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SHORT COMMUNICATION

Allelopathic effects of root and leaf leachates of coconut on selected beneficial microorganisms from coconut rhizosphere

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INTRODUCTION

Coconut is a major perennial oil seed crop in India and its each part is used in agriculture, food, fibre, fuel, medicine and industry. Its productive life lasts for 70-80 years, hence, it needs continuous supply of nutrients from its rhizospheric region. Coconut farmers are predominantly marginal; hence, seldom apply fertilizers due to financial constraints. Under such circumstances, many agriculturally beneficial microbes [diazotrophs (10,20), phosphate solubilizers (22) and plant growth promoting rhizobacteria] provide the minimal nutrition to palms. However, their population and activities are affected by tannins and phenols released from biomass, leachates and root exudates. In coconut gardens, the allelochemicals are added to soil through (i) incorporation of 11.2 tonnes of biomass per year (leaf, inflorescence, husk) (3), (ii) stem and leaf leachates during rainy season (June-October) (1) and (iii) root exudates in 2.0 m diameter of each palm (6). The leachates influence the rhizosphere microflora in coconut based cropping system (11). Therefore, a preliminary *in vitro* study was conducted to determine the effects of different concentrations of leaf and root leachates of adult coconut palm on growth of 10 selected beneficial microorganisms isolated from coconut rhizosphere.

MATERIALS AND METHODS

The study consisted of three factors : (i) coconut leachate/ exudates (leaf leachate, root exudates), (ii) leachate/exudates concentrations (5, 10, 20 %) and (iii) 10 microorganisms [*Azoarcus* sp. Reinhold-Hurek, *Azospirillum amazonense* Magalhaes,

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Azospirillum brasilense corrig.Tarrand, *Herbaspirillum frisingense* Kirchof, *Burkholderia* sp. Yabuuchi, *Bacillus* sp. Cohn, *Pseudomonas* sp. Migula, *Brevibacillus brevis* Migula, *Pseudomonas fluorescens* Migula, *Bacillus coagulans* Hammer J from coconut rhizosphere. The diazotrophs were maintained in LG 1 or Glucose medium (8). *Bacillus* sp. (phosphate solubilizer) was maintained on Pikovskaya's medium (19) and *Pseudomonas* sp., *Brevibacillus brevis*, *Pseudomonas fluorescens*, *Bacillus coagulans* (PGPRs) were maintained on Sabouraud's agar or King's B agar medium (15).

Leachate preparation

Leaves from adult coconut palm var. WCT were washed with deionized water and then air dried for 48 h at room temperature (30 °C). Two hundred g of leaves were taken (70 % green, 30 % senescent) and soaked in 1000 ml deionized water for 72 h. Similarly, fresh live roots from same palms were collected, and their leachates were prepared. This gave 20 % concentration of the leaf and root leachates. The leachates were diluted with deionized water to give 10 % and 5 % concentrations. The effect of leachates was tested on the growth of beneficial microorganisms. One set of leaf and root leachates was used as such, i.e. non-sterile, whereas another set was sterilized through membrane filters.

Organochemical analyses of leachates

The root and leaf leachates of the coconut palm were analysed for total sugars (13), reducing sugars (17), total phenols (7) and total free amino acids (18).

***In vitro* agar cup assay**

Agar media selected for the different groups of microorganisms were poured to the thickness of 2 cm in sterile petriplates and then seeded with microorganisms. The seeded plates were allowed to dry. Then using a sterilized 9 mm cork borer, four holes of 9 mm diameter were made in each plate. In the first, second and third wells, 150 μl of leachates of 5, 10 and 20 % concentrations were added, respectively, Deionized water was added to the fourth well. One set of experiment was conducted with non-sterile and another set with filter-sterilized leachates. All the treatments were replicated five times. The plates were incubated at 30 °C for 5 days, thereafter, the hallow zone of inhibition (mm) around the cup was recorded (12).

RESULTS AND DISCUSSION

The pH value of 20 % root leachate was near to neutral pH than the 20 % leaf leachate (Table 1). Root leachate had more phenols (70%), reducing sugars (74%) and total sugars (70%), respectively, than leaf leachate.

Table 1. Organo-chemical properties of leaf and root leachates of coconut palm variety WCT

Leachates	Organo-Chemical parameters				
	pH	Total phenols (ppm)	Reducing sugars (ppm)	Total sugars (ppm)	Total free amino acids (ppm)
Leaf	4.5-5.2	26.8	106.6	249.6	38.4
Root	5.0-5.7	112.1	403.3	827.0	40.1

Non-sterile leachates

The diazotrophs were found sensitive to both root and leaf leachates, with the latter being more allelopathic (Table 2). Increase in concentration of leachates proved to be more inhibitory to growth of microorganisms. Among the five diazotrophs tested, *Herbaspirillum frisingense* was suppressed most and the inhibition zone was greater at 20% concentration. However, *Burkholderia* sp. proved to be very resistant and was least inhibited by both leachates. The growth of *Azoarcus* sp., *Azospirillum amazonense* and *A. brasilense* with leachates was better than *Herbaspirillum* sp. The leachates were not harmful to phosphate solubilizer and PGPRs (*Pseudomonas fluorescens*, *Brevibacillus brevis* and *Pseudomonas* sp.), rather, there was accumulation of bacterial colonies around the leachate wells. At 5 and 10 % concentration of both root and leaf leachates, the bump of the colonies (overgrowth) was more than that at 20% concentration well and control well. However amongst the PGPRs, *Bacillus coagulans* proved sensitive (as its growth was inhibited by both leachates).

Table 2. Effect of leaf and root leachates on growth of beneficial microorganisms isolated from coconut rhizosphere

Microorganisms	Control	Non-sterile leachate (%)						Filter-sterilized leachate (%)					
		Leaf			Root			Leaf			Root		
		5	10	20	5	10	20	5	10	20	5	10	20
Diazotrophs													
Azoarcus sp.	+	1.5	1.5	2.8	0.5	1.1	1.8	2.1	3.6	4.1	0.9	1.9	2.1
Azospirillum amazonense	+	1.7	2.1	2.9	0.8	1.0	1.2	1.9	2.6	3.2	1.2	1.6	2.1
Azospirillum brasilense	+	1.2	1.7	2.1	0.3	0.9	1.1	1.8	2.2	3.2	0.5	1.2	1.7
Burkholderia sp.	+	1.1	1.2	1.8	0.5	0.8	1.1	1.7	2.3	2.8	1.1	1.7	2.3
Herbaspirillum frisingense	+	3.1	4.8	6.3	3.1	4.3	4.8	3.9	5.1	7.2	4.3	4.9	5.6
Phosphate solubilizer													
Bacillus sp.	+	+	+	+	+	++	++	+	+	0.2	+	0.5	1.1
Plant growth promoting rhizobacteria													
Bacillus coagulans	+	0.8	1.2	1.9	0.5	0.7	0.9	1.5	1.9	2.4	0.9	1.2	2.1
Brevibacillus brevis	+	+	+	+	+	+	++	+	+	+	+	++	+
Pseudomonas fluorescens	+	+	++	+	+	+	++	+	+	+	+	++	+
Pseudomonas sp.	+	+	++	+	+	+	++	+	+	+	+	++	0.3

+ and ++ indicates the degree of enhanced growth of microbial colonies around the wells in agar cup containing the leachates as compared to growth around the well containing water. The other numerical data indicates the radial hallow zone area in mm around the wells where no growth of the microorganisms takes place due to the inhibitory effect of the leachates present in the wells in agar cup, when compared to growth around the well containing water.

Filter sterilized leachates

The sterilized root and leaf leachates were more suppressive than non-sterile. The response pattern of the diazotrophs was similar, i.e. greater inhibition. Among the diazotrophs tested, *Herbaspirillum frisingense* was most sensitive to sterilized root leachate as well as to leaf leachate at all test concentrations. The sterilized leaf leachate suppressed the growth more prominently than root leachate, the 20 % concentrations of both leachates proved inhibitory. *Azospirillum amazonense* was least sensitive to sterilized root leachate and *Burkholderia* towards sterilized leaf leachate. The phosphate solubilizing *Bacillus* sp. grew in 5 % sterilized root leachate and 5 and 10 % sterilized leaf leachate, while got suppressed at 20 % concentration of both leachates. Among the PGPRs, *Pseudomonas* sp., *P. fluorescens* and *Brevibacillus brevis* grew profusely in sterilized root and leaf leachates, particularly at 10 % concentration; whereas *Bacillus coagulans* was suppressed by both leachates (Table 2).

The *in vitro* experiments showed that coconut root and leaf leachates had both positive and negative allelopathic effects on beneficial microorganisms isolated from coconut rhizosphere. The non-sterile leachates exhibited less suppression than sterilized leachate, because the resident microflora present on the phylloplane and rhizoplane adapted to the allelochemicals released by the leaf and root tissues may have already diminished the effective concentration of the allelochemicals by utilizing them for their growth. Sensitivity of diazotrophs to leaf leachates is more, probably due to presence of phenols and nitrogen in both leachates. Though the phenol content is high in roots, but its suppression effect was low, presumably owing to presence of high amount of sugars, which offset the effect of phenols by providing growth nutrients to the organisms. Though the leaves are rich in sugars than phenols, but, the presence of 62.9 μg of highly allelopathic 4-hydroxybenzoic acid per g dry coconut leaves (9), a known anti bacterial component supports our observation of suppressive nature of coconut leaf leachate. Kapusta and Rice (14) reported that seven phenolic acids (identified as allelopathic agents), inhibited the growth of three free living nitrogen fixers *viz.*, *Azotobacter*, *Enterobacter* and *Clostridium*. The effects of root extracts from coconut on certain bacterial species are known (5). The phosphate solubilization and growth promoting abilities of *Bacillus* spp. and *Pseudomonas* spp. overcome the inhibitory effect of leachates by their catabolic property of using phenols for their growth (4). Moreover, native *Bacillus* spp. and *Pseudomonas* spp. are capable of utilizing the polyphenols from coconut (23). Our observation of positive influence of coconut leachates on phosphate solubilizers and PGPRs agrees with Rajappan *et al.* (21). They had found *in vitro* studies that dried leaf extract of *Ipomoea* spp. inhibited the growth of rice sheath rot pathogen, while beneficial microbes (*Bacillus subtilis*, *Pseudomonas fluorescens* and *Trichoderma viride*) were not affected.

This preliminary *in vitro* study establishes the differential response of beneficial microorganisms to the allelochemicals present in coconut root and leaf leachates. Similar reports of differential response to the allelochemicals released from decomposition of leaves stimulating the growth of some soil fungi and bacteria and inhibiting the growth of other types of microorganisms had been reported. (2). Identification of the allelochemical that stimulates the activity of the beneficial microorganisms is of great importance as it could be used to increase the microbial activity and improve the soil fertility (16).

CONCLUSIONS

To understand the allelopathic influence of leaf and root leachates of coconut palm on the growth of 10 beneficial microbes [including diazotrophs, phosphate solubilizers and plant growth promoting rhizobacteria (PGPR)] in its own rhizosphere, an *in vitro* experiment was conducted. The diazotrophs were found sensitive to root and leaf leachates, *Herbaspirillum frisingense* was most sensitive and *Burkholderia* sp. was the least. These leachates do not inhibit growth of phosphate solubilizers (particularly *Bacillus* sp.) and PGPRs (*Pseudomonas fluorescens* and *Pseudomonas spp.*), rather leachates at 5 and 10% concentrations increased their growth over control (deionized water). Thus coconut leachates have both inhibitory and stimulatory allelopathic effects on the beneficial microbes of coconut rhizosphere.

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