODS

Eight plants were selected (Table 1) to estimate the antifungal behaviour against *A. porri* and *S. vesicarium* of onion through poisoned food technique (Nene and Thapliyal, 1979.) The plant species and parts used with their concentration are shown in Table 1.

The fresh leaves and other parts of healthy plants were collected and were thoroughly washed with tap water, and were air dried. Ten grams of plant tissues were ground using pestle and mortar by adding equal amount (10 ml) of sterilized distilled water (1:1 w/v). The extract was filtered through muslin cloth. The supernatant was taken as standard plant extract solution (100%). Further, the extract was diluted by adding sterilized water to get 10% concentration.

Table 1. Evaluation of plant extracts against purple blotch and *Stemphylium* blotch of onion.

S/N	Plant extract	Plant parts used	Concentration (%)	Percent inhibition of mycelial growth (%)	
				PB	SB
1	*A. cepa*	Bulbs	10	23.0	25.20
2	*A. sativum*	Cloves	10	58.05	57.31
3	*Azadirachta indica*	Leaves	10	27.87	26.70
4	*Lantana camara*	Leaves	10	25.20	24.81
5	*Pongamia* species	Seeds	10	26.0	28.42
6	*Ocimum sanctum*	Leaves	10	28.0	27.14
7	*A. vera*	Leaves	10	53.5	47.15
8	*Ginger officinalis*	Rhizome	10	29.87	28.34
-	CD at 1%	-	-	2.84	2.93

PB: Purple blotch; SB: *Stemphylium* blight.

Besides, plant extracts at 10% were also tested *in vitro* by poisoned food technique. The plant extracts were subjected to boiling temperature of 50°C in water bath to avoid contamination and then they were incorporated into potato dextrose agar (PDA) media by transferring 2 ml of each type of plant extract in to a Petri dish containing 20 ml melted warm PDA medium and they were gently shaken for thorough mixing of the extract. The PDA plates containing the plant extracts were inoculated aseptically with *A. porri* and *S. vesicarium* by transferring 8 mm diameter agar disc of 7 days old culture of the pathogen to the centre of the Petri dish. Three replications were maintained for each treatment. The basal medium (PDA) without any phytoextract served as control. All the inoculated Petri dishes were incubated at 25 ± 1°C. The radial growth of the test fungus in the treated plates was measured in all treatments when the pathogen growth touched the periphery in the control Petri dishes. The percent inhibition of fungal growth was estimated by using the formula given by Vincent (1927).

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

where I is the percent inhibition, C is the colony diameter in control, and T is the colony diameter in treatment

The fungal bio-control agents, namely, *Trichoderma viride*, *Trichoderma harzianum* and *Aspergillus niger* were isolated from soil and *Trichoderma hamatum*, *Trichoderma koningii*, *Trichoderma virens* and bacterial antagonists *Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, were evaluated against *A. porri* and *S. vesicarium*. These bio-control agents were screened under *in vitro* conditions against *A. porri* and *S. vesicarium* for their antagonistic activity by using dual culture method as described subsequently. Culture discs (8 mm) 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 cm from the wall of the plate. Similarly, bacteria were streaked on the opposite sides of the pathogen. A check having the test pathogen only was kept for comparison. The Petri dishes were subsequently incubated at 25 ± 1°C till the control plate was completely covered by *A. porri* and *S. vesicarium*. Each treatment was replicated thrice.

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 formula given by Vincent (1927).

Eight fungicides consisting of five systemic, one non-systemic and two combination-products were assayed for their efficacy against *A. porri* and *S. vesicarium* under *in vitro* condition. The systemic fungicides were tested at 0.1% concentration and the rest of the fungicides at 0.2% concentration. The poisoned food technique was adopted for *in vitro* testing of fungicides (Nene and Thapliyal, 1979). The various fungicides evaluated are furnished in Table 3. The calculated quantities of fungicides were thoroughly mixed in the medium before pouring into Petri dishes so as to get the desired concentration of active ingredient of each fungicide separately. Twenty milliliters of fungicide amended medium was poured in each of 80 mm sterilized Petri dishes and was allowed to solidify. The plates were inoculated centrally with 8 mm disc of a 10 day old young sporulating culture of test pathogens. Controls devoid of fungicides were also maintained. The experiment was conducted in randomized block design (RBD) with three replications in each treatment. The inoculated Petri dishes were incubated at room temperature 28°C in the laboratory. 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 (1927).

RESULTS AND DISCUSSION

Antifungal activity of eight botanical extracts was assayed and data of plant extracts on the growth of *A. porri* and S. *vesicarium* is presented in Table 1. The data revealed that significant reduction in growth of *A. porri* and *S. vesicarium* were observed in respect of all the plant extracts tested.

Results indicated that the clove extracts of *A. sativum* at 10% concentration caused significantly maximum inhibition (58.05%) of *A. porri* and 57.31% of *S. vesicarium* followed by leaves extract of *Aloe vera* at 10%, which was found to be promising against *A. porri* (53.5%) and *S. vesicarium* of 47.15%, respectively. Similar results on antifungal activity of aqueous extracts of different botanicals has been reported against onion by Mishra et al. (2008) and Prasad and Barvwal (2004), and also reported in other host by Meena et al. (2003), Saharan et al. (2008) and Upmanyu and Gupta (2005). Eight biological control agents were evaluated under *in*

Table 2. Evaluations of bioagents against purple blotch and *Stemphylium* blight of onion.

S/N	Bioagent	Percent inhibition of mycelial growth (%)	
		PB	SB
1	*T. viride*	55.95	56.15
2	*T. harzianum*	53.17	51.95
3	*T. hamatum*	37.05	35.23
4	*T. konngii*	46.65	45.23
5	*T. virens*	36.87	34.95
6	*A. niger*	41.50	39.15
7	*P. fluoresens*	19.20	20.17
8	*B. subtilis*	29.50	27.85
-	CD at 1%	2.37	2.13

PB: Purple blotch; SB: *Stemphylium* blight.

Table 3. Evaluations of fungicides against purple blotch and *Stemphylium* blight of onion.

S/N	Fungicide	Systemic/Non systemic	Concentration (%)	Percent inhibition of mycelial growth (%)	
				PB	SB
1	Azoxystrobin	S	0.1	95.40	94.23
2	Antracal	S	0.2	75.94	65.54
3	Companian	S+NS	0.2	90.40	97.13
4	Master	S+NS	0.2	95.24	95.00
5	Quintal	S	0.2	87.57	85.94
6	DM-45	NS	0.2	98.94	100.00
7	Hexaconazole	S	0.1	76.14	75.0
8	Propiconazole	S	0.1	89.10	87.14
-	CD at 1%	-	-	0.94	1.02

PB: Purple blotch; SB: *Stemphylium* blight.

vitro conditions against *A. porri* and *S. vesicarium* for their antagonistic activity by using dual culture method. It is apparent from the data presented in Table 2 that all the antagonistic organisms inhibited the growth of *A. porri* and *S. vesicarium* ranging from 19.20 to 55.95%. *T. viride* caused significantly maximum inhibition, that is, 53.17% against *A. porri* and 56.15% against *S. vesicarium* followed by *T. harzianum* (53.17 and 51.95%) and *T. koningii* (46.65 and 45.25%), respectively, while *P. fluorescens* was found to be least effective (19.20 and 20.17%). The inhibitory effect of these bioagents was probably due to competition and/or antibiosis. The antagonism of *T. viride*, *T. harzianum*, *Pseudomonas* and *Bacillus* were also observed in the present studies in tune with the findings of various workers (Mishra et al., 2008; Mahamood et al., 1995). Results on efficacy of different fungicides on the growth of *A. porri* and *S. vesicarium* are presented in Table 3. It was observed that all the systemic and nonsystemic fungicides showed significant differences in their efficacy to inhibit the growth of the pathogen. All the fungicides tested gave significant inhibition of mycalial growth of pathogen over control. Significantly, maximum mean percent inhibition of mycelial growth was recorded (98.94 and 100%) in mancozeb at 0.2%, followed by companion at 0.2% (98.40 and 97.13%) and azoxystrobin at 0.15% (95.40 and 94.23%) as compared to other treatments. Data indicated that mancozeb at 0.2% completely inhibited the mycelial growth (100 and 98.13%), which is statistically at par with companion, azoxystrobin, master, and propiconazole. These fungicides were found to be most effective against *A. porri* and *S. vesicarium*. Among the systemic fungicides, Azoxystrobin (0.1%) gave maximum mycelial inhibition (94.40 and 94.23%), followed by propiconazole at 0.1% (89.10 and 87.14%). The effectiveness of different fungicides against *A. porri* and *S. vesicarium* has been reported by several workers (Gupta et al., 1981; Sugha, 1995; Khosla et al., 2007). The present study revealed that mancozeb at 0.2% was found most effective in inhibiting the growth of *A. porri* and *S. vesicarium*. Azoxystrobin, master, companion and propiconazole at 0.1% were found equally effective and can be used as an alternative to mancozeb. Among the bioagents, *T. viride* was found to be more efficacious in

inhibiting the mycelial growth. Garlic clove extracts at 10% showed maximum growth inhibition among the plant extracts. Thus, the study indicated that suitable integration of more efficient ecofriendly treatments like bioagents and botanicals with lesser use of fungicides may provide a better management of the purple blotch and *Stemphylium* blight of onion.

REFERENCES

Gupta RP, Srivastava PK, Singh L (1981). Bioassay of fungicides against *A. porri* causing purple blotch of onion. Pesticides 15:27-28.

Khosla K, Thakur BS, Bhardwaj SS (2007). Chemical management of stemphylium blight of garlic. Plant Dis. Res. 22(1):47-51.

Mahamood A, Javed N, Ahmad R, Raheel ZI (1995). Biological control of maize leaf blight caused by *Helminthosporium turcicum in vitro*. Pak. J. Phytopathol. 7:62-64.

Meena RL, Rathore RS, Mathur K (2003). Evaluation of fungicides and plant extracts against banded leaf and sheath blight of Maize. Indian J. Plant Prot. 31:94-97.

Mishra RK, Srivastava KJ, Gupta RP (2008). Evaluation of botanical extracts in the management of Stemphylium blight of Onion (*Allium cepa*).Veg. Sci. 35(2):169-171.

Nene YL, Thapliyal PN (1979). Fungicides in Plant Disease Control. 2nd ed. Oxford and IBH pub. Co., New Delhi

Prasad SM, Barvwal MK (2004). Evaluation of plant extracts in management of stemphylium blight of onion. Indian Phytopathol. 57(1):110-111.

Sugha SK (1995). Management of purple blotch of garlic with fungicides. Indian J. Agric. Sci. 65(6):455-458.

Saharan MS, Sharma AK, Singh Surinder (2008). Management of head scab (*Fusarium* spp.) of Wheat with fungicides. Indian J. Agric. Sci. 78(4):328-332.

Tomaz IL, Lima A (1988). An important disease of onion caused by *Stemphylium vesicarium* (Wallr.) Simmons in Portugal. Hortic. Abst. 58:618.

Upmanyu S, Gupta SK (2005). Evaluation of botanicals *in vitro* against *Rhizoctonia solani* Kuhn, the incitant of root rot and Web blight of French bean. Plant Dis. Res. 20(1):66-68.

Vincent JM (1927). Distortion of fungal hyphae in the presence of certain inhibitors. Nature 59:850.