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EVALUATION OF BIO-INTENSIVE DISEASE MANAGEMENT OF FUSARIUM ROT IN SMALL CARDAMOM (BIDMFC) WITH LOCALLY ISOLATED STRAINS

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Abstract: The cultivation of small cardamom and maintenance of healthy plants have become difficult tasks due to the incidence and spread of Fusarium infections in recent years. Fusarium infection in small cardamom have been reported in the form of capsule infection in the field, seed rot and seedling wilt in nurseries, stem rot & stem lodging in plantations, rhizome rot, root tip rot and foliar yellowing. Field experiment was conducted at Santhanpara, Vandanmedu, Parathode and Kattappana to evaluate the individual and combined efficacy of *Pseudomonas fluorescens* (IDK-S-1, IDK-V-2, IDK-P-3, and IDK-k-4), *Bacillus subtilis* (IDK-S-1, IDK-V-2, IDK-P-3, and IDK-k-4), *Trichoderma harzianum* (IDK-S-1, IDK-V-2, IDK-P-3, and IDK-K-4) and AMF(IDK-S-1,IDK-V-2,IDK-P-3,and IDK-k-4) strains to promote the growth and yield parameters of small cardamom and to manage Fusarium wilt disease in field conditions. The dominant pathogen which causes Fusarium wilt of small cardamom was isolated and identified as *Fusarium oxysporum Schlecht*. Five native bacterial antagonists and fungus were isolated from forest and healthy small cardamom plantation soil in different geographical regions. Under in field conditions, the results revealed that the foliar spray and drenching of combined application of PF (Pf-IDK-S-1)+BS(Bs-IDK-V-2) was found to effectively inhibit the mycelial growth of the pathogen (by 60%) when compared to application of individual strains of the bacterial antagonists. The above strains of *P. fluorescens and B. subtilis* (PF (Pf-IDK-S-1) +BS (Bs-IDK-V-2)) were found compatible. The soil combined application of PF (IDK-S-1) +TH (IDK-P-3) exhibited the highest disease reduction. Also, small cardamom plantation treated with PF (IDK-S-1) + BS (IDK-V-2) strains showed a significant stimulatory effect on plant height and increased the yield up to 27% in comparison to the non-bacterized control. The combined strains also increased small cardamom capsule fruit weight. It could be concluded that synergistic consortia of beneficial bacteria isolated from rhizosphere soil are perfectly able to promote plant growth and could be exploited for sustainable management of soil borne diseases especially, Fusarium rot of small cardamom.

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1. INTRODUCTION

Fusarium rot caused by *Fusarium oxysporum* Schlecht is one ofthe most widespread and important fungal diseases of small cardamom (Thomas and Vijayan, 2002). *F. oxysporum* disease may become destructive at all growth stages of the crop under favourable weather conditions and can cause severe infection in this changing climate scenario. Dhanya *et al.* (2018) reported 50 per cent yield loss from poorly managed plants due to the disease. The pathogen survives in soil and crop residues for many years making the management of the pathogen very difficult. The *Fusarium* sp. initially attacks the tiller producing pale discolored lesions leading to dry rotting. The infected tillers are weakened at the point of attack resultingin partial breakage. These tillers bend down and hang from the point of infection (Josephraj kumar *et al.*, 2007). Symptoms include burnt appearance of panicles and rotting of roots from the tip causing yellowing of the plant and thereby reduction in the yield (Murugan *et al.*, 2016). In the scenario of growing concerns over environment pollution and health hazards the need for an integrated approach in the disease management strategy is of high importance. The integrated disease management using various bio-control agents and bio inputs improved the vegetative growth and the yield of the plant in addition to disease management of Fusarium rot of cardamom

(Dhanya *et al*., 2018). The present study entitled as "Evaluation of Bio-Intensive Disease Management of Fusarium Rot in Small Cardamom (BIDDMFC) With Locally Isolated Strains" was undertaken with an objective to assess the

Fusarium rot severity in Idukki district, develop a bio-intensive disease management practices for the disease and to study the impact of these practices on the soil and plant health.

MATERIALS AND METHODS

The location of current study entitled Evaluation of Bio-Intensive Disease Management of Fusarium Rot in Small Cardamom (BIDDMFC) With Locally Isolated Strains" was Small Cardamom Plantation at Santhanpara, Vandanmedu, Parathode and Kattappana in Idukki during the period of 2021-2023. *Fusarium oxysporum* diseases infection in cardamom plantation is prevalent during the summer season, survey was carried out in February- May 2021-2023 in two major cardamom cultivated blocks of Idukki districts *viz*., Santhanpara and Vandanmedu. One Panchayat each of above blocks were surveyed. Three plantations of size 1ha each were selected from each panchayat and the details are given below. Each plantation was divided into four plots each with 250 plants. Ten plants were selected from each plot and five tillers randomly selected from each plant were scored. The following score chart developed by Dhanya *et al.* (2018) used for the study.

0= No disease

1= 1-10 % of tillers or panicles had fungal lesions2= 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 (PDI) was calculated using the formula

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

Percent Disease Index was calculated using the formula of Singh (2002).

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

Root staining for Arbuscular Mycorrhizal Fungi:

At the end of the experiment to study the mycorrhizal colonization pattern of AMF inoculated the plant roots were collected, and washed with water. 10 % KOH (W/V) was added to container and made root samples were submerged but the fluid did not fill more than the half of the container. The tissues were soaked overnight and replaced with fresh KOH before the heat treatment. The solution turned into brown colour by overnight that indicated the tannins were left from tissues. Heat treatment wasgiven by boiling the processed roots in a water bath. The sample solution was removed and rinsed with tap water for three times. After that 5% HCl was added and allowed to retain for about a minute. After that HCl was poured out and added trypan blue stainand examined the stained roots under a microscope (Philip and Haymann, 1970).

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

Five year old well established plantations with yielding plants of most popular localvariety Njallani, which is highly susceptible to the disease, was chosen for the survey. Different treatments of bio-control agents were scheduled into seven treatments as basal applications and foliar spraying. Field experiment was conducted at Santhanpara, Vandanmedu, Parathode and Kattappana to evaluate the individual and combined efficacy of *Pseudomonas fluorescens* (IDK-S-1, IDK-V-2, IDK-P-3, and IDK-k-4),*Bacillus subtilis* (IDK-S-1, IDK-V-2, IDK-P-3, and IDK-k-4), *Trichoderma harzianum* (IDK-S-1,IDK-V-2,IDK-P-3,and IDK-K-4) and AMF(IDK-S-1,IDK-V-2,IDK-P-3,and IDK-k-4) strains to promote the growth and yield parameters of small cardamom and to manage Fusarium wilt disease in 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**
T1-*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)
T2-*Bacillus subtilis*	Spraying of BS-**IDK-S-1** 2.5 ml per L of water (3 times)	Spraying of BS-**IDK-V-2**2.5 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)
T3-Trichoderma harzianum	Basal application of **TH-IDK-S-1** (1kg) with Neem cake (10kg) and FYM(90kg)	Basal application of **TH-IDK-V-2** (1kg) with Neem cake (10kg) and FYM(90kg)	Basal application of **TH-IDK-P-3** (1kg) with Neem cake (10kg) and FYM(90kg)	Basal application of **TH-IDK-k-4** (1kg) with Neem cake (10kg) and FYM(90kg)
T4-AMF	Basal application of **AMF -IDK-S-1** @ 50gm to the root zone at the time of planting (3 times)	Basal application of **AMF IDK-V-2** @ 50gm to the root zone at the time of planting (3 times)	Basal application of **AMF -IDK-P-3** @ 50gm to the root zone at the time of planting (3 times)	Basal application of **AMF -IDK-k-4** @ 50gm to the root zone at the time of planting (3 times)
T5-*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)
T6-*Trichoderma harzianum* + AMF	Basal application of **TH+AMF -IDK-S-1** @ 50gm to the root zone at the time of planting	Basal application of **TH+AMF IDK-V-2** @ 50gm to the root zone at the time of planting	Basal application of **TH+AMF -IDK-P-3** @ 50gm to the root zone at the time of planting	Basal application of **TH+AMF -IDK-k-4** @ 50gm 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 in combination) for the management of the disease in Idukki district of KeralaS in CRD using 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 @ 50gm 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 and Disease incidence and 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
T1	48.05	3.0	13	38	10.00	78.83(84.00)^{e}	36.23(40.00)^{cd}
T2	64.10	3.0	13	37	12.50	46.43(52.50)^{c}	32.89(30.00)^{bc}
T3	69.30	3.0	12	46	10.50	37.81(41.00)^{b}	29.67(19.50)^{a}
T4	75.70	3.0	14	59	11.25	41.56(47.50)^{c}	36.60(27.50)^{bc}
T5	92.00	4.0	17	63	13.66	59.25(65.66)^{d}	39.75(34.50)^{bcd}
T6	96.50	4.0	15	62	12.83	36.27(34.33)^{a}	27.26(19.00)^{a}
T7	40.45	2.0	9	41	7.80	84.63(96.50)^{d}	65.23(80.00)^{cd}

In the present study, about four strains of PGPR were isolated from the different location of small cardamom plantation. The field trials were clearly showed that the bacterial antagonists of *Pseudomonas fluorescens* (IDK-S-1-Santhanpara Local Strains, IDK-V-2-Vandanmedu Local Strains,IDK-P-3-Parathode and IDK-K-4-Kattappana)and *B.Subtilis* strains(IDK-S-1-Santhanpara Local Strains,IDK-V-2-Vandanmedu Local Strains,IDK-P-3-Parathode,IDK-K-4-Kattappana) were compatible and effectively inhibited the growth of Fusarium disease in small cardamom plantation .

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

LS	Plant Height (cm)	No. of Tillers	No. of Leaves	Leaf length (cm)	Leaf width (cm)	DS	DS	RC
IDK-S-1	65.10	3.0	11	46	10.5	42.20	31	65.6
IDK-V-2	77.10	4.0	19	59	11.2	39.27	27	78.2
IDK-P-3	82.00	3.0	18	53	13.2	36.27	29	73.5
IDK-K-4	86.00	4.0	16	52	12.1	56.45	32	69.2
Control	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 (T5 and T6) 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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