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Bio-intensive management of Fusarium rot (*Fusarium oxysporum* Schlecht) in small cardamom

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Abstract
The recent surge of Fusarium infections has made it increasingly difficult to cultivate healthy small-cardamom (*Elettaria cardamomum*) plants. In the field, the pathogen manifests as capsule rot, seed decay, seedling wilt, stem rot and lodging, rhizome and root-tip rot, and leaf yellowing. To address this, a field trial was carried out in Santhanpara, Vandanmedu, Parathode and Kattappana. The objective was to assess the individual and combined effects of several biocontrol agents *Pseudomonas fluorescens* (Strains IDK-S-1, IDK-V-2, IDK-P-3, IDK-K-4), Bacillus subtilis (strains IDK-S-1, IDK-V-2, IDK-P-3, IDK-K-4), *Trichoderma harzianum* (strains IDK-S-1, IDK-V-2, IDK-P-3, IDK-K-4) and arbuscular mycorrhizal fungi (AMF; strains IDK-S-1, IDK-V-2, IDK-P-3, IDK-K-4) on plant growth, yield and suppression of Fusarium wilt. The causal agent of wilt was identified as *Fusarium oxysporum* Schlecht. Five native bacterial antagonists and one fungal antagonist were isolated from forest soil and from healthy cardamom plantations in different regions.
Under field conditions, a combined foliar spray and soil drench of *P. fluorescens* (Pf-IDK-S-1) together with B. subtilis (Bs-IDK-V-2) reduced the pathogen's mycelial growth by 60 % relative to single-strain applications. These two strains were compatible. The greatest disease reduction, however, was achieved by a soil application of *P. fluorescens* (IDK-S-1) together with *T. harzianum* (IDK-P-3). Moreover, plots treated with the *P. fluorescens* + *B. subtilis* mixture showed a significant increase in plant height and a yield gain of up to 27 % compared with untreated control plots.

Keywords: Small cardamom, *F. oxysporum*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma harzianum*

Introduction
India has long been known as the "land of spices," and among the many varieties cultivated there, black pepper holds the title of "king of spices." Spices contribute flavor, colour, aroma and preservative qualities to foods and beverages, and they can be derived from virtually any part of a plant-bark, buds, flowers, fruit, leaves, rhizomes, roots, seeds, stigmas, styles, or whole-plant material. Globally, spices are a staple ingredient in food processing and cooking. Cardamom, often called the "queen of spices," is obtained from the seeds of the perennial herb *Elettaria cardamomum* (family: Zingiberaceae). Although native to the coastal regions of India, cardamom is now cultivated in Guatemala, Tanzania, Sri Lanka, El Salvador, Vietnam, Laos and Cambodia. India remains the leading exporter of dried cardamom. The plant is a tropical herb that grows from a thick rhizome to a height of 1.8-3 m and is indigenous to the evergreen forests of the Western Ghats in southern India. The intensive use of synthetic pesticides to control pests and diseases has led to high residue levels in export-oriented produce, prompting concerns and questioning of market acceptability for both king and queen spices. Consequently, consumer demand for organic spices is rising at about 20 % per year (Krishnakumar, 2015) [30]. Maintaining India's position as the world's spice hub now depends on producing safer, high-quality spices through eco-friendly agricultural practices. Fusarium rot, caused by *Fusarium oxysporum* Schlecht, is a widespread and economically important fungal disease of small cardamom (Thomas & Vijayan, 2002) [29]. Under favourable conditions the pathogen can affect the crop at any growth stage, and climate change is expected to increase its severity. Dhanya *et al*. (2018) [29] reported up to 50 % yield loss in poorly managed fields. The fungus persists in soil and crop residues for many years, making control difficult.

Initial symptoms appear as pale, discoloured lesions on tillers, leading to dry rot. Infected tillers weaken, often breaking or bending at the point of infection. Additional symptoms include a burnt appearance of panicles, root tip rot, and yellowing of foliage, all of which reduce yield (Murugan *et al*., 2016) [29]. Given growing concerns about environmental pollution and human health, an integrated disease-management approach is essential. Combining biocontrol agents with other bio-inputs has been shown to improve vegetative growth, increase yield, and suppress Fusarium rot (Dhanya *et al*., 2018) [29]. The present study, titled "Bio-intensive Management of Fusarium Rot (*Fusarium oxysporum* Schlecht) in Small Cardamom," aims to assess disease severity in Idukki district, develop bio-intensive management strategies, and evaluate their impact on soil and plant health.

Materials and Methods

The research project "Bio-intensive Management of Fusarium Rot (*Fusarium oxysporum* Schlecht) in Small Cardamom (BIDDMFC) with Locally Isolated Strains" was conducted in small-cardamom plantations at Santhanpara, Vandanmedu, Parathode and Kattappana in Idukki district from 2021 to 2023. Fusarium rot typically appears during the summer months, so a survey was performed from February to May across the 2021-2023 seasons in the two main cardamom-growing blocks of Idukki-Santhanpara and Vandanmedu. One panchayat from each block was selected, and three 1-hectare plantations were chosen per panchayat. Each plantation was split into four plots of 250 plants, and ten plants per plot were sampled. From each sampled plant, five tillers were randomly selected and scored using the disease-severity scale described by Dhanya *et al*. (2018) [29].

0 = No disease
1 = 1-10% of tillers or panicles had fungal lesions
2 = 11-25% of tillers or panicle had fungal lesions
3 = 26-50% of tillers had fungal lesions with drying of panicles from tip
4 = 51-75% of tiller stake hold of fungal lesions with partial panicle blight

Based on the data, disease severity was and Percent Disease Index (PDI) was calculated using the formula of Singh (2002).

$$PDI = \frac{\text{Sum of score}}{\text{Total number of tillers observed}} \times \frac{100}{\text{maximum score}}$$

$$\text{Disease Incidence} = \frac{\text{No. of infected plants}}{\text{Total number of plants observed}} \times 100$$

Root staining for Arbuscular Mycorrhizal Fungi

At the conclusion of the trial, root samples from plants that had received arbuscular-mycorrhizal fungal (AMF) inoculation were harvested and rinsed with water. The roots were placed in a container with 10 % KOH (w/v) ensuring the liquid level did not exceed half the container's height. After an overnight soak, the KOH solution was replaced with fresh alkali and the roots were heated in a water bath until the liquid turned brown, indicating the removal of residual tannins. The roots were then rinsed three times with tap water. Next, a 5 % HCl solution was added for about one minute, after which it was discarded. The roots were stained with trypan blue and examined under a microscope following the protocol of Philip and Haymann (1970).

$$\text{Percent root colonization} = \frac{\text{No. of root bits showing colonization}}{\text{Total number of root bits observed}} \times 100$$

A five-year-old, well-established plantation of the locally popular Njallani cultivar known for its high susceptibility to Fusarium wilt was selected for the study. The trial incorporated seven bio-control treatments applied both as basal soil amendments and as foliar sprays. Field trials were carried out at four locations-Santhanpara, Vandanmedu, Parathode and Kattappana to assess the performance of individual and combined applications of Pseudomonas fluorescens (strains IDK-S-1, IDK-V-2, IDK-P-3, IDK-K-4), Bacillus subtilis (strains IDK-S-1, IDK-V-2, IDK-P-3, IDK-K-4), *Trichoderma harzianum* (strains IDK-S-1, IDK-V-2, IDK-P-3, IDK-K-4) and arbuscular mycorrhizal fungi (strains IDK-S-1, IDK-V-2, IDK-P-3, IDK-K-4) on growth, yield and Fusarium wilt suppression in small cardamom under field conditions.

Table 1: Treatment details of different local strains to manage Fusarium rot of small cardamom

Treatments	*IDK-S-1	*IDK-V-2	*IDK-P-3	*IDK-k-4
T_1-*Pseudomonas fluorescens*	Spraying of PF-IDK-S-1 @ 2.5 ml per L of water (3 times)	Spraying of PF-IDK-V-2 2.5 ml per L of water (3 times)	Spraying of PF-IDK-P-3 2.5 ml per L of water (3 times)	Spraying of PF-IDK-k-4 2.5 ml per L of water (3 times)
T_2-*Bacillus subtilis*	Spraying of BS-IDK-S-1 2.5 ml per L of water (3 times)	Spraying of BS-IDK-V-22.5 ml per L of water (3 times)	Spraying of BS-IDK-P-3 2.5 ml per L of water (3 times)	Spraying of BS-IDK-k-4 2.5 ml per L of water (3 times)
T_3-*Trichoderma harzianum*	Basal application of TH-IDK-S-1 (1 kg) with Neem cake (10 kg) and FYM(90 kg)	Basal application of TH-IDK-V-2 (1 kg) with Neem cake (10 kg) and FYM(90 kg)	Basal application of TH-IDK-P-3 (1 kg) with Neem cake (10 kg) and FYM(90 kg)	Basal application of TH-IDK-k-4 (1 kg) with Neem cake (10 kg) and FYM(90 kg)
T_4-AMF	Basal application of AMF-IDK-S-1 @ 50 gm to the root zone at the time of planting (3 times)	Basal application of AMF IDK-V-2 @ 50 gm to the root zone at the time of planting (3 times)	Basal application of AMF-IDK-P-3 @ 50 gm to the root zone at the time of planting (3 times)	Basal application of AMF-IDK-k-4 @ 50 gm to the root zone at the time of planting (3 times)
T_5-*Pseudomonas fluorescens* + *Bacillus subtilis*	Spraying of PF+BS-IDK-S-1 @ 2.5 ml per L of water (3 times)	Spraying of PF+BS-IDK-V-2 2.5 ml per L of water (3 times)	Spraying of PF+BS-IDK-P-3 2.5 ml per L of water (3 times)	Spraying of PF-IDK-k-4 2.5 ml per L of water (3 times)
T_6-*Trichoderma harzianum* + AMF	Basal application of TH+AMF-IDK-S-1 @ 50 gm to the root zone at the time of planting	Basal application of TH+AMF IDK-V-2 @ 50 gm to the root zone at the time of planting	Basal application of TH+AMF-IDK-P-3 @ 50 gm to the root zone at the time of planting	Basal application of TH+AMF-IDK-k-4 @ 50 gm to the root zone at the time of planting
T7 Control (untreated plants)	-	-	-	-

*IDK-S-1-Santhanpara Local Strains, IDK-V-2-Vandanmedu Local Strains, IDK-P-3-Parathode and IDK-K-4-Kattappana.

Results and Discussion

A field experiment was conducted to assess the efficacy of selected bio-agents (individually and combination) for the management of the disease in Idukki district of Kerala. The experiment was laid out using Randomized Block Design (RBD) consisting of seven treatments with three replications. Basal application of TH+AMF-IDK-S-1, TH+AMF-IDK-V-2, TH+AMF-IDK-P-3 and TH+AMF-IDK-k-4 @ 50 gm with the individual of Trichoderma and AMF different strains and combination of TH+AMF to the root zone at the time of planting, Spraying of PF+BS-IDK-S-1, PF+BS-IDK-V-2, PF+BS-IDK-P-3, PF-IDK-k-4 @ 2.5 ml per L of water with individual and combination of bio-agents at monthly interval for three times resulted in average effective disease management (disease incidence: 30% and disease severity: 24.26%) compared to the inoculated control (disease incidence: 90% and disease severity: 59.38%) (Table. 2 & 3).

Table 2: Biometric characters, disease incidence and disease Severity of cardamom plants inoculated with *Fusarium* sp. in response to treatments

Treatment	Plant Height (cm)	No. of Tillers	No. of Leaves	Leaf Length (cm)	Leaf Width (cm)	Disease incidence	Disease severity
T_1	48.05	3.0	11	38	10.00	78.83	36.23
T_2	64.10	3.0	10	37	12.50	46.43	32.89
T_3	69.30	3.0	12	46	10.50	37.81	29.67
T_4	75.70	3.0	14	59	11.25	41.56	36.60
T_5	92.00	4.0	16	63	13.66	59.25	39.75
T_6	96.50	4.0	18	62	12.83	36.27	27.26
T_7	40.45	2.0	10	41	7.80	84.63	65.23
CD	29.39	1.31	5.43	20.62	4.62	23.63	16.30
SE(m)	10.30	0.46	1.90	7.22	1.62	8.28	5.71
SE(d)	14.56	0.65	2.69	10.22	2.30	11.71	8.08

In this investigation, four plant-growth-promoting rhizobacterial (PGPR) isolates were obtained from various small-cardamom fields. Field evaluations demonstrated that the Pseudomonas fluorescens strains (IDK-S-1 from Santhanpara, IDK-V-2 from Vandanmedu, IDK-P-3 from Parathode, IDK-K-4 from Kattappana) and the Bacillus subtilis strains (IDK-S-1, IDK-V-2, IDK-P-3, IDK-K-4) were mutually compatible and markedly suppressed Fusarium wilt development in cardamom plants.

Table 3: Different Local strains of Biometric characters and Disease incidence and severity of cardamom plants inoculated with *Fusarium* sp. in response to treatments

Treatments	Plant Height (cm)	No. of Tillers	No. of Leaves	Leaf length (cm)	Leaf width (cm)	DS	DS	RC
T_1	65.10	3.0	11	46	10.5	42.20	31.00	65.6
T_2	77.10	4.0	19	59	11.2	39.27	27.00	78.2
T_3	82.00	3.0	18	53	13.2	36.27	29.00	73.5
T_4	86.00	4.0	16	52	12.1	56.45	32.00	69.2
T_5	43.45	2.0	8	31	9.6	84.34	65.38	0.00

The aim of the present work is to ascertain that small cardamom plantation increase productivity when AMF(IDK-S-1-Santhanpara Local Strains,IDK-V-2-Vandanmedu Local Strains,IDK-P-3-Parathode and IDK-K-4-Kattappana) and Trichoderma (IDK-S-1-Santhanpara Local Strains,IDK-V-2-Vandanmedu Local Strains,IDK-P-3-Parathode and IDK-K-4-Kattappana) are combined applied, and how such an effect can be ruled. This simultaneous application of a *Trichoderma harzianum* bio-control strain and an AMF formulation produces a significant increase in the colonization by *Trichoderma* and the presence of AMF in Small cardamom plantation. Expression profiling of defense-related marker genes suggests that the phytohormone salicylic acid plays a key role in the modulation of the root colonization process when both fungi are jointly applied. As a conclusion drawn from the data on vegetative characters revealed that application of *Bacillus subtilis* and *P. fluorescens* as single basal application and in combination with *P. fluorescens* spray (T_5 and T_6) showed maximum plant height and leaf length and number of leaves. Sole application of *P. fluorescens* also promoted plant height and number of leaves. There for the treatments comprised of *P. fluorescens* and *Bacillus subtilis* individually and in combination resulted in good vegetative growth of treatment plants.

Conclusion

The present study demonstrated that combined application of bacterial and Fungus native antagonistic (IDK-S-1-Santhanpara Local Strains, IDK-V-2-Vandanmedu Local Strains, IDK-P-3-Parathode and IDK-K-4-Kattappana) is a promising approach for the eco friendly management of Fusarium wilt disease caused by *F. oxysporum* enhancing the growth of the Small cardamom plantation.

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