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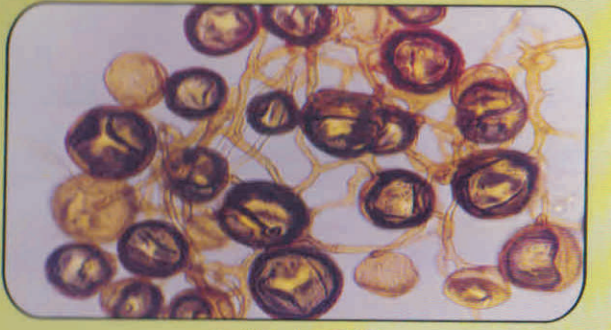

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RHIZOSPHERE BIOTECHNOLOGY: PLANT GROWTH – RETROSPECT AND PROSPECT

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CONTENTS

Chapter - 7

INDUCTION OF SYSTEMIC RESISTANCE IN TEA PLANTS AGAINST ROOT ROT PATHOGEN UPON FIELD APPLICATION OF VAM, PHOSPHATE SOLUBILISING FUNGUS AND BACTERIUM

B.N. Chakraborty[1], U. Chakraborty[1], B.C. Barman[1], L.P. Bhutia[1] and P.K. Ghosh[2]

[1]Immuno-Phytopathology Laboratory, Department of Botany, University of North Bengal, Siliguri-734 430, West Bengal
[2] Cadila Pharmaceuticals Ltd. Cadila Corporate Campus, Ahmedabad-382 210, Gujarat

INTRODUCTION

Tea [*Camellia sinensis* (L.) O. Kuntze] is the most important plantation crop of Darjeeling hills and Terai region and is a perennial plant. Long-term exploitation of soil has led to decreased productivity and increased susceptibility to pests and pathogens, in spite of increasing application of pesticides and external inputs of fertilizers. Hence the use of commercially available bioformulations- 'JOSH' (VAM Product) alone or mixture with 'KALISENA' - made from a soil borne non-symbiotic phosphate solubilising fungus (PSF- *Aspergillus niger*) marketed by Cadila Pharmaceuticals, Agro Division as biofertilizer and a bioprotectant, in combination with a potential strain of *Bacillus megaterium* - plant growth promoting rhizobacterium [PGPR] which was isolated from tea rhizosphere were tested for developing systemic resistance in tea plants against

Fomes lamaoensis causing brown root rot disease. These provides an alternative and effective formulation, not hazardous for the ecological equilibrium, which is the basic nullifying attribute of chemical fertilizers.

PGPR are the soil and rhizosphere bacteria that can benefit plant growth by different mechanism (Glick, 1995). The use of PGPR as natural biofertilizer is advantageous, not only from the economical, but also from the ecological point of view. PGPR has the capability of disease suppression may be due to iron sequestration, production of antibiotics or induction of systemic resistance (Alstrom, 1991; Liu *et al.*, 1995; Reddy *et al.*, 1999; Zehnder *et al.*, 2000; Vidyasekharan *et al.*, 2001). The ability to convert insoluble phosphorus to an accessible form like orthophosphate is an important trait for a PGPR for increasing plant yields (Rossolini *et al.*, 1998).

The present investigation was therefore, undertaken to explore the possibility of using VAM, PSF and PGPR for mobilization of phosphorous in tea soils and also to see their effects on growth and development of tea plants and also suppression of brown root rot disease.

MATERIALS AND METHODS

Plant material

Two varieties (TV-26 and S-449) of *Camellia sinensis* were selected from the Tea Germplasm Bank maintained in the premises of Immuno-phytopathology Laboratory, Department of Botany, NBU.

Fungal Pathogen

Tea root rot pathogen - *Fomes lamaoensis* (Murr.) Sacc & Trott. was obtained from Tocklai Tea Research Institute, Jorhat, Assam.

Bioinoculants

Commercially available VAM product namely 'JOSH' and phosphate solubilising fungus KALISENA produced and marketed by Cadila Pharmaceuticals, Agro division, Ahmedabad, Gujarat were used for this study. *Bacillus megaterium* a potential PGPR was isolated from tea rhizosphere and identified by Diagnostic and

Advisory Service, CABI Bioscience, UK. JOSH was available in powder as well as capsule form. Another commercially available formulation under the product name KALISENA - SD consisting of *Aspergillus niger* (3.28 x 10^8 CFU/gm, Adjuvant 0.5%, Carrier q.s) was prepared as per product specifications. PGPR (*B megaterium*) was multiplied in Nutrient Broth (NB) media for 3-4 days before inoculation.

Application procedure

JOSH was added in rhizosphere of plant in two different ways, either in capsules form or in powder form. In pot condition plants were inoculated with six JOSH capsules/plant. In field condition 10 g of powder was added in each plant rhizosphere. KALISENA-SD was added @ ½ kg/plant at a depth of about 10-15 cm. *B. megaterium* (PGPR) as a suspension (10^6 cfu) was applied to the rhizosphere of tea plants @ 150 ml per pot and application was done thrice at an interval of one month.

Preparation of inoculum and inoculation technique

The inoculum of *F.lamaoensis* was prepared in sand maize meal media (1:1) containing tea root pieces. Three-year-old tea plants grown in earthen pots (12" diameter) containing 5kg soil were inoculated carefully by adding 100 g of inoculum in the rhizosphere of each plant after studying and collecting the data regarding the growth of the plants 60 days following application with bioinoculants. Disease assessment was done 30 days after inoculation. Disease intensity was assessed as rot index on a scale of 0-6, depending on both underground and above ground symptoms.

Population count of VAM

VAM population in soil was determined by wet sieving and decanting method (Gardeman and Nicolson, 1963) and spore collection after 60 days of treatment was performed. The percentage of increase in spore number per gm of soil was determined

Extraction and estimation of phenolics

Phenols were extracted and estimated after 30 days of inoculation following the procedure as described by Mahadevan and Sridhar (1982).

Enzymes

***Phenylalanaine ammonia lyase* (PAL) :** For the extraction of PAL, the method of Bhattacharya and Ward (1987) was followed with modifications.

***Chitinase* :** The Colorimetric assay of Chitinase was carried out according to the Procedure developed by Boller and Mauch (1988). Chitinase activity was expressed as μg/gm N-acetyl D-glucosamine produced/gm tissue/hour.

***β-1,3-Glucanase* :** Assay of activity was done colorimetrically by the Laminarin-Dinitrosalicylate method (Pan *et al.*, 1991). Activity was expressed as μg/min/ gm tissue.

***Poly phenol oxidase* :** For the extraction and assay of poly-phenol oxidase, the method of Mahadevan and Sridhar (1982) was followed.

Extraction of antifungal phenolics and bioassay

Following the method of Daayf *et al.* (1995), antifungal phenolics were extracted from tea roots in methanol, partitioned against diethyl ether, hydrolysed and different fractions were collected and bioassayed by spore germination test.

Estimation of total phosphorous in soil

The Modified Morgan extraction of phosphorus from soil, as described by Knudsen and Beegle (1988) was used. Quantities of phosphorus in soil extract were estimated by comparison with standard curve.

RESULTS AND DISCUSSIONS

VAM population in rhizosphere

Population of VAM spores (*Glomus* sp.) in the rhizosphere after a period of two months following inoculation with Josh showed a significant increase in the range of 60-70% (Table 1).

Plant growth

Effect of inoculations with Josh, Kalisena and *B. megaterium* singly and jointly on growth of tea plants were evaluated in terms of number of leaves, number of branches and height of the plant.

Increase was noted in all cases, of which most significant was obtained by the combined inoculation of Josh and PGPR (Table 2).

Table 1. Percentage increase in spore number of *Glomus* sp. after treatment with Josh

Variety	VAM spores / 10 g of soil		% Increase in spore number following inoculation
	Untreated	Josh inoculated	
TV26	48	83	72
S449	56	88	57

Table 2. Plant growth promotion in tea varieties following different treatments

Treatments	TV-26			S-449		
	Percentage increase in*			Percentage increase in*		
	Height	No. of branches	No. of leaves	Height	No. of branches	No. of leaves
Untreated	20.0	50.0	62.5	52.6	50.0	70.0
Kalisena	72.6	80.0	160.0	61.5	75.0	68.7
Josh	83.3	100.0	166.6	60.0	80.0	86.6
PGPR	90.0	200.0	145.5	50.0	100.0	108.3
Josh + PGPR	105.0	300.0	200.0	110.5	150.0	200.0
Josh + Kalisena + PGPR	88.8	166.6	141.6	77.3	140.0	155.0

* Percent increase calculated after a period of 60 days, taking into consideration initial and final readings

Disease assessment following treatments

Tea plants (TV-26 and S-449) inoculated with Josh, PGPR and Kalisena were further challenge inoculated with brown root rot pathogen, *Fomes lamaoensis*. Disease incidence was calculated. It was observed that all the treatments reduced the disease incidence in relation to untreated inoculated control. Most effective disease control was obtained when joint inoculation with PGPR, VAM and PSF was done (Fig. 1).

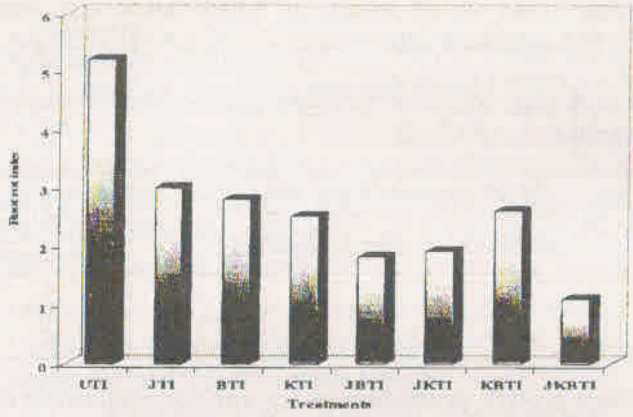

Fig. 1. Root rot index of tea plants inoculated with *Fomes lamaoensis* and subjected to various treatments. UTI = Untreated inoculated (*F. lamaoensis* alone); JTI= Josh + *F. lamaoensis*; BTI = *Bacillus magaterium* + *F. lamaoensis*; KTI= Kalisena + *F.lamaoensis*; JBTI = Josh + *B. megaterium* + *F. lamaoensis*; JKTI = Josh + Kalisena + *F. lamaoensis*; KBTI = Kalisena + *B. megaterium* + *F. lamaoensis*; JKBTI = Josh + Kalisena + *B. megaterium* + *F. lamaoensis.*

Changes in the biochemical components in tea roots following treatments

Phenols **:** An increase in phenol content was obtained in all treatments. Maximum increase was noticed in Josh treated plants (TV-26). But Josh treatment in both the varieties (S-449 and TV-26) resulted in steady increase of phenolics following inoculation with *F. lamaoensis* (Fig. 2).

Antifungal phenolics **:** Extraction and bioassay of antifungal phenolics from roots of untreated healthy and Josh treated plants (TV-26) revealed the presence of antifungal compounds in Fraction III and to some extent in Fraction II of both extracts (Table 3).

Enzymes : Four enzymes involved in defense mechanism, ie., β-1, 3-glucanase (GLU), chitinase (CHT), phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) activities following treatment with Josh, kalisena and PGPR and inoculated with fungal pathogens were assayed. Results reveal that activities of all enzymes increased in the different treatments in both varieties, but greatest in combined treatments. Enzyme activities (CHT, GLU,

PAL and PPO) following treatment with Josh alone following inoculation with root rot pathogen (*F. lamaoensis*) have been presented in Fig. 3.

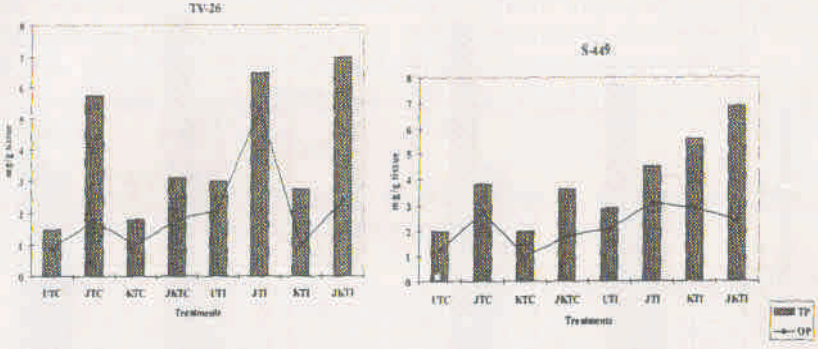

Fig. 2 : Total and O-dihydrox phenol contents in tea roots following various rteatments. UTC= Untreated; JT= Josh treated; KT= Kalisena treated; JKT = Josh + Kalisena treated C= control, I= inoculated with *F. lamaoensis.*

Table 3. Spore germination of different fractions of antifungal phenolics from tea roots

Fractions	% spore germination*
Diethyl ether Control	90.0
Diethyl ether fraction (I)	
UT	80.9
JT	76.4
Ethyl acetate Control	85.0
Ethyl acetate fraction (II)	
UT	80.9
JT	27.2
Ethyl acetate fraction Hydrolyzed (III)	
UT	20.0
JT	00.0
Distilled water control	96.2
Water fraction (IV)	
UT	94.8
JT	92.6

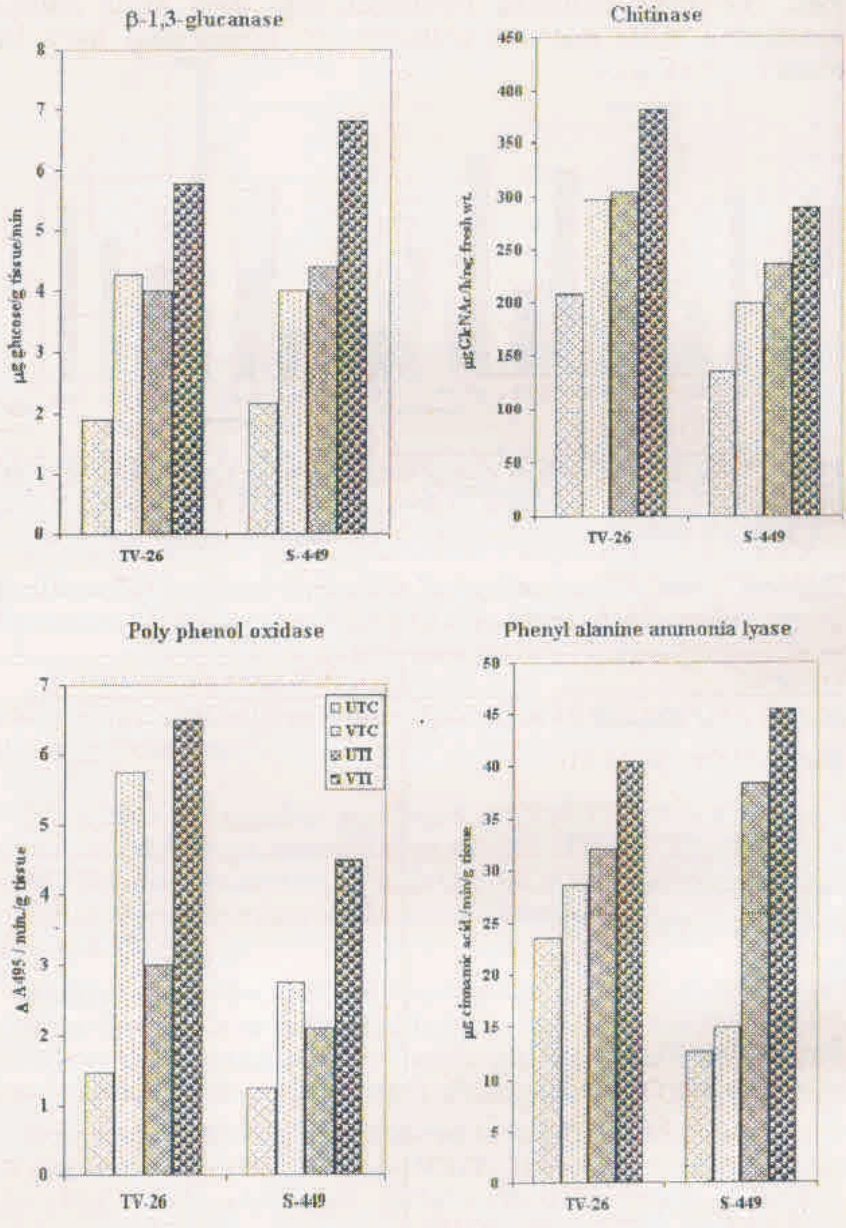

Fig. 3. Changes in defense related enzymes of tea roots following reatments with Josh and *F. lamaoensis*. UT= Untreated; VT = VAM (Josh) treated; C=Control; I=Inoculated with *F. lamaoensis*.

Soil phosphorus : Soil sample from rhizosphere of the two varieties were collected after 60 days treatment and phosphorus content in soil was analyzed for the amount of phosphorus depletion on mobilization after treatment with Josh, Kalisena and PGPR. Phosphorus content in soil has been lowered in the treated block due to excess phosphorus mobilization or utilization by those plants treated with 'Josh', 'Kalisena SD' and combination with PGPR (Table 4).

Table 4. Total phosphorous in rhizosphere soil of tea plants subjected to various treatments

Variety	Treatments	Concentration (mg/lit)
TV-26	Control	0.302
	Josh	0.256
	Kalisena	0.190
	PGPR	0.178
	Josh + PGPR	0.185
	Josh +Kalisena + PGPR	0.180
S-449	Control	0.354
	Josh	0.232
	Kalisena	0.218
	PGPR	0.193
	Josh+PGPR	0.191
	Josh + Kalisena + PGPR	0.195

Considering the result of above experiments it could be said that Josh (VAM), Kalisena (PSF) and *B. megaterium* (PGPR), show ability to increase growth of tea plants and induce resistance. Analysis of biochemical components have shown that these have a certain and obvious capability of inducing certain biochemical changes in the plant tissue system enhancing the production of compounds instrumental in aiding the host defense mechanism. The treatment with Kalisena and the mixture of these did bring about the increase in the defense related compounds like phenols and defense enzymes, yet the biochemical analysis showed certain fluctuations according to various plant material. But VAM treated plants showed steady and increased concentration and activities of phenols and defense related enzymes. Mycorrhizal plants develop extensive root system as compared to non-mycorrhizal plants, which

ensures the plant with increased availability of nutrients there by helping the plant for better growth and development (Carling *et al.*, 1978). Mycorrhizal plants usually have more vascular bundles, hence lignifications in the xylem is greater (Dehne, 1982) which may be instrumental in restricting pathogen proliferation. The possible mechanism may be competition for colonization sites, direct antibiosis and defence reactions colonization of roots by AM fungi induces biochemical changes within host tissue, these include stimulation of phenyl propanoid pathway and activation of defense related genes. Use of commer-cialized products like Josh and Kalisena, are beneficial to the farmers as they are biotic hence eco-friendly, and over all cheap in comparison to other high quality biofertilizer. In this study the use of a native PGPR strain was also found to have the potential of increasing plant growth and which was also a good phosphate solubilizer. Also important fact is that spores of VAM do not easily loose their viability, and germinate again, so once applied to the field, the spores will adhere to the soil lattices and proliferate thus increasing the efficiency of the soil VAM, is an excellent soil aggregator, it changes the macro compounds into micro compounds, which can be easily assimilated by the plant, hence the plant health will increase vigorously. The results obtained in the present investigation have pointed to the possible use of a combination of two or more products commercially in tea plantations to increase productivity and yield without the use of additional chemical inputs.

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