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ORIGINAL ARTICLE

Evaluation of Botanical and Biological Control Strategies Against Onion Purple Blotch Disease in Egypt

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ABSTRACT

Onion is a vital food crop because of its nutritional value and role in food security. However, it remains susceptible to purple blotch disease caused by *Alternaria porri*. This study evaluated the antifungal potential of various botanical aqueous and oil extracts, as well as biological control agents, against *A. porri* through *in vitro*, greenhouse, and field experiments. Garlic extract at 10% demonstrated the highest inhibition rate (37.9%), followed by Bougainvillea extract at 5% and 20%, which achieved inhibition rates of 36.5% and 30.3%, respectively. In contrast, the Moringa extract exhibited minimal antifungal activity. Among the oil extracts tested (25–100 ppm), clove oil was the most effective in vitro, with an EC_{90} value of 2.09%. In greenhouse trials, 10% of Bougainvillea extract resulted in the lowest disease severity (11.5%). Garlic extract at 5% yielded the longest leaves (41.3 cm), whereas Bougainvillea extract at 5% yielded the widest bulbs (25.2 mm). Neem extract at 5% recorded the highest chlorophyll content (69.39%). Garlic oil at 50 ppm reduced disease severity to 21.3% and at 75 ppm resulted in the widest bulb diameter (23.1 mm). Meanwhile, cinnamon oil at 75 ppm produced the longest leaves (45.9 cm). *Trichoderma* sp. was the most effective bioagent in reducing disease severity, whereas *Chaetomium* sp. enhanced leaf length, bulb diameter (26.7 mm), and chlorophyll content. Enzyme assays revealed peak peroxidase activity with *Bacillus* sp. and the highest polyphenol oxidase activity with garlic oil at 75 ppm. Field results confirmed that garlic oil and *Trichoderma* sp. were effective treatments.

Keywords: *Alternaria porri*, Bioagent Control, Botanical extract, Onion, Purple blotch.

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INTRODUCTION

Onion (*Allium cepa* L.) is one of Egypt's most economically important vegetable crops. It demonstrates high adaptability to various soil types, with cooler temperatures and shorter photoperiods favoring vegetative growth, while higher temperatures and longer photoperiods require bulb development. Onion ranks tenth among the top ten agricultural commodities in Egypt, with a production of 380,407,672 tones cultivated over an area of approximately 248,974 Fadden (104,569 hectares) in 2023 (Anonymous, 2025).

Despite its significance, onion cultivation faces challenges from numerous pathogens, including fungi, bacteria, viruses, and nematodes. The prevalence and impact of these pathogens vary depending on seasonal conditions, cultivars, and geographic location (Yazhini *et al*., 2024). Among these, fungal diseases - such as those caused by *Alternaria* spp., *Botrytis* spp., and *Peronospora* sp.- are particularly damaging to both foliage and bulbs (Mahmoud *et al*., 2025).

One of the most destructive fungal pathogens is *A. porri*, the causal agent of PB disease. This disease is especially prevalent in autumn and winter, leading to significant reductions in bulb and seed yield, with losses ranging from 2.5% to as much as 97% under warm and humid conditions (Akter *et al*., 2022). Symptoms typically appear as purple lesions with concentric rings on the leaves and flower stalks. The pathogen survives in plant debris as mycelium, which produces

conidia at the start of the season and spreads via wind and water. Fungal diseases in onions are commonly managed using chemical fungicides (Wadiphasme *et al*., 1991; Ishak *et al*., 2023). However, the intensive use of these chemicals raises concerns regarding environmental contamination, toxicity, and disruption of soil microbial communities. Consequently, integrated disease management strategies are increasingly advocated. These approaches combine cultural practices, balanced plant nutrition, and environmentally friendly alternatives. Natural microbial interactions, including competition and hyperparasitism, also play a role in suppressing harmful pathogens and supporting ecological balance (Haas and Défago, 2005).

In recent years, there has been a growing interest in botanical and biological control methods due to their multiple advantages—such as broad-spectrum activity, cost-effectiveness, and beneficial effects on soil health. Several studies have confirmed the efficacy of botanicals and bioagents in the management of fungal diseases in onions (Mishra and Gupta, 2012; Gondaliya *et al*., 2020).

This study aimed to isolate and identify the fungal strains responsible for purple blotch disease in onions and evaluate the effectiveness of selected botanical extracts, oil extracts, and bioagents in disease management through laboratory, greenhouse, and field experiments.

MATERIALS AND METHODS

Sources of Pathogens and Bioagents

An *Alternaria porri* isolate, kindly provided by Abo-Zaid, was obtained and purified from onion leaves exhibiting purple blotch symptoms (Abo-Zaid *et al*., 2020). The isolate was identified using slide culture technique, as described by Woudenberg *et al*. (2014), Lawrence *et al*. (2016), and Ravichandra *et al*. (2025). Two antagonistic fungi, *Trichoderma harzianum* and *Chaetomium globosum*, along with three antagonistic bacteria, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Actinomyces* sp., were isolated from pathogen-infected leaves and utilized accordingly. Fungal isolates were purified and identified based on their morphological characteristics, including the structure of mature perithecia, terminal hairs, asci, and ascospores, following the descriptions provided by Abdel-Azeem (2019). In a similar manner, the bacterial isolates were identified based on their morphological traits and standard biochemical tests, following the procedures described by Mansour *et al*. (2023).

Botanical Materials

A. Preparation of the aqueous extracts

Fresh and healthy leaves of four plant species were collected from the Botanical Garden, Faculty of Agriculture, Ain Shams University (Table 1). The leaves were washed, surface-sterilized, air-dried, and ground into a fine powder (Khatri *et al*., 2025). The powder was mixed with distilled water at a 1,1 (w/v) ratio and shaken for 24 h. The mixture was then filtered and centrifuged at 5000 rpm for 20 min at 4°C. The supernatant was sterilized by passing it through a 0.20 µm bacterial filter (Seitz) (Sallam *et al*., 2021).

B. Source of the oil extracts

Oil extracts from four plant species were obtained from Haraz Co. Ltd, Cairo, Egypt. (Table2).

Table 1. Sources of the aqueous plant extracts evaluated for their antifungal activity against *A. porri* in onion

Binomial name	Common name	Family
Azadirachta indica A. Juss.	Neem	Meliaceae
Allium sativum L.	Garlic	Amaryllidaceae
Moringa oleifera Lam.	Moringa	Moringaceae
Bougainvillea Commers	*Bougainvillea*	Nyctaginaceae

Table 2. Sources of the botanical extracts from selected medicinal plants evaluated for their antifungal activities against *A. porri*

Binomial name	Common name	Family
Cinnamon zylanicum Blume	Cinnamon	Lauraceae
Syzygium aromaticum (L.) Merr.	Clove	Myrtaceae
Nigella sativa L.	Black seed	Ranunculaceae
Allium sativum L.	Garlic	Amaryllidaceae

***In vitro* experiments**

Effect of Aqueous Extracts on *A. porri* using the Poisoned Food Technique

The antifungal activity of leaf extracts at concentrations of 5, 10, 15, and 20% were evaluated using the poisoned food technique. Potato dextrose agar (PDA) plates were prepared with the extract and inoculated with 7-day-old fungal cultures. Plates were incubated at 25°C with five replicates per treatment. Control plates contained no extracts. Fungal growth was measured after 7 days, and the inhibition percentage was calculated (Kwodaga *et al.*, 2019; Abo-Zaid *et al*., 2020).

Effect of oil extracts on *A. porri* using the well diffusion method

The antifungal activity of the four oil extracts was tested at concentrations of 25, 50, 75, and 100 ppm using the well diffusion method (Pawar and Thaker, 2007). Sterilized PDA medium was poured into Petri dishes under aseptic conditions and then centrally inoculated with the pathogen. Wells (5 mm diameter) were prepared using a flame-sterilized Cork borer and filled with oil solutions containing 0.5% Tween-20. Each treatment was replicated five times. Plates were incubated at 25 ± 1°C for 5 days, and inhibition zones were measured to assess antifungal efficacy (Fayzalla *et al*., 2011; Abo-Zaid *et al*., 2020).

Evaluation of Bioagents Using the Dual Culture Method

The antagonistic effects of fungal and bacterial bioagents on *A. porri* linear growth were assessed. Two fungi (*T. harzianum* and *C. globosum*) and three bacteria (*B. subtilis*, *P. fluorescens*, and *Actinomyces* sp.), isolated by Abo-Zaid *et al*. (2020), were tested. Additionally, both surface-associated and endophytic fungi were evaluated using the dual culture technique (Abo-Elyousr *et al*., 2014; Adolf, 2016).

Effect of Fungal Bioagents on A. porri growth

Sterilized PDA (20 mL) was poured into 9-cm Petri dishes and cooled to 45°C. Mycelial discs (5 mm) from 7-day-old cultures of both the pathogen and the antagonistic fungi were placed 1 cm from the rim on opposite sides of the plates. Treatments were repeated five times and incubated at 25 ± 1°C. The control plates contained only the pathogen. Once control plates reached full growth, colony diameters were measured, and inhibition percentage was calculated as follows (Ayoubi *et al.*, 2012):

Inhibition (%) = (C – T) / C × 100

Where,

C = radial growth in the control

T = radial growth with treatment.

Based on the inhibition zone, the antagonistic effects were categorized as follows:

0 = no effect,

OG = overgrowth on pathogen;

N = narrow (<3 mm),

M = moderate (3–7 mm),

B = broad inhibition (>7 mm).

Effect of Bacterial Bioagents on A. porri growth

First Technique

Each bacterial isolate was streaked 1 cm from the edge of the plate, with the pathogen placed on the opposite side. Controls included only the pathogen. This setup was used to evaluate direct antagonistic interactions.

Second Technique

Bacterial overnight cultures were centrifuged, washed twice with sterile water

and adjusted to 10^4 cells/mL using a Scepter™ automated cell counter (Millipore Sigma, USA). Ten µL drops were inoculated at five equidistant points on a 9-cm PDA plate. A five mm disc of *A. porri* was placed at the center of the plate. Plates were incubated at 25°C for 4–6 days. Colony diameters were measured in two perpendicular directions aligned with bacterial drops (Herrera *et al*., 2016; Whitaker and Bakker, 2019). Inhibition was calculated using the same formula and classification scale as in the fungal assay.

***In vivo* experiments**

Greenhouse Experiments

Effect of Treatments on Vegetative Parameters

Experiments were conducted in a greenhouse in Shoubra El-Kheima, Qalyubia. Aqueous extracts were applied at 5 and 10%, oil extracts at 50 and 75 ppm, and spore/cell suspensions of *T. harzianum*, *C. globosum*, *B. subtilis*, and *Actinomyces* sp. Were adjusted 10^6 and 10^4 CFU/mL. Onion seedlings (cv. Giza Red) were grown in 20 cm pots, with eight replicates per treatment. Treatments were applied 48 h after pathogen inoculation, beginning 60 days after planting, and repeated five times at intervals of 15–20 days. The leaf length (cm) and bulb diameter (mm) were measured at harvest using a digital caliper.

Effect on Chlorophyll Content

On fully developed leaves, chlorophyll content was measured using a Minolta SPAD-502 Plus meter, following Baquy *et al*. (2017). Measurements were taken from three leaves per plant.

Enzyme Assays

Fresh leaf samples from 120-day-old plants were collected for determination the activity peroxidase (EC 1.11.1.7) and polyphenol oxidase (EC 1.14.18.1). Enzyme extraction was carried out as mentioned by Abo-Zaid (2020) for POD and PPO.

Field Experiment

A field trial was conducted at the Horticulture Department, Faculty of Agriculture, Ain Shams University, Egypt, to evaluate three treatments—Bougainvillea aqueous extract, *Allium sativum* oil extract, and *Trichoderma harzianum* bioagent—on two onion cultivars (Giza Red and Giza 20), selected based on greenhouse results. Spraying began 60 days after planting, before the appearance of symptoms. The leaf length (cm) and bulb diameter (mm) were recorded at harvest using a digital caliper.

Disease Assessment

Disease symptoms were evaluated between 4 and 6 days post-inoculation. Severity was rated using a modified 0–5 scale (Yildiz, 2000; Abo-Zaid *et al.*, 2020) based on the percentage of leaf area affected,

0. No visible symptoms
1. Minor spots near the tip, ~10% of the leaf area
2. Multiple purplish-brown lesions (up to 20%)
3. Numerous patches with light borders (up to 40%)
4. Central splitting or streaks up to 75%
5. The entire leaf is dried or broken from the center.

Disease severity was calculated as follows (Yildiz *et al*., 2007; Abo-Zaid *et al*., 2024):

$$\text{Percent disease severity} = \frac{\sum(\text{Class rating} \times \text{class frequency})}{\text{total number of samples} \times \text{highest rating}} \times 100$$

Statistical Analysis

Statistical analyses were conducted using the SAS software (SAS Institute, 1992). Data were subjected to one-way analysis of variance, and treatment means were compared using the least significant difference test at a 95% confidence level. Additionally, Pearson correlation coefficients were calculated to evaluate the relationships among the variables under study.

RESULTS

In vitro experiments

Effect of Aqueous Extracts on *A. porri* Using the Poisoned Food Technique

All the four evaluated botanical extracts exhibited significant antifungal activity against *A. porri* (Table 3). Garlic extract at a concentration of 10% demonstrated the highest inhibition rate (37.9%), significantly surpassing all other treatments. The next most effective treatment was *Bougainvillea* extract at concentrations of 5, 10, and 20%, with inhibition percentages of 36.5, 30.1, and 30.3%, respectively. In contrast, the least inhibition was recorded for neem extract (7.9%) at 20% concentration. Interestingly, *Bougainvillea* extract at 15% concentration promoted the mycelial growth of *A. porri*, as shown in Fig. (1). Overall, botanical extracts at 5 and 10% concentrations were more effective than those at 15 and 20%. The aqueous moringa extract displayed the lowest efficacy percentages (Table 3).

Table 3. Effect of different concentrations of aqueous plant extracts on the linear growth of *A. porri* using the poisoned food technique on PDA at 25 ± 1°C for 7 days.

Treatment	Conc. (%)	Inhibition %	EC_{50} %	EC_{90} %	Y = a + bX	Coeff. of Determination (R^2)
Moranga	5	17.5	0.023	1.92	y = 0.6732x + 4.9847	0.8284
	10	26.9				
	15	21.2				
	20	27.1				
Garlic	5	18.0	0.12	1.51	y = 0.9266x + 4.8851	0.6411
	10	37.9				
	15	14.1				
	20	11.6				
Neem	5	25.5	0.21	1.49	y = 0.9986x + 4.79	0.4451
	10	29.0				
	15	7.9				
	20	24.0				
Bougainvillea	5	36.5	0.15	2.93	y = 0.5967x + 4.912	0.8029
	10	30.1				
	15	21.8				
	20	30.3				
Control		0.0				

Y: Probit values of the mean inhibition percentage (%); **X**: Log-transformed values of the mean concentrations of the aqueous extracts tested. **EC_{50}**: Half-maximal effective concentration. **EC_{90}**: Effective concentration at 90% inhibition. **Coeff. of Determination (R^2)**: indicates the regression model's goodness of fit

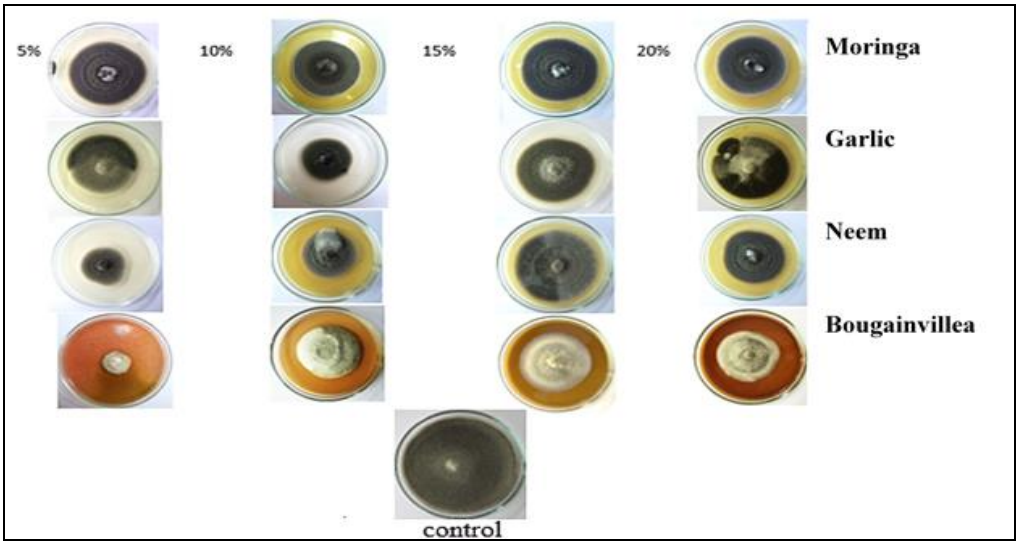

Fig. 1. Effect of different concentrations (5, 10, 15, and 20%) of aqueous plant extracts on the mycelial growth of *A. porri on PDA medium using the poisoned food technique.*

Effect of Oil Extracts on *A. porri* Mycelial Growth Using the Well Diffusion Method

Oil extracts from cinnamon, clove, black seed, and garlic were evaluated at concentrations of 25, 50, 75, and 100 ppm. As shown in Table (4) and Fig. (2), clove and garlic oils demonstrated the most effective antifungal activity. Notably, the EC_{50} values of garlic and black seed oil were 0.37 and 0.71%, while the EC_{90} values for cinnamon, garlic, and black seed oils were1.55, 1.75 and 1.79% indicating strong efficacy even at low concentrations.

Evaluation of Bioagents Using the Dual Culture Method

The inhibitory effects of five biocontrol agents *T. harzianum*, *Chaetomium globosum*, *B. subtilis*, *P. fluorescens*, and *Actinomyces* sp. on *A. porri* were examined in vitro using two dual culture techniques. As illustrated in Figs. (3 and 4), all biocontrol agents significantly inhibited fungal growth. The mechanisms of inhibition included direct competition through overgrowth and antibiosis, as evidenced by the clear inhibition zones shown in Fig. (5).

Table 4. Effect of Different Concentrations of Oil Extracts on *A. porri* Linear Growth on PDA at 25 ± 1°C for 7 Days.

Treatment	Conc. (ppm)	Inhibition %	EC_{50} %	EC_{90} %	Y = a + bX	Coeff. of Determination . (R^2)
Cinnamon	25	1.1	1.34	1.55	y = 5.9994x + 3.0368	0.7446
	50	2.4				
	75	4.2				
	100	21.1				
Cloves	25	11.1	1.24	2.09	y = 1.5085x + 3.1231	0.8993
	50	15.2				
	75	31.5				
	100	37.5				
Black seed	25	6.4	0.71	1.79	y = 1.1834x + 4.1648	0.6374
	50	6.5				
	75	11.6				
	100	24.2				
Garlic	25	16.1	0.37	1.75	y = 0.9298x + 4.6544	0.852
	50	9.2				
	75	6.1				
	100	7.8				
Control		0.0				

Y: Probit values of the mean inhibition percentage (%); **X**: Log-transformed values of the mean concentrations of the aqueous extracts tested. **EC_{50}**: Half-maximal effective concentration. **EC_{90}**: Effective concentration at 90% inhibition. **Coeff. of Determination (R^2)**: indicates the regression model's goodness of fit.

In vivo experiments

Greenhouse Experiments

Effect of Treatments on Vegetative Parameters and Chlorophyll Content

As shown in Table (5), *Bougainvillea* extract at a concentration of 10% resulted in the lowest disease severity (11.5%) in infected onion plants. Garlic extract at 5% notably enhanced leaf length (41.3 cm). No significant differences in the number of leaves were observed among aqueous extracts. Neem extract at 5% recorded the highest total chlorophyll content (68.60%) in infected onions. Additionally, *5% of Bougainvillea produced* the largest bulb diameter (25.2 mm) among the treatments.

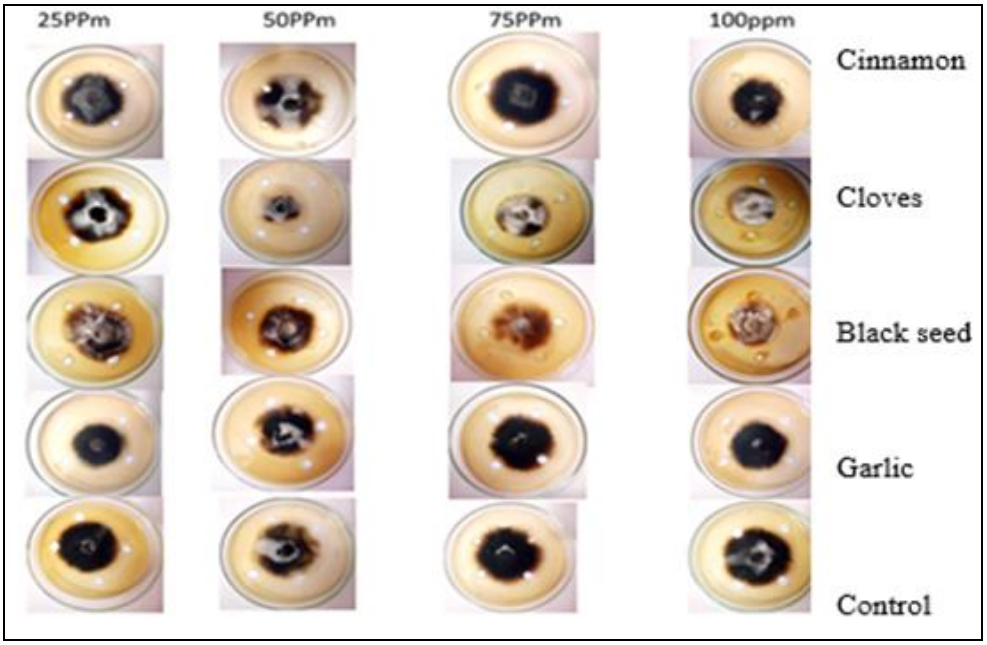

Fig. 2. Effect of different concentrations (25, 50, 75, and 100 ppm) of oil extracts from cinnamon, clove, black seed, and garlic on the mycelial growth of *A. porri on PDA medium using the poisoned food technique.*

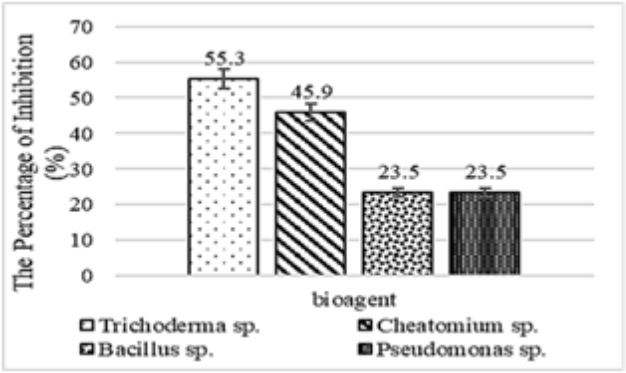

Fig. 3. In vitro efficacy of bioagents (*T. harzianum*, *C. globosum*, *B. subtilis*, *P. fluorescens*) against *A. porri* (First technique)

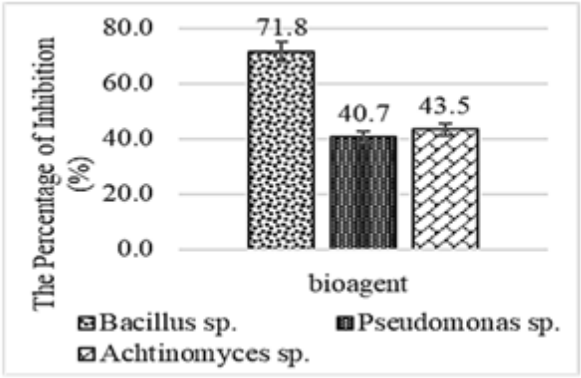

Fig. 4. *In vitro* efficacy of bioagents (*B. subtilis*, *P. fluorescens Actinomyces* sp.) against *A. porri* (Second technique)

The results in Table (6) show that garlic oil at 50 ppm exhibited the lowest disease severity (21.3%). Cinnamon oil at 75 ppm resulted in the longest leaf (45.9 cm). The number of leaves was not significantly vared among the oil treatments. Garlic oil at 75 ppm had the highest chlorophyll content (66.04%) and produced the largest bulb diameter (23.1 mm). Data presented in Table (7) show that *T. harzianum* resulted in the least disease severity. In contrast, *C. globosum* had the most pronounced effect on leaf length. No significant differences were observed in the number of leaves among bioagents treatments. The highest chlorophyll content (57.76%) and largest bulb diameter (26.7 mm) were observed in plants treated with *C. globosum.*

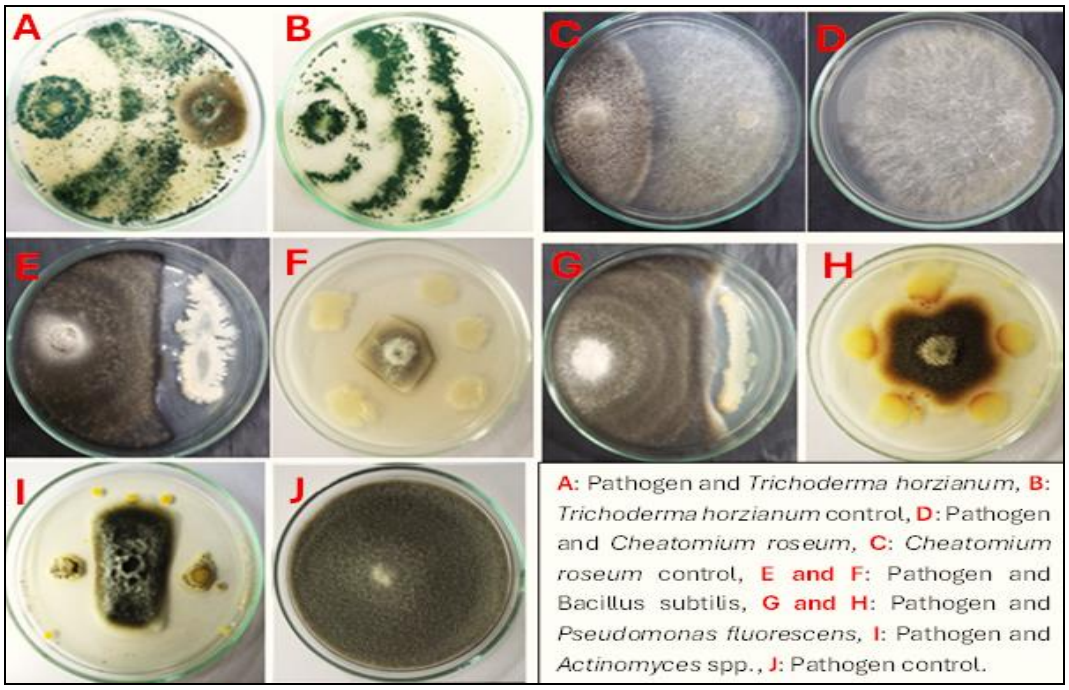

Fig 5. *In vitro* antagonistic activity of various bioagents against *A. porri.*

Table 5. Effects of different aqueous plant extracts on the vegetative growth and chlorophyll content of onion plants in pot experiments

Treatment	Conc. %	Leaf length (cm)	No. leaves	Chlorophyll Quantity (SPAD)	Bulb Size (mm)	Disease severity %
Neem	5	37.7 ± 3.15 [a]	5 ± 0.31 [a]	69.39 ± 3.15 [a]	22.7 ± 1.12 [a]	14.9 ± 4.82 [a]
	10	35.2 ± 2.70 [a]	6 ± 0.32 [a]	63.35 ± 3.85 [a]	22.2 ± 1.36 [a]	20.6 ± 2.92 [a]
Garlic	5	41.3 ± 2.05 [a]	6 ± 0.33 [a]	66.75 ± 2.71 [a]	24.6 ± 0.96 [a]	25.4 ± 2.89 [a]
	10	36.2 ± 2.42 [a]	5 ± 0.18 [a]	65.88 ± 4.36 [a]	23.1 ± 1.54 [a]	13.3 ± 4.31 [a]
Moringa	5	39.8 ± 2.05 [a]	6 ± 0.38 [a]	67.15 ± 3.37[a]	24.3 ± 1.19 [a]	26.2 ± 5.68 [a]
	10	39.6 ± 1.26 [a]	5 ± 0.23 [a]	68.60 ± 1.73 [a]	22.9 ± 0.61 [a]	15.4 ± 6.14 [a]
Bougainvillea	5	40.6 ± 2.05 [a]	5 ± 0.38 [a]	63.61 ± 4.88 [a]	25.2 ± 1.19 [a]	17.3 ± 5.68 [a]
	10	38.6 ± 1.26 [a]	5 ± 0.23 [a]	58.83 ± 8.74 [a]	22.7 ± 0.61 [a]	11.5 ± 6.14 [a]
Control	------	41.0 ± 2.15 [a]	5 ± 0.26 [a]	57.19 ± 2.69 [a]	22.7 ± 0.95 [a]	44.8 ± 3.39 [b]

Means followed by the same letter are not significantly different according to Duncan's multiple range test at **P ≤ 0.05**. *n* = 8). Values are presented as means ± standard error. **SPAD**: A device used to measure chlorophyll content in plant leaves.

Table 6: Effect of different oil extracts on the vegetative growth and chlorophyll content of onion plants grown in pots under greenhouse conditions.

Treatment	Conc. (ppm)	Leaf length (cm)	No. leaves	Chlorophyll Quantiy (SPAD)	Bulb Size (mm)	Disease severity %
Garlic	50	39.0 ± 1.33 [a]	5 ± 0.1.9 [ab]	65.73 ± 4.42 [ab]	21.7 ± 0.66 [a]	21.3 ± 5.81 [a]
	75	40.9 ± 2.28 [a]	5 ± 0.30 [ab]	66.04± 3.17 [ab]	23.1 ± 1.20 [a]	27.8 ± 6.23 [ab]
Cinnamon	50	38.5 ± 2.05 [a]	5 ± 0.33 [ab]	57.36± 1.85 [ab]	22.7 ± 1.29 [a]	25.1 ± 4.91 [a]
	75	45.9 ± 0.88 [a]	5 ± 0.33 [ab]	62.84± 1.97[ab]	22.5 ± 1.82 [a]	30.9 ± 2.08 [ab]
Cloves	50	43.5 ± 2.72 [a]	5 ± 0.16 [ab]	61.11 ± 4.29[ab]	22.7 ± 1.55 [a]	26.5 ± 4.92 [a]
	75	42.6 ± 2.09 [a]	4 ± 0.18 [b]	56.10 ± 2.55 [b]	21.4 ± 0.90 [a]	24.5 ± 4.00 [a]
Black seed	50	39.7 ± 2.68 [a]	5 ± 0.32 [ab]	59.15 ± 1.01 [bc]	21.5 ± 1.98 [a]	21.5 ± 3.81 [a]
	75	44.2 ± 3.53 [a]	5 ± 0.35 [ab]	59.91 ± 1.43 [bc]	21.8 ± 1.41 [a]	40.7 ± 2.68 [c]
Control	------	41.0 ± 2.15 [a]	5 ± 0.26 [a]	57.19 ± 2.69 [a]	22.7 ± 0.95 [a]	43.8 ± 3.39 [c]

Means followed by the same letter are not significantly different according to Duncan's multiple range test at **P ≤ 0.05**. *n* = 8). Values are presented as means ± standard error. **SPAD**: A device used to measure chlorophyll content in plant leaves.

Enzyme activity assays in greenhouse-grown plants

Results in Fig. (6) demonstrate that most treatments enhanced the activity of both peroxidase and polyphenol oxidase enzymes in infected onion leaves. The highest peroxidase activity was observed in plants treated with Bacillus subtilis, whereas the lowest was observed in those treated with garlic oil at 50 ppm. For polyphenol oxidase, garlic oil at 75 ppm exhibited the highest enzyme activity, whereas plants treated with 10% moringa extract exhibited the lowest activity.

Field Experiment

Field trials indicated that garlic oil treatment reduced disease severity in the Giza Red onion cultivar 120 days after transplantation. Similarly, T. harzianum reduced disease severity in Giza 20 cultivar under the same conditions. As shown in Table (8), Giza 20 shows greater increases in bulb diameter than Giza Red. All treatments improved the bulb diameter relative to the control. The highest values were recorded in plants treated with Bougainvillea extract, whereas the lowest values were recorded in garlic-treated plants.

Table 7. Effect of different bioagents on the vegetative growth and chlorophyll content of onion plants grown in pots under greenhouse conditions.

Treatment	Leaf length (cm)	No. leaves	Chlorophyll Quantity (SPAD)	Bulb Size (mm)	Disease severity %
***T. harzianum* (Tri. sp.)**	41.3 ± 2.47 ^{a}	6 ± 0.18 ^{bc}	52.58 ± 2.99 ^{a}	25.6 ± 1.83 ^{ab}	19.4 ± 4.58 ^{a}
***C. globosum* (Ch. sp.)**	46.6 ± 1.99 ^{a}	7 ± 0.31 ^{a}	57.76 ± 0.95 ^{a}	26.7 ± 0.87 ^{a}	42.4 ± 3.24 ^{b}
***Actinomyces* sp. (Ac. sp.)**	41.9 ± 2.11 ^{a}	6 ± 0.30 ^{abc}	53.46 ± 2.76 ^{a}	20.3 ± 1.06 ^{c}	34.9 ± 6.27 ^{b}
***B. subtilis* (Ba. sp.)**	43.7 ± 1.57 ^{a}	6 ± 0.46 ^{ab}	54.44 ± 2.83 ^{a}	22.2 ± 1.54 ^{bc}	46.0 ± 3.45 ^{b}
Control	41.0 ± 2.15 ^{a}	5 ± 0.26 ^{c}	57.19 ± 2.69 ^{a}	22.7 ± 0.95 ^{b}	43.8 ± 3.39 ^{b}

Means followed by the same letter are not significantly different according to Duncan's multiple range test at **P ≤ 0.05**. *n* = 8). Values are presented as means ± standard error. **SPAD**: A device used to measure chlorophyll content in plant leaves.

Table 8. Effect of different treatments on disease severity and bulb size of two onion cultivars under field conditions..

Treatment	Conc.	Disease severity %		Bulb size (mm)	
		Giza 20	Giza Red	Giza 20	Giza Red
***B. Commers* (Bou. Sp.)**	10%	57.33± 1.59 ^{a}	62.70±1.19 ^{b}	38.34± 0.75 ^{a}	35.14± 0.80 ^{a}
Garlic	50 ppm	62.31± 1.13^{b}	58.89±1.12 ^{a}	35.56 ± 0.85 ^{ab}	35.47± 0.78 ^{a}
***T. harzianum* (Tri. sp.)**	106 cfu/ml	57.29± 1.29 ^{a}	61.84± 1.38 ^{ab}	36.96±1.12 ^{a}	31.33± 0.92 ^{b}
Control	----------	66.48± 0.99 ^{c}	65.05± 1.17 ^{b}	32.92± 1.15 ^{b}	30.94± 1.24 ^{b}

Means followed by the same letter are not significantly different according to Duncan's test at **P ≤ 0.05**. Values are presented as mean ± standard error.

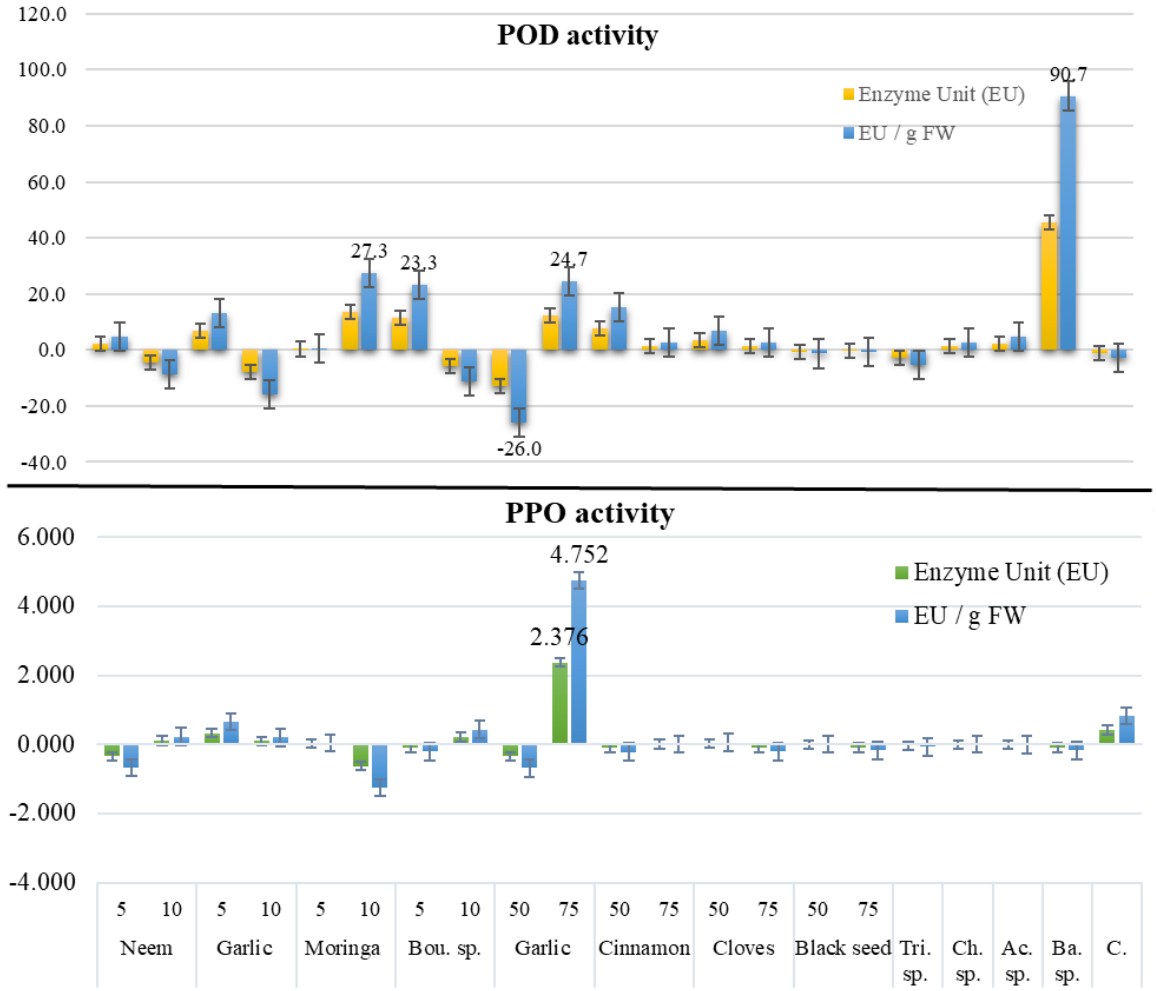

Fig. 6. Peroxidase (POD) and polyphenol oxidase (PPO) activities in onion leaves, expressed as the rate of hydrogen peroxide conversion per fresh mass, following different treatments at 120 days after transplantation.

DISCUSSION

The results of this study demonstrate that the application of both aqueous and oil-based plant extracts can effectively manage *A. porri* infection in onion plants. Garlic oil and *Bougainvillea Commers* extracts exhibited strong antifungal activity, significantly reduced disease severity. These findings are consistent with those of previous studies highlighting the potent antifungal properties of garlic oil against a range of phytopathogens.

In this study, the antifungal efficacy of botanical extracts was significantly influenced by both plant species and the applied concentration. Among all tested botanicals, garlic extract at a concentration of 10% exhibited the highest inhibition rate (37.9%), corroborating earlier research that attributes the antimicrobial potency of garlic to allicin—a sulfur-containing compound known for its broad-spectrum action (Mohan *et al*., 2001). Conversely, neem extract at a concentration of 15% demonstrated minimal inhibitory activity (7.9%), possibly due to oxidative degradation or antagonistic interactions at higher concentrations.

Bougainvillea Commers extracts also exhibited substantial inhibition at lower concentrations (36.5% at 5% and 30.3% at 10%). Interestingly, Bougainvillea extract appeared to promote fungal growth at 15% concentration, showing a possible hermetic effect**,** where low doses inhibit while higher

concentrations stimulate biological activity (Tiwari and Srivastava, 2004).

Among the various botanical formulations tested in previous studies, 5% neem seed kernel and pongamia seed extracts proved to be superior to others, such as periwinkle leaves, sweet flag rhizomes, wild tobacco, and garlic–green chili mixtures (Gowdar and Reddy, 2006). Ravichandra (2025) also demonstrated that 5% garlic clove extract resulted in 100% inhibition of *A. porri*, followed by 10% ginger and pongamia leaf extracts with over 90% effectiveness.

Oil extracts of garlic, clove, cinnamon, and black seed were evaluated across a concentration gradient (25–100 ppm). Despite their promising phytochemical profiles, garlic, cinnamon, and black seed oils showed relatively low efficacy in vitro, with EC_{50} and EC_{90} values below 2.0%. This demonstrated that while these oils have antifungal components, their volatility, solubility, or interactions with the media might reduce their effectiveness under laboratory conditions.

These findings emphasize the importance of optimizing both the concentration and extraction method when formulating botanical treatments. Lower concentrations (5–10%) were generally more effective than higher concentrations, which diminished efficacy or even stimulated fungal growth in some cases.

In field trials, garlic oil showed consistent efficacy in reducing disease severity, particularly in the Giza Red cultivar. This is supported by previous studies (Awad, 2016; Saleh *et al*., 2025) that demonstrated the ability of garlic oil to enhance plant resistance, resulting in improved agronomic traits, including yield components and oil content. Allicin, the principal active compound in garlic, has broad-spectrum antimicrobial activity both in vitro and in planta (Curtis *et al*., 2004). Garlic extracts are known to promote plant growth due to their content of growth-promoting compounds, such as starch, vitamins, and diallyl disulfide (Martins, 2016). Hayat *et al*. (2018) further improved tomato growth following garlic extract application.

Trichoderma harzianum exhibited the most pronounced antagonistic effect against *A. porri*, validating its status as a potent bioagents. This is consistent with earlier findings (Ahmed *et al*., 2017), where *T. harzianum* was found to significantly suppress fungal pathogens, including *Alternaria* species. *Chaetomium* sp. also displayed substantial antifungal effects, corroborating results from Karthika *et al*. (2020) and Lewaa and Zakaria (2023), who highlighted its efficacy in controlling plant diseases.

Conversely, *Pseudomonas fluorescens* was less effective in this study. This aligns with previous reports (Mathivanan *et al.*, 2000) that identified its limited efficacy against *A. porri*, indicating the need for further research into optimizing its use, potentially through synergistic combinations with other agents.

In terms of growth parameters, garlic oil at 50 ppm yielded the highest chlorophyll content, supporting the findings of Fayzalla *et al*. (2011) and Attia *et al*. (2020). Plant-derived biostimulants enhance photosynthetic activity and regulate nutrient metabolism by activating phytohormonal signaling pathways (Yasmeen *et al*., 2013; Yasmeen *et al*., 2014; Elzaawely *et al*., 2017). Therefore, the enhanced chlorophyll content observed with garlic treatment may reflect a dual role in disease suppression and physiological enhancement. Similarly, *Bougainvillea* extract significantly increased bulb diameter (25.2 mm), consistent with the findings of Mohan *et al*. (2001), who reported improved plant growth using specific plant extracts.

Enzyme assays revealed significant changes in peroxidase (POD) and polyphenol oxidase (PPO) activities in response to different treatments. *B. subtilis* triggered the highest peroxidase activity, suggesting a strong resistance response. This supports previous research (Akram and Anjum, 2011; Rais *et al*., 2017) indicating that *Bacillus* appendances oxidative enzyme production during pathogen challenge. However, garlic oil at 50 ppm showed the lowest peroxidase activity, showing that its

antifungal effect may operate through direct toxicity rather than host defense enzyme activation.

Interestingly, the highest PPO activity was observed with garlic oil at 75 ppm, supporting the findings of Diken (2020), who found that garlic-based treatments upregulated PPO in multiple crops. PPO contributes to the synthesis of defense-related phenolics, limiting the spread of pathogens. Conversely, 10% moringa extract resulted in the lowest PPO activity, indicating that although moringa possesses antifungal properties (Goss *et al*., 2017), it may not robustly trigger the plant's enzymatic defense system. Moringa bioactivity is often attributed to compounds such as zeatin, quercetin, β-sitosterol, caffeoylquinic acid, and kaempferol (Ashfaq *et al*., 2012).

In field conditions, garlic oil and *Trichoderma* sp. significantly reduced disease severity, particularly in Giza Red and Giza 20 onion cultivars. These results are in line with the findings of Bocate *et al.* (2021) and Hasna (2021), who highlighted the role of these agents in enhancing field-level disease resistance and crop performance. Notably, Giza 20 cultivar exhibited a greater increase in bulb diameter than the Giza Red cultivar across treatments. This aligns with the findings of Ahmed (2017), who reported improved onion bulb size in response to integrated treatments.

Among all treatments, *Bougainvillea* sp. extract produced the largest bulbs, whereas garlic although effective against disease, resulted in smaller bulb sizes. Ankita (2019) reported similar observations, demonstrating that while garlic is an effective antimicrobial agent, its role in promoting growth may be limited relative to other botanicals. These findings highlight the need for combining growth-promoting botanicals, such as Bougainvillea, with potent bioagents, such as *Trichoderma*, to achieve comprehensive disease management and enhanced productivity.

CONCLUSION

Overall, this study highlights the potential of integrating botanical extracts and biological control agents for the effective and sustainable management of *A. porri* in onion cultivation. Garlic oil and *Bougainvillea* sp. emerged as promising candidates under both controlled and field conditions. Future research should focus on the synergistic effects of combining such treatments and optimizing application protocols under diverse agro-ecological conditions.

AUTHOR CONTRIBUTIONS

A.A. conceptualized the study, performed data analysis, and K.H. supervised methodology and edited the manuscript. A.A. and M.E. provided overall supervision and approved the final version. A.A., H.A., M.E. and K.H. read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests. The contents of the manuscript have neither been published nor under consideration for publication elsewhere.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author (s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

STATEMENT AND ETHICS DECLARATIONS

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article. Ethical approval was not required for this study.

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