

In vitro evaluation of commercial fungicides and biocontrol agents for the management of shallot anthracnose disease

^{1,*}Ahmad, S., ¹Koyube, M.N.K., ¹Rashid, M.S.A., ¹Laboh, R. and ²Sa'at, N.H.M.

¹*Pest and Disease Management Programme, Horticulture Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia*

²*Plant Breeding Programme, Horticulture Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia*

Article history:

Received: 3 May 2024

Received in revised form: 22 May 2024

Accepted: 14 September 2025

Available Online: 3 November 2025

Keywords:

Anthracnose,
Shallot,
Fungicides,
Trichoderma spp.,
Colletotrichum spp.

DOI:

[https://doi.org/10.26656/fr.2017.9\(S3\).4](https://doi.org/10.26656/fr.2017.9(S3).4)

Abstract

Anthracnose disease, caused by the fungus *Colletotrichum* spp., has been found in the shallot cultivation areas of MARDI Bachok Station and MARDI Serdang in Malaysia. This disease poses a significant threat to shallot production, especially in humid areas, leading to substantial yield losses. It is therefore critical to address this disease before initiating large-scale shallot cultivation in Malaysia. The objective of the study was to assess, through in vitro assays, the efficacy of commercial fungicides and the antagonistic activity of *Trichoderma* spp. against *Colletotrichum* spp. The eight fungicides tested in this study were chlorothalonil, propiconazole, hexaconazole, mancozeb, copper oxychloride, benomyl, azoxystrobin, and tebuconazole. The fungus was grown on potato dextrose agar (PDA) with three concentrations (10, 50, and 100 ppm) of each fungicide. Additionally, sixteen isolates of *Trichoderma* spp. were assessed for their antagonistic activity against *Colletotrichum* spp. using the dual culture technique. The in vitro tests of fungicides revealed that three active ingredients, propiconazole, benomyl, and tebuconazole, had significant inhibitory effects on the mycelial growth of *Colletotrichum* spp. At 91.18%, propiconazole showed the highest percentage of inhibition, followed by benomyl at 84.7%, and tebuconazole at 83.14%. Conversely, copper oxychloride and chlorothalonil had the least effect on fungal growth. All sixteen of the *Trichoderma* isolates showed rapid growth and significant inhibition of *Colletotrichum* spp., with inhibition percentages ranging from 56.66% to 82.16% on day 8. These findings indicated that *Trichoderma* spp. has significant antagonistic potential against *Colletotrichum* spp., which requires further confirmation through in vivo applications. In conclusion, the study suggested that both certain fungicides and *Trichoderma* isolates could play important roles in managing anthracnose disease in shallots. These findings can be incorporated into effective disease management strategies to help develop a competitive and sustainable shallot industry in Malaysia.

1. Introduction

Shallot (*Allium cepa*), belonging to the Liliaceae family, is one of the most important commodities in the world, including Malaysia. It is a staple in every cuisine in Malaysia, causing demand to rise annually in tandem with the country's growing population. Due to the absence of commercial shallot cultivation in Malaysia, most shallots are imported. The top five countries that export shallots to Malaysia are India (38%), Pakistan (23%), China (16%), the Netherlands (8%) and Thailand (8%). Malaysians consume approximately 17 kg of shallots per capita annually, which translates to about 1.4

kg per month. This results in a monthly requirement of nearly 36,000 tonnes, as reported by the Ministry of Domestic Trade and Consumer Affairs in 2020. However, global market price volatility can significantly burden consumers. To address this issue and reduce dependency on imports, the Malaysian Agricultural Research and Development Institute (MARDI) has initiated the development of shallot production technology. This initiative aims to create a competitive and sustainable shallot industry in Malaysia, thereby enhancing local supply and stabilising market prices.

Shallot (*Allium cepa*) crops, like other agricultural

*Corresponding author.

Email: suhanna@mardi.gov.my

products, are susceptible to diseases caused by fungi, bacteria, viruses, and nematodes. Anthracnose, purple blotch, basal rot, and downy mildew are major diseases affecting shallot crops, resulting in economic losses. These diseases have been reported in countries such as Indonesia and India (Dinakaran *et al.*, 2013; Safitri *et al.*, 2019). Anthracnose disease is a major concern in shallot cultivation, particularly in humid regions and can reduce yield by up to 90% (Mishra *et al.*, 2014, Bambang *et al.*, 2014). Anthracnose, caused by the fungus *Colletotrichum* spp., has been found in shallot cultivation areas at MARDI Bachok Station in Kelantan and the MARDI headquarters in Serdang, Selangor in Malaysia. Symptoms of anthracnose include white spots on leaves that turn brown, and concentric rings of acervuli with orange conidial masses in necrotic areas. As the disease progresses, infected leaves twist, break, droop, and the lesion on the bulb becomes visible, resulting in bulbs rotting and plant death (Dutta *et al.* 2022). The identification of *Colletotrichum* spp. as the cause of anthracnose provides essential information for developing disease management strategies to reduce losses in shallot production in Malaysia. However, because Malaysia's shallot industry is still in its early stages, there is limited information available on the prevalence and impact of this disease. Understanding the types of diseases that affect shallots is crucial for implementing effective preventive and control measures. The current study aimed to evaluate the efficacy of fungicides and *Trichoderma* isolates against anthracnose disease. The findings will contribute to the development of future disease management practices, resulting in a strong and sustainable shallot industry in Malaysia.

2. Materials and methods

2.1 Isolation and maintenance of pathogen and antagonist

Trichoderma spp. was cultured on Potato Dextrose Agar (PDA). The PDA plates were incubated at room temperature for 5 to 7 days to allow sufficient growth for subsequent experiments. The pathogen causing anthracnose, *Colletotrichum* spp., was isolated from infected shallot leaves. The diseased tissues were washed and dipped for 1 min in 70% ethanol to surface sterilise, followed by re-washing with sterile distilled water. The sterilised tissues were then plated onto PDA. Mycelia that emerged from the tissue were sub-cultured to ensure pure pathogen cultures. A 5mm diameter disc was transferred from these cultures to fresh PDA plates using a cork borer to obtain pure cultures of *Colletotrichum* spp.

2.2 In vitro evaluation of fungicides against *Colletotrichum* spp.

A total of eight (8) different commercial fungicides (Table 1) were evaluated in vitro using the food poison technique to determine their efficacy against *Colletotrichum* spp. The fungicides were tested at three different concentrations of 10, 50, and 100 parts per million (ppm). A mycelium disc of *Colletotrichum* spp. was punched from a 10-day-old colony using a sterilized cork borer. The mycelium disc was placed in the centre of each PDA plate containing the fungicides. PDA plates without fungicides were used as a control. The plates were incubated at 28°C for 10 days, and each concentration was replicated three times. After 10 days of incubation, the diameter of the fungal colony on each plate was measured. The percentage inhibition of the fungus was calculated using the formula provided by Wonglom (2019):

$$\text{Percentage inhibition (\%)} = \left(\frac{C - T}{C} \right) \times 100$$

Where C = Colony diameter in control and T = Colony diameter in treatment

Table 1. Eight (8) evaluated fungicides for in vitro evaluation against *Colletotrichum* spp.

No	Active ingredient	Trade Name	Concentrations (ppm)
1	Chlorothalonil	Daconil	10, 50 and 100
2	Propiconazole	Tilt	10, 50 and 100
3	Hexaconazole	Anvil	10, 50 and 100
4	Mancozeb	Kencozeb	10, 50 and 100
5	Copper oxychloride	Coprantol 870	10, 50 and 100
6	Benomyl	Kenlate	10, 50 and 100
7	Azoxystrobin	Amistar	10, 50 and 100
8	Tebuconazole	Folicur	10, 50 and 100

2.3 In vitro evaluation of bioagents against *Colletotrichum* spp.

The mycelial discs obtained from the 10-day-old culture of *Colletotrichum* spp. were placed 1.5 cm from the centre of the PDA plate, while the mycelial disc of *Trichoderma* spp. was placed 3 cm away from the mycelial disc of *Colletotrichum* spp. on the same plate. Control plates contained only *Colletotrichum* spp. without *Trichoderma* to serve as a baseline for comparison. All dual-culture plates were prepared in triplicate and incubated at 28 °C for 7 days. The response of *Colletotrichum* spp. against *Trichoderma* spp. was observed. The percentage inhibition of radial growth (PIRG) of *Colletotrichum* spp. in the presence of *Trichoderma* spp. was calculated using the formula from Jinantana *et al.* (1998):

$$\% \text{PIRG} = \left(\frac{R1 - R2}{R1} \right) \times 100$$

Where R1 = Radial growth of *Trichoderma* in the absence of the antagonist; R2 = Radial growth of *Trichoderma* in the presence of the antagonist.

2.4 Statistical analysis

All experiments were carried out in completely randomised design (CRD) with three replicates. All data were subjected to variance analysis (ANOVA) using SAS 9.4. Means values were compared by LSD to determine the effect of treatments.

3. Results and discussion

3.1 Evaluation of fungicides

Three of the eight fungicides tested in vitro were effective in inhibiting the growth of *Colletotrichum* spp. at all three concentrations (10 ppm, 50 ppm, and 100 ppm) when compared to the control. Propiconazole inhibited *Colletotrichum* spp. growth at the highest rate (91.18%), followed by benomyl at 84.7% and tebuconazole at 83.14% (Table 2 and Figure 1). All fungicides performed best at 100 ppm, with the lowest inhibition observed at 10 ppm. Figure 2 shows the experimental effects of different fungicides on the colony growth of *Colletotrichum* spp.

Similar findings have been reported in other studies. Ranjitha et al. (2019) found that propiconazole was 85% effective against *C. gloeosporioides*. Ravi et al. (2020) observed 100% mycelial inhibition of *C. gloeosporioides* by both tebuconazole and propiconazole, followed by hexaconazole with an 85.41% inhibition rate. These findings confirm that fungicides containing propiconazole, benomyl, and tebuconazole are effective at inhibiting the growth of *Colletotrichum* spp. These findings suggest that incorporating these fungicides into disease management strategies can effectively control anthracnose disease in shallots, reducing the economic impact of this pathogen.

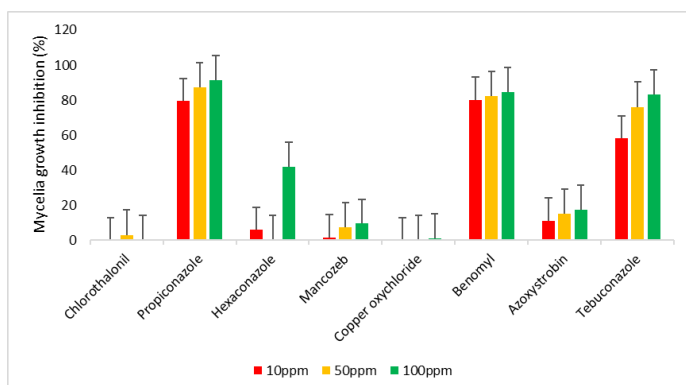


Figure 1. In vitro evaluation of fungicides against *Colletotrichum* spp.

Table 2. Percentage inhibition of the growth of *Colletotrichum* spp. Against three different concentrations (10, 50 and 100 ppm) of eight different fungicides tested in vitro at day 10.

Active Ingredient	Mycelial growth inhibition (%)		
	Fungicide concentration (ppm)		
	10	50	100
Chlorothalonil	0 ^k	2.94 ^{jk}	0 ^k
Propiconazole	79.41 ^{bc}	87.25 ^{ab}	91.18 ^a
Hexaconazole	5.88 ^{ijk}	29.22 ^f	41.96 ^e
Mancozeb	1.57 ^{jk}	7.26 ^{hijk}	9.4 ^{hij}
Copper oxychloride	0 ^k	0 ^k	0.79 ^k
Benomyl	80.2 ^{bc}	82.35 ^{bc}	84.7 ^{ab}
Azoxystrobin	11.17 ^{ghi}	14.9 ^{gh}	17.45 ^g
Tebuconazole	58.24 ^d	76.08 ^c	83.14 ^{bc}

Values are presented as means. Values with different superscripts are statistically significantly different ($p < 0.05$) using LSD.

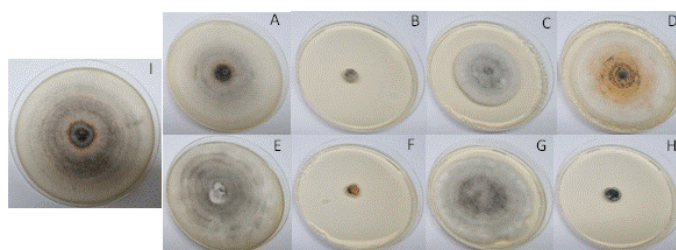


Figure 2. The effect of eight (8) different fungicide on the growth of a *Colletotrichum* spp. colony at 100 ppm. A. Chlorothalonil B. Propiconazole. C. Hexaconazole. D. Mancozeb. E. Copper oxychloride. F. Benomil. G. Azoxystrobin. H. Tebuconazole. I. Control

3.2 Evaluation of bioagents

Most *Trichoderma* spp. grew rapidly in dual culture experiments and showed significant inhibition abilities against *Colletotrichum* spp., with varying efficiencies as shown in Table 3. The dual culture experiments revealed that all 16 *Trichoderma* spp. isolates inhibited the growth of *Colletotrichum* spp. to varying degrees, ranging from 56.66% to 82.16%. Inhibition began 24 hours after the experiment started and gradually increased.

Trichoderma asperellum (TR15) had the greatest inhibitory effect on *Colletotrichum* spp. growth, with an 82.16% reduction and completely covering the pathogen colony after 10 days (Figure 3). During the dual culture test, *T. asperellum* grew faster than *Colletotrichum* spp., giving it a competitive advantage for nutrients and space. According to Reynaldo et al. (2018), this effect could be attributed to a mycoparasitism mechanism, as *T. asperellum* produces a lot of chitinases, which are enzymes that degrade fungal cell walls. Other research has shown that *Trichoderma* spp. can produce antibiotics and volatile compounds that inhibit pathogen growth

(Stracquadiano et al., 2020), Ruangwong et al., 2021).

The promising results of *T. asperellum* (TR15) in vitro indicate for further evaluation through in vivo applications to determine its efficacy in controlling anthracnose in shallots. While good in vitro results are encouraging, they do not always imply beneficial antagonistic effects in vivo. Future research will focus on testing these promising *Trichoderma* isolates in the field to confirm their potential as biocontrol agents.

Table 3. In vitro evaluation of *Trichoderma* spp. against mycelial growth of *Colletotrichum* spp.

Code	Strain	Isolates of <i>Trichoderma</i>	Percentage of inhibition (%)
TR1	1510	<i>T. asperellum</i>	77.65 ^{ab}
TR2	1509	<i>T. asperellum</i>	76.08 ^{ab}
TR3	1505	<i>T. harzianum</i>	56.66 ^d
TR4	1417	<i>T. harzianum</i>	72.35 ^{ab}
TR5	1451	<i>T. harzianum</i>	61.17 ^{cd}
TR6	1450	<i>T. virens</i>	61.18 ^{cd}
TR7	1430	<i>T. harzianum</i>	70.78 ^{abc}
TR8	1447	<i>T. resei</i>	72.55 ^{abc}
TR9	1428	<i>T. virens</i>	67.45 ^{bcd}
TR10	1501	<i>T. harzianum</i>	74.90 ^{ab}
TR11	1448	<i>T. asperellum</i>	74.71 ^{ab}
TR12	1514	<i>T. harzianum</i>	76.27 ^{ab}
TR13	1615	<i>T. asperellum</i>	67.84 ^{bcd}
TR14	1470	<i>T. virens</i>	56.86 ^d
TR15	ADBT	<i>T. asperellum</i>	82.16 ^a
TR16	LOD24	<i>T. harzianum</i>	66.47 ^{bcd}

Values are presented as means. Values with different superscripts are statistically significantly different ($p < 0.05$) using LSD.

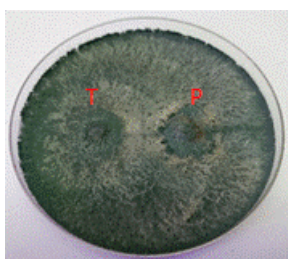


Figure 3. Antagonism of *T. asperellum* (TR15) against *Colletotrichum* spp. Colony of *Colletotrichum* spp. completely destroyed by *T. asperellum* after 10 days.

4. Conclusion

The study found that the fungicides propiconazole, benomyl, and tebuconazole effectively inhibited the growth of *Colletotrichum* spp., the cause of anthracnose disease in shallots. Additionally, *T. asperellum* (TR15) showed promising results as a biocontrol agent for anthracnose disease. Integrating fungicide treatment and the use of *Trichoderma* spp. can significantly reduce the

economic losses caused by shallot anthracnose. *Trichoderma*-based products offer a safe and environmentally friendly alternative to chemical fungicides, making them a viable option for farmers. They pose no risk of chemical residues, which can be a concern with excessive use of chemical fungicides, potentially exceeding maximum allowable residue levels and posing health risks.

In conclusion, while fungicides such as propiconazole, benomyl, and tebuconazole can effectively control *Colletotrichum* spp., incorporating *T. asperellum* (TR15) as a biocontrol agent provides a sustainable and safe approach to managing anthracnose disease in shallots. This integrated disease management strategy can help to ensure farmer safety as well as the environmental sustainability of shallot cultivation in Malaysia.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research was supported by the 12th Malaysia Plan (RMK-12) MARDI Development Fund numbered P21400010170526.

References

- Bambang, H.I. and Khusnul, M. (2014). Effectiveness of resistance and biopesticide induction on *Cercospora* and Anthracnose leaves in chili (*Capsicum annum* L.). *Planta Tropika Journal of Agro Science*, 2(2), 106-114
- Dinakaran, D., Gajendran, G., Mohankumar, S., Karthikeyan, G., Thiruvudainambi, S., Jonathan, E.I., Samiyappan, R., Pfeiffer, D.G., Rajotte, E.G., Norton, G.W., Miller, S. and Muniappan, R. (2013). Evaluation of integrated pest and disease management module for shallots in Tamil Nadu, India: A farmer's participatory approach. *Journal of Integrated Pest Management*, 4(2), B1-B9. <https://doi.org/10.1603/IPM12019>
- Dutta, R.K.J., Nadig, S.M., Manjunathgowda, D.C., Gurav, V.S. and Singh, M. (2022). Anthracnose of onion (*Allium cepa* L.): A twister disease. *Pathogens*, 11(8), 884. <https://doi.org/10.3390/pathogens11080884>
- Jinantana, J. and Sariah, M. (1998). Potential for biocontrol of Sclerotium foot rot of chilli *Trichoderma* spp. *Pertanika*, 21, 1 – 10.
- Ministry of Domestic Trade and Consumer Affairs. (2020). Bekalan bawang mencukupi dan stabil.

- Retrieved from website: <https://www.kpdn.gov.my/en/media-kpdnhep/beritakpdn/berita-terkini/2020-berita-terkini/782-bekalan-bawangmencukupi-dan-stabil>
- Mishra, R.K., Jaiswal, R.K., Kumar, D., Saabale, P.R. and Singh, A. (2014). Management of major diseases and insect pests of onion and garlic: A comprehensive review. *Journal of Plant Breeding and Crop Science*, 6(11), 160–170. <https://doi.org/10.5897/JPBCS2014.0467>
- Ranjitha, N., Devappa, V. and Sangeetha, C.G. (2019). Evaluation of fungicides against *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. the incitant of mango anthracnose. *Journal of Pharmacognosy and Phytochemistry*, 8(3), 812-814.
- Ravi, M., Yenjerappa, S.T., Amaresh, Y.S., Sreedevi, S.C. and Jaiprakash, N.R.P. (2021). Efficacy of different fungicides by in vitro against *Colletotrichum gloeosporioides*, the causal agent of mango anthracnose. *International Journal of Chemical Studies*, 9(1), 3408-3412. <https://doi.org/10.22271/chemi.2021.v9.i1av.11762>
- Reynaldo, D.C.Q., Sevastianos, R., Raul, R.H., Daniel, H.C. and Cristobal, N.A. (2018). Growth inhibition of *Colletotrichum gloeosporioides* and *Phytophthora capsica* by native Mexican *Trichoderma* strains. *Karballa International Journal of Modern Science*, 4 (2), 237-243. <https://doi.org/10.1016/j.kijoms.2018.03.002>
- Ruangwong, O.-U., Wonglom, P., Suwannarach, N., Kumla, J., Thaochan, N., Chomnunti, P., Pitija, K. and Sunpapao, A. (2021). Volatile Organic Compound from *Trichoderma asperelloides* TSU1: Impact on Plant Pathogenic Fungi. *Journal of Fungi*, 7, 187. <https://doi.org/10.3390/jof7030187>
- Safitri, Y.A., Hasanah, U., Salamiah, S. and Pramudi, M.I. (2019). Distribution of major diseases of shallot in South Kalimantan, Indonesia. *Asian Journal of Agriculture*, 3, 33–40. <https://doi.org/10.13057/asianjagric/g030201>
- Stracquadanio, C., Quiles, J.M., Meca, G. and Cacciola, S.O. (2020). Antifungal activity of bioactive metabolites produced by *Trichoderma asperellum* and *Trichoderma atroviride* in liquid medium. *Journal of Fungi*, 6(4), 263. <https://doi.org/10.3390/jof6040263>
- Wonglom, P., Ito, S. and Sunpapao, A. (2020). Volatile organic compounds emitted from endophytic fungus *Trichoderma asperellum* T1 mediate antifungal activity, defense response, and promote plant growth in lettuce (*Lactuca sativa*). *Fungal Ecology*, 43, 100867. <https://doi.org/10.1016/j.funeco.2019.100867>