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Efficacy of Botanical Extracts, Biocontrol agents and Fungicides against Alternaria brassicicola Causing Leaf Spot of Cabbage (*Brassica oleracea* var. *capitata* L.)

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A B S T R A C T

This study investigated the in vitro efficacy of various management strategies against *Alternaria brassicicola* infecting cabbage (*Brassica oleracea* var. *capitata*). Aqueous extracts from *Barleria prionitis* L. and *Solanum virginianum* L. leaves exhibited concentration-dependent antifungal activity, with *Barleria prionitis* extract demonstrating the highest inhibition (80.75%) at 6% concentration. GC-MS analysis of the *Barleria prionitis* extract identified β-sitosterol, oleanolic acid, lupeol, and barlerin, all of which have documented antifungal properties. Similarly, *Solanum virginianum* extract displayed significant inhibition (82.44%) at 8% concentration, with phytol and solanidine identified as potential antifungal constituents. Commercially available fungicides (Bavistin, Mancozeb, Carbendazim and Captan) also inhibited *A. brassicicola* growth, with Bavistin exhibiting the strongest activity (100% inhibition at all tested concentrations). *Trichoderma viride* displayed superior antagonistic potential (83.09% inhibition) compared to *Trichoderma harzianum* (64.31%) against the pathogen. The findings suggest the potential of botanical extracts and biocontrol agents as eco-friendly alternatives for managing *Alternaria brassicicola* in cabbage cultivation. Further research is needed to explore the fungicidal mechanisms of identified bioactive compounds and optimize their application strategies for field efficacy.

Introduction

Cole crops, including cabbage (*Brassica oleracea* var. *capitata*), cauliflower, kale, Brussels sprouts, broccoli, and kohlrabi, are essential components of a healthy diet due to their rich nutrient content and dietary fiber (Guerena, 2006). These vegetables are susceptible to various fungal diseases, with *Alternaria brassicicola* causing significant yield losses worldwide (Saharan *et al.,* 2016). The disease manifests as necrotic lesions on leaves, hindering photosynthesis and ultimately impacting crop productivity (Chowdhury *et al.,* 2013). While conventional management relies on chemical fungicides, their indiscriminate use raises concerns about environmental pollution, human health risks, and potential development of fungicide resistance in pathogens (Ahmad and Ashraf, 2016). In recent years, there has been a growing interest in exploring eco-friendly alternatives for disease control in agricultural crops. Plant-based extracts have emerged as promising

candidates due to their inherent bioactivity, minimal environmental impact, and potential for cost-effectiveness (Kavita and Dalbeer, 2015; Khalse *et al.,* 2017). Additionally, biological control agents like *Trichoderma* species offer an attractive option for suppressing plant diseases through various mechanisms (Hajieghrari *et al.,* 2008; Kumar *et al.,* 2011). This study aimed to evaluate the efficacy of various management strategies against *Alternaria brassicicola* infecting cabbage. We investigated the antifungal activity of commercially available chemical fungicides and botanical extracts derived from readily available plant materials. We further explored the biocontrol potential of *Trichoderma sp.*, a well-known antagonist of several fungal pathogens. The findings of this research could contribute to the development of a sustainable and effective disease management strategy for *Alternaria* spot in cabbage cultivation.

Materials and Methods

The experiment was conducted in the Plant Pathology Laboratory of the Department of Botany at JES College, Jalna, Maharashtra. We employed the poisoned food technique (Thaware *et al.,* 2010; Roopa *et al.,* 2014) to screen the antifungal activity of various treatments against the target fungus. 0.Four commercially available fungicides, Mancozeb, Bavistin, Carbendazim, and Captan, were evaluated at four different concentrations: 500 ppm, 1000 ppm, and 1500 ppm.

Additionally, aqueous extracts prepared from the leaves of *Barleria prionitis* L. and *Solanum virginianum* L. were tested at four concentrations: 2%, 4%, 6%, and 8%.To assess the biocontrol potential of *Trichoderma harzianum* and *Trichoderma viride*, a separate dual culture technique was employed (Babu *et al.,* 2000). All treatments were replicated three times to ensure statistical robustness.

Isolation of fungal specimen

The fungal pathogen, *Alternaria brassicicola* was isolated from the leaf spot infected leaves of cabbage plant (Figure 1 - A) collected from a cabbage farm in Rohanwadi, Jalna, following the tissue segmentation method. The pure culture of the isolated pathogen (Figure 1 B) was maintained on Potato Dextrose Agar slants at 4±1ºC. On the basis of morphological characters of conidia as described by Yu (2015); Corlett and Mac Latchy (1996a, 1996b) pathogen was identified as *Alternaria brassicicola*.

Preparation of Botanical Extracts and Fungicide Solutions

Botanical extracts were prepared from fresh leaves of *Barleria prionitis* L. and *Solanum virginianum* L. collected locally. The Soxhlet extraction method was employed to isolate bioactive compounds using ethanol as the solvent (Gupta *et al.,* 2012; Tesfaye and Tefera, 2017). After the extraction was complete, the ethanol solvent was evaporated using a rotary evaporator at a reduced pressure and controlled temperature (e.g., 40°C) to minimize thermal degradation of the extracted compounds (Baskaran *et al.,* 2018). The concentrated extract residue was then weighed to determine the yield. The dried extract was subsequently dissolved in a known volume of sterilized distilled water (e.g., 100 mL) to prepare the 100% stock solution for further GCMS analysis and antifungal assays at 2%, 4%, 6%, and 8% concentration. Similar procedures were followed for preparing the chemical fungicide solutions. Calculated amounts of stock solutions of Mancozeb, Bavistin, Carbendazim, and Captan were mixed with sterilized Potato Dextrose Agar (PDA) to achieve final concentrations of 500 ppm, 1000 ppm, and 1500 ppm. Twenty milliliters of either the amended PDA media with the botanical extracts or the fungicide solutions were poured into individual sterilized 90 mm petri plates and allowed to solidify. Control plates contained PDA without any added botanical extracts or fungicides.

Mycelial Inoculation and Incubation

A 7 mm diameter disc of fungal culture (*Alternaria brassicicola*) obtained from a 9-day-old culture was aseptically cut using a sterilized cork borer and inoculated in the center of each solidified media plate (amended with botanical extracts, fungicides, or control). Each treatment was replicated in three petri plates. The inoculated plates were then incubated at 27 ± 1°C for seven days.

Evaluation of Biocontrol Potential using Dual Culture Technique

The antagonistic activity of *Trichoderma harzianum* and *Trichoderma viride* isolates was assessed against *Alternaria brassicicola* using the dual culture technique. Twenty milliliters of sterilized Potato Dextrose Agar (PDA) were poured into each petri plate. A 7 mm diameter mycelial disc from a actively growing culture of *Alternaria brassicicola* was placed on one side of the

plate, approximately 1 cm from the edge. On the opposite side of the plate, at a similar distance from the edge, a 7 mm diameter mycelial disc from a 7-day-old culture of either *T. harzianum* or *T. viride* was placed. Control plates were inoculated with only the pathogen (*Alternaria brassicicola*) without the addition of any biocontrol agent. Each treatment was replicated in three petri plates to ensure statistical robustness. All plates were incubated at 27 ± 1°C for seven days.

Determination of antifungal activity

Following incubation for seven days at 27°C, the extent of fungal growth in each treatment was measured using a vernier caliper. The percentage inhibition of mycelial growth relative to the control was calculated using the formula described by Vincent (1947).

$$PGI = \frac{C - T}{C} X100$$

Where, PGI- Percent growth inhibition, C - Growth of hyphae in control (mm) and T = Growth of hyphae in treatment (mm).

Gas Chromatography-Mass Spectrometry (GCMS) analysis

Gas Chromatography-Mass Spectrometry (GCMS) analysis GC-MS analysis of plant leaf extract (Ethanol) was done at the Sophisticated Analytical Instrument Facility (SAIF) labs, MIT CARS, Department of agriculture Engineering College, Aurangabad, Maharashtra using standard GCMS mode. The procedure followed was of Dandekar *et al.,* (2015).

Results and Discussion

Antifungal Activity of Botanical Extracts

This study investigated the in vitro antifungal activity of aqueous extracts from *Barleria prionitis* L. and *Solanum virginianum* L. leaves against *Alternaria brassicicola*. Both extracts exhibited concentration-dependent inhibition of the fungal pathogen (Table 01). The highest level of growth inhibition (80.75%) was observed with *Barleria prionitis* extract at a concentration of 6%. *Solanum virginianum* extract displayed a maximum inhibition of 76.31% at 8% concentration. These findings support previous research demonstrating the potential of plant extracts for controlling fungal pathogens. For example, Singh *et al.,* (2011) reported complete inhibition of *Alternaria* sp. in *A. calamus* at a concentration of 1% (v/v). Similarly, Goussous *et al.,* (2010) observed remarkable antifungal effect against *A. solani* mycelial at concentrations exceeding several medicinal plants extracts. Additionally, studies by Sasode *et al.,* (2012) and Singh *et al.,* (2013) documented significant antifungal activity of some plant extracts against *Alternaria brassicae* and *Alternaria brassicicola*. However, our results regarding *Solanum virginianum* extract differ from findings reported by Shrestha and Tiwari (2009) and Biswas and Gosh (2018). These discrepancies may be attributed to inherent physiological variations among different *Alternaria* species. As suggested by Shrestha and Tiwari (2009), the antifungal activity of various botanical extracts can also be influenced by the specific content of active antifungal compounds present in the extracts.

Antifungal Activity of Chemical Fungicides

The effectiveness of four commercially available fungicides (Mancozeb, Bavistin, Carbendazim, and Captan) against *Alternaria brassicicola* was evaluated using the poisoned food technique. All fungicides significantly inhibited fungal growth compared to the control (90 mm average diameter).

The extent of inhibition increased with increasing fungicide concentration (Table 2). Among the tested fungicides, Bavistin displayed the most potent antifungal activity, achieving complete growth inhibition (100%) at highest tested concentrations. Carbendazim followed closely, with inhibition ranging from 62.72% to 82.17% depending on concentration. Mancozeb exhibited moderate activity, with inhibition ranging from 73.84% to 76.18%. Captan was the least effective fungicide, with a maximum inhibition of only 51.66% at the highest concentration (1500 ppm).

Our findings on the relative efficacy of these fungicides partially align with previous research. Madadi *et al.,* (2021) reported complete inhibition of *A. alternata* using hexaconazole, followed by mancozeb and then carbendazim. Similarly, Das *et al.,* (2023) observed complete inhibition of *A. brassicicola* by hexaconazole at higher concentrations.

Table.1 *Invitro* efficacy of plant extracts/botanicals against *Alternaria brassicicola*

Tr. No	Treatments	Colony Dia. *(mm) at Conc. of				Av. (mm)	% Inhibition* at Conc. of				Av. Inhibition (%)
		2%	4%	6%	8%		2%	4%	6%	8%	
T1	*Barleria prionitis* L.	24.12	21.23	17.32	20.32	20.74	73.2	76.41	80.75	77.42	76.95
T2	*Solanum virginianum* L.	32.12	28.32	23.21	21.32	26.24	64.43	68.53	74.21	76.31	63.76
T3	Control	90	90	90	90		90	90	90	90	

Table.2 *Invitro* efficacy of fungicide against *Alternaria brassicicola*

Tr. No	Treatments	Colony Dia. *(mm) at ppm of			Av. (mm)	% Inhibition* at ppm of			Av. Inhibition (%)
		500	1000	1500		500	1000	1500	
T1	Bavistin	08.36	00.00	00.00	02.78	90.71	100	100	96.91
T2	Mancozeb	23.54	28.35	21.44	24.44	73.84	68.5	76.18	70.62
T3	Carbendazim	33.55	22.45	16.05	24.02	62.72	75.06	82.17	73.32
T4	Captan	48.21	43.23	43.23	45.55	46.33	49.74	51.66	49.38
T5	Control	90	90	90	90	00.00	00.00	00.00	00.00

Table.3 *Invitro* efficacy of Bioagents against *Alternaria brassicicola*

Treatment	Colony diameter of test pathogen (mm)	Control (mm)	% Inhibition
T. harzanium	32.12	90	64.31
T. viride	15.22	90	83.09

GC-MS Analysis of *Barleria prionitis* L. Leaf Extract

Table.4 Putative Antifungal Compounds Identified in *Barleria prionitis* L. Leaf Extract

Compound Name	Retention Time (min)	Peak area %	Molecular Formula	Molecular Weight (g/mol)	Reference
β-sitosterol	9.793	7.6839	$C_{29}H_{50}O$	414.7	Khan and Javaid (2020).
Oleanolic acid	12.06	9.68	$C_{30}H_{48}O_3$	456.7	Choi *et al*., (2017)
Lupeol	17.37	8.93	$C_{30}H_{50}O$	426.7	Javed, *et al*., (2021); Do *et al*., (2022)
Barlerin	19.37	82.636	$C_{19}H_{28}O_{12}$	448.42	Gangaram *et al*., (2021)

Table.5 Putative Antifungal Compounds Identified in *Solanum virginianum* L. Leaf Extract

Compound Name	Retention Time (min)	Peak area %	Molecular Formula	Molecular Weight (g/mol)	Reference
Phytol	5.987	25.52	$C_{20}H_{40}O$	128.17	Liu *et al*., (2017)
Solanidine	7.239	1.95	$C_{27}H_{43}NO$	397.6	Kwon *et al*., (2003); El-Naggar *et al*., (2010)

Figure.1 (A) Symptoms of leaf spots on cabbage with concentric rings on lesions; (B) 7-day-old culture of *A. brassicicola* on PDA agar; (C) conidia of *A. brassicicola*

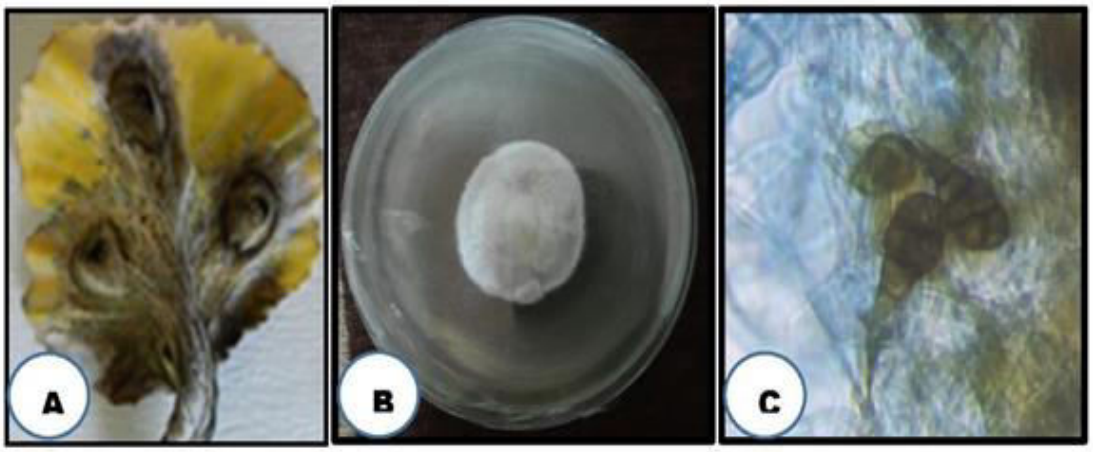


Figure.2 GC-MS chromatogram of ethanolic extract of leaf of *Barleria prionitis* L.

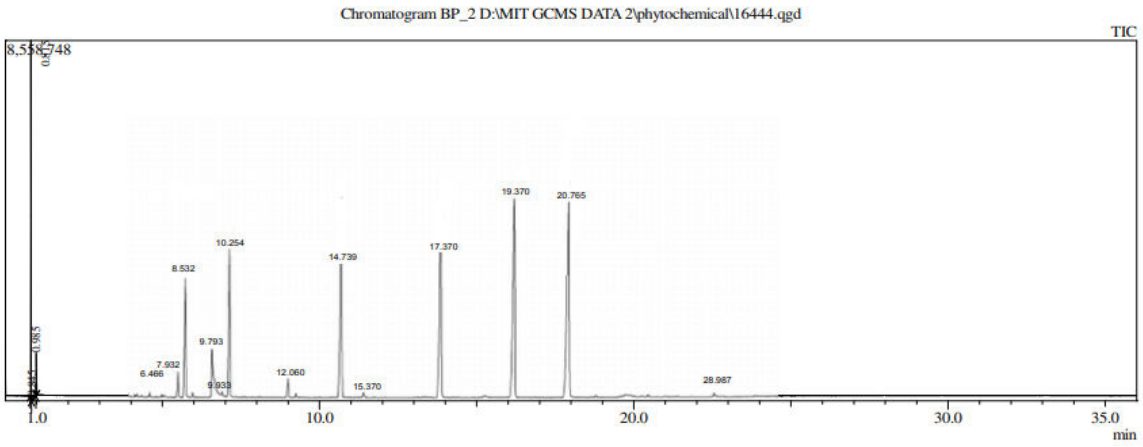
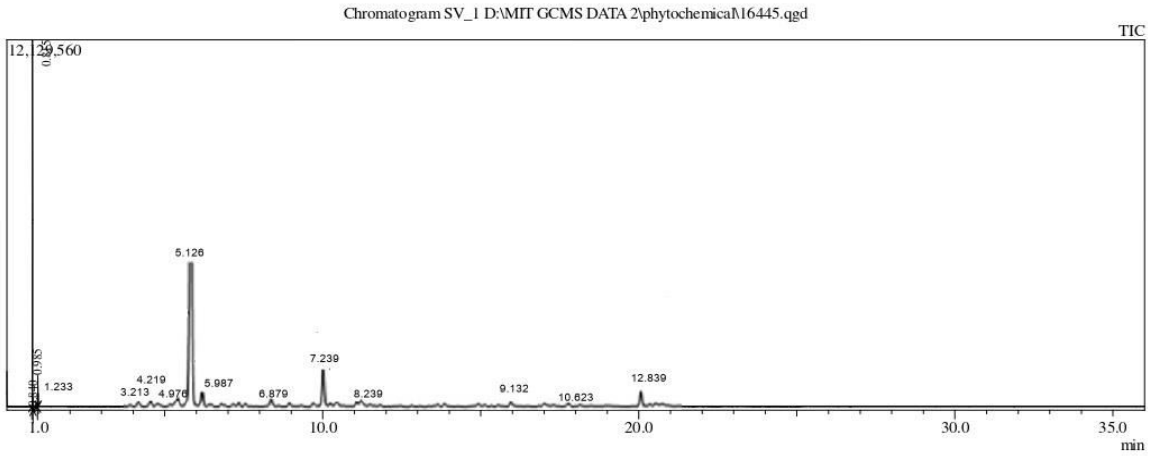


Figure.3 GC-MS chromatogram of ethanolic extract of leaf of *Solanum virginianum* L.



However, our results differ regarding carbendazim, which showed higher efficacy in some prior studies (Ishieze *et al.,* 2023) and (Pun *et al.,* 2020). These discrepancies may be attributed to factors such as the specific *Alternaria* species studied and inherent differences in fungal susceptibility.

Antifungal Activity of *Trichoderma* Isolates

The antagonistic potential of *Trichoderma viride* and *Trichoderma harzianum* isolates obtained from soil was evaluated against *Alternaria brassicicola* using the dual culture technique. After seven days of incubation, *T. viride* exhibited the highest level of growth inhibition (83.09%) against the pathogen, followed by *T. harzianum* (64.31%), compared to the control (Table 3). These findings are consistent with previous research by Wongamthing and Sainamole (2023), who reported 71.85% inhibition of *A. solani* by *Trichoderma* sp. Similarly, Metz and Hausladen (2022) and Meena *et al.,* (2017) documented the inhibitory effect of *T. harzianum* against various fungal pathogens. The antagonistic activity of *Trichoderma* species is attributed to a combination of mechanisms. Production of volatile and non-volatile antibiotics, such as acetaldehyde (Kithan and Daiho, 2014), has been demonstrated to play a role could be involved in the mycoparasitic colonization of the fungal host by *Trichoderma* isolates (Mukherjee *et al.,* 2022).

To elucidate the potential bioactive constituents responsible for the observed antifungal activity of the *Barleria prionitis* L. leaf extract, gas chromatography-mass spectrometry (GC-MS) analysis was employed. The ethanolic extract was subjected to GC-MS analysis, resulting in the identification of 14 major components within the chromatogram (Figure 2).

Tentative identification of these components was achieved by comparing their mass spectra to established libraries within the NIST database. Compounds with documented or predicted antifungal properties from the identified list are presented in Table 4.

The GC-MS analysis identified a diverse range of compounds in the ethanolic extract of *Barleria prionitis* L. leaves. Several studies of Rahman and Choudhary (2001); Liu *et al.,* (2017); Wang *et al.,* (2005); El-Naggar and Ghanem (2010) stated that these compounds, including (β-sitosterol, Oleanolic acid, Lupeol, Barlerin) have been reported to possess antifungal activity in previous studies. The presence of these potential antifungal compounds in the *Barleria prionitis* extract may contribute to its observed inhibitory effect on *Alternaria brassicicola* growth.

GCMS analysis of *Solanum virginianum* L. Leaf Extract

Similar to the analysis of *Barleria prionitis* L., GC-MS was employed to characterize the bioactive constituents potentially responsible for the antifungal activity observed in the ethanolic extract of *Solanum virginianum* L. leaves. The analysis identified a complex profile of compounds, with a total of 13 major peaks evident in the chromatogram (Figure 3). The mass spectra of these

peaks were compared against reference libraries within the NIST database for tentative identification. Table 5 summarizes the compounds with potential antifungal properties among identified compounds.

The GC-MS analysis revealed a rich tapestry of bioactive compounds within the *Solanum virginianum* L. leaf extract. Interestingly, some of the identified compounds, such as Phytol and Solanidine, have been previously linked to antifungal activity.

Yadav and Koshi (2022) demonstrated that Phytol and Solanidine exhibits antifungal properties against various fungal species. The presence of these potential antifungal compounds in the *Solanum virginianum* extract might contribute to its inhibitory effect on *Alternaria brassicicola* growth. Further research is necessary to isolate, purify, and characterize the antifungal compounds identified in this study.

This study explored eco-friendly alternatives for controlling *Alternaria brassicicola*, the fungus causing devastating leaf spot disease in cabbage. Aqueous extracts from *Barleria prionitis* L. and *Solanum virginianum* L. leaves displayed concentration-dependent antifungal activity, with GC-MS analysis revealing potential antifungal compounds like β-sitosterol and phytol within them. While commercial fungicides like Bavistin showed strong inhibition, this research highlights the need for sustainable solutions. *Trichoderma viride*, a natural biocontrol agent, demonstrated superior antagonistic potential against the pathogen. Future investigations to isolate and characterize these antifungal compounds and conduct in vivo field trials are crucial to validate their efficacy and pave the way for a more sustainable future for cabbage cultivation, minimizing reliance on chemical fungicides and safeguarding the environment.

Author Contribution

Sara Shaikh and Rutuja Ghone: Conceptualization, Methodology, Investigation, Data Curation, Yogesh Urdukhe: Formal analysis, Writing – Original Draft, Writing – Review and Editing, Umesh Mogle: Supervision.

Data Availability

This includes data collected from experiments, such as inhibition zone measurements, fungal growth data, GC-MS chromatograms, and mass spectra.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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