

## Evaluation of the *in vitro* efficacy of selected plant extracts against strawberry anthracnose disease in highlands of Sri Lanka

Kalupahana Pushpanjie<sup>1\*</sup>, Arulappu George Chandra Babu<sup>2</sup>, Karuna Pedige Harsha Chathuranga<sup>3</sup>, Herath Kumara Bandarage Muditha Indrajith Karunarathna<sup>4</sup>

<sup>1</sup>Agricultural Research Station, Department of Agriculture Thelijjawila, Matara 81060, Sri Lanka

<sup>2</sup>Climate Smart Irrigated Agriculture Project, Ministry of Agriculture, Killinochchi 44000, Sri Lanka

<sup>3</sup>Agricultural Research Station, Department of Agriculture, Seetha Eliya, Nuwara Eliya 22200, Sri Lanka

<sup>4</sup>Government Seed and Planting Material Production Office, Department of Agriculture, Murunkan 41000, Sri Lanka

\*Email address: pushpanjie@yahoo.com

(Received: 20 October 2025, Revision accepted: 11 March 2026)

**Citation:** Pushpanjie, K., Babu, A. G. C., Chathuranga, K. P. H., & Karunarathna, H. K. B. M. I. (2026). Evaluation of the *in vitro* efficacy of selected plant extracts against strawberry anthracnose disease in highlands of Sri Lanka. *Jurnal Lahan Suboptimal : Journal of Suboptimal Lands*. 15(1): 1–6. <https://doi.org/10.36706/JLSO.15.1.2026.758>

### ABSTRACT

Anthracnose was one of the major diseases of strawberry cultivation in Sri Lanka, caused by a fungal pathogen *Colletotrichum acutatum* Simmonds, and the application of synthetic fungicides was the well-known method used in disease management. Since there was a requirement to find alternative methods to control the disease, six plant extracts were tested as an organic management approach. The study aimed to evaluate the *in vitro* efficacy of selected plant extracts against *Colletotrichum acutatum* isolated from strawberry and to identify promising botanical alternatives to synthetic fungicides for sustainable strawberry cultivation in the highlands of Sri Lanka. In well diffusion test, sterilized distilled water, garlic (0.25 g/mL), Mint (0.25 g/mL), rosemary (0.25 g/mL), turmeric (0.25 g/mL), ginger (0.25 g/mL), cinnamon oil (0.25 mL/mL), Fungicide (Chlorothalonil 500 g/L SC (0.003 mL/mL) were the tested treatments. Cinnamon oil suspension (0.25 g/mL) and garlic bulb extract (0.25 g/mL) were able to control *C. acutatum* under *in vitro* conditions. However, according to the sensory evaluation test of plant extracts treated fruits, the most preferable plant extract application was garlic bulb extract, which is having significantly similar “overall acceptance” with untreated fresh strawberry fruits. Based on the results, garlic bulb extract is suggested as an organic disease management method which can be used in an anthracnose management method in organic strawberry production.

Keywords: anthracnose disease, garlic bulb extract, organic management method, sensory evaluation test, strawberry

### INTRODUCTION

Strawberry (*Fragaria × ananassa* Duch.) is a high-value temperate fruit crop cultivated worldwide due to its nutritional, economic, and nutraceutical importance. Global strawberry production has steadily increased over the last decade due to rising consumer demand for fresh and processed berries (FAOSTAT, 2023). In Sri Lanka, strawberry cultivation is mainly concentrated in the Nuwara Eliya district in the central highlands, where cool temperatures and moderate humidity favor crop growth. According to AGSTAT (2020), Sri Lanka exported

approximately 8 tons of strawberries in 2019, highlighting its emerging economic value.

Anthracnose disease, caused primarily by the *Colletotrichum acutatum* species complex, is one of the most destructive diseases affecting strawberries worldwide. Under warm and humid conditions, disease incidence can exceed 50%, resulting in severe yield and quality reduction (Dean et al., 2012; Damm et al., 2012). Recent phylogenetic and molecular investigations have further clarified that the *C. acutatum* species complex consists of genetically diverse lineages with variable pathogenicity and fungicide sensitivity, complicating management strategies (Vieira et al., 2020; Jayawardena et al., 2021). In

tropical and subtropical production systems, climate variability, especially increased rainfall and humidity associated with climate change, has intensified anthracnose outbreaks (Sharma et al., 2022).

Currently, anthracnose management largely depends on synthetic fungicides such as chlorothalonil, azoxystrobin, carbendazim, and other strobilurin and triazole compounds. However, repeated and indiscriminate fungicide applications have resulted in the emergence of resistant *Colletotrichum* populations in several strawberry-producing countries (Forcelini et al., 2016; Oliveira et al., 2022). Additionally, concerns regarding environmental contamination, non-target effects, residue accumulation, and consumer health risks have increased regulatory pressure to reduce synthetic pesticide usage. Since strawberries are harvested at short intervals, maintaining recommended pre-harvest intervals is challenging, thereby increasing the risk of fungicide residues on fresh fruits.

Consequently, there is growing global interest in environmentally friendly and sustainable disease management approaches, particularly in organic and residue-free fruit production systems. Botanical extracts and essential oils derived from medicinal and aromatic plants have gained attention as promising alternatives due to their antifungal, biodegradable, eco-friendly, and relatively low-toxicity properties. Recent studies (2020–2024) demonstrate that plant-derived compounds such as allicin (from garlic), cinnamaldehyde (from cinnamon), eugenol, thymol, and curcumin possess strong inhibitory effects against *Colletotrichum* spp. by disrupting cell membranes, inhibiting spore germination, and interfering with fungal enzymatic systems (Raveau et al., 2020; Kakar et al., 2021; Sharma & Tripathi, 2022; Adeyemi et al., 2023).

Garlic (*Allium sativum*) extract shows significant inhibition of mycelial growth and spore germination of anthracnose pathogens in several fruit crops due to sulfur-containing bioactive compounds. Similarly, cinnamon (*Cinnamomum verum*) essential oil, rich in cinnamaldehyde, has demonstrates broad-spectrum antifungal activity against post-harvest pathogens, including *Colletotrichum* species (Tzortzakis & Economakis, 2020; Ribeiro-Santos

et al., 2021). Extracts from ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), mint (*Mentha* spp.), and rosemary (*Salvia rosmarinus*, formerly *Rosmarinus officinalis*) are also reported to suppress fungal growth through antioxidant and antimicrobial mechanisms (Prakash et al., 2021; Kheawka et al., 2023). These findings indicate the strong potential of plant-based bio-fungicides as components of integrated disease management programs.

Despite extensive international research, limited information is available regarding the efficacy of locally available plant extracts against *C. acutatum* infecting strawberry under Sri Lankan highland conditions. Considering the increasing demand for organic strawberries and sustainable production systems, evaluating indigenous botanical resources is both economically and environmentally important. Therefore, the objective of this research was to evaluate the *in vitro* efficacy of selected plant extracts against *Colletotrichum acutatum* isolated from strawberry and to identify promising botanical alternatives to synthetic fungicides for sustainable strawberry cultivation in the highlands of Sri Lanka.

## MATERIALS AND METHODS

### Preparation

The *in vitro* study was conducted at the Agricultural Research Station, Seetha Eliya, Nuwara Eliya, Sri Lanka, in the division of plant pathology. The causal agent of strawberry anthracnose disease (*C. acutatum*) was isolated from strawberry fields at the Agricultural Research Station, Seetha Eliya, Sri Lanka.

### Implementation

Six plant extracts were evaluated *in vitro* against the fungal pathogen i.e.: - garlic bulbs (*Allium sativum*), mint plants (*Mentha spicata*), rosemary plants (*Rosmarinus officinalis*), turmeric powder (*Curcuma longa*), ginger rhizome (*Zingiber officinale*) and cinnamon oil (*Cinnamomum verum*). The experiment was designed in a Completely Randomized Design with three replicates. Treatments were prepared with water as follows: - T1-sterilized distilled water, T2-garlic (0.25 g/mL), T3-Mint (0.25

g/mL), T4- rosemary (0.25 g/mL), T5-turmeric (0.25 g/mL), T6- ginger (0.25 g/mL), T7-cinnamon oil (0.25 mL/mL), T8-Fungicide (Chlorothalonil 500 g/L SC (0.003 mL/mL). Concentration of plant extracts was decided by a preliminary study and the rate of fungicide was used as DOA recommendation. Garlic, mint, rosemary, and ginger plant materials were cleaned properly by washing with running tap water. Small pieces of the plant materials were crushed, with sterilized distilled water using a sterilized mortar and pestle according to the above ratio, and cinnamon oil and turmeric powder were mixed with water. 0.01% v/v Tween 20 was added to each mixture for even

distribution of components and the mixture was homogenized well by vortexing. Crushed plant materials were centrifuged (8,000 rpm, 15 min, 4 °C) to separate the aqueous phase from solid plant particles. Then, the aqueous solutions were filtered and sterilized using a 0.2 µm cellulose acetate syringe filter to remove any microbial contamination in the plant extracts. 0.01% v/v Tween 20 was added to each plant extract to disperse the plant extract evenly in water. Those aqueous solutions were directly used for *in vitro* study without concentrating them. Well-diffusion method was used to evaluate *in vitro* activity of plant extracts against *C. acutatum* (Egamberdieva et al., 2017).

$$\text{Inhibition} = \frac{\text{Colony diameter of control} - \text{Colony diameter of selected treatment}}{\text{Colony diameter of control}} \times 100 \text{ of Growth\%}$$

Potato dextrose agar (PDA) plates were used in this experiment, and two wells were prepared (0.5 cm in diameter) on opposite sides of petri dish, 2 cm away from the edge of the petri dish. The fungal culture disc (0.5 cm) was kept in the middle of the same petri dish. 200 µL of the plant extract was added to each well in the PDA plate. All those activities were done under aseptic conditions. Culture plates were incubated at 27±1 °C and the fungal culture diameter (cm) of each plate was measured at 3, 5, and 7 days after inoculation by using a ruler. The diameter of fungal culture was measured at the center of fungal culture where the culture and two plant extract wells were in same line. The percentage inhibition of mycelia growth in each treatment was calculated by using the above formula (Thomidis & Filotheou, 2016). Based on the results of the *in vitro* study, the best-performing plant extracts were applied to detached, ripened strawberry fruits. The strawberry fruits were surface sterilized by dipping them in 1% w/v Sodium Hypochlorite solution for one minute and those fruits were washed three times with sterilized distilled water. The surfaced sterilized fruits were air dried in the laminar air flow cabinet, before using them for the experiment (Hosseini et al., 2020). The experiment was designed in Completely Randomized Design with five replicates (three fruits per replicate). Those treated fruits were kept on a bench top of the

laboratory at room temperature (22±1°C) for five days. Then the sensory evaluation of those treated fruits was done. A five point scale was used for evaluation (1= very weak, 2= weak, 3= intermediate, 4= good, and 5= very good) (Lu et al., 2017). Four sensory parameters were evaluated ie: flavors, odor, appearance, and overall acceptance. The sensory evaluation was conducted with trained panelists (five males and five females, aged 20-40 years). Based on that evaluation, the most preferable plant extract for strawberry fruits was selected.

### Data Analysis

Data analysis was done using SAS 9.2 statistical software using one-way ANOVA, and mean separation was done using the Least significant difference (LSD) test.

## RESULTS

According to the results of this *in vitro* study, cinnamon oil (0.25 mL/mL) followed by garlic bulb extract (0.25 g/mL) was able to affect *C. acutatum* fungal mycelia growth (Figure 1). These plant extracts were effective in controlling the pathogenic fungus of the anthracnose disease in Strawberries. After 03 days incubation period, cinnamon oil showed the highest percentage of inhibition of growth in fungal mycelium (45.09%) followed by garlic bulb extract

(26.47%) among the plant extracts. In the meantime, Chlorothalonil fungicide was able to reduce mycelia growth with the highest percentage of inhibition of growth (60.73%) in fungal mycelium among all the treatments. While the incubation period was extended up to 07 days, inhibition of fungal growth % due to cinnamon oil and garlic extract was reduced, but the effectiveness of the fungicide remained as previously. Even though the cinnamon oil treatment was effective under *in vitro* conditions,

based on the sensory evaluation test results, it revealed that cinnamon oil treatment has given a poorer appearance to strawberry fruits comparing to garlic bulb extract treatment and untreated control. Also overall acceptance of the strawberry fruit were high in untreated fruit and garlic bulb extract applied fruit compared to cinnamon oil treated strawberry fruit (Figure 2). Meantime taste of strawberry fruits in all the treatments remains as same in each treatment.

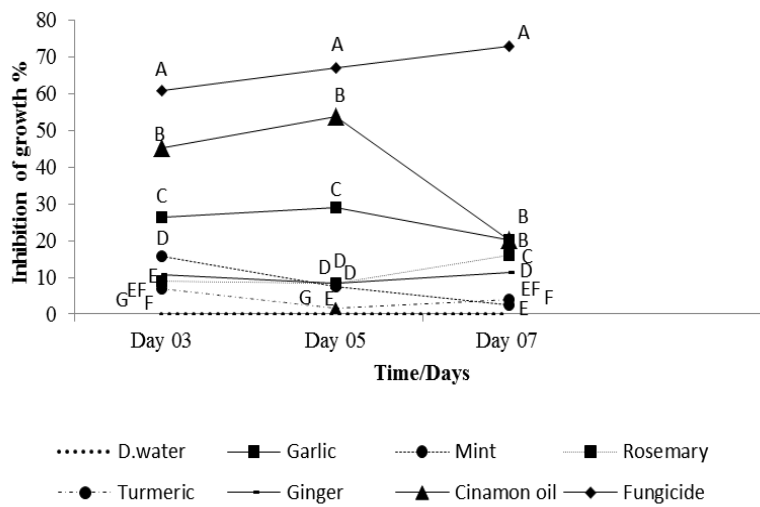


Figure 1. Inhibition of growth (%) of fungal colony during the incubation period

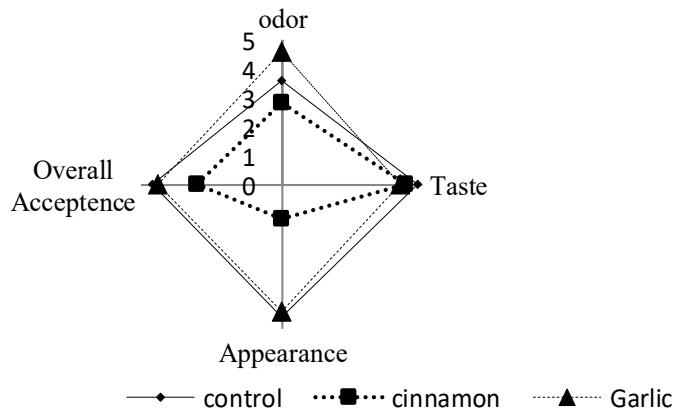


Figure 2. Influence of *A. sativum* and *C. verum* aquase extract application on sensory attributes of strawberry fruits storage at (22±1 °C) for 5 days storage

## DISCUSSION

According to previous *in vitro* studies on *C. acutatum* mycelial growth against concentration series of cinnamon essential oil, 0.100  $\mu\text{L}/\text{mL}$  level was able to inhibit mycelial growth up to 48% (He et al., 2018) which is compatible with the results of this experiment. Cinnamon bark essential oils were effective against *C. acutatum* in both *in vitro* and *in vivo* conditions (Duduk et al., 2015). A study about anthracnose disease in chili, also found that the cinnamon leaf extract was able to control the disease under *in vitro* and field conditions (Alahakoon et al., 2013).

Several experiments showed that some essential oils contained in plant extracts are important to control fungal growth. Cinnamon bark contains essential oils such as trans-cinnamaldehyde, and trans-cinnamyl acetate. Garlic essential oils contain diallyl trisulfide (39.79%) as the main component of essential oils in its' bulbs extract (Gong et al., 2017), and ( $\alpha$ -pinene (15.779%) in rosemary. It also said that those essential oils can inhibit the mycelia growth and conidial germination of *C. nymphaeae* and essential oils from garlic were more effective compared to the rosemary one (Hosseini et al., 2020). Even though the cinnamon oil treatment was effective under *in vitro* condition, based on the sensory evaluation test results, it revealed that cinnamon oil treatment can affect the appearance and overall acceptance of the strawberry fruit compared to untreated fruit and garlic bulb extract applied fruit (Figure 2). The appearance of strawberry fruit is very important, since it is consumed as fresh fruit. So in future studies, sensory evaluation result should be considered together with *in vitro* fungal controlling ability results of plant extract/ their essential oils. It will be useful to develop sustainable non chemical method to control anthracnose disease in Strawberry.

## CONCLUSION

Among the tested plant extracts, cinnamon oil suspension (0.25 g/mL) (by mixing 0.25 mL of cinnamon oil with 1mL of sterilized water and added 0.01% Tween 20 to it) and garlic bulb

extract (0.25 g/mL) (by crushing 0.25 g of garlic with 1mL of sterilized water and added 0.01% Tween 20 to it) was able to control *C. acutatum* under *in vitro* conditions. However, according to the sensory evaluation of treated fruits, the most preferable plant extract application was garlic bulb extract. Based on that this botanical can be used in organic strawberries. Cultivation to control anthracnose disease. Also, this can be used on strawberry fruits to prevent anthracnose disease in the post-harvest stage. Further studies on the efficacy of the active compounds of the plant extract against the pathogen, under field and storage conditions need to be done.

## ACKNOWLEDGEMENTS

We would like to extend our gratitude to all the staff members of Agriculture Research Station Seethaeliya, Nuwaraeliya, Sri Lanka for the contributions, directions, suggestions and providing research facility all through my process.

## REFERENCES

- Adeyemi, M. M., Adegboye, M. F., Akinyemi, A. J., & Oladipo, I. C. (2023). Plant-derived antifungal compounds as sustainable alternatives to synthetic fungicides. *Plants*, 12(7), 14–56. <https://doi.org/10.3390/plants12071456>
- AGSTAT. (2020) (XVII). Socio Economics and Planning Center, Department of Agriculture.
- Alahakoon, P. W., Jayawardana, N. H., Nilmini, R. K., & Munasinghe, R. (2013). Effect of cinnamon, clove and nutmeg leaf extracts on the control of anthracnose disease in chilli. *Annals of the Sri Lanka Department of Agriculture*, 15, 195–203.
- Damm, U., Cannon, P. F., Woudenberg, J. H. C., & Crous, P. W. (2012). The *Colletotrichum acutatum* species complex. *Studies in Mycology*, 73, 37–113. <https://doi.org/10.3114/sim0010>
- Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G. D. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, 13(4), 414–430. <https://doi.org/10.1111/j.1364-3703.2011.00783.x>
- Duduk, N., Markovic, T., Vasic, M., Duduk, B., Vico, I., & Obradovic, A. (2015). Antifungal activity of three essential oils against *Colletotrichum acutatum*, the causal agent of strawberry anthracnose. *Journal of Essential Oil Bearing Plants*, 18(3), 529–537.
- Egamberdieva, D., Wirth, S., Behrendt, U., Ahmad, P., & Berg, G. (2017). Antimicrobial activity of medicinal plants correlates with the proportion of antagonistic endophytes. *Frontiers in Microbiology*, 8, 1–99. <https://doi.org/10.3389/fmicb.2017.00199>

- FAO. (2023). *Food and Agriculture Organization of the United Nations statistical database*. FAO. <https://www.fao.org/faostat/>
- Forcelini, B. B., Seijo, T. E., Amiri, A., & Peres, N. A. (2016). Resistance in *Colletotrichum acutatum* from strawberry to QoI fungicides. *Plant Disease*, *100*(10), 2050–2056. <https://doi.org/10.1094/PDIS-02-16-0193-RE>
- Gong, X., Su, X., Liu, H., Chen, J., & Zhang, J. (2021). Diallyl trisulfide, the antifungal component of garlic essential oil and the bioactivity of its nanoemulsions formed by spontaneous emulsification. *Molecules*, *26*(23), 71–86. <https://doi.org/10.3390/molecules26237186>
- He, J., Wu, D., Zhang, Q., Chen, H., Li, H., Han, Q., Lai, X., Wang, H., Wu, Y., Yuan, J., Dong, H., & Qin, W. (2018). Efficacy and mechanism of cinnamon essential oil on inhibition of *Colletotrichum acutatum*. *Frontiers in Microbiology*, *9*, 12–88. <https://doi.org/10.3389/fmicb.2018.01288>
- Hosseini, S., Amini, J., Saba, M. K., Karimi, K., & Pertot, I. (2020). Preharvest and postharvest application of garlic and rosemary essential oils for controlling anthracnose and quality assessment of strawberry fruit during cold storage. *Frontiers in Microbiology*, *11*, 18–55. <https://doi.org/10.3389/fmicb.2020.01855>
- Jayawardena, R. S., Hyde, K. D., Damm, U., Cai, L., Liu, M., Li, X., & Zhang, W. (2021). Diversity and global distribution of *Colletotrichum* species associated with anthracnose diseases. *Mycosphere*, *12*(1), 1–40.
- Kakar, M. U., Nawaz, A., Saeed, M., Shah, S. A., & Iqbal, Z. (2021). Antifungal mechanisms of plant essential oils against plant pathogenic fungi. *Microbial Pathogenesis*, *152*, 104620. <https://doi.org/10.1016/j.micpath.2020.104620>
- Kheawka, P., Soyong, K., & Kanokmedhakul, S. (2023). Antifungal activity of plant extracts against *Colletotrichum* species causing fruit anthracnose. *Journal of Fungi*, *9*(4), 4–12. <https://doi.org/10.3390/jof9040412>
- Lu, H., Ban, Z., Wang, K., Li, D., Poverenov, E., & Li, L. (2017). Aroma volatiles sensory and chemical attributes of strawberry (*Fragaria × ananassa* Duch.). *International Journal of Food Science & Technology*, *52*, 2614–2622.
- Oliveira, M. S., Amiri, A., & Peres, N. A. (2022). Fungicide resistance in *Colletotrichum* spp.: Current status and management strategies. *Crop Protection*, *158*, 106008. <https://doi.org/10.1016/j.cropro.2022.106008>
- Prakash, B., Kedia, A., Mishra, P. K., & Dubey, N. K. (2021). Plant essential oils as sustainable alternatives for postharvest disease management. *Food Control*, *123*, 107736. <https://doi.org/10.1016/j.foodcont.2020.107736>
- Raveau, R., Fontaine, J., & Lounès-Hadj Sahraoui, A. (2020). Essential oils as potential alternative biocontrol products against plant pathogens. *Molecules*, *25*(14), 32–86. <https://doi.org/10.3390/molecules25143286>
- Ribeiro-Santos, JA dos Santos Oliveira, AP Ferreira, AD Polli, AA da Silva, A da Silva Ribeiro, JL Azevedo, Acta Scientiarum. Technology. (2021). Plant growth-promoting activity of wild-type and bromate-resistant mutant of the endophytic fungus *Colletotrichum karstii*.
- Sharma, R. R., Singh, D., & Singh, R. (2022). Climate change impact on fruit crop diseases and management strategies. *Scientia Horticulturae*, *293*, 110695. <https://doi.org/10.1016/j.scienta.2021.110695>
- Sharma, S., & Tripathi, P. (2022). Botanical fungicides for sustainable crop protection: Recent advances and future prospects. *Biological Control*, *172*, 104971. <https://doi.org/10.1016/j.biocontrol.2022.104971>
- Thomidis, T., & Filotheou, A. (2016). Evaluation of five essential oils as biofungicides on the control of *Pilidiella granati* rot in pomegranate. *Crop Protection*, *89*, 66–71.
- Tzortzakis, N., & Economakis, C. D. (2020). Antifungal activity of essential oils against postharvest pathogens. *Agronomy*, *10*(6), 765. <https://doi.org/10.3390/agronomy10060765>
- Vieira, S. A. W., Bezerra, A. P., Silva, A. C., Veloso, S. J., Camara, S. M., & Doyle, P. V. (2020). Optimal markers for the identification of *Colletotrichum* species. *Molecular Phylogenetics and Evolution*, *143*, 106694. <https://doi.org/10.1016/j.ympev.2019.106694>