ZANCO Journal of Pure and Applied Sciences

TYPE Original article
DOI http://dx.doi.org/10.21271/ZJPAS.37.1.6

Antifungal Activity of Selected Medicinal Plants Against Grey Mold Disease and Extension of Strawberry Shelf Life

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RECEIVED :21 /08 /2024
ACCEPTED :25/11/ 2024
PUBLISHED :28/ 02/ 2025

KEYWORDS:
Amethyst garlic, pelargonium, fruit shelf life, biocontrol efficacy and *Botrytis cinerea.*

ABSTRACT

Botrytis cinerea is the causal agent of grey mold disease in many plant species and presents serious losses cost problems globally; hence, it has to be controlled. This research work was aimed at ascertaining how aqueous extracts of selected medicinal plants work against *B. cinerea* and extend the shelf life of strawberry fruits. For this study, the following plant materials were selected: amethyst garlic, cinnamon, cloves, sumac, ginger and pelargonium plants with the potential to inhibit the growth of the newly isolated and identified *B. cinerea* (accession number PQ590382) using the well diffusion method. Two concentrations were utilized: 10 and 15 mg/mL. Subsequently, Strawberry fruits were immersed in these extracts for five minutes to assess their effects on shelf life and pathogen protection. Pelargonium and amethyst garlic manifested huge antifungal effects against the growth of *B. cinerea*. The plant extracts have considerably extended the shelf life of fungal-inhibited strawberries, reduced their spoilage, and maintained quality compared to controls. In another application, 15 mg/mL concentration of effective extracts was sprayed on seedlings of strawberry plants to determine their effect on the incidence and severity of the disease and biocontrol efficacy. Amethyst garlic and pelargonium exhibited a considerable reduction in grey mold incidence and disease severity. The biocontrol efficacy increased to about 85% when both plants were combined. These results indicate that the extracts of medicinal plants could act as natural and efficient substitutes of synthetic fungicides for grey mold management and the extension of strawberry postharvest life.

1.Introduction

Botrytis cinerea, which causes gray mold disease, is a common problem for several crops, but more intensified among strawberries (Petrasch *et al.*, 2019). This pathogen does not only lower the yield and quality of the crops but is also responsible for the dramatic decrease in the shelf life of fruits, so it leads to economic losses for producers and retailers (Elmer and Michailides, 2007). *B. cinerea* causes a number of important diseases on a wide range of host plants, including fruits, vegetables, and ornamentals. This pathogen is responsible for considerable economic losses among yield and quality, even in storage and transport, of crops such as strawberries, tomatoes, grapes, and roses (Nakajima and Akutsu, 2014). In spite of their effectiveness, there are a number of deficits including a potential health risk to humans and the environment, and the development of fungicide-resistant strains. Therefore, traditional fungicides spawn interest in natural alternatives for controlling of grey mold (Zhang *et al*., 2020). It is a fact that plants have medicinal value, and such plants are rich in bioactive substances with antibacterial properties. These natural compounds open one route to the potential development of eco-friendly antifungal agents. Plant extracts can be an appropriate substitute for the synthetic chemicals, since they have many advantageous effects in disease management in plants. These natural extracts have rich bioactive compounds having strong antibacterial property, such as tannins, alkaloids, flavonoids, and essential oils (Saboon *et al*., 2019). The plant extracts alter the incidence and intensity of diseases by growth suppression of harmful bacteria and fungi in crops. Besides, most plant extracts normally induce their antifungal effect through several pathways, including cell membrane rupture, the arrest of spore germination, and interference with pathogen metabolism; hence, reducing the possibility of developing resistance (Sood *et al*., 2021). Some plant extracts can result in systemic resistance in plants, increasing the activities from natural products that protect them from a wide range of pathogens and their direct effects against pathogens (Burketova *et al*., 2015). Moreover, the eco-friendly application of the plant extracts is because it is biodegradable and have no or negligible detrimental effects on human health, beneficial insects, and soil microorganisms. This sustainable means promotes organic farming methods and growth of healthier crops in addition to aiding in preservation of ecological balance (Verma *et al*., 2018).

This allows for the use of some plant extracts in postharvest protection of fruits, which is natural and sustainable, in order to prolong their shelf life (Shahbaz *et al*., 2022). This makes such extracts, which are derived from a range of plants, herbs, and spices, possess antibacterial and antioxidant qualities, which may prevent the multiplication of bacteria that leads to spoilage and consequently goes on to influence the oxidation process (El Khetabi *et al*., 2022).

More recently, edible films and coatings from natural plant-based products have found higher use in the food packaging industry. The coating materials prevent microbial decay, reduce mass loss during storage, improve the appearance of the fruits, and protect their phytochemicals and biochemical quality in the postharvest storage. The extracts create a thick, rich barrier that protects the produce from moisture loss and fungal infections, maintains firmness, and preserves nutritional value. This is a strategy toward promoting the overall health and environmental sustainability of shelf life and safety of the produce, thereby meeting consumer requirements on natural and chemical-free food preservation methods. This study was established by Jiang *et al*. (2021).

There are a number of reports in which plant extracts have been tested for their effectiveness in vitro against *B. cinerea*. However, their in vivo use in improving the shelf life in strawberry fruits is very poorly documented. In order to evaluate the antifungal effect of certain medicinal plant products on *B. cinerea*. Thus, this research was conducted to elucidate the ability of aqueous extract of selected plants against *B. cinerea* and prolonging the shelf life of strawberry fruits. In addition, this research aimed at reducing the use of synthetic fungicides through the development of a sustainable method for the management of grey mold disease and the improvement of quality and longevity of strawberry plants and fruits.

2. Materials and methods:

2.1. isolation and identification of Botrytis cinerea

Strawberry fruits with gray mold symptoms were selected and divided into small pieces then surface sterilized with alcohol 70% after that they were rinsed with sterile distilled water three times. The specimens placed on the potato dextrose agar (PDA) (Difco™) in Petri dishes, and incubated at 28±2°C in the incubator. Gray and fuzzy colonies of Botrytis were selected and sub-cultured to obtain pure isolates. Universal primers ITS1 and ITS4 were used to amplify Polymerase chain reaction (PCR) of pathogen isolate to identify and confirm the species identity. The obtained sequence was then matched with reference sequences from the genetic database at NCBI to verify Botrytis cinerea identification. When its identification was verified, it was submitted for an accession number to NCBI (Rashid *et al*., 2016).

2.2. Preparation of Plant extracts:

Five different plants [sumac (*Rhus coriaria*) fruit pericarp, cinnamon (*Cinnamomum verum*) sticks, Amethyst garlic (*Allium amethystinum*) leaves, cloves (*Syzygium aromaticum*) buds, and pelargonium (*Pelargonium roseum*) leaves] were selected and purchased from herb store. The plants were confirmed by medicinal plant department college of Agricultural Engineering Sciences/ Salahaddin University. The plant materials were cleaned to remove dirt on the plant tissues. The cleaned ones were ground into fine powder with the mechanical grinder. The powdered material was weighed and an aqueous extraction was carried out by adding the plant powder in a ratio of 100:1000; w/v. The mixture was stirred gently to ensure full submersion of the plant material and was allowed to soak for 48 hours at room temperature. The soaked mixture was filtered on Whatman filter paper to discard the solid residues. The filtrate was transferred into the rotary evaporator to remove water content gradually under reduced pressure at 40°C, which concentrated this extract to completely dried form. The concentrated plant extract was preserved in an airtight container at 4°C until further use in subsequent assays or applications (Rashid *et al*., 2022).

2.3. Antifungal activity of plant extract against Botrytis cinerea

The well diffusion method was used to evaluate the antifungal activity of plant extracts against *B. cinerea*. Aqueous extracts of each plant were prepared at a concentration of 100 mg/ml. Five mm plugs of *B. cinerea* were placed in the center of Petri plates containing PDA. Two wells were made on each side of the plug, and 20 µl of each plant extract was added to these wells, while wells with sterilized distilled water served as the negative control. The plates were incubated at 25°C until the control plates were nearly full. Each treatment was replicated five times and repeated twice. After one week the radial growth measured as converted to the percentage of inhibition following the following formula:

$$\%\ \text{of Inhibition} = \frac{R1 - R2}{R1} \times 100$$

R1 is the radial growth of control and R2 is the radial growth of treated plates.

2.4. Strawberry Shelf life extending by different extracts

Healthy strawberry samples were selected and purchased after that thoroughly washed to remove surface contaminants for utilizing plant extracts to extend the shelf life of strawberry in the lab. These strawberries were then divided into treatment groups, including a control group that receives no treatment. The strawberry fruit samples were treated with plant extracts using a dipping method. The strawberries were immersed in the extract solution at two different concentrations (10 mg/mL and 15 mg/mL) for five minutes to ensure thorough contact. Then the strawberries were allowed to air dry to eliminate any leftover solution. Following this, they were put in sterile containers lined with tissue and kept in a lab setting. The strawberries were checked every until 10 days for indications of rotting, fungal infection, and general quality characteristics like firmness and color during the storage period. By contrasting the treated strawberries with a control group dipped in sterile distilled water, the effectiveness of the plant extracts in extending shelf life was ascertained. The experiment was repeated twice, using a Completely Randomized Design (CRD), with five replications of each treatment, each replication containing five fruits.

2.5. In vivo study:

Two of the most effective plants (pelargonium and amethyst garlic) was selected and tested for preventing grey mold disease in strawberry plants grown in a greenhouse was determined. Healthy strawberry seedlings were selected (Jewel cultivar) and purchased from a nursery around 10 cm. The positive control was treated with Pristine fungicide (BASF: Germany) at the recommended manufacturer's concentration (5 g/5 liters) was prepared and sprayed, while the negative control received distilled water. The treatment groups were sprayed with a concentration of 15 mg/ml of pelargonium and amethyst garlic alone and in combination. A suspension of *B. cinerea* (10^6 spores/mL) was uniformly sprayed onto the chosen groups after giving the extracts two days to absorb. Over a period of several weeks, the plants were observed for the emergence of disease, with particular attention paid to the frequency and intensity of symptoms like blight and leaf spots. This experiment was repeated twice. The temperature ranged between 20-30°C, with a 12–16-hour natural light cycle, and a relative humidity maintained at around 80–85%. Using a Completely Randomized Design (CRD), each treatment consisted of five replications (one plant/plot) and was repeated twice. The percentage of the disease severity and incidence, and the percentage of biocontrol efficacy were conducted using the following formulas.

Disease severity (DS) was measured weekly from the first week until the eighth week using a 0-to-4 scale: 0 = no spot and blight symptoms; 1 = 1–25 % of leaves were infected; 2 = 26–50 %; 3 = 51–75 %; 4 = 76–100 % (Parafati *et al.*, 2015).

$$\text{Disease severity (\%)} = \frac{\sum (\text{number of infected plants in each scale} \times \text{disease scale}}{(\text{total plants analyzed} \times \text{highest disease scale})} \times 100$$

Subsequently the disease incidence and biocontrol efficacy were estimated accordingly (Xue *et al.*, 2009):

$$\text{Disease incidence (\%)} = \frac{\text{Number of Infected Plants}}{\text{Total Number of Plants Assessed}} \times 100$$

$$\text{Biocontrol efficacy (\%)} = \frac{\text{Disease incidence of control} - \text{Disease incidence of treated group}}{\text{Disease incidence of control}} \times 100$$

2.6. Statistical analysis:

Statistical analysis was conducted using ANOVA to evaluate the differences between treatment groups. The SAS (Statistical Analysis System) software was employed to perform the ANOVA, ensuring robust and reliable statistical computations. For further analyze the means, Duncan's multiple range test was applied.

3. Results and discussion:

3.1. Pathogen isolation and identification

Botrytis cinerea was successfully isolated from affected strawberry fruit samples and subsequently subjected to molecular identification. The genetic sequences obtained were compared with those available in the NCBI database, showing a 100% match to the reference sequence (MW820601) which ensuring accurate identification of the pathogen. The accession number PQ590382 was obtained from the NCBI database following sequence submission.

3.2. Antifungal activity of plant extracts against Botrytis cinerea

From the five plant extracts tested, four demonstrated weak antifungal activity. Among them, pelargonium exhibited the highest antifungal activity at 25%, followed by Amethyst garlic at 20% with significance differences compared to other treatments. Sumac and clove showed very weak activity, at 12.5% and 10% respectively. In contrast, cinnamon and ginger did not show any antifungal activity (Fig. 1).

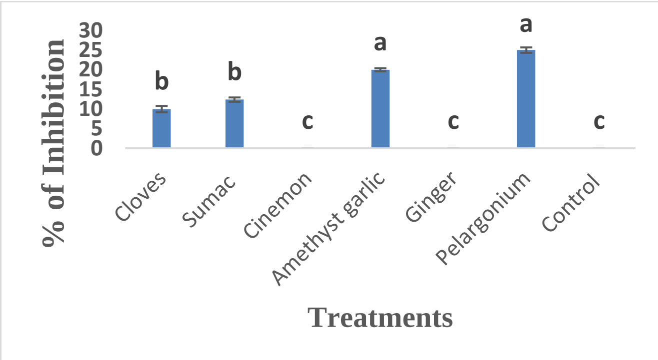

Fig 1. The inhibition Percentage of *Botrytis cinerea* by various plant extracts

Numerous studies have reported the antimicrobial activity of *Pelargonium* spp. For instance, *Pelargonium* sp. leaf oil has shown fungicidal properties against *Cryptococcus neoformans* and *Candida albicans* (Rath *et al.*, 2005). Dzamic *et al*. (2014) observed complete inhibition of *Candida albicans* growth due to the anticandidal activity of *P. graveolens* oil. Lalli *et al.* (2008) demonstrated the inhibitory activity of *P. pseudoglutinosum* against *Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae,* and *Candida albicans*. The essential oils of *Pelargonium roseum* were active against *C. albicans, Pseudomonas aeruginosa*, and *S. aureus* (Carmen and Hancu, 2014). Extracts from *Pelargonium zonale* stalks exhibit activity against *C. albicans* (Lewtak *et al*., 2014). Oils from various Pelargonium species exhibited strong activity against *Alternaria alternata, Aspergillus flavus, A. terreus, A. niger, A. versicolor, A. ochraceus, Aureobasidium pullulans, Cladosporium cladosporioides, C. fulvum, Fusarium sporotrichoides, F. tricinctum, Mucor mucedo, Penicillium funiculosum, P. ochrochloron, Phoma macdonaldii, Phomopsis helianthi, Trichoderma viride*, and *C. albicans* (Dzamic *et al*., 2014). In 2023, Allagui *et al*. reported that *P. asperum* essential oils by Amethyst garlic and Sumac.

completely inhibited the growth of *Alternaria alternata* and *B. cinerea*. Additionally, (Roman *et al.*, 2023) reported that Pelargonium essential oil exhibited significant antibacterial activity against *K. pneumoniae, S. aureus*, and *E. coli* strains, as well as antifungal activity against *Candida* spp.

In addition, different studies were reported the antifungal effect of different Allium species. For instance, The Mahmoudabadi and Nasery (2009) reported the antifungal activity of *Allium ascalonicum* fresh crude juice against *C. albicans, Microsporum gypseum, Trichophyton mentagrophytes, Epidermophyton floccosum, Syncephalastrum, A. niger, Penicillium sp., Paecilomyces sp., Scopulariopsis sp., Cladosporium sp., Alternaria sp.,* and *Drechslera sp.*. Also, *Allium roseum* var. *grandiflorum* subvar appeared to be able to inhibit *Fusarium solani* f. sp. *cucurbitae*, *Rhizoctonia solani, B. cinerea, F. oxysporum f. sp. niveum, A. solani* and *Pythium ultimum* (Rouis-Soussi *et al*., 2014).

Extending the shelf life of Strawberry by different plant extracts

Fig 2 shows the effect of all tested plant extracts at 10 mg/mL concentration on shelf-life of strawberry fruits. Pelargonium treatment shows the slowest increase in affected fruits, reaching 8 fruits by day 3 and 4, and still three fruits were healthy by the day 7 and 8, which indicates the best performance in reducing the number of affected fruits. The number of fruits in control group affected increases rapidly, peaking at 12 fruits by day 4, indicating the highest susceptibility compared to all treatments. Amethyst garlic showed a decrease in the number of affected fruits over time. From day 3, the number of affected fruits started to rise, reaching around 8 by day 3. This number continued to increase, reaching approximately 8 affected fruits by day 4. Pelargonium was the most effective treatment in minimizing the number of affected fruits over the observed period, followed by Amethyst garlic and Sumac.

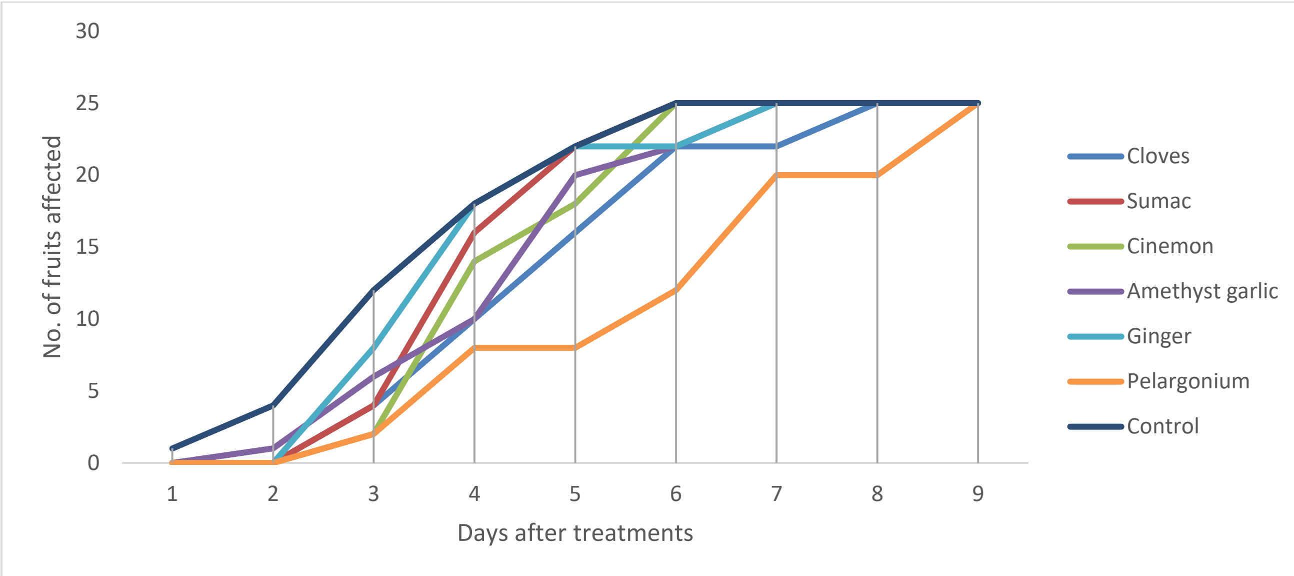

Fig 2. Shelf life of strawberry fruits after plant extracts treatment by 10 mg/mL concentration.

At a concentration of 15 mg/mL, during the first two days, most of the extracts did not show any symptoms of Botrytis infection except ginger one fruit affected. However, after three days, Botrytis began to affect the fruits treated with all plant extracts. Cinnamon and pelargonium treatments showed signs of infection after three days, with two fruits affected by the third day for Amethyst garlic and cinnamon. The pelargonium extracts notably reduced the infection progression which on the 3rd day, two fruit were affected, by the 4th until 5th days, eight fruits were affected, on the 6th day, 12 fruits were affected, By the 7th day, 20 fruits were affected and by the 9th day, all fruits were affected. After seven days, all treatments resulted in all twelve fruits being affected, except for the pelargonium treatment, where only 20 fruits were affected, leaving two fruits still healthy, these two fruits took nine days to rot completely (Fig 3). Also, the treated fruits exhibited less color deterioration over the storage period, indicating enhanced visual appeal.

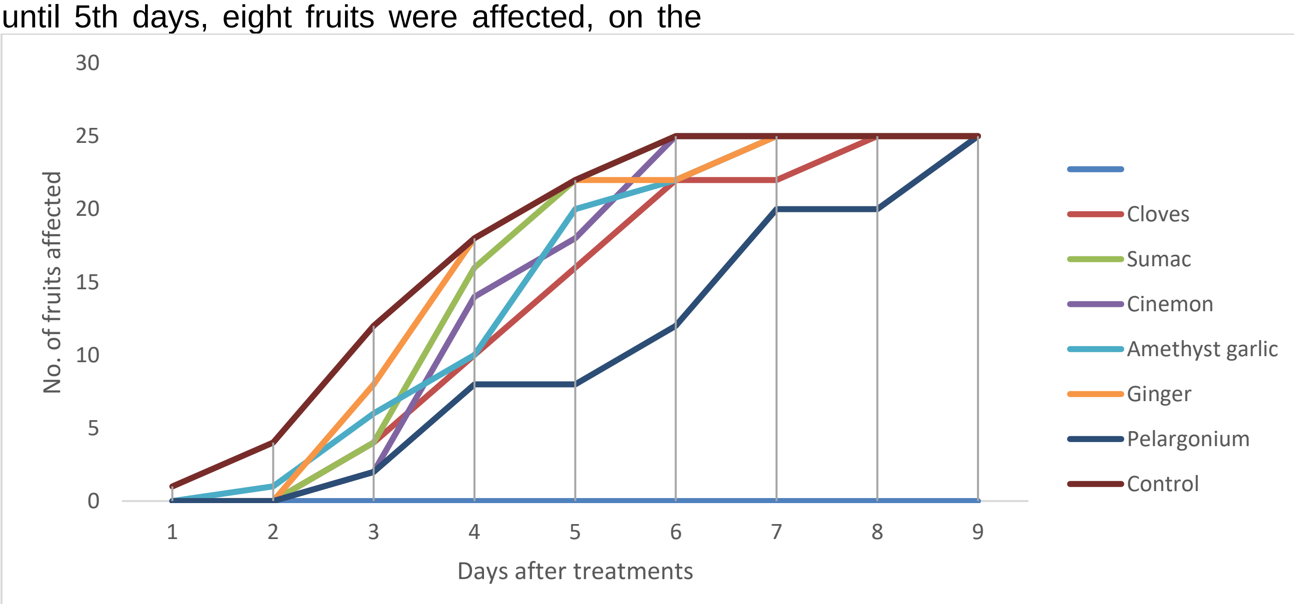

Fig 3. Shelf life of strawberry fruits after plant extracts treatment by 15 mg/mL concentration.

Similarly, various studies have explored the use of plant extracts to extend the shelf life of strawberries. For instance, pomegranate and grape seed extracts had a beneficial effect on maintaining the quality of fresh strawberries throughout the storage period (Duran *et al*., 2016). *Prosopis juliflora* water-soluble leaf ethanolic extract, described by (Saleh and Abu-Dieyeh, 2022), exhibited strong antifungal activity. Enriched gum Arabic coatings also provided positive results for preserving the qualitative parameters of strawberries. Samples coated with the extract showed the best maintenance of qualitative parameters after 14 days, with lower decay rates and good consumer acceptability (De Bruno *et al*., 2023). Novel edible coatings based on basil seed gum enriched with echinacea extract were monitored for their effects on the quality characteristics of fresh strawberries over a storage period of 20 days (Moradi *et al*., 2019). Moringa and aloe vera gel coatings were also studied, with current results revealing that a 20% aloe vera gel coating increased firmness by 60% over a 5-day ambient storage period (Moradi *et al*., 2019).

In vivo treatments:

Fig 4 displays the percentage of infection in strawberry plants under different treatment conditions after two months of treatment. The plants inoculated with pathogen and treated with Pelargonium extract show an infection rate of around 40%. Similar to the Pelargonium treatment, the Amethyst garlic treatment followed by pathogen exposure also shows about 40% infection. When both Amethyst garlic and pelargonium extracts were used together before pathogen exposure, the infection rate drops to approximately 20%. The control group inoculated with the *B. cinerea* shows the highest infection rate at around 100%. This indicates the high virulence of the pathogen in the absence of any treatment to inhibit its growth. the plants treated with a fungicide after pathogen exposure show an infection rate of about 20%, demonstrating the effectiveness of the fungicide in significantly reducing the infection rate.

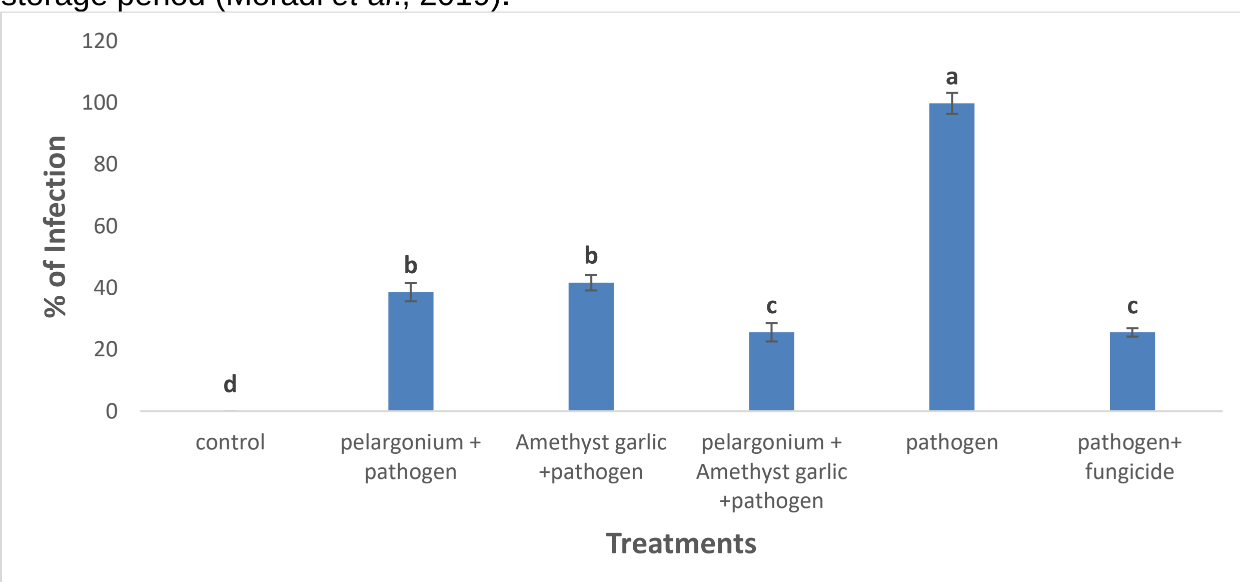

Fig 4. The effect of pelargonium and Amethyst garlic alone and in combination on the percentage of infection in strawberry plants.

Fig 5 Shows the percentage of disease incidence in strawberry plants under various treatment conditions. The plants treated with Pelargonium extract inoculated with pathogen show a disease incidence of around 20%. Similarly, the Amethyst garlic treatment followed by pathogen exposure results in a disease incidence of about 25%. When both plant extracts mixed and sprayed the plants shows a lower disease incidence, around 10%. The control group inoculated with the pathogen and not treated exhibits the highest disease incidence at around 100%, indicating the high virulence of the pathogen in the absence of any treatment. The plants inoculated with the pathogen and treated with a fungicide showed a disease incidence of approximately 15%, demonstrating the effectiveness of the fungicide in significantly reducing disease incidence.

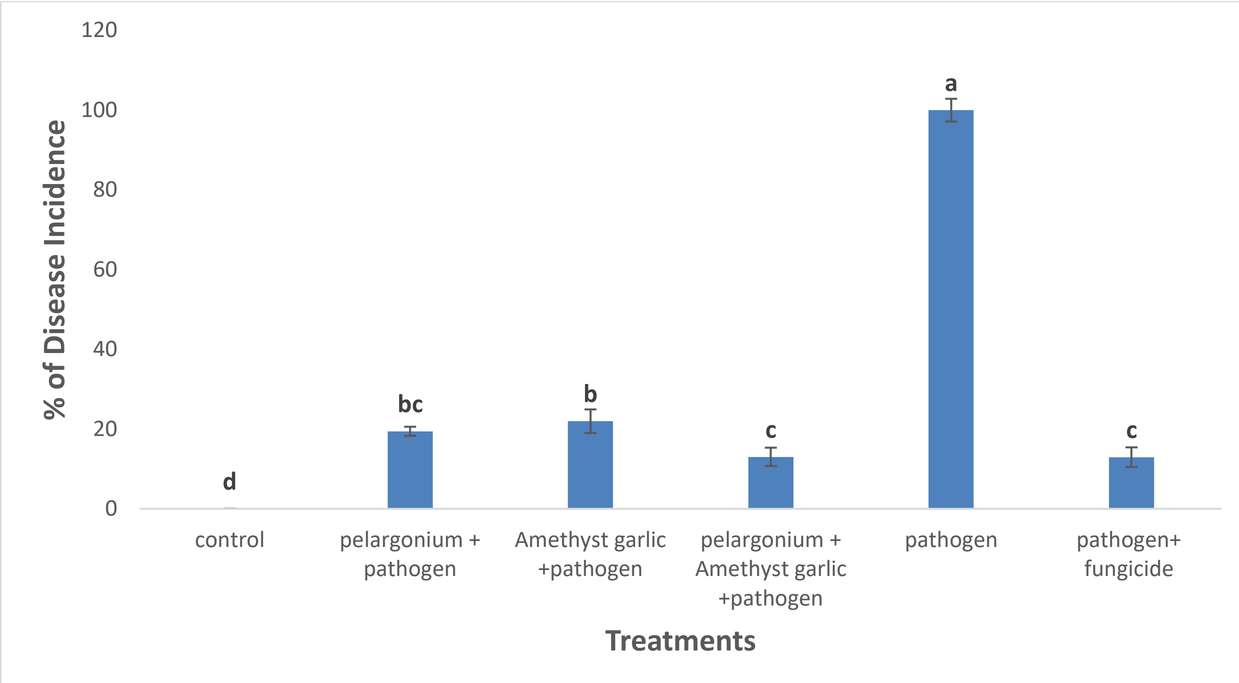

Fig 5. The effect of pelargonium and Amethyst garlic on the disease incidence in strawberry plants infected with *Botrytis cinerea*

The Fig 6 shows the percentage of biocontrol efficacy of Pelargonium and Amethyst garlic alone and in combination against grey mold disease on strawberry plants. The biocontrol efficacy for Pelargonium treatment inoculated with the *B. cinerea* 75% was recorded. The Amethyst garlic treatment shows a biocontrol efficacy of about 70%. When both plants combined showed higher percentage of biocontrol efficacy around 85% which was as effective as fungicide. This result is comparable to the combined Amethyst garlic and pelargonium treatment, highlighting the effectiveness of fungicides in controlling the pathogen.

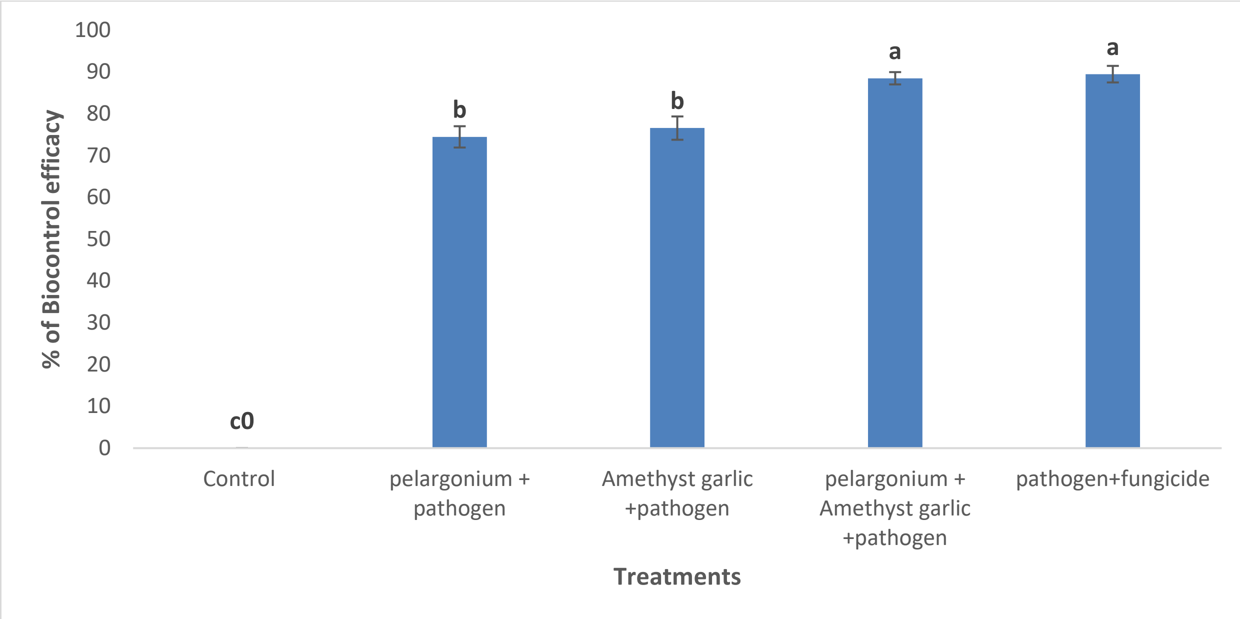

Fig 6. The percentage of biocontrol efficacy of Amethyst garlic and pelargonium against the *B. cinerea* in strawberry plants.

Although there are no reports on the use of these two plants in vivo in the study of their role in reducing plant diseases, several other plants tested in vivo have been reported to act like natural fungicides, hence reducing disease incidence. For instance, tea tree oil significantly reduced the total incidence of strawberry fruit rots (Washington *et al*., 1999; Šernaitė *et al.*, 2019) reported that Allspice extract demonstrate higher inhibition of *B. cinerea* on PDA and strawberry leaves. The aqueous neem extract resulted in an 89.11% reduction in disease severity compared with the control (Gholamnezhad, 2019). Clove extract showed was effective as a biocontrol product (Šernaitė *et al.*, 2020). Additionally, Coriander seeds essential oil as reported by Dėnė *et al.* (2023) that decreased disease severity of strawberry grey mold.

Conclusion

The present study investigates the effect of various medicinal plant extracts on B. cinerea and prolongs the shelf life of strawberry fruits. Strawberry fruits quality was successfully improved, and their shelf life was extended by the use of Amethyst garlic and Pelargonium extracts. In summary, the application of pelargonium and amethyst garlic extracts offers a viable option in grey mold disease management and extends the shelf life of strawberries. The process may help farmers to reduce chemicals and increase fruit quality, making it more environmentally friendly.

Acknowledgements: We acknowledge the invaluable assistance and plant identification provided by Dr. Ali Mala Galaly.

Conflict of interest: The authors declare that they have no competing interests.

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