



ECOFRIENDLY MANAGEMENT OF STEMPHYLIUM BLIGHT (*Stemphylium botryosum*) OF GARLIC BY PLANT EXTRACT AND BIOAGENTS

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ABSTRACT: *In vitro* screening of six extract of plant species viz. *Azadirachta indica*, *Datura metel*, *Lantana camara*, *Parthenium hystorophorus*, *Ocimum spp.*, *Argimone mexicana* and five bioagents viz. *Trichoderma harzianum*, *T. viride*, *Aspergillus niger*, *Penicillium citrinum* and *Gliocladium virens* were tested against *Stemphylium botryosum*. Among plant extracts *Azadirachta indica* (66.5 per cent) and *Datura metel* (64.5 per cent) were the best in restricting the growth of pathogen over control and in evaluation of bioagents, *S. botryosum* + *T. harzianum* (81.2 per cent) and *S. botryosum* + *T. viride* (74.5 per cent) were significantly inhibited the growth of pathogen. Under field condition suppression of pathogen by *T. harzianum*, treating the garlic cloves (0.2 per cent) along with two foliar sprays (0.2 per cent) at 15 days interval found to be most effective for management of this disease.

Keywords : Garlic, blight, plant extract, bioagent.

Garlic (*Allium sativum* L.) is an important of both vegetarian and non vegetarian people with spicy use. It possesses medicinal value too. Due to presence of volatile oil and other sulphur compound it has antiseptic and antibiotic action. Garlic is the second most widely cultivated crop after onion. It is regarded as one of the important bulb crop grown and used as spice or condiment through out India (Singh and Srivastava, 7). India ranks second in world's total area under garlic and third in production. Madhya Pradesh is the leading state in India with around 30 per cent of both area and production (Tambi *et al.*, 8).

Garlic is affected by several fungal diseases such as purple blotch, downy mildew, smut, black mould, and *stemphylium* blight. Out of them, *Stemphylium* blight is an important disease of garlic crop. Incidence of this disease is ranged between 5.0-43.2 per cent (Jakhar *et al.* 4). Since garlic is consumed from green leaf to dried mature cloves and the use of chemical is being discouraged, now a day for the reason that the fungicides are not ecofriendly for being hazardous to mammalian group and responsible for creating the environmental pollution in air, soil and water.

Therefore, keeping in view the damage and wide spread occurrence of the disease, plant extract and bioagent against this pathogen *in vitro* and *in vivo* were tested for ecofriendly management of this disease.

MATERIALS AND METHODS

Isolation of the pathogen and pathogenicity test

The leaf spot and lesion showing the initial and distinct characteristic symptom were selected for isolation of pathogen. Naturally infected leaves showing the characteristic symptom of *Stemphylium* leaf blight collected from infected field of garlic and maintained in P.D.A. (Potato Dextrose Agar) medium.

Collection and maintenance of antagonist

The antagonistic bioagent available in Department of Plant Pathology, C.S. Azad University of Agri. & Tech., Kanpur were utilized in present study. All antagonist then incubated for 48 h at $28 \pm 1^\circ\text{C}$. The culture of both bacterial and fungal antagonists were then preserved in refrigerator and periodic transfer was made for their maintenance.

I. *In vitro* evaluation of plant extracts

The relative efficacy of six plants extracts viz. *Azadirachta indica*, *Datura metel*, *Lantana camara*, *Parthenium hysterophorus*, *Ocimum spp.* and *Argemone maxicana* were tested against the pathogen in laboratory. Fresh and healthy leaves of all six test plants were collected from the surrounding University field for the preparation of plant extract. The leaves were first washed under running tap water to remove dust material adhering to surfaces and then in distilled water. One hundred grams (100 g) leaves from each sample were then ground with sterile water (100 ml) at 1:1 w/w in a pestle and mortar. After thorough grinding the extract was filtered through muslin cloth and then through Whatman filter paper no.1. Later the extract was passed through sieve filter to free them from bacterial contamination. The extract is then used as standard plant extract solution of 100 per cent concentration of 1:1 ratio. Prepared plant extract was treated at 60°C for 15 minutes for destruction of other microorganism contamination. Five ml of each extract was incorporated in sterilized molten 100 ml of P.D.A. medium and poured into sterilized petri plates (20 mm in size). Each treatment having three replications were maintained and allowed to solidify. A circular disc of 5 mm diameter was taken from 15 days old culture of the pathogen, cut by sterilized cork borer and placed in the centre of each petri plate containing solidified plant leaves extract. The plants were incubated at 25 ± 1°C. The efficacy of plant leaves extract were assessed by measuring the growth of colony diameter in mm and interpreted in per cent inhibition over control. The per cent inhibition over control was calculated by formula reported by Bliss (2).

$$\text{Per cent inhibited over control} = \frac{C - T}{C} \times 100$$

Where,

C=Growth of fungus in control

T=Growth of fungus in treatment

II. *In vitro* evaluation of bioagents

Five bioagents viz. *Trichoderma harzianum*, *T. viride*, *Aspergillus niger*, *Penicillium citrinum* and *Gliocladium virens* were tested *in vitro* against pathogen and the culture media devised of any bioagents served as control. Five mm disc of test fungus was placed before 72 hours of bioagent placement on P.D.A. (Potato Dextrose Agar) medium in petri-plates. The test fungus and bioagents were placed opposite to each other at a distance of 5 mm from the periphery. Each treatment was replicated three times and incubated at 25 ± 1°C. The data were recorded after 96 hours at bioagents placement, when the inhibition zones were formed and expressed as per cent inhibition. The percentage of inhibition of pathogen was calculated by the formula of Bliss (2).

Table 1: Effect of plant leaf extracts on colony growth of *S. botryosum* *in vitro*.

S. No.	Leaf extract (Treatment)	Average colony growth	Per cent inhibition over control
1.	<i>Azadirachta indica</i>	29.4	66.5
2.	<i>Datura metel</i>	31.2	64.5
3.	<i>Lantana camara</i>	34.8	64.5
4.	<i>Parthenium hysterophorus</i>	52.0	40.8
5.	<i>Ocimum sp.</i>	63.5	27.7
6.	<i>Argemone maxicana</i>	68.6	2.9
7	Control	87.8	-
	CD (P=0.05)	2.57	-

III. Evaluation of effective bioagents in field

The most effective bioagent in laboratory evaluation were employed for clove treatment and also for foliar spraying when the appearance of diseases. The susceptible variety of garlic G-15 was sown on 16th October. Three replications were kept for each treatment and untreated cloves sown similarly served as control. Observations were recorded at fortnightly interval.

RESULTS AND DISCUSSION

I. *In vitro* effect of plant extract

The results presented in Table 1 show that out of six plant extracts, *Azadirachta indica* (66.5 per cent inhibition over control) statistically at par with *Datura metel* was proved to be most effective for inhibiting fungal growth. The next in superiority was *D. metel* extract which gave 31.2 mm radial growth and 64.5 per cent inhibition over control. Prasad and Barnwal (6) also evaluated that effect of

leaf extract of *Azadirachta indica*, *Pongamia pinnata*, *Datura metel*, *Ocimum sanctum* (*O. tenuitissimum*), *Eucalyptus citriodora* and *Mentha arvensis* on *Stemphylium* blight of onion (cv. N-53) in field trial. Datar (3) reported that, maximum reduction of purple blotch on onion caused by *Alternaria porii* was observed with leaf extract of *Polyanthia longifolia* followed by *Eucalyptus citriodora*, *Datura alba*, *Ipomea carnea*, *Tridax procumbens* and *Tabernemontana coronaria* under field conditions.

Table 2: Effect of the bioagents on the growth of *S. botryosum* *in vitro*.

S.No.	Leaf extract (Treatment)	Average diameter of fungal colony (mm)	Per cent inhibition over control
1.	<i>Stemphylium botryosum</i> + <i>Trichoderma harzianum</i>	16.5	81.2
2.	<i>S. botryosum</i> + <i>T. viride</i>	22.4	74.5
3.	<i>S. botryosum</i> + <i>Aspergillus niger</i>	27.8	68.3
4.	<i>S. botryosum</i> + <i>Penicillium citrinum</i>	32.6	62.9
5.	<i>S. botryosum</i> + <i>Gliocladium virens</i>	37.4	57.4
6.	Control	87.8	-
	CD (P=0.05)	1.81	-

II. *In vitro* evaluation of bioagents

Table 2 showed that out of five bioagents, the maximum (81.2 per cent) colony growth inhibition of *Stemphylium botryosum* was in *T. harzianum* (Kanpur isolates) followed by 74.5, 68.3, 62.9 and 57.4 per cent in *T. viride* (Kanpur isolate), *A. niger* (Delhi isolate), *P. citrinum* (Lucknow isolate) and *G. virens* (Pantnagar isolate), respectively. The radial growth of the pathogen ranged between 16.5 mm (*T. harzianum*) to 37.4 mm (*G. virens*). Montensions *et al.* (5) also reported on screening of bacterial antagonists against *Stemphylium vesicarium* *in vitro* of 2 + 7 strain of *Pseudomonas fluorescens*, 21 strains inhibited the growth of *Stemphylium vesicarium*.

III. Effect of bioagents (Cloves treatment and foliar spray) against disease and yield of garlic *in vivo*

It is evident from Table 3 that all the treatments including control were statistically different with each other. The minimum disease infestation was recorded on clove treatment (0.2 per cent) plus two foliar spray of *Trichoderma harzianum* (18.5 per cent) and 19.2 per cent during 2003-04 and 2004-05, respectively. Minimum increase in yield over control was observed in this treatment proving thereby its superiority in efficacy. Barnwal *et al.* (1) have also reported the efficacy of *P. fluorescens* and hexacenoazole against *Stemphylium botryosum* causing blight in onion. All the treatments reduced severity of disease as

Table 3: Effect of bioagent by cloves treatments and foliar spray against disease and yield of garlic in vivo.

S. No.	Treatment	Average disease intensity (%)			Average yield (q/ha)			Increase in yield over control (%)		
		2003-04	2004-05	Average	2003-04	2004-05	Average	2003-04	2004-05	Average
1.	Clove treatment	32.00 (34.43)	30.80 (33.68)	31.40 (34.06)	97.20	99.80	98.50	14.10	15.00	14.55
2.	Clove treatment + single foliar spray	27.20 (31.41)	26.40 (30.89)	26.80 (31.15)	108.50	110.00	109.25	27.30	26.70	27.00
3.	Clove treatment + two foliar sprays	18.50 (25.47)	19.20 (25.99)	18.85 (25.73)	116.40	115.80	116.10	36.60	33.40	35.00
4.	Control	48.00 (43.85)	45.80 (42.58)	46.90 (43.22)	85.20	86.80	80.00	-	-	-
	C.D. (P=0.05)	3.52	4.30	-	12.42	15.85	-	-	-	-

compared to control with hexaconazole treatment resulting good crop yield and lower disease severity as compared to *P. fluorescens*.

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