Original Research Article

Unravelling the fungal bioagents and botanicals efficacy for the *invitro* management of leaf blight disease of *Stevia rebaudiana* caused by *Alternaria alternata* (Fr.) KEISSLER

Abstract

Stevia rebaudiana, a herbaceous perennial prized for its natural sweetness, has gained global acclaim and found in various parts of India including Karnataka. Investigation on leaf spot disease (*Alternaria alternata* (FR.) Keissler) of Stevia under south Karnataka condition was carried out to find out suitable management strategies. Symptoms initially appeared as small circular spots, light brown in colour. Later, became irregular and dark brown to grey, while others remained circular with concentric rings or zones. On severely affected leaves several spots coalesced to form large necrotic areas. On older leaves concentric spots were more common at the tips. Leaf spots varied from 2-18 mm in diameter. Conidial dimensions varied from 10–40 × 6-12 mm, mid to dark brown or olive-brown in colour, short beaked, borne in long chains, oval and bean shaped with 3–5 transverse septa. Due to hazardous effect of chemical fungicides, search for safer alternative to control the pathogen is better choice. This led to trials on the use of bioagents to control the pathogen. The six known bioagents were evaluated by dual culture, pathogen at periphery and pathogen at the center technique to monitor antagonistic effect. The results revealed that out of all the six bioagents used, two bioagents viz., *Trichoderma viride* (Bangalore isolate) (74.77%, 69.04% and 79.45%) and *T. harzianum* (71.25%, 59.96% and 74.78%) showed maximum growth inhibition in dual culture, pathogen at periphery and pathogen at the center methods, respectively. Unraveled the strong antagonistic effect to inhibit the mycelia growth of the pathogen significantly.

Key word: Stevia, Leaf spot, *Alternaria alternata*, Bioagent, Trichoderma

1. Introduction

Stevia [*Stevia rebaudiana* (Bertoni)] belongs to the family Asteraceae. A herbaceous perennial prized for its a natural sweetener with nutritional, therapeutic and industrial importance is being used across the globe. It is native of Paraguay and South-West Brazil also called as sweet leaf, sugar leaf, sweet honey leaf and methi tulsi. Stevia leaf contains more than 20 steviol glycosides, *viz.*, stevioside and rebaudioside A, B, C and D etc. It is 100–300 times sweeter than sucrose and widely used as zero calorie sweetener all over the world on commercial scale (5). The sweetening property of stevia is well documented in literature and its extracts (stevioside and rebaudioside–A) are considered safe for human consumption without any side effects (4). In India, cultivation of stevia started due to more demand from diabetic medicine market and now the country's total annual production is

nearly about 600 tonnes of dry leaf. The climatic conditions in most parts of India are quite favourable for stevia cultivation and it is mainly grown in Rajasthan, Punjab, Uttar Pradesh, Madhya Pradesh, Karnataka, Chhattisgarh and West Bengal (11). However, the cultivation of this remarkable herb is not without challenges. One of the key concerns is leaf spot disease, primarily caused by *Alternaria alternata* (FR.) Keissler. It is one of the most destructive diseases, commonly prevailing in almost all stevia growing pockets of India.

Investigation on alternaria leaf spot disease of Stevia under South Karnataka condition was carried out to find out suitable management strategies. The pathogen affects all the above ground parts *viz.,* leaf, petiole and stem. The symptoms include small, dark brown necrotic lesion. Symptoms initially appeared as small circular spots, light brown in colour. As the infection progresses, these spots became irregular and darken to shades brown to grey, while others remained circular with concentric rings or zones. Severely affected leaves can coalesce, forming large necrotic areas. Older leaves are more prone to concentric spots, often concentrated at the tips. Under favourable condition, it results in defoliation, drying off of twig. The pathogen is both air and soil borne (REFERENCE?).

Due to hazardous effects of chemical fungicides on both the environment and human health have necessitated a quest for safer alternatives to manage the *Alternaria alternata* pathogen in Stevia cultivation. This shift towards sustainable and environmentally friendly practices led to the evaluation of bioagents as potential biological control agents against *Alternaria alternata* was carried out in this study.

2. Materials and Methods

2.1 Collection and isolation of the pathogen

The leaves of stevia showing typical symptoms of the leaf blight symptoms were collected from GKVK, UAS, Bengaluru. The standard tissue isolation technique was followed to isolate the pathogen. Dilute spore suspension was prepared; spreader on agar surface and incubated at 27±2°C; single spore was isolated and used for morphological characterization to identify the pathogen.

2.2 *In vitro* evaluation of botanical extracts on the mycelial growth of *A. alternata*

To evaluate the antifungal activity of botanicals fresh samples were washed in tap water and finally washed thrice using sterilized distilled water. They were crushed in a sterilized pestle and mortar by adding little quantity of alcohol (1:1 w/v) just enough to moisten the samples so that it was easy to crush. The extracts were strained through the two layers of muslin cloth. Finally, filtrates thus obtained from the leaves were used as stock solution (1). To study the antifungal mechanism of plant extracts, poisoned food technique was followed as suggested by (7). Mean colony diameter in each case was recorded. The efficacy of the botanicals was expressed as per cent inhibition of mycelial growth over control which was calculated by using the formula as given by (12). The botanicals used in this study were given in table 1.

$$I = \frac{C-T}{C} \times 100$$

I = Per cent inhibition

C = No. of spores germinated in control

T = No. of spores germinated in treatment

Table 1 : Botanical and common name of species

Sl. No.	Botanical name	Common name	Plant parts used
1	*Azadirachta indica*	Neem	Leaves
2	*Bougainvillea spectabilis*	Bougainvillea	Leaves
3	*Lantana camara* L.	Lantana	Leaves
4	*Psidium guajava*	Guava	Leaves
5	*Allium sativum* L.	Garlic	Bulb
6	*Moringa oleifera*	Moringa	Leaves
7	*Cymbopogan ambiguous*	Lemon grass	Leaves
8	*Allium cepa* L.	Onion	Bulb
9	*Zingiberis officinalis*	Ginger	Rhizome

2.3 *In vitro* evaluation of bioagents on the mycelial growth of *A. alternata*

Bio-agents were evaluated for their efficacy through dual culture technique. Twenty ml of sterilized and cooled potato dextrose agar medium was poured into sterilized Petri plates. Fungal antagonists were evaluated by inoculating a pathogen at one side of the Petri plate and the antagonist at exactly opposite side of the same plate by leaving about 4 cm gaps. For this, actively growing cultures were used. After required period of incubation i.e., when the growth in control plate recorded 90 mm in diameter, the radial growth of the pathogens was measured. Per cent inhibition over control was worked out according to the equation given by (12). The six different fungal antagonistic organisms were used against stevia leaf spot pathogens was given in table 2.

Sl. No.	Bio-agents	Isolates	Source
1	*Trichoderma viride*	TV	Department of Microbiology, GKVK
2	*Trichoderma viride*	TV-2	Department of Plant Pathology, GKVK

3	*Trichoderma viride*	TV-3	Department of Plant Pathology, GKVK
4	*Trichoderma harzianum*	Th-41	Department of Plant Pathology, GKVK
5	*Trichoderma harzianum*	Th-44	Department of Plant Pathology, GKVK
6	*Trichoderma harzianum*	Th	Department of Plant Pathology, Chintamani

Table 2: List of fungal bio-agents evaluated against *A. alternata*

3 Results and discussion

3.1 Collection and isolation of the pathogen

In culture, the fungal colony was initially white, cottony with profuse aerial mycelium which gradually turned greenish grey. Aged culture appeared completely brown with no aerial mycelium. The microscopic studies of the isolated fungus revealed that conidia of the pathogen were three - four celled, Conidiophores short to long, simple or branched arising singly.Conidiophores were hyaline to golden brown coloured. Conidia are typically muriform, dark brown, thick walled, in long chains. Based on the characters of colony and morphological characters of conidiophores and conidia, the fungus was identified as *Alternaria alternata* (9) (10) (8).

3.2 *In vitro* evaluation of botanicals against *A.alternata*

The study was carried out to know the antifungal activity nature of different plant extracts against *A.alternata* by poison food technique. Based on the observation of radial growth of the fungus, the per cent inhibition was calculated. The effectiveness of different plant extracts in reducing the mycelial growth of *A.alternata* varied greatly noted in fig 1 and plate1.

The results presented in Table 3 and figure 1 revealed statistical difference between plants extract per cent inhibition at three different concentrations with three replications. Neem (36.63 **%**) was found to be most effective and statistically on par with Ginger (36.42 %) and lemon grass (36.41 %). The least inhibition of mycelial growth was observed in Moringa (18.76 %) .

The findings are in collaboration with the earlier findings (2) reported that plant extract of *Azadirachta indica* exhibited maximum mycelial inhibition of 80.53 mm at 15 per cent concentration, whereas *Allium sativum* showed 21.60 mm of mycelial inhibition which was significantly lower over rest of plant extracts.

Naik *et al.* (2020) evaluated botanicals to know the antifungal potential against *A. solani* by poisoned food technique at 10 per cent concentration. In case of crude extraction method, the maximum inhibition was obtained by *Azadirachta indica* (60.49 %), *Zingiber officinale* (54.73 %) and *Ocimum sanctum* (53.09 %), whereas, acetone extraction method

showed slight increase in inhibition of mycelial growth of *A. solani* by *A. indica* (64.24 %), *O. sanctum* (58.62 %) and *Z. officinale* (57.32 %).

Sl. No.	Botanicals	Per cent inhibition over control			
		Concentration (%)			
		10 %	**15 %**	**20 %**	**Mean**
1	Ginger	23.33 (28.87) *	38.89 (38.56) *	47.04 (43.28) *	36.42 (33.09) *
2	Garlic	22.22 (28.11)*	32.22 (34.57)*	35.56 (36.59) *	30.00 (33.09) *
3	Lantana	18.89 (25.75) *	30.00 (33.20)*	34.44 (35.92)*	27.77 (31.62)*
4	Guava	16.67 (24.09)*	22.22 (28.11)*	31.11 (33.89)*	23.33 (28.69)*
5	Onion	28.89 (32.50) *	32.59 (34.80)*	38.89 (38.56)*	33.44 (35.28)*
6	Neem	30.37 (33.43)*	36.67 (37.25)*	40.00 (39.22)*	36.63 (36.63)*
7	Moringa	8.52 (16.96)*	19.63 (26.29)*	28.15 (32.03)*	18.76 (25.09)*
8	Lemon grass	32.22 (34.57)*	34.81 (36.15)*	42.22 (40.51)*	36.41 (37.07)*
9	Bougainvillea	10.00 (18.43)*	21.85 (27.86)*	27.78 (31.79)*	19.87 (26.02)*
Mean		21.23 (26.96)*	29.87 (32.97)*	36.13 (36.86)*	29.18 (31.84)*
		Botanicals (B)	**Concentration(C)**		**Interaction(B ×C)**
S.Em ±		0.22	0.12		0.40
CD @ 1 %		0.86	0.50		1.50

Table 3: *In vitro* evaluation of botanicals against *A.alternata*

Fig 1: *In vitro* evaluation of botanicals against *A. alternata*

T0: Control,T1: Ginger, T2: Garlic, T3: Lantana,T4: Guava, T5: Onion, T6: Neem,

T7: Moringa, T8: Lemon grass, T9: Bougainvillea

3.3 Evaluating the efficacy of *Trichoderma* spp. as bioagents

Six known bioagents were tested for their efficacy in controlling *Alternaria alternata* through dual culture, pathogen at the periphery, and pathogen at the center techniques. The results were promising, with two bioagents, *Trichoderma viride* (TV) (79.45 %) was found highly superior in inhibiting the mycelial growth followed by *Trichoderma harzianum* (TH) (74.78 %). The *T. viridae* 2 (TV2), demonstrating the highest levels of growth inhibition in dual culture which was on par with *Trichoderma harzianum* (TH). The least inhibition was recorded in *T. harzianum*-41 (56.96 %). These findings unveil the strong antagonistic effect of *Trichoderma* spp., significantly inhibiting the mycelial growth of the *Alternaria alternata* pathogen (table 4 and figure 2).

Sl. No.	Fungal bioagent	Per cent inhibition over control*
1	*T. harzianum*-41 (TH-41)	56.96 (48.98)*
2	*T. harzianum* -44 (TH-44)	71.25(57.55)*
3	*T. harzianum* (TH)	74.78 (59.83)*
4	*Trichoderma viride* 2 (TV2)	74.77 (59.82)*
5	*Trichoderma viride* -3 (TV3)	69.45 (56.42)*
6	*Trichoderma viride* (TV)	79.45 (63.02)*

	SEm ±	0.46
	CD @ 1%	1.98

Table 4: *In vitro* evaluation of bioagents against *A.alternata*

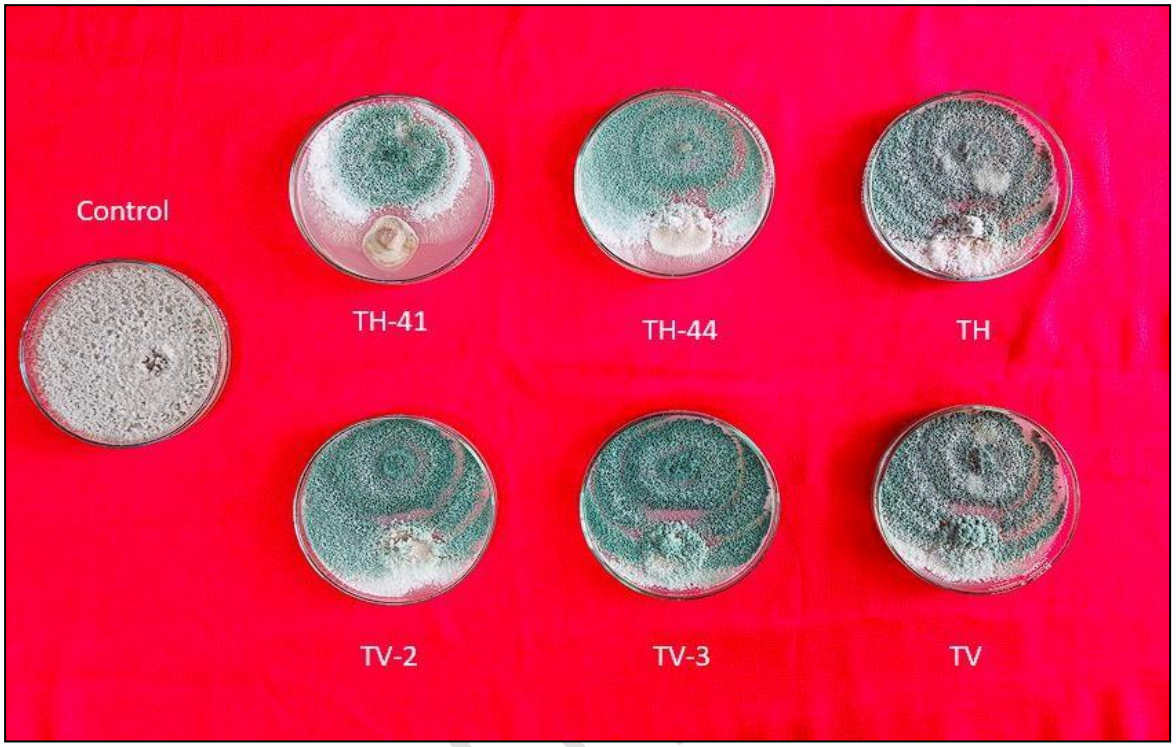

Plate 1: *In vitro* evaluation of fungal bio agents against *A. alternata*

The findings are in collaborate with the earlier findings of The findings are in collaborate with the earlier findings of (3) tested. All the seven fungal and two bacterial antagonists, exhibited significant mycelial growth inhibition of *A. alternata*. However, *T. viride* recorded significantly highest mycelial growth inhibition (86.85 %) of the test pathogen, followed by *T. hamatum* (82.04 %) and *A. niger* (81.11 %).

4. Conclusion

The prevalence of *Alternaria alternata*-induced leaf spot disease poses a significant threat to Stevia cultivation in South Karnataka. However, this study's findings offer hope for effective and sustainable management strategies by harnessing the biological control potential of *Trichoderma viride* and *T. harzianum*. By reducing the reliance on chemical fungicides and embracing bioagents, Stevia cultivators can mitigate the impact of leaf spot disease and promote a healthier and more environmentally friendly agricultural ecosystem in the region. Further research and implementation of these biological control methods hold promise for the future of Stevia production in South Karnataka.

5.Reference

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