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Interactive effect of biocontrol agents in the management of *Fusarium* rot in cardamom and its impact on plant defense mechanism

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Abstract

Cardamom plantations are subjected to constant threat due to the *Fusarium* rot disease caused by *Fusarium oxysporum* Schlecht which is pronounced during summer months. The current study deals with the identification of an effective and ecofriendly management practice for the disease through the use of biocontrol agents. Survey conducted between February and May 2019 revealed maximum disease severity and incidence in Pampadumpara panchayat of Nedumkandam block (84.40% and 100%) and minimum in Erattayar panchayat of Kattappana block (50.40% and 60.00%). A pot culture experiment was conducted to study the effect of three bioagents (*Glomus fasciculatum*, *Trichoderma asperellum* and *Pseudomonas fluorescens*) individually as well as in combinations. Root inoculation of *G. fasciculatum* with basal application and spray with *P. fluorescens* as well as root inoculation of *G. fasciculatum* along with basal application of *T. asperellum* and *P. fluorescens* spray were identified to be effective against the disease. Disease suppression by the above bioagents was facilitated by reduced pathogen antagonist ratio in the soil, high level of mycorrhizal colonization in the roots and enhanced biochemical activity of defense enzymes like peroxidase, poly phenol oxidase and phenylalanine ammonia lyase in the plants.

Keywords: cardamom, defense enzymes, *G. fasciculatum*, *F. oxysporum*, *P. fluorescens*, *T. asperellum*

Introduction

Cardamom, commonly referred to as the "queen of spices", is one of the most valued spice crops in the world. In India, Kerala's western ghats is the main production home of this crop. Sustainable cardamom production has been challenged by several diseases that cause quantitative and qualitative crop loss. Fusarium rot caused by *Fusarium oxysporum* Schlecht is one of the most widespread and important fungal diseases of small cardamom

(Thomas & Vijayan 2002). The disease may become destructive at all growth stages of the crop under favorable weather conditions (mainly during summer season) as well as in the changing climatic scenario. Dhanya *et al.* (2018) reported that yield loss in poorly managed plantations accounts to about 50 per cent due to this disease. The pathogen's capability to survive in the soil and crop residues makes it very difficult to manage.

Murugan *et al*. (2016) reported that cardamom plantations in southern India have been receiving heavy doses of chemical pesticides due to which the pesticide consumption of the crop has increased by several folds during the last 50 years. In Kerala, an array of pesticides are banned due to various issues that arose due to their injudicious use. The recent trend is to encourage organic cultivation of cardamom for various reasons including environmental and health perspectives. Nowadays farmers are using different bio-agents and various organic inputs individually and in combinations for the management of *Fusarium* rot. In addition to disease management, good vegetative growth and yield were obtained from plantations that follow organic management practices against Fusarium rot in cardamom (Dhanya *et al.* 2018).

However, studies on individual and interactive effect of bioagents and their mode of action in the management of cardamom diseases are lacking. Considering this, the present work was carried out to identify an effective management practice against *Fusarium* rot of cardamom and to study its impact on pathogen antagonist ratio in soil, per cent mycorrhizal colonization in roots and biochemical defense mechanisms in plants.

Materials and methods

Surveys were carried out during February - May of 2019 in two blocks of Idukki district, where cardamom cultivation is more prevalent. Based on this, three plantations each from Pampadumpara and Erattayar panchayats of Nedumkandam and Kattappana blocks respectively were identified as hotspot area for the disease. Each plantation was divided into four plots each with 250 plants. Twenty five plants were selected from each plot for the study. Five tillers, randomly selected from each plant, were subsequently scored for the disease using the score chart developed by Dhanya *et al*. (2018). The disease severity was worked out as per the method described by Singh (2002).

Isolation of the pathogen

The pathogen was isolated from infected panicles and pseudostem by adopting standard procedures. The pathogenicity was confirmed under greenhouse conditions.

Pot culture experiment

Cardamom suckers were raised in pots (10 Kg capacity) filled with solarised soil. Vermiculite based inoculum of *G. fasciculatum* (Kerala Agricultural University strain) was applied to the root zone at the time of planting. All the other treatments including chemical check were applied to the soil after the establishment of plants (one month after planting) in the pots. First spraying of the treatments (T4-T7) was given along with soil application and repeated at monthly intervals two times (Table 1). Three weeks after soil application of the treatments, pathogen innoculum (cfu 2×10^6 g^{-1}) multiplied in sterilized sand maize medium prepared in the ratio 9:1 was applied to the base of all experimental plants @ 0.5 per cent pot^{-1}.

The disease severity in the experimental plants in response to above treatments was recorded with a 0-4 scale score chart.

0 - No disease

1 - 1-10% area of the tillers had fungal lesions

2 - 11-25% area of the of tiller had fungal lesions

3 - Root tip rotting as well as fungal lesions on 26-50% area of the of tiller

4 - Lesions on >50% area of tiller as well as root rotting/ broken tillers at the point of infection

Table 1. Treatment details of pot trial to manage *Fusarium* rot of cardamom

Treatment	Basal application	Spraying (3 times)
T1	*G. fasciculatum* @ 2 % pot $^{-1}$ to the root zone at the time of planting	-
T2	*T. asperellum* (KAU strain) in neem cake FYM mixture @ 1% pot $^{-1}$	-
T3	*P. fluorescens* (KAU strain) in cowdung slurry @ 2% pot $^{-1}$)	-
T4	*G. fasciculatum* (2 % pot $^{-1}$)	*P. fluorescens* (2%) @ 0.5 L plant^{-1}
T5	*G. fasciculatum* (2%) + *T. asperellum* (enriched FYM mixture @1% pot $^{-1}$) + *P. fluorescens* (2% pot $^{-1}$)	*P. fluorescens* (2%) @ 0.5 L plant^{-1}
T6	*G. fasciculatum* (2%) + *P. fluorescens* (2% pot $^{-1}$)	*P. fluorescens* (2%) @ 0.5 L plant^{-1}
T7	Carbendazim 50WP (0.2% pot $^{-1}$)	Carbendazim (0.1%) @ 0.5 L plant^{-1}
T8	Control (untreated plants)	

Based on this, disease severity (PDI) was worked out using the formula described by Singh (2002)

Disease incidence (DI) was worked out as follows

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

Population dynamics of Fusarium *sp. and bio control agents in the soil*

The population of the pathogen and biocontrol agents in soil was recorded one month after pathogen inoculation and repeated at monthly intervals two times using the serial dilution technique (Waksman 1922). The dilutions 10^{-3}, 10^{-4} and 10^{-6} were taken for enumeration of *F. oxysporum* and *T. asperellum* and *P. fluorescens* on Martin rose bengal agar (MRBA), *Trichoderma* specific media and Kings B media respectively.

Per cent colonization of arbuscular mycorrhizal fungi

Roots of cardamom plants inoculated with *G. fasciculatum* were collected at the end of the experiment and stained as per the protocol of Philip and Haymann (1970). The per cent colonization was worked out as follows.

$$\text{Per cent root colonization} = \frac{\text{No. of root bits having mycorrhizal colonization}}{\text{Total no. of root bits observed}} \times 100$$

Induction of defence enzymes in cardamom plants in response to bio control agents

Phenol (Bray & Thrope 1954), OD phenol (Johnson & Sachaal 1952), peroxidase (Srivastava 1987), polyphenol oxidase (Mayer & Harel 1979) and phenylalanine ammonia lyase (Dickerson *et al.* 1984) were estimated from the leaves of treated plants as per the standard protocol at the end of the experiment.

Results and Discussion

Typical eye shaped lesions on the pseudostem and blighting of panicles were recorded from the surveyed plots with which the disease incidence and severity were worked out. These were reported to be the characteristic symptoms of *F. oxysporum* infection in cardamom (Vijayan *et al*. 2013, 2014). Disease

incidence (%) varied from 60-100 per cent and disease severity (%) ranged from 50.40-84.80 in the surveyed plants. Highest disease severity (84.80 %) in Nedumkandam block was recorded from Pampadumpara panchayat and that in Kattappana block was from Erattayar panchayat (77.40%). Thomas and Vijayan in a survey of 37 plantations of Idukki district during 2000-2002 observed DI ranging from 3-27.5%. Further Vijayan *et al.* (2011) reported DI of 68 per cent during 2010-11.

Based on the pathogenicity test the pseudostem isolate (Fs_1) was found more virulent compared to panicle isolate (Fp_1). Therefore $Fs_1$was used in the pot culture studies. In the pot culture experiment, all the characteristic symptoms of the disease (root tip rot, foliar yellowing and pseudostem rot) were observed in the control plants. Disease severity was minimum in plants treated with the combination of *G. fasciculatum*, *T. asperellum* and *P. fluorescens* (T5 and T6) (Table 2). Yursan *et al.* (2009) noticed that disease severity of *F. oxysporum* Schlecht f. sp *radicis-lycopersici* in tomato was reduced significantly by the combined application of AMF and *P. fluorescens*. The present study also revealed that basal application of AMF with

Table 2. Disease incidence and severity of cardamom plants inoculated with *Fusarium* sp. in response to treatments (pot culture)

Treatment*	Disease incidence	Disease severity
T1	88.83 (100.00)	39.23 (40.00)
T2	46.43 (52.50)	32.89 (30.00)
T3	55.25 (67.66)	37.75 (37.50)
T4	43.56 (47.50)	31.60 (27.50)
T5	39.81 (41.00)	24.67 (17.50)
T6	36.27 (35.33)	24.26 (17.00)
T7	40.10 (41.50)	29.88 (25.00)
T8	88.34 (100.00)	69.38 (87.50)
CD(0.05)	3.08	8.89
C.V	24.8	28.57

*Details of treatments (T1 to T8) is provided in Table 1

(Values in parenthesis are Arc sine transformed)

Table 3. Population dynamics of *Fusarium* sp. and biocontrol agents in soil

Treatment*	One month after pathogen inoculation			Two months after pathogen inoculation			Three months after pathogen inoculation		
	F. oxysporum (10^3)	*P. fluorescens* (10^6)	*T. asperellum* (10^4)	*F. oxysporum* (10^3)	*P. fluorescens* (10^6)	*T. asperellum* (10^4)	*F. oxysporum* (10^3)	*P. fluorescens* (10^6)	*T. asperellum* (10^4)
T1	16.50^{bc}	-	-	19.50^{a}	-	-	8.50^{b}		
T2	16.50^{bc}	-	8	15.50^{c}	-	2.5	4.00^{de}	-	
T3	15.00^{cd}	5	-	17.00^{b}	2	-	5.00c	1	-
T4	16.00^{bc}	3	-	12.00^{d}	1	-	3.50^{ef}	1	-
T5	12.75^{d}	3	8	11.00^{de}	3	5.5	2.00^{f}	5	6
T6	15.00^{cd}	3	-	10.50^{c}	4	-	2.00^{f}	6	-
T7	10.50^{e}	-	-	12.50^{d}	-	-	2.00^{f}	-	-
T8	19.00^{a}	-	-	20.50^{a}	-	-	22.00^{a}	-	-
CD(0.05)	1.67			2.73			1.16		

*Details of treatments (T1 to T8) is provided in Table 1

either *T. asperellum* or *P. fluorescens* reduced the disease severity by 69.50 and 70.00 per cent respectively. Similar to this, Srivastava *et al.* (2010) observed that combined application of AMF, *Trichoderma* sp. and *P. fluorescens* was effective in reducing the Fusarium wilt of tomato (*F. oxysporum* f. sp. *lycopersici*) by 63.00 per cent. While conducting the compatibility studies between *Trichoderma* sp. and AMF, synergistic nature of these bioagents was confirmed by Camprubi *et al.* (1995). According to Mbuthia *et al.* (2019) the complimentary effect of the above bioagents in combination might be due to their additive or synergistic nature. Field study conducted by Dhanya *et al.* (2018) confirmed the effective role of bio agents like *Trichoderma* sp., *P. fluorescens* and AMF as combination against the Fusarium rot of cardamom. From the present study it was also concluded that root inoculation of *G. fasciculatum* along with *P. fluorescens* (as basal application and spray) (T6) are equally effective and statistically on par with the above treatment. The study also provides a clear cut idea about the effectiveness of the above bioagents in Fusarium rot management when used individually and in combination.

The pathogen - antagonist ratio in soil when treated with combinations of *G. fasciculatum* and *T. asperellum* as well as *G. fasciculatum* and *P. fluorescens* (Table 4) reduced considerably and became minimum in third analysis.

Table 4. Colonization per cent of AMF in cardamom roots when applied alone and in combination with *T. asperellum* and *P. fluorescens*

	Treatment	Colonization per cent
T1	*G. fasciculatum*	50.55
T5	*G. fasciculatum* + *T. asperellum*	68.76
T6	*G. fasciculatum* + *P. fluorescens*	80.00
T9	Control	0.00

Similar to this, Khan *et al.* (2004) reported a drastic reduction in the population of soil wilt pathogen (*Fusarium oxysporum* f sp. *ciceri*) when *Trichoderma* sp. was applied to chickpea plants. From the present study it was concluded that when the bioagents were used in combination, (*i.e. G. fasciculatum* with either *T. asperellum* or *P. fluorescens*) their interactive effect enhanced the population of both biocontrol agents in the soil (Table 3). Dehariya *et al* (2004) also reported that when AMF and *Trichoderma* sp were given in combination against *Fusarium udum* in pigeon pea, both AMF colonization in plant roots and *Trichoderma* population in soil were triggered. Sangeetha *et al.* (2013) reported high population of *P. fluorescens* in soil even after 75 days, if applied along with AMF compared to its sole application. All these research findings strongly support the results of the present study.

Root staining was done for the AMF treated plants at the end of the experiment and colonization was observed in all treated plants. Colonization percentage and number of vesicles were comparatively higher when AMF was given in combination either with *T. asperellum* or *P. fluorescens* (Fig.1). Sarma *et al*. (2014) opined that *Trichoderma* sp. induced an enhanced colonization of AMF in vascular plants which supports the present results. Boer *et al.* (2005) also reported 50-60% enhanced mycorrhizal colonization in roots when co-inoculated with *P. fluorescens*. Kumar *et al.* (2012) obtained similar results in sorghum. Gamalero *et al.* (2013) found significant colonization of AMF in tomato roots inoculated with *P. fluorescens*. Colonization per cent obtained by combined application of AMF with *T. asperellum* was 68.76 % in the present study (Table 4). Yadav & Aggarwal (2015) also reported similar result in ground nut. In the study, soil application of *G. fasciculatum* with *T. asperellum* and *P. fluorescens* resulted in 77.8% colonization of the roots. An evaluation study with different *P. fluorescens* strains and AMF strains in sorghum resulted in 50.90 to 72.80% colonization (Kumar *et al*. 2011).

AMF with *T. asperellum* and *P. fluorescens*

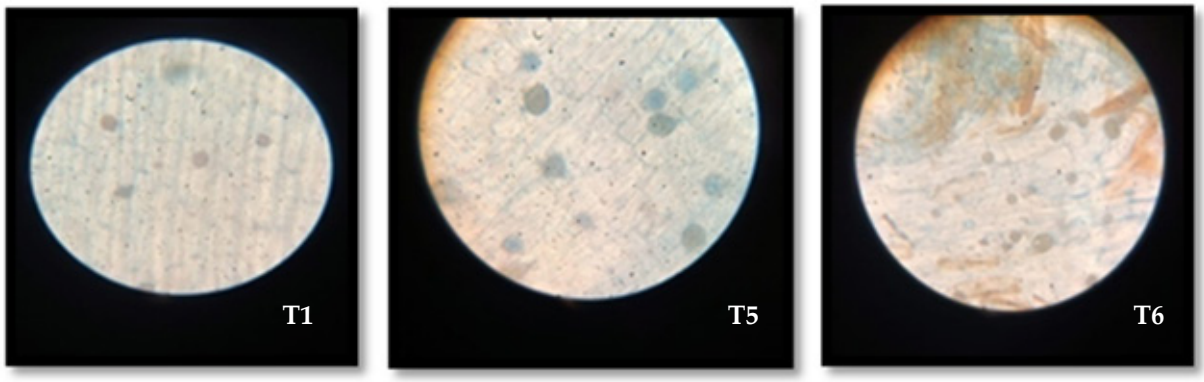

Fig 1. **Root Colonization of *G. fasciculatum* in treatment plants**

(T5) increased the level of defense related biochemical activities like phenol (7.05 μgl^{-1}), OD phenol (36.03 μgl^{-1}), PO (30.82 $\mu g\ min^{-1}g^{-1}$) and PPO (1.17 $\mu g\ min^{-1}g^{-1}$) in cardamom leaves three months after treatment (Table 5). Allay and Chakraborty (2013) reported induction of defence enzymes like β-1, 3-glucanase and peroxidase in mandarin against *F. solani* by dual application of AMF and *T. asperellum.* Doley *et al.* (2014) also reported the role of *G. fasciculatum* and *T. viride* in managing *Macrophomina phaseolina* infection in ground nut through the induction of phenol, peroxidase and poly phenol oxidase. Duc *et al.* (2017) observed that, application of *Trichoderma* sp., AMF and *P. fluorescens* accelerated the activities of poly phenol oxidase in Kapria cultivar and peroxidase in Karpex cultivar of pepper. Basal application of *G. fasciculatum* and *P. fluorescens* along with *P. fluorescens* spray (T6) enhanced OD phenol (7.95 μgl^{-1}) and peroxidase (23.09 $\mu g\ min^{-1}g^{-1}$) activities of cardamom leaves. Mohamed *et al.* (2019) observed high level of PO in common bean against *Sclerotium rolfsi* when treated with *P. fluorescens* and AMF. High concentration of OD phenol (90.6μg) was observed in *Solanum viarum* seedlings (Hemashenpagam and Selvaraj, 2011) when combined inoculation of AMF and *T. harzianum* was given. Sangeetha

Table 5. Estimation of biochemical parameters in cardamom plants subjected to different treatments

Treatment*	Phenol (μgl^{-1})	OD-phenol (μgl^{-1})	PO ($\mu g\ min^{-1}g^{-1}$)	PPO ($\mu g\ min^{-1}g^{-1}$)	PAL ($\mu gmin^{-1}\ g^{-1}$)
T1	10.10	38.05	12.85	0.49	0.19
T2	4.70	42.45	11.00	0.60	0.21
T3	4.25	31.83	16.95	0.92	0.16
T4	6.60	40.56	20.14	2.39	0.14
T5	7.05	36.03	30.82	1.17	0.01
T6	7.95	45.61	23.09	2.03	0.02
T7	5.87	38.43	1.10	0.37	0.15
T8	2.90	20.85	3.06	0.26	0.006
CD (0.05)	0.79	1.68	1.65	0.39	0.03
CV	17.12	16.08	6.70	23.03	19.71

*Details of treatments is provided in Table 2

et al. (2013) reported that treatment with AMF and *Pseudomonas* sp. induced good level of phenol on 75th day of inoculation in maize. Therefore it is evident that combination of bioagents imparts good management of the disease through less pathogen antagonist ratio, increased root colonization by *G. fasciculatum* and activation of bio chemical defence mechanisms in plants.

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