INTERACTION OF CERTAIN ANTAGONISTS OF RUBBER PATHOGENS WITH FUNGICIDES USED IN RUBBER CULTIVATION

Kochuthresiamma Joseph, P. K. Bijitha and Roshni Susan Elias

Rubber Research Institute of India, Kottayam-686 009, Kerala, India

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The antagonistic activity of 77 bacterial isolates collected from the rhizosphere and non rhizosphere soil and tissues of different clones of rubber was tested against the five major pathogens of rubber, *viz*. *Phytophthora meadii*, *Corynespora cassiicola*, *Colletotrichum acutatum*, *Corticium salmonicolor* and *Phellinus noxius* by dual inoculation and selected five isolates showing larger zone of inhibition of each of the pathogens. *Pseudomonas* spp. and *Bacillus* spp. were the main antagonistic bacterial groups which included the endophytes and rhizosphere colonizers. They produced various antipathogenic and plant growth promoting metabolites. The compatibility of the antagonists with four systemic fungicides *viz*. tridemorph (Calixin), hexaconazole (Contaf), propiconazole (Tilt) and carbendazim (Bavistin) and two contact fungicides *viz*. mancozeb (Indofil M-45) and wettable sulphur (Sulfex) commonly used in rubber plantations was studied at different concentrations under *in vitro* conditions. Carbendazim was the only systemic fungicide tested which was safe to all the isolates studied. The contact fungicide mancozeb was inhibiting the growth of all the antagonists even at the lowest level studied while wettable sulphur was not harmful to most of the isolates even at double the recommended level. *Pseudomonas* spp. in this study were more tolerant than *Bacillus* spp., to the fungicides used in rubber cultivation.

Keywords: Antagonists, Fungicides, *Hevea* pathogens, Interaction

INTRODUCTION

Pesticide application is an inevitable operation in agricultural practices for crop protection and for maintaining high crop productivity. The excessive dependence on chemical pesticides have many limitations such as the development of resistance in pathogens and outbreak of secondary pathogens/biotypes, high cost, environmental pollution and health hazard problems (Bagyaraj, 2011). The lethal effects of pesticides are not usually confined to target pathogen alone but in the course the beneficial non target organisms also get affected. Systemic fungicides are readily translocated by plants and affect the pathogens as well as the microbial population in the rhizosphere (Dar, 2010) and the plant endophytes and their activities. Considering these limitations, there has been a growing awareness to develop such management practices, which alone or in combination with other practices could bring about a reasonably good reduction of disease incidence. Biological

Correspondence: Kochuthresiamma Joseph (Email: kochuthressia@rubberboard.org.in)

control using antagonistic microorganisms is an important approach in this direction. Microbial antagonists are living organisms which are highly dependent on the environmental conditions. The biotic and abiotic factors in the field and various cultural operations influence their establishment and activities.

Natural rubber (*Hevea brasiliensis*), at all stages of its growth is affected by various fungal pathogens. Leaf diseases caused by *Phytophthora* spp., *Corynespora* sp., *Colletotrichum* spp. and *Oidium* sp., stem disease by *Corticium* sp. and root disease by *Phellinus* sp. are the major diseases occurring in rubber plantations in India. These diseases are controlled by chemical fungicides. Repeated use of these chemicals may adversely affect the environment and beneficial organisms in the field. Microorganisms antagonistic to rubber pathogens were found to present in rubber plantations (Joseph *et al*., 2009). In this study, certain bacterial antagonists of rubber pathogens were selected and their various beneficial activities were estimated. The isolates were identified based on biochemical characterization. The interaction of these isolates with commonly used fungicides was studied to find out their sensitivity and compatibility for integrated management of diseases.

MATERIALS AND METHODS

The antagonistic activity of 77 bacterial isolates taken from the cultural collections of Rubber Reseach Institute India was studied. They were collected from the rhizosphere and non rhizosphere soil and tissues of different clones of rubber and were tested against the five major pathogens of rubber, *viz*. *Phytophthora meadii*, *Corynespora cassiicola*, *Colletotrichum acutatum*, *Corticium salmonicolor* and *Phellinus noxius* by dual inoculation in potato dextrose agar plates. The zone of growth inhibition of pathogens by the isolates was measured after seven days of co-inoculation. Five isolates showing larger zones of inhibition of each of the pathogens were selected for studying their beneficial activities and sensitivity to fungicides. These isolates were identified based on their reaction to different biochemical tests. The production of antifungal metabolites like volatile organic compounds (VOCs) (Bhatia *et al*., 2003), hydrogen cyanide (HCN) (Wei *et al*., 1992), ammonia (Cappuccino and Sherman, 1999) and siderophores (Yeole *et al*., 2001) were assessed. Their ability to solubilize insoluble phosphates (Nguyen *et al*., 1992), to produce indole acetic acid (IAA), the growth promoting hormone (Sheng *et al*., 2008), and polysaccharides (Tank and Saraf, 2003) were also studied.

The compatibility of the antagonists with commonly used fungicides in rubber plantations was studied by poisoned food technique. Four systemic fungicides *viz*. tridemorph (Calixin), hexaconazole (Contaf), propiconazole (Tilt) and carbendazim (Bavistin) and two contact fungicides *viz*. mancozeb (Indofil M-45) and wettable sulphur (Sulfex) were tested by incorporating to the bacterial growth media at 25, 50,75,100 and 200 per cent recommended levels. The growth of the isolates in these media was compared with their growth in control plates without the fungicides to record their sensitivity.

RESULTS AND DISCUSSION

From the 77 bacterial isolates tested, five each showing larger zone of growth inhibition of the different pathogens are listed in Table1. The highest zone of inhibition of *P. meadii* was recorded in RB 66 (3.6 cm), *C. cassiicola* in K 47 (2.6 cm),

Table 1. **Antagonists selected for the different pathogens and inhibition zone (cm).**

Sl. No	*P. meadii*	*C. cassiicola*	*C. acutatum*	*C. salmonicolor*	*P. noxius*
1	RB 66 (3.6)	K 47 (2.6)	K 25 (2.2)	PP 36a (2.5)	K 18 (0.9)
2	Ri 31 (2.7)	F 1 (2.3)	PP 38 (2.0)	RB 88 (2.4)	Ps 24 (0.8)
3	A 1 (2.7)	F 4 (2.0)	L53 (1.8)	K 25 (2.3)	1 L (0.0.8)
4	PP 36a (2.6)	L 5 (2.0)	A 1 (1.5)	PP 38 (2.2)	F 14 (0.5)
5	L 54 (2.4)	PP 38 (1.8)	Ps 3 (1.3)	K 24 (2.0)	F 4 (0.5)

C. acutatum in K 25 (2.2cm), *C. salmonicolor* in RB 88 (2.4 cm) and *P. noxious* in K18 (0.9 cm.) (Fig.1). Four of the selected isolates (K 25, A 1, PP 36a and F4) showed antagonism against two of the pathogens. The isolate PP38 inhibited the growth of three pathogens *viz. C. cassiicola, C. acutatum* and *C. salmonicolor*. Hence a total of 19 isolates were selected for further study.

The identification of the 19 isolates based on Gram staining and biochemical tests along with their source of isolation are given in Table 2. Out of the 19 isolates, ten belonged to *Bacillus* spp., five to *Pseudomonas* spp., two to *Proteus* spp. and one each to *Alcaligens* and *Micrococcus*. Of these, 12 isolates were rubber endophytes (7 *Bacillus* spp., 3 *Pseudomonas spp.*, 1 *Micrococcus* and 1 *Proteus*) and the rest were from the rhizosphere of rubber. *Pseudomonas* spp. and *Bacillus* spp. are the major bacterial groups found to survive in different plant parts and soil. They have been reported to control several soil borne, seed borne and air borne diseases of crops (Vidhyasekaran, 1998).

The isolates were found to produce various metabolites which help in plant protection and growth promotion (Table 3). All the tested isolates produced siderophores at different levels. Siderophores are low molecular weight molecules secreted by microorganisms to sequester iron from the environment (Hofte, 1993). Their mode of action in suppression of diseases was thought to be based on competition for iron with the pathogens (Duijff *et al.*, 1999) and there are some evidence on their involvement in microbially mediated induced systemic resistance (De Meyer and Hofte, 1997).

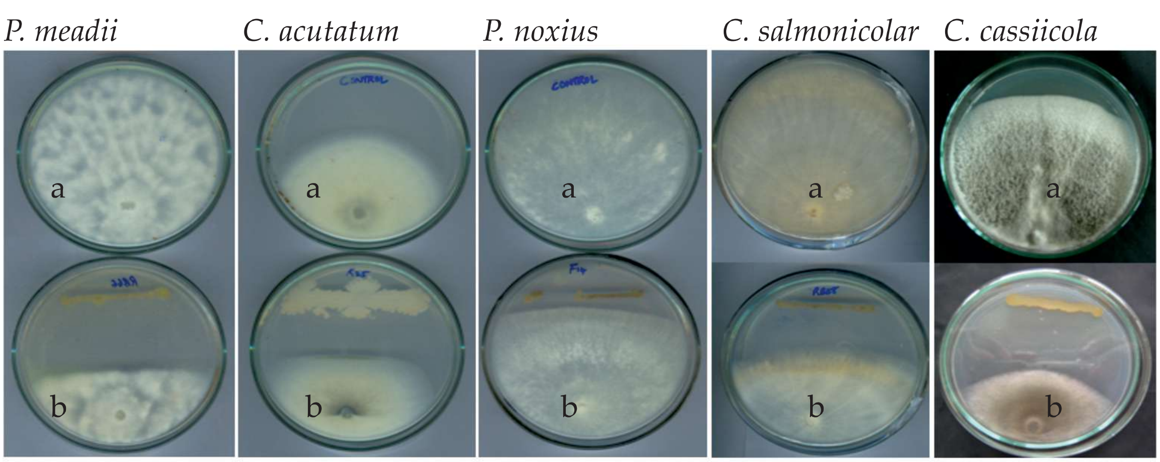

Fig.1. Antagonists of the different pathogens (a. pathogen alone; b. pathogen with antagonist)

Table 2. **The isolates selected and their source**

Sl. No.	Isolate no.	Organism	Source	Clone
1	F 1	*Bacillus* sp	rhizosphere	RRII 105
2	F 4	*Bacillus* sp.	root	RRII 414
3.	F 14	*Pseudomonas* sp.	root	RRII 414
4	K 25	*Proteus* sp.	bark	RRII 105
5	K 18	*Bacillus* sp.	bark	RRII 105
6	K 24	*Bacillus* sp.	leaf	RRII 105
7	K 47	*Bacillus* sp.	stem	RRIM 600
8	L 5	*Alcaligens* sp.	rhizosphere	GT 1
9	L 53	*Micrococcus* sp.	root	RRII 105
10	L 54	*Bacillus* sp.	root	GL 1
11	1 L	*Bacillus* sp.	leaf	GT 1
12	A 1	*Bacillus* sp.	root	RRII 105
13	Ps 3	*Pseudomonas* sp.	rhizosphere	RRII 105
14	Ps 24	*Pseudomonas* sp.	rhizosphere	RRII 105
15	PP 38	*Bacillus* sp.	rhizosphere	RRII 105
16	PP 36a	*Bacillus* sp.	rhizosphere	RRII 105
17	RB 88	*Pseudomonas* sp.	root	RRII 422
18	RB 66	*Pseudomonas* sp.	root	RRII 105
19	Ri 31	*Proteus* sp.	rhizosphere	RRII 105

Siderophore producing bacteria also promote plant growth by chelating the limited iron in the rhizosphere and making it available to the plant roots (Glick, 1995). The response of pathogens to growth inhibiting volatile compounds produced by the antagonists also varied. The growth of *P. meadii* and *P. noxius* were not reduced by the VOC. The VOC produced by all the selected antagonists reduced the growth of *C. acutatum* (24 - 44% reduction), *C. salmonicolor* (52 -72% reduction) and *C. cassiicola* (53-73% reduction). The VOC produced by A 1, a common antagonist of *P. meadii* and *C. acutatum*, reduced the growth of the latter (44%) only. Similarly the VOCs produced by the antagonist F 4 reduced the growth of *C. cassiicola* (70%) and not *P. noxius* showing the differential response of the pathogens to these chemicals. Vespermann *et al.* (2007) reported that the strength of fungal growth inhibition by the VOCs depends on the rhizobacterial isolate and different fungi show individual inhibition patterns. VOCs produced by plant growth promoting rhizobacteria were found to elicit plant growth promotion and induced resistance in *Arabidopsis* (Rhu *et al*., 2003). Microbial VOCs have been shown to affect both fungal mycelial growth and enzyme activity (Mackie and Wheatley, 1999). Bacteria isolated from canola and soybean plants produced VOCs that inhibited sclerotia and ascospore germination and mycelial growth of *Sclerotinia sclerotiorum*. (Fernando *et al*., 2005). They identified six inhibitory compounds *viz*, benzothiozol, cyclohexanol,

Table 3. **Production of beneficial metabolites by the antagonists**

Sl. No.	Isolate	Siderophore	VOC	NH_3	IAA ($\mu g\ mL^{-1}$)	Polysaccharides ($mg\ mL^{-100}$)	PO_4 SE (%)
1	F 1	low	+ve	medium	15.90	08.9	118
2	F 4	medium	+ve	medium	52.50	08.9	130
3.	F 14	high	-ve	medium	7.20	05.2	190
4	K 25	low	+ve	medium	10.20	00.5	110
5	K 18	low	-ve	high	10.90	05.1	124
6	K 24	low	+ve	nil	13.40	03.3	107
7	K 47	medium	+ve	medium	9.48	10.8	110
8	L 5	low	+ve	nil	4.24	04.1	113
9	L 53	low	+ve	low	8.20	10.3	138
10	L 54	low	-ve	high	2.04	01.1	110
11	1 L	low	-ve	medium	4.96	00.6	125
12	A 1	low	+ve	high	9.04	-	163
13	Ps 3	high	+ve	medium	1.80	00.8	-
14	Ps 24	medium	-ve	low	5.32	12.1	207
15	PP 38	medium	+ve	medium	3.12	13.6	138
16	PP 36a	medium	+ve	medium	2.92	11.0	125
17	RB 88	high	+ve	low	6.92	16.3	208
18	RB 66	medium	-ve	medium	6.68	06.7	126
19	Ri 31	medium	- ve	medium	9.80	27.7	

n-decanal, dimethyltrisulphide, 2-ethyl-1-hexanol and nonanal. Except the isolate L 5 and K 24 the antagonists produced low, medium or high levels of ammonia. Microorganisms are able to excrete traces of ammonium ion into their environment as antifungal compound which was found to inhibit fungal growth (Howel *et al*., 1988). The production of HCN, a volatile antifungal compound produced by many antagonistic bacteria was not shown by the isolates tested.

In addition to antagonism, these isolates also had some plant growth promoting activities. All the antagonists produced IAA, the growth promoting hormone. The isolate F 4 showed higher production of IAA (52.5 $\mu g\ mL^{-1}$) than the other isolates which ranged from 1.8- 15.9 $\mu g\ mL^{-1}$. Except the antagonist A 1, all other isolates produced polysaccharides which ranged from 0.5 -27.7 $mg\ mL^{-100}$. Solubilisation of phosphate in Apatite agar as indicated by the zone of clearance around the colony was shown by antagonists other than F 1 and Ps 3. The PO_4 solubilising efficiency (SE) of the isolates ranged from 107- 208 per cent. Hallmann (2001) also reported the beneficial plant growth promoting effects of endophytic bacteria in addition to their antagonistic activity towards plant pathogens. Plant growth promoting rhizobacteria *viz, Pseudomonas fluorescens, P. putida, Bacillus pumilis etc* were found to colonise roots and protect plants against foliar diseases by induced systemic resistance (Kloepper *et al*., 1999).