

Original Research Article

Effect of *Trichoderma harzianum* and botanicals on Purple blotch disease of garlic (*Allium sativum* L.) caused by *Alternaria porri* (Ellis) Cif.

Comment [WU1]: There are some grammar and language mistakes that should be corrected in the whole paper.

ABSTRACT

Comment [WU2]: The abstract should contain a concise introduction, the aim of the work, a concise method of work, brief results, and recommendations.

This study was aimed to determine the effect of botanicals and bio-agent on purple blotch disease of garlic caused by *Alternaria porri*. The research work was undertaken at Central Research Farm (CRF) Sam Higginbottom University of Agriculture, Technology And Sciences, SHUATS, Naini, Prayagraj during *rabi* season in 2022-23. A randomized block design with eight treatments was employed including T₀ (Control - untreated), T₁ (Azoxystrobin@0.1% as treated check), T₂ (*Trichoderma harzianum*@10g/litre), T₃ (*Lantana camara* leaf extract@10%), T₄ (*Azadirachta indica* leaf extract @10%), T₅ (Henna leaf extract @2% + Licorice root extract@2% + Rosemary leaf extract @2%), T₆ (Licorice root extract @10%) and T₇ (Neem cake @50g/m²). All botanicals and bio-agent were tested significantly beneficial in reducing the disease intensity of purple blotch disease. The results revealed that among the treatments, T₇ showed minimum disease intensity (4.33, 18.74 and 26.35%) at 30, 60 and 90 DAS respectively; while maximum disease intensity was recorded in T₅ (9.46, 24.21 and 31.31%). T₇ also recorded maximum plant height (24.02, 34.01 and 45.71cm) at 30, 60 and 90 DAS, respectively and highest yield of 4.46t/ha. Maximum number of leaves were recorded with T₃ (5.53, 7.5 and 9.4) at 30, 60 and 90 DAS respectively.

Comment [WU3]: Any abbreviation should be written in detail for the first time mentioned. That should be applied on the whole paper.

Keywords: *Alternaria porri*, Bio-agent, Garlic (*Allium sativum*), Plant extracts, Purple blotch.

1. INTRODUCTION

Comment [WU4]: Introduction should be ended with clear aim of the work.

Garlic (*Allium sativum* L.) belongs to the family Amaryllidaceae and is the second most important bulb crop after onion (Rubatzky and Yamaguchi, 1997) [1]. Garlic is one of the important spice crops which is used as culinary item and is also used to prevent or cure various diseases / disorders in respect of human health. Whenever an epidemic has emerged, garlic has been the first preventive and curative remedy.

Garlic (*Allium sativum*) is a perennial flowering plant growing from a bulb. It has a tall, erect flowering stem that grows up to 1 m. The leaf blade is flat, linear, solid, and approximately 1.25–2.5 cm wide, with an acute apex. The economic yield is obtained from underground develop part known as bulbs. Often the bulb contains 10 to 20 cloves that are asymmetric in shape. The small central cloves are not usually used because they produce small plants and hence small bulbs (Purseglove, 1975) [2].

In 2020, China had the highest production of garlic throughout the Asia-Pacific region, in which over 20.7 million tons of garlic was produced. Madhya Pradesh recorded the highest production of garlic across India in financial year 2022, amounting to over two million metric tons followed by Rajasthan, Uttar Pradesh and Gujarat. The country produced over 3.1 million metric tons of the garlic in 2021 (Minhas, 2023) [3].

Alternariaporri causing purple blotch of garlic is a common saprophyte on plant debris and plant tissue. It leads to considerable loss in yield and quality of garlic. It is the most destructive disease of *Allium* spp. (onions, garlic, shallots, leeks, scallions and chives). The pathogen of *Allium* was first designated as *Macrosporiumporri* (Cooke and Ellis, 1879) [4] and *Alternariaallii* (Nolla, 1927) [5]. Later both species were called *Alternariaporri* (Ciferri, 1930) [6].

The pathogenicity of *Alternariasp.* is due to production of host specific or nonspecific toxins that may induce disease. These toxins are mainly secondary metabolites that destroy susceptible cultivars by leaf necrosis (Mamgain *et al.*, 2013) [7]. The disease usually affects the leaves and bulbs of a plant, and reducing their yield up to 97% (Kareem *et al.*, 2012) [8].

Purple blotch disease is severe in high humidity and moderate temperature of 80–90% and 25–30°C, respectively. The initial symptoms on host leaves and floral stalks are white flecks which enlarge and produce sunken purple lesions sometimes surrounded by a yellow to pale brown border. The leaves shrivel, usually from the tip. The fungus does not persist in the soil. Air-borne spores often invade plants through wounds, bruises or uncured neck tissue. The pathogens survive in infected cloves (Dicklow, 2013) [9]. Since the leaves are the only photosynthetic organ, this foliar pathogen directly influence the bulb yield. Significant reduction in bulb yield (25-60 %) due to drying of leaves has been observed in garlic (Bisht and Agarwal, 1993) [10].

Conidia of *A. porri* are normally ovoid, obclavate, ellipsoid and sub cylindrical with multiple transverse and longitudinal septa. The body of the conidium is oblong with its formal end protruded out and the terminal part tapered into a beak. It is produced from a bud formed by the conidiophores. Conidiophores are solitary or found in groups. They are purple when young and brown when old. They are 120 µm long and 5-10 µm thick with one or numerous well defined conidial scars (Dar *et al.*, 2020) [11].

In the absence of resistant cultivars farmers must rely on repeated application of fungicides, which has significant environmental and economic consequences. In order to reduce fungicide use, an assessment needed to be made of how much disease can be tolerated without significant yield loss, and the amount of loss attributable to the disease also needed to be defined accurately (Bisht and Agarwal, 1994) [12]. Many types of composted material have been shown to suppress diseases like cattle manure and FYM (Reuveniet *et al.*, 2002) [13]. Recently, trends to use extracts of many higher plants have an effective and safe role against plant fungal diseases (Drazet *et al.*, 2019; Ragupathiet *et al.*, 2020) [14] [15]. Biological control of plant pathogens through antagonistic microorganisms is proved as an effective, not harmful to the environment and a suitable strategy other than an optimistic alternative of chemical uses (Kumar and Palakshappa, 2008) [16]. Plant extract possess an anti-fungal activity in opposition to a wide range of plant pathogenic fungi. These are less phytotoxic, biodegradable and host metabolism stimulatory. Various experiments were undertaken over the past many years to manage purple blotch disease through bio-agents, botanicals and fungicides (Ali *et al.* 2016; Jhalaet *et al.*, 2017; Yadavet *et al.*, 2017) [17-19].

2. MATERIALS AND METHODS

The experiment was carried out at Crop Research Farm, Department of Plant Pathology, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj (UP) during the *Rabi* season of 2022-23. Field experiment was laid out in Randomized Block Design with eight treatments having three replications.

2.1 Field preparation

The selected field area was well prepared and plot marked as per the layout plan. The selected field was ploughed, cleaned and the soil was well pulverized after which the total area was divided into sub-plots. A total of 24 sub-plots were made (8 treatments × 3 replications).

2.1.1 Sowing

Garlic was sown manually with spacing of 15cm between row to row and 10cm between plant to plant. Sowing of cloves was done at a depth of 5cm keeping their growing heads upwards. Then the cloves were covered with soil and light irrigation was given.

2.2. Isolation and identification of pathogen

The diseased garlic leaves that had typical symptoms of purple blotch were collected from the experimental field area. They were washed thoroughly with tap water to remove dirt. Then the leaves were cut into small pieces and surface sterilized with sodium hypochlorite solution 1% for 30 seconds. The surface sterilized pieces were washed with sterilized distilled water for 3 times and dried on filter papers, then placed on Potato Dextrose Agar medium in Petri plates. The

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Petri plates were incubated at 27±2°C for 48 hours. To obtain pure culture of fungus, single spore technique was used. The focus point of dissecting microscope was adjusted to the surface of media to find germinating spores, a small piece of media with the target spore attached was picked up using a sterilized syringe or glass needle and transferred onto a 60 mm media plate. Around 10 spores were picked and transferred individually in each Petri plate and cultured under room temperature (25°C) to get pure colonies. Examination of fungal colony characteristics was done through microscopic examination. Using a sterile needle, a small portion of the culture was taken and placed on a glass slide. One drop of lactophenol was dropped over it and then it was stained using cotton blue. Then, the microscope was used for the examination of morphology and culture characteristics of fungal structures (Grahovac *et al.*, 2012) [20].

2.3. Plant materials and preparation of extracts

Fresh and healthy leaves of *Azadirachta indica*, *Lantana camara*, Rosemary (*Salvia rosmarinus*), Henna (*Lawsonia inermis*), roots of licorice (*Glycyrrhiza glabra*) were collected from local area. The collected leaves and roots of licorice were first washed under running tap water to remove extraneous materials and then washed in distilled water.

2.3.1. Azadirachta indica and Lantana camara

One hundred grams (100 g) leaves from each sample were mixed with sterile water (100 ml) at 1:1 (w/w) with the help of mortar and pestle, after that the extract was filtered through muslin cloth followed by Whatman filter paper. The extract is used as standard plant extract solution of 100% concentration (stock solution). Prepared plant extract was heated at 60°C for 15 minutes to inactivate the microbial contaminants. The final volume was adjusted to 1000 ml with distilled water.

2.3.2. Licorice (Glycyrrhiza glabra), Rosemary (Salvia rosmarinus)

All plant materials were air dried and crushed in the electrical mill to prepare plant powder. Ten grams of plant powder of licorice and rosemary were soaked in 100 ml of distilled water and shaken thoroughly. Supernatant of each extract was separated from the residue by squeezing through double layers of muslin cloth and then obtained aqueous extracts were filtered through Whatman filter paper no. 1. (Satish *et al.*, 2007) [21]. The final volume was adjusted to 1000 ml with distilled water.

2.3.3. Henna (Lawsonia inermis)

The leaves of henna were obtained from local market. They were air dried and crushed in the grinder to prepare plant powder. The aqueous extract of henna was prepared by soaking 10 grams of henna powder in 100 ml of distilled water and then boiled for 20 minutes (Kumar and Kathireswari, 2016) [22]. After cooling, supernatant of extract was separated from the residue by squeezing through double layers of muslin cloth and then obtained aqueous extracts were filtered through Whatman filter paper no. 1. The final volume was adjusted to 1000 ml with distilled water.

2.4. Spraying of treatments on garlic plants

After the initial appearance of disease at 30 days after sowing (DAS), the foliar spray of botanicals was applied given for 3 times at 15 days interval.

2.5. Effect of treatments on disease intensity and plant growth parameters

Data were noted on disease intensity (%), plant height (cm), number of leaves per plant at 30, 60 and 90 DAS respectively. Five randomly selected plants were chosen from each plot to study these parameters. Total yield (t/ha) was determined for each experimental plot after harvesting. The garlic plants were solar dried for 10 days before determining the yield.

2.5.1. Disease assessment

Disease intensity (%) was recorded according to Mayee and Datar, (1986) [23]. (Fig.1).



Fig.1. Disease rating scale (0-9) of purple blotch (Mayee and Datar, 1986)

Table1: Rating scale for disease intensity on foliage

| 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 |

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all diseased ratings}}{\text{Total number of rating} \times \text{Maximum disease grade}} \times 100$$

2.6. Statistical analysis

The Data recorded throughout the course of investigation was subjected to Statistical analysis using analysis of variance (ANOVA) for randomized block design (RBD) (Fischer and Yates, 1963) [24]. Whenever 'F' test was found significant for comparing the means of two treatments, a critical difference (C. D. at 5%) was worked out.

Table 2: Details of Treatments:

| S.No. | Treatments | Treatment Details | Application |
|-------|----------------|--|---|
| 1 | T ₀ | Control (Untreated) | Distilled water spray |
| 2 | T ₁ | Azoxystrobin @0.1 % (Check) | Foliar Spray |
| 3 | T ₂ | <i>Trichodermaharzianum</i> @10g/litre | Foliar Spray |
| 4 | T ₃ | <i>Lantana camara</i> @10% | Foliar Spray (leaf extract) |
| 5 | T ₄ | <i>Azadirachtaindica</i> @10% | Foliar Spray (leaf extract) |
| 6 | T ₅ | Henna Powder(2%)+ Licorice Powder(2%)+ Rosemary Powder(2%) @6% | Foliar Spray (leaf and root extract) |
| 7 | T ₆ | Licorice Powder @10% | Foliar spray (root extract) |
| 8 | T ₇ | Neem Cake @ 50g/m ² | Soil Application |

3. RESULTS AND DISCUSSION

Effect of treatments on disease severity of purple blotch in garlic

The data presented in table 3 reveal that the minimum disease intensity (%) was recorded in treatment T₇ - Neem cake @50g/m² (26.35), which was superior to overall treatments, followed by T₄ - *Azadirachta indica* @10% (27.65), T₃ - *Lantana camara* @10% (28.42), T₂ - *Trichoderma harzianum* @10g/L (29.45), T₆ - Licorice @10% (30.48), T₅ - Henna + Licorice + Rosemary @6% (31.31) as compared to treated check T₁ - Azoxystrobin @0.1% (25.36) and T₀ - Control untreated check (34.68). Similar findings are in accordance with the studies conducted by Singh *et al.* (2010 a), Singh *et al.* (2010 b) and Bhonde *et al.* (1999) [25-27] who showed that neem has anti-microbial activity. Neem has been reported to exhibit antifungal, antibacterial and insecticidal properties. The ethanolic extract of neem leaves stops the growth of fungi.

Effect of treatments on plant height (cm) of garlic

The data presented in table 4 reveal that the maximum plant height (cm) was recorded in treatment T₇ - Neem cake @50g/m² (45.71), which was superior to overall treatments, followed by T₄ - *Azadirachta indica* @10% (43.81), T₃ - *Lantana camara* @10% (40.81), T₂ - *Trichoderma harzianum* @10g/L (38.03), T₆ - Licorice @10% (35.43), T₅ - Henna + Licorice + Rosemary @6% (33.93) as compared to treated check T₁ - Azoxystrobin @0.1% (47.30) and T₀ - Control untreated check (20.04). The result is in agreement with the study conducted by Katyayan (2012) [28] who reported that neem cake supplies available N for long time in soil resulting in promoting vegetative growth. Neem cake also reduces nitrification. Neem seed cake acts as a nutrient reservoir providing favourable pH, aeration and improving other physical and chemical properties of soil.

Effect of treatments on number of leaves of garlic

The data presented in table 5 reveal that the maximum number of leaves were recorded in treatment T₃ - *Lantana camara* @10% (9.4), which was superior to overall treatments, followed by T₄ - *Azadirachta indica* @10% (9.1), T₂ - *Trichoderma harzianum* @10g/L (8.8), T₇ - Neem cake @50g/m² (8.6), T₆ - Licorice @10% (8.3), T₅ - Henna + Licorice + Rosemary @6% (8.4) as compared to treated check T₁ - Azoxystrobin @0.1% (9.7) and T₀ - Control untreated check (8.1). Study found that the application of *Lantana camara* had a positive effect on the number of leaves of garlic, which is similar to the findings of Ambika *et al.* (2003) [29] who reported that the *Lantana camara* showed positive allelopathy effect and this effect depended on allelochemicals concentrations and it was also specific to different plant species. Different parts of *Lantana* weed have various allelochemicals like carbohydrates, flavonoids and tannins. Such chemicals or compounds enhance or reduce growth and yield of different crops.

Effect of treatments on average yield of garlic

The data presented in table 6 reveal that all the treatments application has significantly increased the total yield. The maximum yield of garlic was recorded in treatment T₇ - Neem cake @50g/m² (4.46), which was superior to overall treatments, followed by T₄ - *Azadirachta indica* @10% (4.06), T₃ - *Lantana camara* @10% (3.7), T₂ - *Trichoderma harzianum* @10g/L (3.35), T₆ - Licorice @10% (3.06), T₅ - Henna + Licorice + Rosemary @6% (2.96) as

compared to treated check T₁ - Azoxystrobin @0.1% (5.56) and T₀ - Control untreated check (2.62). The probable reason for such findings may be that neem cake is a rich source of organic carbon and hence organic matter content which is rich source of plant nutrients. This was in agreement with the findings of Shivakumaret al. (2011)[30] who reported an increase in organic carbon content from 0.03 to 0.06% after application of neem seed cake in finger millet. Similar results were ascribed by Uma Singh and Pokhriyal (1997) and Kumar et al. (2009) [31-32]who reported thatneem seed cake acts as nutrient reservoir and on decomposition release organic acids and the crops might absorb ions through their roots for the entire growth period leading to higher yields.

Table 3: Effect of treatments on disease severity of purple blotch in garlic at 30, 60 and 90 DAS

| Treatment Combinations | | 30 DAS | 60 DAS | 90 DAS | MEAN |
|------------------------|-----------------------------|-------------------------|--------------------------|--------------------------|---------------------------|
| T ₀ | Control | 10.42 ^a | 25.52 ^a | 34.68^a | 23.54 ^a |
| T ₁ | Azoxystrobin | 3.69 ^h | 16.52 ^g | 25.36^h | 15.19 ^f |
| T ₂ | <i>Trichodermaharzianum</i> | 7.30 ^d | 21.49 ^c | 29.45^d | 19.413 ^c |
| T ₃ | <i>Lantana camara</i> | 6.47 ^e | 20.6 ^d | 28.42^e | 18.497 ^{cd} |
| T ₄ | <i>Azadirachtaindica</i> | 5.48 ^f | 19.55 ^e | 27.65^f | 17.56 ^d |
| T ₅ | Henna + Licorice+ Rosemary | 9.46 ^b | 24.21 ^b | 31.31^b | 21.66 ^b |
| T ₆ | Licorice | 8.36 ^c | 23.58 ^b | 30.48^c | 20.807 ^b |
| T ₇ | Neem Cake | 4.33^g | 18.47^f | 26.35^g | 16.383^e |
| C.D. (5%) | | 0.590 | 0.715 | 0.394 | 0.967 |
| S.Em(±) | | 0.053 | 0.078 | 0.024 | 0.143 |

*Mean of three replications

*Figures followed by similar alphabets in same column indicates non-significant to each other at 5% level

Table 4: Effect of treatments on plant height (cm) of garlic at 30, 60 and 90 DAS

| Sr. no. | Treatments | 30 DAS | 60 DAS | 90 DAS |
|------------------|-----------------------------|--------------------------|--------------------------|--------------------------|
| T ₀ | Control | 15.57 ^f | 22.31 ^g | 29.04 ^h |
| T ₁ | Azoxystrobin | 26.19 ^a | 35.43 ^a | 47.30 ^a |
| T ₂ | <i>Trichodermaharzianum</i> | 18.10 ^d | 28.50 ^{de} | 38.03 ^e |
| T ₃ | <i>Lantana camara</i> | 21.20 ^c | 29.27 ^d | 40.81 ^d |
| T ₄ | <i>Azadirachtaindica</i> | 22.43 ^c | 32.06 ^c | 43.81 ^c |
| T ₅ | Henna + Licorice + Rosemary | 16.65 ^{ef} | 26.64 ^f | 33.93 ^g |
| T ₆ | Licorice | 17.06 ^{de} | 27.75 ^{ef} | 35.43 ^f |
| T ₇ | Neem cake | 24.02^b | 34.01^b | 45.71^b |
| C.D. (5%) | | 1.425 | 1.407 | 1.470 |
| S. Em (±) | | 0.312 | 0.304 | 0.331 |

*Mean of three replications

*Figures followed by similar alphabets in same column indicates non-significant to each other at 5% level

Table 5: Effect of treatments on number of leaves of garlic at 30, 60 and 90 DAS

| Sr. no. | Treatments | 30 DAS | 60 DAS | 90 DAS |
|------------------|------------------------------|--------------------------|-------------------------|------------------------|
| T ₀ | Control | 4.33 ^f | 6.26 ^e | 8.1 ^g |
| T ₁ | Azoxystrobin | 5.60 ^a | 7.8 ^a | 9.7 ^a |
| T ₂ | <i>Trichoderma harzianum</i> | 5.07 ^{cd} | 7.06 ^{bc} | 8.8 ^d |
| T ₃ | <i>Azadirachta indica</i> | 5.27 ^{bc} | 7.33 ^{ab} | 9.1 ^c |
| T ₄ | <i>Lantana camara</i> | 5.53^{ab} | 7.5^{ab} | 9.4^b |
| T ₅ | Henna + Licorice + Rosemary | 4.53 ^e | 6.4 ^{de} | 8.4 ^{ef} |
| T ₆ | Licorice | 4.60 ^e | 6.53 ^{de} | 8.3 ^{fg} |
| T ₇ | Neem cake | 4.80 ^{de} | 6.93 ^{cd} | 8.6 ^{de} |
| C.D. (5%) | | 0.241 | 0.470 | 0.218 |
| S. Em (±) | | 0.008 | 0.033 | 0.007 |

*Mean of three replications

*Figures followed by similar alphabets in same column indicates non-significant to each other at 5% level

Table 6: Effect of treatments on average yield of garlic

| TREATMENTS | YIELD (t/ha) |
|---|-------------------------|
| T ₀ Control (untreated) | 2.62 ^h |
| T ₁ Azoxystrobin | 5.56 ^a |
| T ₂ <i>Trichoderma harzianum</i> | 3.35 ^e |
| T ₃ <i>Lantana camara</i> | 3.7 ^d |
| T ₄ <i>Azadirachta indica</i> | 4.06 ^c |
| T ₅ Henna + Licorice + Rosemary | 2.96 ^g |
| T ₆ Licorice | 3.06 ^f |
| T ₇ Neem Cake | 4.46^b |
| C.D. (5%) | 2.84 |

| | |
|----------|------|
| S.Em (±) | 1.24 |
|----------|------|

*Mean of three replications

*Figures followed by similar alphabets in same column indicates non-significant to each other at 5% level

4. CONCLUSION

From the present study, it can be concluded that the treatment of garlic plants with 'Neem cake' was significant in decreasing purple blotch disease as minimum disease intensity (26.35%) was observed in treatment Neem cake @50g/m². Maximum plant height (45.71cm) and highest yield of garlic (4.46t/ha) was also obtained with Neem cake @50g/m². *Lantana camara*@10% was found significantly best to increase no. of leaves per plant (9.4). The present investigation was limited to one crop season (*Rabi*), under the agro climatic conditions of Prayagraj (U.P). Therefore, to substantiate the present result, more research is needed to be done in different seasons at different locations.

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