Induction of Defense Mechanisms in Coffee (*Coffea arabica* L.) Against Coffee Leaf Rust Caused by *Hemileia vastatrix* by Native Rhizobacterial Isolates

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ABSTRACT. *In coffee (Coffea arabica* L.*), leaf rust caused by Hemileia vastatrix is one of the most destructive diseases. The present study was attempted to identify the effects of microbial consortium (i.e. combination of Azospirillum, Azotobacter, Phosphobacteria, Gluconacetobacter and Pseudomonas) along with Arbuscular Mycorrhizal (AM) fungi on the control of coffee leaf rust disease, defense related enzyme activities and biochemical parameters viz., phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO) activities and total phenol contents of treated and untreated coffee plants under field conditions. The results indicated that, two applications of microbial consortium (20 g/plant) and AM fungi (10 g/plant) in coffee plants under field conditions recorded higher PAL, PO, PPO activities and total phenols in response to the coffee leaf rust compared to that of soil and foliar application of Pseudomonas fluorescens (PFK 9) or authentic Pseudomonas fluorescens (Pf 1) alone. The same treatment was found suppressing the development of coffee leaf rust disease under field conditions.*

INTRODUCTION

Coffee (*Coffea arabica* L.), being a perennial crop, is subject to heavy losses in potential production due to the ravages of various diseases. Among many diseases in coffee, the leaf rust caused by the fungus *Hemileia vastatrix* is the most destructive disease causing premature leaf fall, yield loss and sometimes death of plants when the attack is severe (Fernandez *et al*., 2004). In most of the coffee producing countries including India, this disease is being managed by using chemicals and also by adopting some cultural practices. However, the complete control of this disease is very difficult due to the emergence of new physiological races of the rust pathogen as the time progresses. At present, more than 45 physiological races of the rust pathogen have been identified (Rodrigues *et al.*, 1993).

Hence, enhancing the resistance of coffee to the rust fungus has now become the first and foremost priority for economic and sustainable arabica coffee production. Among available strategies for improving plant resistance to parasites, those based on induction of host defense (Hammond Kosack and Parker, 2003) may offer a promising alternative to chemical control. Induced resistance is an important mechanism of biological control by a number of strains of plant growth promoting rhizobacteria applied to roots or seeds or through foliar application in many crops (Ramamoorthy *et al*., 2001). Soil has enormous untapped potential antagonistic microbes, which are helpful in reducing pathogen

inoculation through different modes of action such as competition for nutrients and space, antibiosis, mycoparasitism, production of siderophores and lytic enzymes (Rangaswami, 1975). At present, a similar type of approach in coffee to control rust disease is very much essential. The present study aims to identify the effect of native biological agents on disease suppression and various biochemical parameters to induce disease resistance in the host plant system.

MATERIALS AND METHODS

A field experiment was conducted during 2005-2006 at Regional Coffee Research Station, Tamil Nadu, India. The cultivar selected was fifteen years old S.795 planted at a spacing of 2 m x 2 m. In the experiment, the following efficient isolates screened from different coffee growing regions in India *viz*., *Azospirillum* - ASW 13, *Azotobacter* - AZK 9, *Gluconacetobacter* - GDT 13, Phosphobacteria - PBY 23, *Pseudomonas fluorescens* - PKK 9 and Arbuscular Mycorrhizal (AM) fungi like *Glomus mosseae*, *Glomus fasciculatum* and *Glomus* spp. were used for the study. The authentic strain *Pseudomonas fluorescens* - Pf 1 obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India was used as standard check for the experiment. In AM fungi, the mixed cultures were multiplied using sterile vermiculite as substrate and maize as host plant. These root parts and soil containing chlamydospores were used as inocula (40-50 spores/g of inoculums) for the experiment.

The selected bacterial isolates were multiplied in nutrient medium and with cell load of 10^8 cfu/ml were used for inoculant preparation. Lignite (Neyveli Lignite Corporation Limited, Tamil Nadu, India) was used as a carrier material. The microbial consortium was prepared by mixing equal volumes of nutrient medium (above mentioned five bacterial isolates from coffee plantations) with carrier material. Similarly, *P. fluorescens* - PFK 9 and *P. fluorescens* - Pf 1 were prepared separately and used in the experiment. The details of treatments are given in Table 1 and were applied during the month of June 2005. The treatment was replicated four times and Randomized Block Design was used.

Table 1. Details of different treatments used for the experiment.

Treatment	Description of treatments
T_1	Control (un inoculated)
T_2	Soil application of *Pseudomonas fluorescens* - PFK 9
T_3	Soil application of authentic *P. fluorescens* - Pf 1
T_4	Foliar application of *P. fluorescens* - PFK 9
T_5	Foliar application of authentic *P. fluorescens* - Pf 1
T_6	Soil application of microbial consortium
T_7	Soil and foliar application of *P. fluorescens* - PFK 9
T_8	Soil and foliar application of authentic *P. fluorescens* - Pf 1

At the time of initiation of experiment, as per the treatments, soil application of *P. fluorescens* - PFK 9, *P. fluorescens* - Pf 1 and microbial consortium was given at the rate of 20 g/plant and then second application was given during the month of August 2005. The foliar application of *P. fluorescens* - PFK 9 and Pf 1 at the rate of 0.5% was given at the time of soil application. In microbial consortium treatment, AM fungi were inoculated at the rate of 10 g/plant. The rust incidence level was observed in all the treatments at monthly interval up to January 2006. Observation on leaf rust incidence was recorded by counting the total number of leaves and infected leaves and calculated percentage of coffee rust incidence at monthly intervals from June 2005 to January 2006. The observed values of percentage of coffee rust incidence were transformed in to arc sine transformation and the same values were used for statistical analysis.

To study the Induced Systemic Resistance (ISR), the enzyme activities *viz*., Peroxidase (PO), Polyphenol Oxidase (PPO), Phenylalanine Ammonia Lyase (PAL) and defense inducing chemicals (total phenols) for rust disease in coffee plants were assessed. From treated plants, leaves were collected randomly at 0, 12 h, 1, 3, 7, 14 and 21 days after second application of *Pseudomonas* and microbial consortium. The samples were subjected to biochemical analysis *viz.,* PAL (Dickerson *et al*., 1984), PO (Hammerschmidt *et al*., 1982), PPO (Mayer *et al*., 1965) and total phenol content (Zieslin and Ben-Zaken, 1993) by adopting standard methods.

RESULTS

In general, coffee rust incidences were in the range of 4.8 to 23.1% during 2005-2006 seasons in control. In all the treatments, the disease incidences were low up to July and subsequently increased from September to November, and thereafter, a declining trend was noticed. Among the treatments, soil application of microbial consortium (T_6) showed a lower level of disease incidence from September to January (8.5-11.6%) followed by soil and foliar application of *P. fluorescens* - PFK 9 (4.8-14.5%) and *P. fluorescens* - Pf 1 (4.8-15.2%), respectively (Table 2).

The results of the enzyme activities indicated that among all the treatments, soil application of microbial consortium significantly increased the enzyme activities *viz.,* PO, PPO and PAL after 12 h to three days compared to other treatments (Figs 1, 2 and 3). In general, the enzymes PO, PPO and PAL activities increased up to three days followed by a declining trend up to 21 days in all the treatments (Figs 1, 2 and 3). Similar to enzyme activities, the accumulation of total phenols started one day after application of either foliar or soil application of *P. fluorescens* or microbial consortium (Table 3).

In control, there was no perceptible difference in the accumulation of phenol in leaves from 0 h to 21 days (111.6 to 113.6 µg of catechol/g of fresh leaf tissue), where as in other treatments, the total phenol content increased up to three days, thereafter the increased levels were maintained up to 21 days. The soil application of microbial consortium recorded significantly higher amount of total phenols in the range of 136.2 to 186.6 µg of catechol/g of fresh leaf tissue when compared to other treatments.

Table 2. Rust incidences from June 2005 to January 2006 in arabica coffee (S. 795) treated with *Pseudomonas fluorescens* and microbial consortium under field conditions.

Treatment	Rust incidences (%) from June 2005 to January 2006							
	June 2005	July	Aug	Sep	Oct	Nov	Dec	Jan 2006
T_1	5.2 (13.21)	6.9 (15.20)	9.6 (18.02)	15.6 (23.22)	21.3 (27.43)	23.1 (28.70)	19.8 (23.39)	15.6 (23.22)
T_2	5.2 (13.13)	6.8 (14.83)	8.6 (15.83)	14.0 (20.28)	19.6 (24.74)	21.4 (25.99)	18.2 (23.65)	13.9 (20.11)
T_3	5.1 (12.74)	6.8 (15.06)	8.4 (16.29)	13.5 (20.39)	19.1 (24.99)	20.9 (26.38)	17.7 (24.07)	13.4 (20.32)
T_4	5.1 (13.04)	6.5 (14.75)	6.9 (15.24)	11.7 (19.98)	15.9 (23.46)	16.8 (24.19)	14.5 (22.36)	11.5 (19.80)
T_5	5.3 (13.24)	6.5 (14.72)	7.3 (15.60)	11.5 (19.75)	16.2 (23.67)	17.2 (24.43)	15.9 (23.42)	11.7 (19.94)
T_6	5.0 (12.88)	6.2 (14.43)	4.8 (12.48)	08.6 (15.41)	11.6 (19.63)	11.1 (19.24)	09.4 (17.86)	08.5 (15.03)
T_7	4.8 (12.61)	6.3 (14.53)	6.3 (14.51)	11.2 (19.48)	13.3 (21.32)	14.5 (22.29)	11.9 (20.13)	10.8 (19.12)
T_8	4.8 (12.61)	6.3 (14.50)	6.7 (14.96)	11.4 (19.70)	14.8 (23.83)	15.2 (22.90)	12.5 (20.67)	11.1 (19.39)
SEd	0.58	0.68	0.73	0.99	1.14	1.26	1.13	0.98
CD (P=0.05)	1.18	1.39	1.49	2.04	2.33	2.59	2.32	2.01

Note: Figures in the parentheses are arc sine transformed values; SEd - Standard Error of difference; CD - Critical Difference.

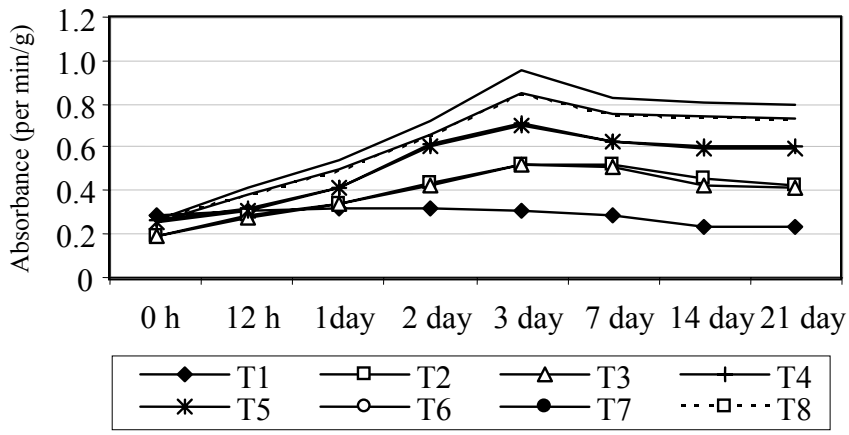

Fig. 1. Changes in peroxidase activity in arabica coffee (S. 795) under field condition against coffee leaf rust (*Hemileia vastatrix*).

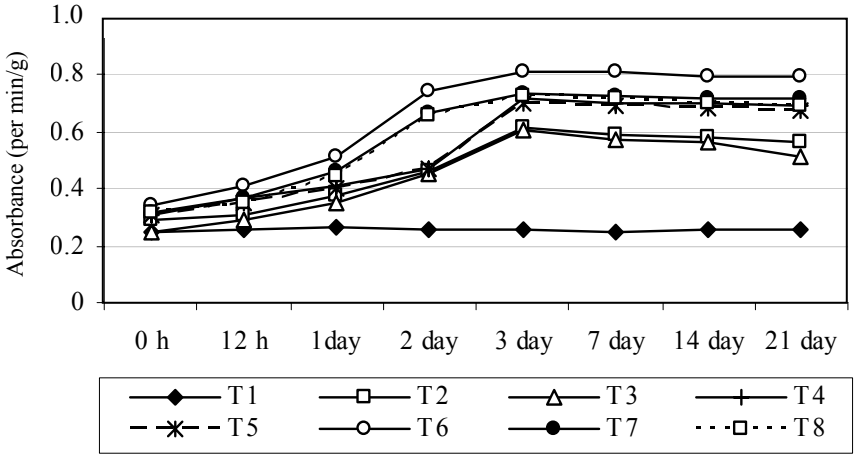

Fig. 2. Changes in polyphenol oxidase activity in arabica coffee (S. 795 under field condition against coffee leaf rust (*Hemileia vastatrix*).

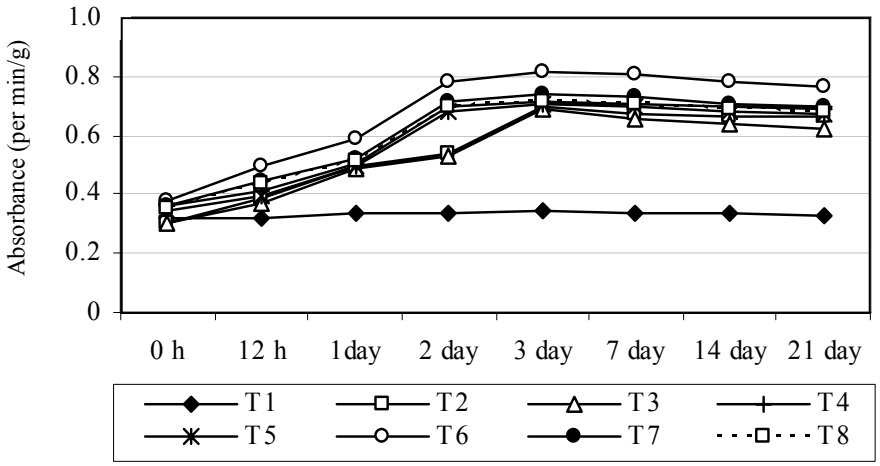

Fig. 3. Changes in phenylalanine ammonia lyase activity in arabica coffee (S. 795) under field condition against coffee leaf rust (*Hemileia vastatrix*).

Note:

T_1 - Control
T_2 - Soil application of *P.fluorescens* - PFK 9
T_3 - Soil application of *P.fluorescens* - Pf 1
T_4 - Foliar application of *P.fluorescens* - PFK 9
T_5 - Foliar application of *P.fluorescens* - Pf 1
T_6 - Soil application of microbial consortium
T_7 - Soil and Foliar application of *P.fluorescens* - PFK 9
T_8 - Soil and Foliar application of *P.fluorescens* - Pf 1

Results of the change in total phenol content of coffee leaves indicated that all the treatments recorded significantly higher total phenol contents in all the months when compared to the control. Among the treatments, the microbial consortium application recorded significantly higher amount of total phenol (160.8 to 199.8 µg/g of fresh leaf

tissue) followed by soil and foliar application of either *P. fluorescens* - PFK 9 or *P. fluorescens* - Pf 1 (Table 4).

Table 3. Total phenol contents in arabica coffee (S. 795) treated with *Pseudomonas fluorescens* and microbial consortium under field conditions against coffee leaf rust (*Hemileia vastatrix*).

Treatment	Total phenol content (µg of catachol/g of fresh leaf tissue)							
	0 h	12 h	1day	2nd day	3rd day	7th day	14th day	21st day
T_1	113.6	113.6	113.4	113.3	113.2	112.8	112.2	111.6
T_2	130.1	131.0	137.6	153.8	154.4	152.6	153.2	153.2
T_3	129.8	130.0	136.5	151.6	153.8	150.2	152.2	152.6
T_4	130.6	132.0	141.0	159.8	161.2	160.8	160.6	156.4
T_5	130.2	131.8	139.7	156.2	160.3	157.2	159.2	155.4
T_6	136.2	142.4	158.4	184.2	186.6	183.0	182.4	179.2
T_7	130.4	133.6	149.3	172.6	175.3	171.4	169.8	166.4
T_8	130.8	132.4	141.6	169.8	171.2	168.6	163.4	162.6
SEd	3.62	3.76	4.03	4.93	5.04	4.93	4.94	4.85
CD (P=0.05)	7.39	7.67	8.22	10.05	10.28	10.05	10.07	9.90

Note: SEd - Standard Error of difference; CD - Critical Difference.

Table 4. Total phenol contents in arabica coffee (S. 795) treated with *Pseudomonas fluorescens* and microbial consortium under field conditions against coffee leaf rust (*Hemileia vastatrix*).

Treatment	Total phenol content in leaves (µg of catachol/g of fresh leaf tissue)							
	Jun 2005	Jul	Aug	Sep	Oct	Nov	Dec	Jan 2006
T_1	110.2	108.4	112.6	113.2	116.4	120.8	114.8	114.2
T_2	149.6	143.8	147.2	148.1	149.3	153.2	150.3	139.6
T_3	148.2	141.3	144.4	114.6	145.4	151.6	151.0	138.2
T_4	152.3	146.2	149.2	150.6	153.8	159.2	156.0	141.2
T_5	150.6	145.7	148.2	148.6	151.6	158.0	155.7	141.0
T_6	160.8	169.3	171.8	181.9	193.2	199.8	184.2	181.2
T_7	156.4	161.2	166.4	169.4	171.6	176.4	161.4	160.2
T_8	154.6	160.5	161.2	165.4	170.2	172.6	159.8	158.4
SEd	9.82	9.46	9.75	9.04	9.96	10.52	10.14	9.06
CD (P=0.05)	20.72	19.97	20.57	19.08	21.02	22.19	21.39	19.12

Note: SEd = Standard Error of difference; CD = Critical Difference.

DISCUSSION

In the present investigation, soil application of microbial consortium before the onset of monsoon (June and August) showed a lower level of disease incidence from September to January (8.5-11.6%) followed by soil and foliar application of either *P. fluorescens* - PFK 9 or Pf 1 under field conditions when compared to other treatments. Based on the scientific information available from other crops, it can be inferred that synergistic interaction between rhizobacteria and AM fungi might have resulted in decreasing rust incidence level. Induction of systemic resistance by growth promoting rhizobacteria against *Eucalyptus* spp. rust caused by *Puccinia psidii* has been reported by Teixeira *et al.* (2005), that the rhizobacterial isolates FL2 and MF4 were significantly more efficient in reducing *Eucalyptus* spp. rust severity.

At present, inducing self defense mechanisms of plants by prior application of a biological inducer are thought to be a novel plant protection strategy. Induction of systemic resistance by *P. fluorescens* has earlier been reported by several researchers (Zehnder *et al.,* 2000). Cucumber seed treatment with *P. fluorescens* suppressed the foliar pathogen by inducing systemic resistance (Wei *et al.,* 1996). In the present study, the maximum activity level of all the three enzymes *viz*., PO, PPO and PAL were observed in coffee plants inoculated with microbial consortium followed by soil and foliar application of either *P. fluorescens* PFK 9 or *P. fluorescens* Pf 1. These results clearly indicated that the increased activity of defense enzymes (PO, PPO and PAL) in coffee plants might have suppressed the development of rust on the coffee leaves.

In coffee, Ram *et al.* (2002) reported that the constitutive level of phenylalanine ammonia lyase (PAL), lipoxygenase (LOX) and peroxidase (PO) activity in progeny population derived from Ligenoides and Hibrido de Timor (HdeT) was manifesting high rust resistance. They also stated that PAL and LOX are good indicators of coffee leaf rust resistance at the constitutive level of activity and it can be effectively utilized for the identification of rust resistance.

In the present study, change in total phenol content of coffee leaves was assessed and the results indicated that in treated coffee plants, the increasing accumulation of phenol was directly proportional to the rust incidence. Among the treatments, the microbial consortium application recorded significantly higher amount of total phenol followed by soil and foliar application of either *P. fluorescens* - PFK 9 or Pf 1. These results are in accordance with the earlier findings of many researchers in other crops. Phenols are toxic to fungi in nature and increase the physical and mechanical strength of the host plant cell walls.

Guedes *et al.* (1994) reported the total phenol and phytoalexins accumulation in coffee infected with *Hemileia vastatrix* and *Pseudomonas syringae*. In the present study, the microbial consortium and AM fungi played a similar role in controlling pathogens in coffee plants. Phenolic compounds are known as antifungal substances produced in the host due to colonization by AM fungi. The AM fungi colonized roots expressed more than double the concentration of phenols (130 mg/g fresh weight) when compared to control (62 mg/g fresh weight) which is responsible for the suppression of the pathogen, *Sclerotium rolfsii* (Krishna and Bagyaraj, 1983). Arbuscular Mycorrhizal colonization lead to the induction and accumulation of isoflavanoids and expression of defense gene transcripts. Association of *Glomus versiforme* in the roots of *Medicago truncatul* increased the levels of PAL and

defense gene transcripts consistent with isoflavanoid accumulation (Harrison and Dixon, 1993).

CONCLUSIONS

The present investigation indicated that the two applications of microbial consortium (20 g/plant) and Arbuscular Mycorrhizal fungi (10 g/plant) in coffee plants under field conditions was found to be suppressing the development of coffee leaf rust disease. This finding also showed the use of native rhizobacterial isolates in combination was effective in inducing host plant resistance to coffee leaf rust disease infection. Microbial consortium along with Arbuscular Mycorrhizal fungi showed induction of defense related enzymes in coffee against coffee leaf rust and those could be utilized in integrated disease management strategy as an eco-friendly and inexpensive method.

ACKNOWLEDGEMENTS

The financial assistance (in the form of study leave) by Coffee Board, Bangalore, India is gratefully acknowledged.

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