Comparative efficacy of plant extracts *and Trichoderma harzianum against* purple blotch *(Alternaria porri*) of garlic (*Allium sativum* L.)

Abstract

Garlic (*Allium sativum* L.), commonly known as "Lahsun," is a crucial spice from the Amaryllidaceae family and is the second most widely cultivated *Allium* species after onions.. *Alternaria porri* inflicts severe damage on crops and significantly reduces yields, remaining a major concern for farmers and agricultural researchers. This study is aimed to evaluate the Comparative efficacy of plant extracts *and Trichoderma harzianum against* purple blotch *(Alternaria porri*) of garlic (*Allium sativum* L.). A laboratory and field experiment were conducted at the research plot of the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj, Uttar Pradesh in *rabi* season of 2023-24 to evaluate the effect bio- agents and plant extracts against purple blotch of garlic and to increase the growth parameters. A total of eight treatments namely *Trichoderma harzianum*@10%, *Lantana camara* leaf extract@10%, Eucalyptus leaf extract@10%, Tulsi leaf extract @10%, Datura leaf extract @10%, Neem leaf extract @10% including control as non-treated check and Mancozeb +Tebuconazole @0.2% as a treated check were replicated three times. Among all the tested treatments, Eucalyptus leaf extract was found significantly superior over control along with all other treatments which recorded minimum disease intensity and maximum yield followed by Neem leaf extract. In *in vitro* conditions, the bio agent *Trichoderma harzianum* were tested by dual culture method and plant extracts were evaluated by poison food technique. In the present study, *Trichoderma harzianum* among the bio-agent and Neem leaf extracts among the plant extracts shows maximum mycelial growth inhibition as compared to control. Evaluating plant extract contributes to the scientific validation of traditional knowledge and combining biological control with botanicals can be an effective component of IDM programs, reducing the need for synthetic inputs

Keyword – *Alternaria porri*, Disease intensity, Garlic, Plant extracts, *Trichoderma harzianum*

Introduction

As the second most widely grown Allium species after onions, garlic (*Allium sativum* L.), often known as "Lasun," is one of the most significant spices in the Amaryllidaceae family. In comparison to other Allium crops, it has a higher nutritional value. Carbohydrates (29%) proteins (6.3%) minerals (0.3%) and essential oils (0.1–0.4%) are all abundant in garlic. It also has trace levels of vitamin C, sulfur compounds, and lipids. Ascorbic acid is abundant in green garlic in particular. Due to its natural properties, garlic oil and extracts are being utilized as potential bio-pesticides, serving as effective insecticides and fungicides in organic farming practices **(Memane *et al.* 2008)**. According to **Etoh and Simon (2002)**, garlic is thought to be indigenous to Southern Europe and Western Asia. It has been used for more than 5,000 years in China and India, and for more than 4,000 years in Egypt **(Kamenetsky and Rabinowitch 2001).** Numerous diseases and insect pests, such as purple blotch, Botrytis leaf blight, downy mildew, Fusarium basal rot, white rot, and stem and bulb nematodes, can affect garlic (*Allium sativum* L.). These diseases and pests affect the crop both during its growth in the field and post-harvest storage, resulting in significant losses in yield and a decline in marketable quality **(Prahlad *et al.* 2021).**

Alternaria porri is the cause of purple blotch, a serious foliar disease that affects garlic. It grows best in warm, humid environments with relative humidity levels between 80 and 90% and temperatures between 25 and 30 °C **(Dar *et al*. 2020).** The disease begins with the appearance of small, whitish flecks on the leaves and flower stalks. These spots gradually expand into sunken, purple-colored lesions bordered by yellow to pale brown margins **(Nolla *et al.* 1927).**

The pathogen remains viable in infected garlic cloves but does not persist in the soil **(Dicklow *et al*. 2013)**. Crop losses as high as 60% have been documented, primarily due to the drying of leaves (Bisht and Agarwal 1993). *Alternaria porri* produces obclavate conidia with several septa, formed on purple to brown conidiophores that display clear conidial scars **(Dar *et al.* 2020).** This disease poses a major challenge in garlic-producing areas across the globe **(Hausbeck *et al.* 1999; Bisht and Thomas 1992).**

Materials and methods

The current study was conducted at Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj, at the Central Research Farm, Department of Plant Pathology

Isolation and identification of fungi

Garlic leaves displaying distinctive indications of purple blotch were gathered and properly cleaned under running water. The existence of Alternaria species was then confirmed by microscopically examining the symptomatic tissues. Small pieces with both healthy and diseased parts were removed under sterile conditions, surface sterilized for one minute with 1% sodium hypochlorite (NaOCl), and then rinsed three times with sterile distilled water. As explained by **(Tuite 1969),** these tissue fragments were air-dried on sterile filter paper before being put (4–5 pieces per plate) onto sterilized Petri plates filled with potato dextrose agar (PDA) treated with streptomycin at 100 ppm to prevent bacterial development. In a BOD incubator, the plates were incubated at 27 ± 2 °C. After three days, fungus development became apparent, and single hyphal tip cultures were extracted using a cork borer to produce pure cultures. The fungal pathogen was identified based on its colony morphology, spore characteristics, and comparison with established taxonomic keys.

Dual culture technique

Twenty milliliters of autoclaved, cooled potato dextrose agar (PDA) media were added to sterilized *Petri plate*. The pathogen was infected on one side of the *Petri plate* and the antagonist was positioned on the opposite side, leaving about 4 cm between them, in order to evaluate the efficacy of fungal antagonists. Following incubation, the radial development of the pathogen in the treatment plates was assessed once the fungal growth in the control plate had grown to a diameter of 90 mm **(Vincent 1947).**

The percentage inhibition of mycelial growth of the test pathogen was calculated using the following formula:

$$I = \frac{C - T}{C} x100$$

Where,

I = % reduction in growth of test pathogen

C = Radial growth (mm) in control

T = Radial growth (mm) in treatment

Evaluation of botanical extracts on the colony growth of test fungus

The experimental procedure followed the method described by Nene and Thapliyal (1993). Forty milliliters of sterilized and cooled potato dextrose agar (PDA) medium were carefully combined with a specified volume of plant extract. Sterilized glass *Petri plate* was filled with 15–20 milliliters of this modified medium, which was then left to harden at room temperature. Three separate tests were conducted for each botanical extract and its corresponding concentration. The control was PDA-containing plates devoid of any botanical extract. A 5 mm mycelial disk was positioned in the center of each treatment and control plate to aseptically inoculate them once the media had solidified. This disc was extracted from a pure culture of *Alternaria porri* that was one week old and vigorously developing. For a maximum of seven days, or until the mycelial growth in the control plates fully covered the surface, the inoculated plates were incubated at 25 ± 1 °C. Up to the seventh day, the amount of *Alternaria porri* growth inhibition (measured in millimeters) was noted every day. Two diameters collected at right angles across the colony were averaged to determine the radial mycelial growth in each plate.

Poisoned food technique

Each *Petri plate* holding the botanicals at the necessary concentration dissolved in PDA has a five mm diameter *Alternaria porri* culture disc in the middle. There were three replications kept up to date. Colony diameter was measured after the plates were incubated for seven days at 27±2˚C (Vincent 1947).

The following formula was used to determine the percentage inhibition of mycelial growth:

$$I = \frac{C-T}{C} x100$$

where,

I = Percent inhibition,

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

T=Growth (mm) of test fungus in treated plate

Field experiment

The experimental field was well-prepared and organized according to the designated layout and design. Healthy seedlings, aged 25–30 days, were transplanted into pre-marked hills. Gap filling was done 15 days following transplanting in order to preserve a consistent plant population. Weeds were controlled through regular manual weeding, which also helped in conserving soil moisture. Foliar spray treatments were initiated at the first appearance of disease symptoms, with a total of three applications made at 15-day intervals. Spraying was conducted in the evening using a hand sprayer to enhance treatment effectiveness.

Management of disease through botanicals and *Trichoderma harzianum*

The Department of Plant Pathology at SHUATS conducted a field evaluation of the effectiveness of a few chosen botanicals and their commercial formulations during the *rabi* season of 2023–2024. Three replications of the experiment were carried out using a Randomized Block Design (RBD).

Three foliar treatments were administered at 15-day intervals following the onset of purple blotch symptoms. At weekly intervals from the onset of disease for each treatment, the incidence of disease was visually evaluated in each plot.

Observation recorded

Observations recorded on purple blotch of garlic were disease intensity (%), Plant height (cm), Number of leaves per plant and yield.

Disease Intensity

Every week, beginning with the onset of symptoms for each therapy, the disease incidence in each plot was visually observed and documented. The gathered information was statistically examined. The Wheeler (1969) formula was used to determine the disease incidence.

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total no. of rating} \times \text{Maximum disease grade}} \times 100$$

Chart 1 Disease rating scale

Grade	Leaf area covered (%)
0	No disease symptoms
1	1%
2	2-5%
3	6-10%
4	11-15%
5	16-25%
6	26-40%
7	41-60%
8	61-75%
9	>75% in addition to dryness of most leaves

(Mayee and Datar, 1986)

Results and discussion

Evaluation of *Trichoderma harzianum* and plant extracts on *Alternaria porri in vitro*

Under laboratory conditions, the experiment was conducted using a completely randomized block design (CRD). Dual culture with the test pathogen was used to assess the effects of the bioagents. Using the poison food technique, all plant extracts were assessed at a 10% concentration.

The present finding revealed significant reduction in T_1 – Neem leaf extract (22.70mm) followed by T_4 - Lantana leaf extract (26.42mm), T_3 – Eucalyptus leaf extract (30.77mm) T_4 – Datura leaf extract (31.18 mm), T_5 – Tulsi leaf extract (36.03mm) as compared to untreated check control T_0 – (90mm).

The current investigation found that T_3 (neem leaf extract) had the highest mycelial growth inhibition, most likely as a result of the presence of bioactive substances such limonoids, nimbin, azadirachtin, and salin. These components have the ability to break down fungal cell membranes and block important enzymes that are necessary for fungal growth. According to **Kumar *et al*.(2024)** neem has been shown to most important active constitute is azadiractine and the others are nimboline, nimbin, nimbidin, which are effective for the control of purple blotch of onion attributed the inhibitory activity to azadirachtin's ability to penetrate spores and exert fungitoxic effects and neem and its chemicals play role in scavenging of free radiation generation and prevention of disease pathogenesis. **Vijaykumar *et al*. (2022) and (Priya *et al*. 2018)** also confirmed the through antimicrobial activity, it inhibits microbial growth or potential to break the

cell wall of pathogen `and efficacy of *Azadirachta indica* extracts, reporting a minimum inhibitory concentration (MIC) of 0.19 mg, which was more effective than the MIC of 0.78 g for metalaxyl + mancozeb respectively.

Table 1 Effects of selected plant extracts on radial growth (mm) of *A. porri*

Sr. no.	Treatments	Concentration	Mean Colony diameter (mm)	Percent inhibition
T_0	Control (untreated check)	-	90.00	0.00
T_1	Datura leaf extract	10%	31.18[a]	65.68[a]
T_2	Tulsi leaf extract	10%	36.03	59.95
T_3	Neem leaf extract	10%	22.77	74.69
T_4	*Lantana* leaf extract	10%	26.42	70.64
T_5	Eucalyptus leaf extract	10%	30.77[a]	65.78[a]
		SE(m)±	0.34	0.36
		CD (5%)	1.02	1.01

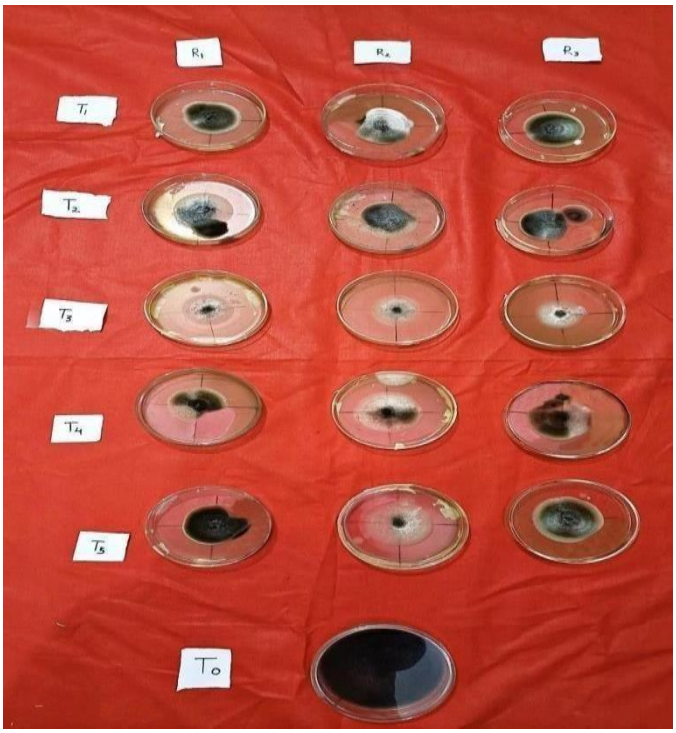

Fig.1 Efficacy of plant extract on percent mycelial inhibition of *Alternaria porri* by poisoned food technique

Effect of *T. harzianum* on mycelial growth of *A. porri*

In comparison to the untreated control (0%), the data showed that the bioagent tested demonstrated antifungal action against *A. porri* and greatly suppressed its growth. The test pathogen exhibited the greatest mycelial growth suppression (76.30 mm), indicating that *T. harzianum* was the most effective antibiosis controlling *Trichoderma harzianum.* **(Sairam *et al.*,2025)** *Trichoderma* spp. was tested for efficacy using the dual culture technique, and it was discovered that they produced secondary metabolites such as 6-pentyl- alpha-pyrone (6pp), iso-cyanide derivatives, acids (heptadic and koningic acid), peptaibols, and cell wall degrading enzymes (CDWE), all of which are involved in the growth inhibition of many phytopathogenic fungi. Similarly, several studies show that Trichoderma has antagonistic activity against *Alternaria porri* and that there is a difference between species and even between the same species of Trichoderma. The antagonistic activity of Trichoderma is due to various mechanisms, such as its high capacity for competition for nutrients compared to that presented by other fungi, to the production of compounds called siderophores that catch iron and stop the growth of other fungi and by its ability to generate ATP for its growth from different carbon sources **(Camacho-luna *et.al*., 2021; Cornejo *et al.,* 2016)**

(%) percent inhibition					
Treatment No.	**Treatment details**	**R_1**	**R_2**	**R_3**	**Mean**
T_1	*Trichoderma harzianum*	76.30	75.10	75.50	75.63
T_0	Control	0.00	0.00	0.00	0.00
	SE(m)				0.36
	CD (5%)				1.14

Table-2 Effect of *T. harzianum* against mycelial inhibition of *Alternaria porri*

Fig.2 *T. harzianum* shows mycoparasitism over *Alternaria porri*

Effects of treatments on plant height at 90 DAS

In the present findings, the data on plant height at 90 DAS reveals that plant height(cm) significantly increased in- T_3 (*T. harzianum* + Eucalyptus leaf extract – 46.35cm) followed by T_6 (*T. harzianum* + Neem leaf extract –42.54cm), T_1 (*T. harzianum* –41.91cm), T_2 *(T. harzianum* + Lantana leaf extract – 40.11cm), T_5 (*T. harzianum* + Datura leaf extract – 39.01cm) and T_4 (*T. harzianum* + Tulsi leaf extract – 38.05cm) as compared to T_7 (Treated Check) (Mancozeb + Tebuconazole – 51.30cm) as well as non- treated check -T_0 (control- 32.14cm).

Effects of treatments on number of leaves at 90 DAS

In the present findings, the data on plant height at 90 DAS reveals that the number of leaves significantly increased in- T_3 (*T. harzianum* + Eucalyptus leaf extract – 9.73) followed by T_6 (*T. harzianum* + Neem leaf extract –9.40), T_1 (*T. harzianum* –9.06), T_2 (*T. harzianum* + Lantana leaf extract –8.80), T_5 (*T. harzianum* + Datura leaf extract –8.40) and T_4 (*T. harzianum* + Tulsi leaf extract – 8.26) as compared to T_7 (Treated Check) (Mancozeb + Tebuconazole – 10.13) as well as non-treated check -T0 (control-7.73).

Effects of treatments on disease intensity at 75 DAS

In the present findings, the data on disease intensity at 75 DAS reveals that the disease intensity (%) significantly decreased in -T_3 *(T. harzianum* + Eucalyptus leaf extract – 28.34%) followed by T_6 *(T. harzianum* + Neem leaf extract – 30.08%), T_1 (*T. harzianum* – 31.11%), T_2 (*T. harzianum* + Lantana leaf extract – 32.37%), T_5 (*T. harzianum* + Datura leaf extract – 33.52%) and T_4 (*T.*

harzianum + Tulsi leaf extract – 34.90%) as compared to T_7 (Treated Check) (Mancozeb + Tebuconazole – 25.88%) as well as non-treated check -T_0 (control-39.20%).

Effects of treatments on yields (t ha-1)

In the present findings, the data on yield reveals that after harvest, maximum yield was observed in -T_3 (*T. harzianum* + Eucalyptus leaf extract –5.41t/ha) followed by T_6 (*T. harzianum* + Neem leaf extract – 11.50t/ha), T_1 (*T. harzianum* –10.10t/ha), T_2 (*T. harzianum* + Lantana leaf extract – 9.00t/ha), T_5 (*T. harzianum* + Datura leaf extract –8.00 t/ha) and T_4 (*T. harzianum* + Tulsi leaf extract –7.30t/ha) as compared to T_7 (Treated Check) (Mancozeb + Tebuconazole – 14.17t/ha) as well as non-treated check - T_0 (control-5.41t/ha)

Table 3 Effects of treatments on growth parameters

		Per cent disease intensity			Plant height (cm)	No. of leaves	Yield (t/ha)
Treatments	**Treatment details**	**45 DAS**	**60 DAS**	**75 DAS**	**90 DAS**	**90 DAS**	
T_0	Control	12.33	30.45	39.20	32.14	7.73	5.41
T_1	*Trichoderma harzianum* + *Trichoderma harzianum* 10g/kg (S.T.) +10% (F.S.)	7.40^{b}	23.11^{cd}	31.11^{cd}	41.91^{a}	9.06^{a}	10.10^{a}
T_2	*Trichoderma harzianum* + Lantana leaf extract 10g/kg (S.T.) +10% (F.S.)	8.17	24.06^{bc}	32.37^{bc}	40.11	8.80^{a}	9.00
T_3	*Trichoderma harzianum* + Eucalyptus leaf extract 10g/kg (S.T.) +10% (F.S.)	5.34	19.77	28.34	46.35	9.73	12.27
T_4	*Trichoderma harzianum* +Tulsi leaf extract 10g/kg (S.T.) +10% (F.S.)	9.26^{a}	26.09^{a}	34.90^{a}	38.05^{b}	8.26^{b}	7.30^{b}
T_5	*Trichoderma harzianum* +Datura leaf extract 10g/kg (S.T.) +10% (F.S.)	8.98^{a}	25.12^{ab}	33.52^{ab}	39.01^{b}	8.40^{b}	8.00^{b}
T_6	*Trichoderma harzianum* +Neem leaf extract 10g/kg (S.T.) +10% (F.S.)	6.66^{b}	22.10^{d}	30.08^{d}	42.54^{a}	9.40	11.00^{a}
T_7	Mancozeb +Tebuconazole (Treated check)	3.73	16.51	25.88	51.30	10.13	14.17

The study conducted by **Sudhanshu *et.al* (2024)** Eucalyptus leaf extract control due to it may have involved in the prevention of hyphal growth and sporulation, interruption in nutrient uptake and metabolism, induction of lysis. They might be responsible for the alternation in fungal physiology by inducing changes in cell wall composition, plasma membrane disruption, mitochondrial structure disorganization and interference which might have affected the respiratory enzymatic reactions of the mitochondrial membrane of pathogen.

Similarly results align with the studies carried out by **Kanta *et al.* (2018).** Eucalyptus globes have a good allelopathy impact, and their extracts are mostly composed of bioactive chemicals such phenolic acid and phytochemical makeup.

Through causing modifications to the composition of the cell wall, disruption of the plasma membrane, disarray of the mitochondrial structure, and interference with the respiratory enzymatic processes of the mitochondrial membrane, they are also accountable for the variation in fungal physiology. Numerous phytochemicals present in eucalyptus leaf extract, including phenolic diterpenes, flavonoids, organic compounds, and phenolic acid, disrupt the pathogen's cell and reduce the activity of specific enzymes required for fungi to multiply in the wild, ultimately leading to their death **(Hedfi *et al.* 2014).** Eucalyptus may therefore have an indirect effect on plant development and other growth-related factors.

Conclusions

According to the findings of the current study, " Comparative efficacy of plant extracts *and Trichoderma harzianum against* purple blotch *(Alternaria porri*) of garlic (*Allium sativum* L.)," the bio-agent *Trichoderma harzianum* and Neem leaf extract outperformed the other plant extracts *in vitro* against *Alternaria porri*. The information shows that *Trichoderma harzianum* (S.T.) + Eucalyptus leaf extract (F.S.) had the highest plant height (cm), the most leaves, the yield (t/ha), and the lowest disease intensity (%). The findings concluded to integrated disease management programs and inspire further research in the field of biological control and plant pathology. The valuable insights into the use of plant extracts and the biocontrol agent *Trichoderma harzianum* as eco-friendly alternatives to chemical fungicides. Such approaches are crucial for developing sustainable disease management strategies that minimize environmental risks and reduce chemical dependence.

Acknowledgement

The division of Plant Pathology at SHUATS, Prayagraj, is acknowledged by the authors for providing the lab space required to carry out the study.

Declaration Conflict of interest on behalf of all authors, the corresponding author states that there is no conflict of interest.

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