Short communication

In vitro study on the effectiveness of fungicides and bioagents against three new fungal pathogens in small cardamom [*Elettaria cardamomum* (L.) Maton]

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Abstract

Three new fungal diseases caused by *Phoma* sp.*, Sarocladium kiliense* and *Marasmiellus* sp. affecting cardamom have been reported from various parts of cardamom hill reserves of Idukki district in Kerala. An attempt was made to study the effectiveness of two contact, five systemic and four combination fungicides and biocontrol agents against these pathogens under *in vitro* conditions. Fungicides under triazole group (Propiconazole 0.1% and Hexaconazole 0.1%) and the combination products *viz.,* Captan + Hexaconazole (0.05%) and Propiconazole+ Difenaconazole (0.1%) showed complete inhibition of the growth of *Phoma* sp. The maximum inhibition (100%) in the mycelial growth of *S. kiliense* was observed with the fungicides Mancozeb (0.3%), Carbendazim (0.1%), Propiconazole (0.1%) and Hexaconazole (0.1%), and the combination products Captan + Hexaconazole (0.05%), Propiconazole+ Difenaconazole (0.1%) and Carbendazim + Mancozeb (0.25%). The mycelial inhibition of *Marasmiellus* sp. was highest with the chemicals Mancozeb (0.3%), Copper oxychloride (0.2%), Propiconazole (0.1%) and Hexaconazole (0.1%), and combination products Captan + Hexaconazole (0.05%) and Propiconazole+ Difenaconazole (0.1%). The biocontrol agent *Trichoderma* sp. (MTCC 5694) also showed better antagonism (>55%) against all the three pathogens when compared to *Pseudomonas fluorescens* (PN025).

Key words: *Marasmiellus* sp., *Phoma* sp., *Sarocladium kiliense.*

Small cardamom [*Elettaria cardamomum* (L.) Maton], the "Queen of Spices", which belongs to the ginger family (Zingiberaceae) is the third most expensive spice in the world, sought after saffron and vanilla. Cardamom hill reserves (CHR) surrounded by the evergreen forest in the Western Ghats are considered to be the "hot spot" area for cardamom. Adverse climatic conditions, especially prolonged drought and excessive wetness, drastically affect its yield potential. Due to high relative humidity in the plantation areas, cardamom is prone to fungal diseases *viz.,* capsule and panicle rot or *azhukal* disease caused by *Phytophthora meadii*, rhizome rot caused by the combined infection of *Pythium vexans, Rhizoctonia solani* and *Fusarium oxysporum,* Fusarium rot incited by *F. oxysporum* and leaf blotch caused by *Phoeodactylium venkatesanam.* Three new diseases caused by *Phoma* sp. (MN962956)*, Sarocladium kiliense* (MN962925) and *Marasmiellus sp.* (MN962926) were identified recently from the cardamom growing hill tracts of Idukki district. Even though these pathogens are new to small cardamom, several reports revealed the presence of

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Table 1. Details of the locations in Idukki district showing the diseases

Sl. No.	Locations	Disease incidence (%)	Pathogens identified	Cultivars of cardamom affected	GPS location		Altitude (feet) (MSL)
					Latitude	Longitude	
1.	Kattappana municipality	80	*Phoma* sp. and *Sarocladium kiliense*	*Njallani*	9° 39' 51.768'' N	77° 5' 50.9028'' E	2,870
2.	Nedumkandam panchayath	60	*Phoma* sp.	*Njallani*	9° 49' 9.66'' N	77° 7' 53.94'' E	3,900
3.	Chakkupallam panchayath	50	*Phoma.* sp.	*Njallani*	9° 50' 51.036'' N	76° 58' 51.24'' E	3,900
4.	Pampadumpara panchayath	60	All three	*Njallani*	9° 50' 51.036'' N	76° 58' 51.24'' E	4000
5.	Karunapuram panchayath	50	*S.kiliense*	*Njallani*	9° 45' 42.3'' N	77° 12' 50.472'' E	3200
6.	Erattayar panchayath	60	*S. kiliense* and *Marasmiellus* sp.	*Njallani*	9° 50' 51.036'' N	76° 58' 51.24'' E	850
7.	Konnathady panchayath	55	*Marasmiellus* sp.	*Njallani*	9° 50' 51.036'' N	76° 58' 51.2544'' E	

these pathogens in many monocotyledonous crops (Kilaru and Hasenstein, 2005; Adedji, 2006; Miller et al., 2010; Saju et al., 2011; Lanoiselet et al., 2012; Pong et al., 2012; Thiruchchelvan et al., 2013; Tamur et al., 2018). These diseases pose new threat to cardamom cultivation. So far, no studies have been conducted on the management of these diseases. Hence, an attempt was made to identify the most effective chemical as well as bio control agent against these pathogens through an *in vitro* evaluation.

The disease symptoms incited by the above pathogens were noticed during routine field visits to various cardamom plantations of Idukki district (Table 1). Symptoms were recorded in *Njallani* variety which has been cultivated widely in CHR region. The samples were brought to laboratory and the pathogens were isolated using standard procedures.

The sensitivity of the fungal pathogens to eleven commonly used fungicides in cardamom plantations were evaluated by employing Poisoned Food Technique (Grover and Moore, 1962). Treatments in this experiment included contact, systemic and combination fungicides (Table 2). Potato Dextrose Agar medium without any fungicide was kept as the control for comparison, and per cent inhibition over control was recorded when the growth in the control plate was completed. Furthermore, the potential of two biocontrol agents *viz., Trichoderma* sp. strain T6 (MTCC5694) and *Pseudomonas fluorescens* (PN 025) were also evaluated following the dual culture technique (Dennis and Webster, 1971a). Petri plates inoculated with each pathogen alone served as control for comparison. Three replications were maintained and the growth of the pathogen in treatment plates was compared against the control plate. The per cent inhibition over control was calculated using the formula given below

Table 2. Details of the fungicides and bioagents used in the study

Sl. No.	Fungicides and Bioagents	Trade/Strain name	Dose (g or ml L^{-1})
1.	Carbendazim 50 WP	Benomyl	1 g
2.	Mancozeb 75 WP	Indofil M 45	3 g
3.	Captan 70+ Hexaconazole 5 WP	Taqat	0.5 g
4.	Copper oxychloride 50 WP	Fytran	2 g
5.	Propiconazole13.9+ Difenaconazole 13.9 EC	Task	1 ml
6.	Propiconazole 25 EC	Tilt	1 ml
7.	Potassium phosphonate	Akomin	3 ml
8.	Fosetyl –Al 80 WP	Alliette	1 g
9.	Cymoxanil 8 + Mancozeb 64 WP	Moximate	1 g
10.	Carbendazim 12+ Mancozeb 63 WP	SAAF	2.5 g
11.	Hexaconazole 5 SC	Contaf	1 ml
12.	*Trichoderma sp.*	MTCC 5694	10gL^{-1}
13.	*Pseudomonas fluorescens*	PN025	20g L^{-1}

(Vincent 1947).

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

Where I is the percent inhibition, C is the colony diameter in control and T is the colony diameter in treatment.

Production of volatile metabolites by *Trichoderma* sp. was studied by "inverted plate technique" as suggested by Dennis and Webster (1971b). Three replications were kept for each pathogen and Petri plates with each pathogen alone were maintained as control. The per cent inhibition was calculated using the same formula which was used in dual culture technique.

Production of non-volatile secondary metabolites by *Trichoderma* sp. against the pathogens was evaluated by following the modified methods put forward by Dennis and Webster (1971b). The incubation period was five days for *Marasmiellus* sp. as well as *Phoma* sp. and 14 days for *Sarocladium kiliense.*

Symptomatolgy of the cardamom plants infected with the disease was studied and the symptoms were described. Disease symptoms caused by *Phoma* sp. started as irregular to elongated reddish brown lesions at the base of leaf petioles and spread along the pseudostem. Gradually, the lesions spread to several inches in length resulting in splitting of the affected tillers. The split areas of pseudostem showed lesions with straw coloured centre and dark brown margin. The infection further proceeded to the inner sheaths of pseudostem causing a vertical splitting of the infected leaf sheaths. Ultimately the plants broke at the affected portion and collapsed. Symptoms associated with *S. kiliense* included rotting of the outer leaf sheath of the tiller. Symptoms began as dark brown blotches on the pseduostem. Subsequently, these lesions enlarged and coalesced to cover the outer sheath. In some plants combined infection of *Sarocladium* and *Phoma* sp. was also observed.

The disease symptoms caused by *Marasmiellus* sp. were manifested as brown lesions on the tillers, which later spread towards the inner sheaths. The symptoms of the disease developed as white powdery growth within the outer sheaths of the affected plants. The infection gradually spread to the inner sheaths resulting in drying and death of the plants. Saju et al. (2011) have reported the incidence of *Phoma hedericola* on large cardamom. The infection appeared on leaves as round water-soaked lesions with typical grey coloured centre. According to Lanoiselet et al. (2012) *Sarocladium oryzae* produced oblong pale to dark brown lesions on the leaf sheaths of infected rice plants. Infection with *Marasmiellus* sp., a soil borne fungal pathogen, resulted in rotting at collar region and development of white mycelial growth near the soil line of banana plant (Thiruchchelvan et al., 2013b). All the above-mentioned symptoms were found similar to the disease symptoms quoted under the current study and was observed in monocotyledonous host plants as well.

The plants showing the above typical symptoms of the diseases were brought to the laboratory and the pathogens were isolated using standard procedures (Nelson et al., 1983). The isolation was carried out on the PDA medium and the fungi were stored on same medium at 4°C for further use.

The fungicide and biocontrol agent can be considered to be effective when the fungal growth is diminished to an extent of 50 per cent or more (Saju et al., 2012). On *Phoma* sp. grown on fungicide amended medium, the inhibitory effect of the treatments was observed to range from 44.81 to 94.44 per cent (Table 3; Fig. 1). Complete inhibition (100 %) was recorded for the systemic fungicides Propiconazole 25 EC (0.1%) and Hexaconazole 5 SC (0.1%) as well as for the combination fungicides Captan 70+ Hexaconazole 5 WP (0.05%) and Propiconazole 13.9+ Difenaconazole 13.9 WP (0.1%). Minimum inhibition (less than 45%) was observed when the pathogen was grown in potassium phosphonate (0.3

Figure 1. In vitro assessment of efficacy of fungicides against *Phoma* sp.

%) amended medium (Table 3). Earlier studies by Koelsch et al. (1995) and Eckert et al. (2010) showed that triazole fungicides had complete inhibition of mycelia growth as well as germination of the conidia of *Phoma*. Triazole group of fungicides were reported to be potent inhibitors of ergosterol synthesis, the major constituent of cell membrane (Dupont et al., 2012; Connerton et al., 1991). Griffiths et al. (2003) detailed the sterol composition on the cell wall of *Leptosphaeria maculans,* the teleomorph of *Phoma lingam*. Giri (2017) found better control of the pathogen using Propiconazole (0.1 %) and Carbendazim (0.1 %). Patil (2007) also noticed the effectiveness of Carbendazim 50 WP (0.05%), Propiconazole 25 EC (0.1%) and the combination product, Carbendazim 12 % + Mancozeb 63 % (0.2%), against the growth of *Phoma* sp. The protectant or contact fungicides prevented only spore germination, while the systemic fungicides inhibited fungal growth and sporulation. Thus, the combination products were revealed to have the highest inhibiting effect against the fungi compared to other fungicides (Gisi et al., 1985).

In our study, *Trichoderma* sp. showed more than 55 per cent inhibition of mycelial growth of *Phoma* sp. (Table 3; Fig. 2).In agreement with this, Mokhtar and Dehimat (2015) and Dawidziuk et al. (2016) reported the antagonistic effect of *Trichoderma* sp. against *Phoma* sp. A clear inhibition zone was formed between the pathogen and the biocontrol agent during dual culture study. Lopez et al. (2019) also observed an inhibition zone between *Phoma* sp. and *Trichoderma* sp. when grown as dual culture.

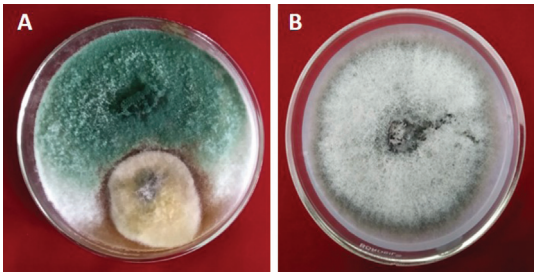

Figure 2. Dual culture with *Trichoderma* sp. and *Phoma* sp. A) *Trichoderma* sp. X *Phoma* sp. B) *Phoma* sp. (Control)

Table 3. Evaluation of the efficacy of fungicides and biocontrol agents against the pathogens

Sl. No.	Fungicides & biocontrol agents	Per cent inhibition over control (%)		
		Phoma sp.	*S.kiliense*	*Marasmiellus* sp.
1.	Carbendazim	57.40(7.60)^{bc}	100.00(10.00)^{a}	0.00 (0.701)^{g}
2.	Mancozeb	94.44(9.73)^{a}	100.00(10.00)^{a}	91.86(9.593)^{ab}
3.	Captan + Hexaconazole	100.00(10.03)^{a}	100.00(10.00)^{a}	100.00(10.029)^{a}
4.	Copper oxychloride	59.63(7.75)^{b}	68.52(8.28)^{c}	100.00(10.029)^{a}
5.	Propiconazole + Difenaconazole	100.00(10.023)^{a}	100.00(10.00)^{a}	100.00(10.029)^{a}
6.	Propiconazole	100.00(10.03)^{a}	100.00(10.00)^{a}	100.00 (10.029)^{a}
7.	Potassium phoshonate	44.82(6.65)^{d}	58.52(7.59)^{d}	39.25(6.306)^{f}
8.	Fosetyl –Al	55.56(7.49)^{bc}	74.07(8.60)^{bc}	74.818(8.678)^{d}
9.	Cymoxanil + Mancozeb	50.75(7.14)^{bcd}	77.78(8.82)^{bc}	88.14(9.401)^{b}
10.	Carbendazim + Mancozeb	46.29(6.83)^{cd}	100.00(10.00)^{a}	85.93(9.297)^{bc}
11.	Hexaconazole	100.00(10.03)^{a}	100.00(10.00)^{a}	78.14(8.860)^{cd}
12.	*Trichodermasp.*	58.52(7.68)^{b}	79.25(8.89)^{b}	55.93(7.516)^{e}
13.	*P. fluorescens*	0.00(0.70)^{e}	24.07(4.90)^{e}	0.00 (0.701)^{g}

The secretion of extracellular enzymes like exo chitinases could have resulted in the formation of such clear zone (Kullnig et al., 2000; Brunner et al., 2003). These enzymes dissolved the host cell fragments which in turn induced the production of further enzymes and triggered a cascade of physiological changes, stimulating rapid and directed growth of *Trichoderma* sp. (Zeininger et al., 1999).The antagonistic potential of *P. fluorescens* against *Phoma* was also evaluated by employing dual culture technique. The biocontrol agent exhibited poor antagonism and the pathogen completely smothered the antagonist (Table 3). Hammoudi et al. (2012) also reported weak antagonistic potential of *P. fluorescens* against *Phoma* sp. when the culture extract of the bioagent was amended in the growth medium.

While growing *S. kiliense* on fungicide amended media, there was growth inhibition of the fungus ranging from 58.52 to 100 per cent. Cent per cent inhibition of the fungal growth was noticed for systemic fungicides Carbendazim (0.1%), Propiconazole (0.1%), and Hexaconazole (0.1%), contact fungicide Mancozeb (0.2%) as well as the combination products Captan + Hexaconazole (0.05%) and Propiconazole + Difenaconazole (0.1%). The least inhibition (58.52%) was recorded when the fungus was grown in potassium phosphonate (0.3%) amended medium (Table 3; Fig.3). Similar results were reported by Viswanathan and Narayanasamy (1990) as well as Venkateswarlu and Chauhan (2005) wherein they observed better growth inhibition of the pathogen when grown in Carbendazim and Mancozeb (0.25%) amended media.

Figure 3. In vitro assessment of efficacy of fungicides against *S. kiliense*

Dual culture study with *S. kiliense* and *Trichoderma* sp. showed more than 79 per cent inhibition of the pathogen (Table 3; Fig. 4). The results were in congruence with the study carried out by Panneerselvam and Saravanamuthu (1996) in which they noticed maximum mycelium inhibition of

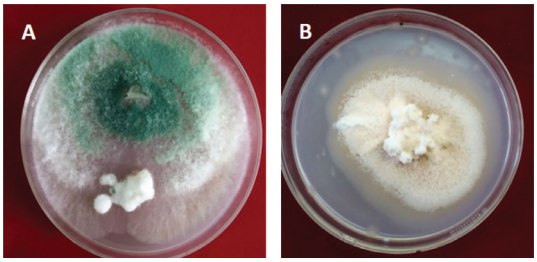

Figure 4. Dual culture with *Trichoderma* sp.and *S. kiliense. A)Trichoderma* sp. *X S. kiliense, B)S. kiliense* (control)

Sarocladium oryzae by *T. viride*. Similar reports were put forth by Srinivas and Ramakrishanan (2003), Gopalakrishnan and Valluvaparidasan (2006) and Kalaiselvi and Panneerselvam (2011). The growth inhibition of the fungus might have been due to the higher growth rate of the antagonist. *Sarocladium* sp., owing to slow growth rate, failed to completely fill the Petri plate during the incubation period. They also observed 78 per cent inhibition of the mycelial growth of *S. oryzae* and development of a clear zone between the pathogen and antagonist when grown as dual culture with *T. viride*. Similarly, Gveroska (2013) reported overgrowth of *Trichoderma* sp. on *Phytophthora parasitica* due to the slow growth rate of the pathogen.

P. fluorescens showed only 24.07 per cent inhibition in the mycelial growth of *S. kiliense* within 21 days of incubation period. The antagonist exhibited least inhibition of the pathogen as the pathogen

completely overgrew the biocontrol agent (Table 3). Kumar and Patibanda (2015) revealed that none of the isolates of *P. fluorescens* manifested any inhibition in the growth of *S. oryzae,* the incitant of rice sheath rot disease.On the contrary, Bora and Ali (2019) reported an inhibition of 82.06 per cent in the mycelial growth of *S. oryzae* by *P. fluorescens.* The cell wall of majority of the fungi consisted of chitin (Latge, 2007). Most of the fluorescent pseudomonads were weak producers of chitinase enzyme (Vasudevan et al., 2002) and that might be the reason for the poor antagonistic potential of this biocontrol agent.

The study showed that the fungicides evaluated against *Marasmiellus* sp. resulted in an inhibition per cent ranging from 39 to 100. Cent per cent inhibition was shown by contact fungicides Mancozeb 75 WP (0.3%) and copper oxychloride 50 WP (0.2%) as well as systemic fungicides Propiconazole 25 EC (0.1%) and Hexaconazole 5 SC (0.1%) and combination products Propiconazole 13.9+ Difenaconazole 13.9 WP (0.1%) and Captan 70+ Hexaconazole 5 WP (0.05%). Least inhibition (39.26 %) was observed when the pathogen was grown in potassium phosphonate (0.3%) amended media (Table 3; Fig. 5). Similar results were reported by Thiruchchelvan et al. (2013a) who reported a complete inhibition of growth of *Marasmiellus* sp.

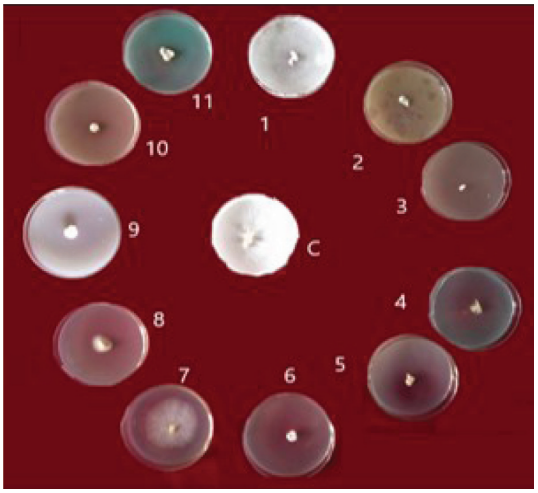

Figure 5. In vitro assessment of efficacy of fungicides against *Marasmiellus* sp.

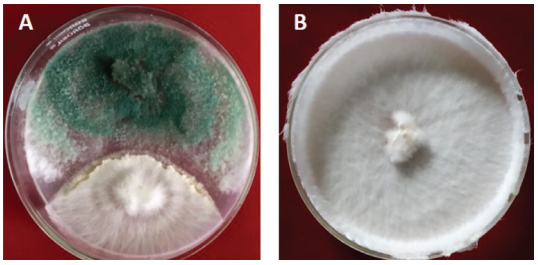

Figure 6. Dual culture with *Trichoderma* sp. and *Marasmiellus* sp. A) *Trichoderma* sp. X *Marasmiellus* B) *Marasmiellus* sp. (control)

when grown on Mancozeb and copper fungicide amended media. Amoako-Attah et al. (2016) also observed a reduction in growth of *M. scandense* when treated with copper-based fungicides. No published results are available on the effect of potassium phosphonate on *Marasmiellus* sp. Aguin et al.(2006) reported the lower efficacy of potassium phosphonate against *Armillaria mellea*, a basidiomycetous fungus similar to *Marasmiellus* sp. The results of the dual culture study of *Marasmiellus* sp. with *Trichoderma* sp. showed more than 55 per cent inhibition in mycelial growth of the pathogen (Table 3; Fig. 6), whereas, Thiruchchelvan et al. (2013b) noticed 80 per cent growth inhibition when grown against *T. harzianum.* In the present study, we used a different species of *Trichoderma* and that might have been the reason for the lower inhibition compared to the earlier published results. *P. fluorescens* is known to enhance plant growth promotion and reduce severity of many fungal diseases (Hoffland et al., 1996) which might be due to the production of a number of secondary metabolites including antibiotics, siderophores and hydrogen cyanide (O'Sullivan and O'Gara, 1992). However, in this study *P. fluorescens* showed no inhibition of the growth of the pathogen (Table 3). In fact, the pathogen completely smothered the biocontrol agent when grown in dual culture. There are no reports on the biocontrol efficiency of *Pseudomonas* sp. against *Marasmiellus* sp. However, Fox et al. (1991) reported poor antagonistic effect of *Pseudomonas* sp. against *Armillaria mellea,* a basidiomycetous fungus similar to *Marasmiellus* sp.

There was poor/no inhibition of the growth of the pathogens inverted plate technique was conducted, owing to the lower efficacy of volatile metabolites on the growth of the pathogens. However, all three pathogens *viz.*, *Phoma* sp., *S. kiliense* and *Marasmiellus* sp. showed 71.11, 100 and 95.19 per cent inhibition respectively of the mycelial growth when grown in the culture extract of the antagonist. The biocontrol effect imparted by *Trichoderma* sp. confirms the role of non-volatile metabolites in pathogen inhibition and was due to antibiosis, competition for nutrients and the secretion of cell wall lysing enzymes (Kumar, 2013). In congruence with our findings, Khaledi and Tahari (2016) also observed that non-volatile metabolites secreted by *T. harzianum* caused more growth inhibition of the pathogenic fungi than the volatile metabolites. Reino et al. (2008) have detailed the production of non-volatile secondary metabolites such as terpenes by *Trichoderma* spp. against plant pathogens. In addition to this, several metabolites like trichodecenins, trichorovins and trichocellins have also been reported from *T. viride* (Fujta et al., 1994; Wada et al., 1995).

In vitro studies showed that systemic fungicides Propiconazole 25 EC and Hexaconazole 5 SC, and combination products Captan 70 + Hexaconazole 5 WP and Propiconazole 13.9 + Difenaconazole 13.9 EC were highly effective (100 % inhibition) against all the three fungi. The bioagent *Trichoderma* sp. also showed good inhibition (>55 %) of the pathogens. In addition to these, Mancozeb (0.3%), Carbendazim (0.1%) and combination product Carbendazim + Mancozeb (0.25%) also exhibited good inhibition of *S. kiliense*, whereas the contact fungicides Mancozeb (0.3%) and copper oxychloride (0.2%) proved very effective against *Marasmiellus* sp. The performance of these chemicals as well as bioagents was assessed by *in vitro* studies. Since these pathogens are new to cardamom plantations, field level studies of the best *in vitro* treatments including the bioagent (*Trichoderma* sp.) have to be carried out to evaluate their performance in field conditions.

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