PLANT GROWTH PROMOTING RHIZOBACTERIA, THEIR CHARACTERIZATION AND MECHANISMS IN THE SUPPRESSION OF SOIL BORNE PATHOGENS OF COLEUS AND ASHWAGANDHA

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1. INTRODUCTION

Coleus forskohlii (Wild) Briq. [Synonym *C. barbatus* (Andr.) Benth.] is a plant of Indian origin (Valdes *et al*., 1987) and belongs to the family Lamiaceae (previously Labiatae). It is the most important species of genus Coleus popularly known as Pashan Bhedi in Sanskrit, Patharchur in Hindi, Makandi or Mangani beru in Kannada, Garmalu in Gujarathi and Mainmul in Marathi. It is one of the most important potential medicinal crops of the future, as its pharmacological properties have been discovered recently.

It is a subtropical and warm temperate crop, naturally grown at 600-1800 m elevation. It is cultivated in India, Pakistan, Sri Lanka, tropical East Africa, Brazil, Eygpt, Nepal, Arabia and Ethiopia. In India, it is found in the subtropical Himalayan regions from Kumaon to Nepal, Bihar and on the Deccan plateau of southern India upto an altitude of 2500 M above mean sea level. It is cultivated in parts of Rajasthan, Maharashtra, Gujarat, Karnataka and Tamil Nadu and is being grown in an area of more than 2500 hactres for its tuberous roots.

Tuberous roots of *Coleus forskohlii* resembling carrot in shape and brown in colour contain a diterpenoid forskolin (Syn. Coleonol) (Shah, *et al.* 1980) an important constituent which ranges from 0.1 to 0.8 per cent (Valdes *et al*., 1987). The Indian herb *C. forskohlii* is the only known source of forskolin (De Sauza and Shah, 1988). One gram of forskolin costs US $110, showing the importance of this crop. The alkaloid has the unique property of activating all hormone-sensitive adenylate cyclase enzymes in biological systems. Traditionally, the tuberous roots of coleus have been used as condiments and in the preparation of pickles (Anon., 1950). They are known for a number of medicinal properties: used in the Ayurvedic schools of medicines (Ammon and Muller, 1985). They are used as antihelmentic and few drops of root extract in milk is administered to infants suffering from constipation and abdominal pain (Singh *et al*., 1980).

The therapeutic properties of forskolin are utilized in treating cardiac insufficiency, hypertension, glaucoma, thrombosis, cancer, asthama and metastatic conditions (Seamon, 1984). Further, pharmacological and biochemical investigations established that forskolin possesses multifaceted biological activities such as positive inotropic, antihypertensive, branchospasmolytic, anti-thrombotic, platelet aggregation inhibiting, antiglaucoma, and adenylate cyclase stimulation (Rupp *et al*., 1986). The novel feature of forskolin is its unique mechanism of generating cyclic adenosine monophosphate (AMP) in the cells through the direct activation of the catalytic unit of adenylate cyclase enzyme, which made the pharmaceutical industry to recognize the plant as most medicinally and economically important.

Ashwagandha (*Withania somnifera* L. Dunal), also known as Indian ginseng, belongs to the family Solanaceae, is an important ancient medicinal plant, used in the Indian traditional systems of medicine, ayurveda and unani. It is popularly known as ashwagandha and varahakarni in Sanskrit, asgandh and punir in Hindi, hiremaddinagida, panneru and ashwagandha in Kannada, amukkira in Tamil and winter cherry in English.

The local name, ashwagandha seems to have been derived form the Sanskrit name, which means smelling like a horse. It is a widespread species having disseminated from the southern mediterranean area to the different parts of Africa, India, Isreal, Jordan, Egypt, Sudan, Iran, Afghanistan and Pakistan. It grows in dry parts in sub-tropical regions. Rajasthan, Punjab, Haryana, Uttar Pradesh, Gujarat, Maharashtra, Tamil Nadu and Madhya Pradesh are the major ashwagandha producing states of the country. In Madhya Pradesh alone it is cultivated in more than 5000 ha. The estimated annual production of ashwagandha roots in India is more than 2000 tonnes, against annual requirement of about 7000 tonnes (Patra *et al*., 2004).

Ashwagandha is regarded as the treasure of biochemical constituents serving as remedy for many health problems. Roots contain several pyrazole alkoloids like withasomnine, withaferin A, withanolides, withaninol and steroidal lactones, starch and reducing sugars. Withaferin is the chief constituent (0.13 to 0.31%) having bacteriostatic and

antitumerous properties. It is considered to be one of the best rejuvenating agents in Ayurveda (Farooqi *et al.*, 2003).

Its roots, seeds and leaves are used in ayurvedic and unani medicines. Roots are used for treatment of rheumatic pain, inflammation of joints, nervous disorders and epilepsy, and also used as tonic for hiccup, cold, cough and female disorders. The leaves are also useful for carbuncles, inflammation, swellings and conjunctivitis. Bark decoction is taken to cure asthama and bed sores. Ashwagandha and its extracts are used in preparation of herbal tea, powders, tablets and syrups which help in reducing arthritis, disability, fatigue, high cholesterol and stress, increase healing processes, have positive effect against impotence and normalize the sugar content of the blood.

India could be termed the "Botanical Garden" of the world. Indian system of medicine (ISM) exploits 25,000 species of medicinal plants spanned over 1000 genera. Phyto-pharmaceutical companies require a wealth in knowledge on biological diversity in rhizosphere and non-rhizosphere of medicinal plants (Karthikeyan *et al*., 2007). The pharmaceutical industries are mainly dependent upon the wild population of these plants for the supply of tuberous roots for forskolin and withafarin extraction. The large scale and indiscriminate collection of the wild material from the forests and inadequate attempts either to allow its replenishment or its cultivation have led these two important medicinal plants to be listed as endangered species (Gupta, 1988; Vishwakarma *et al*., 1988). Among the major constraints for growing these two crops diseases like root rot/collar rot/wilt complex disease, caused by *Fusarium chlamydosporum*, *Rhizoctonia bataticola*, *Sclerotium rolfsii,* bacterial wilt caused by *Ralstonia solanacearum* and root-knot by *Meloidogyne incognita*, stem blight caused by *Phytophthora* and leaf spot and blight caused by *Botryodiplodia* and *Rhizoctonia* respectively on coleus whereas root rot and wilt caused by *Fusarium solani*, root-knot caused by *Meloidogyne incognita* and leaf blight by *Alternaria tenius* are most important on ashwagandha.

Many effective pesticides have been tested against soil borne pathogens but not considered as long term solution because of concerns about exposure risks, health and environmental hazards, expensiveness, residue persistence, development of resistance to pesticides and elimination of natural enemies. The non-availability of efficient appliances and pesticides (fungicide, bactericide and nematicide), and lack of resistant varieties also aggravate the problem. Therefore, a need for alternative methods of control of soil borne pathogens has become vital. Unfortunately, there are no effective ecologically sound multiple disease management practices especially for nematodes. Development of biological control for soil borne diseases is accepted as a durable and ecofriendly alternative for agrochemicals.

The disease root rot and wilt complex of coleus and ashwagandha is caused by soil borne pathogens. In nature, plants are rarely exposed to the influence of single pathogen. Fawcett (1931) recognized that "nature does not work with pure cultures' and that many plant diseases are influenced by associated organisms. Plant parasitic nematodes often play a major role in disease interactions, for they contribute substantially to variability in crop growth and resulting heavy crop losses. It seems reasonable to expect that infection by one pathogen may alter the host response to subsequent infection by another (Zacheo, 1993). Bacteria that colonize roots effectively are termed rhizobacteria (Schroth and Hancock, 1982). The rhizosphere zone of 1-2 mm around plant roots is rich in nutrients and provides niches different from there in bulk soil for bacteria to thrive. Root colonization is the process where bacteria survive on seeds, multiply in spermosphere in response to seed exudates rich in carbohydrates and aminoacids (Kloepper *et al*., 1989), attach on to the root surfaces (Suslow, 1982) and colonize the developing root system. One of the uses of bacteria from soil is for biological control of soil borne plant diseases (Kloepper and Beauchamp, 1992). Rhizobacteria may play a significant role in reducing their inoculum level in the soil. Hence, it was found necessary to test the native antagonistic rhizobacteria from the amended soil against fungi (*Fusarium* spp.), bacteria (*Ralstonia solanacearum*) and nematode (*Meloidogyne incognita*).

Rhizobacteria have also been studied as plant growth promoters for increasing agricultural production and as biocontrol agents against plant diseases (Burris, 1998; Chen *et al*., 1996).

Plant growth promoting rhizobacteria (PGPR) exhibit direct and indirect mechanisms as plant growth promoters and biological control agents. Direct mechanisms by PGPR, include the provision of bioavailable phosphorus for plant uptake, nitrogen fixation for plant use, sequestration of iron for plant by siderophores, production of plant hormones like auxins, cytokinins and gibberellins and lowering plant ethylene levels using ACC deaminase that accumulate during biotic and abiotic stresses (Glick 1995; Glick *et al*, 1999; Mayak *et al*., 2004). Indirect mechanisms of PGPR include production of antibiotics, *viz.* 2,4-Diacetyl phloroglucinol (DAPG), phenazine, pyoluteorin and pyrrolnitrin against pathogenic fungi and bacteria, reduction of iron available to phytopathogens in the rhizosphere, synthesis of fungal cell wall and insect-gut membrane lysing enzymes, chitinase enzyme for hydrolysis of chitin layer of the eggshell of nematode and also competition with detrimental microorganisms for sites on plant roots and induction of systemic resistance against various pathogens and pests in plants (Ramamoorthy *et al*., 2001).

Finally to use antibiotic production, growth promotion, biocontrol potential and induced responses of PGPR as an effective pathogens management tool, we should evaluate their effects on plant performance and yield under agricultural settings.

With this supportive background information and keeping these points in view, the present investigations on soil borne pathogens of coleus and ashwagandha was undertaken with following objectives to evolve the efficacious PGPR bioformulations against fungi, bacteria and nematode diseases under glasshouse and field conditions. The specific objectives were :

(1) Survey for the collection and isolation of plant growth promoting rhizobacteria (PGPR) from major coleus and ashwagandha growing areas of Karnataka

(2) *In vitro* screening of effective PGPR strains against the major soil-borne pathogens of coleus and ashwagandha

(3) To asses the plant growth promotion of the selected efficient PGPR strains on coleus and ashwagandha under glasshouse conditions

(4) Development of talc-based bioformulations and evaluating their efficacy against fungi, bacteria and nematode diseases under glasshouse conditions

(5) Elucidation of mechanisms of efficient PGPR strains

(6) To study the effect of PGPR strains on growth promotion and biocontrol potential on coleus and ashwagandha under field conditions.

2. REVIEW OF LITERATURE

A perusal of literature on diseases of coleus and ashwagandha showed that wilt complex is the major constraint in the production of coleus and ashwagandha, important medicinal plants. Wilt complex is caused by a multitude of pathogens either alone or in combination and distributed, wherever coleus and ashwagandha cultivation are persued intensively. Wilt complex as the name indicates results ultimately in wilting with drying of foliage and tubers. The major pathogens involved are almost similar in both the crops and as such, the disease management in general would be similar. The major pathogens involved in wilt disease are species of *Fusarium, Rhizoctonia*, *Sclerotium* and as well as a bacterium *Ralstonia solanacearum* (Smith) and root-knot caused by *Meloidogyne incognita* (Kofoid and White) Chitwood. All the pathogens are known to form complexes with nematodes, aggravating the disease. Nematodes alone are also potential pathogens of coleus and ashwagandha.

The following account summarizes present knowledge on the diseases about symptoms, pathogens, their interactions, the effect of bioagents and their mechanisms in disease suppression as well as plant growth promotion.

Amongst the rhizopshere microorganisms, the plant growth promoting rhizobacteria (PGPR) have been considered important in sustainable agriculture due to their plant growth promotional ability as well as biocontrol potential. PGPR have emerged as the biggest group of beneficial soil microorganisms, involved in the control of a number of plant diseases and pests by virtue of their ability to synthesize a wide range of antagonistic secondary metabolites. These ubiquitous microorganisms can be a significant component of management practices to achieve sustainable yields. Literature pertaining to the plant growth promotion, biocontrol activity, mechanisms of actions of PGPR is reviewed herein.

2.1 SURVEY, DISTRIBUTION, ISOLATION AND IDENTIFICATION OF PATHOGENS OF COLEUS AND ASHWAGANDHA

2.1.1. Coleus

2.1.1.1 *Fusarium chlamydosporum*

The literature on survey of disease complexes on crop plants involving plant parasitic nematodes and soil borne pathogens under field conditions is very limited (Sharma and McDonald, 1990).

Merterhazy and Vojtovics (1977) reported *Fusarium fusarioides* on maize for the first time from Hungary. Sherkar and Utikar (1982) noticed the leaf spot disease caused by *F. fusarioides* on pomegranate (*Punica grantum* L.). Bazalar and Delgado (1981) reported that *F. fusarioides* caused typical wilt symptoms on the terminal leaflets of cotton in Peru.

Aneja *et al.* (1993) conducted a series of surveys throughout Haryana during 1988-92 to identify naturally occurring fungal pathogens of water hyacinth leaves. It was mainly caused by *Fusarium chlamydosporum*.

Shyla (1998) isolated pathogen from diseased roots of *C. forskohlii* which consistently yielded *F. chlamydosporum*. El-Arabi and Abughania (1998) conducted the study on fusarium wilt disease of tomato in some coastal regions of Libya. Several *Fusarium* spp. were isolated from tomato plants and soils and were identified as *F. oxysporum* f.sp. *lycopersici* and *F. fusarioides* (*Fusarium chlamydosporum*).

Boby and Bagyaraj (2003) isolated *Fusarium chlamydosporum* from diseased root fragments of *C. forskohlii*.

2.1.1.2 Symptomatology

Symptoms of coleus wilt caused by *F. chlamydosporum* was described by Shyla (1998). In the field, the infected plants were characterized by gradual yellowing marginal necrosis and withering of leaves followed by loss in vigour and premature death. Such plants showed discolouration of roots and complete decaying of tap and lateral root system. The bark of such plants easily peeled off. There was extensive sloughing off and shredding of affected bark. Such affected plants were finally killed due to severe root and collar rot. The infected tubers showed rotting and emitting bad odour.

2.1.1.3 *Ralstonia solanacearum*

The incidence of the bacterial wilt of coleus caused by *Ralstonia solanacearum* (Smith) was reported for the first time from Brazil (Netto and Assis, 2002).

Netto and Assis (2002), reported for the first time the bacterial wilt of *Coleus barbatus* caused by *Ralstonia solanacearum* from Brazil and they identified it as *R. solanacearum*, biovar N_2, race 1, which also causes wilt in tomato, potato, sweet pepper, egg plant and necrosis in cucumber plants.

Survey conducted by Aggarwal *et al.* (2006) reported the prevalence of bacterial wilt of solanceous vegetables in Himachal Pradesh during 2001 and 2002 in the months of May and June. Maximum wilt incidence was observed in Kangra district (100%) in Naveen variety of tomato. They also reported that traditional tomato belt of Jamanabad area changed cropping to other non-solanaceous crops due to severe attack of this wilt pathogen.

Bacterial wilt incited by *Ralstonia solanacearum* is one of the devastating diseases of solanaceous vegetables occurring in tropical, subtropical and temperate area of the world. In Himachal Pradesh, it was a sporadic disease since 1990, restricted upto certain pockets of Kangra district. But it spread at an alarming rate to other parts of the state (Aggarwal *et al.*, 2006).

2.1.1.4 Symptomatology

James *et al.* (1993) observed *P. solanacearum* producing water soaked, glistening golden brown patches on the leaves with irregular margin. Brown discolouration of root and stem tissue with decaying of roots and sometimes shredding of roots was seen in nutmeg infected with *P. solanacearum*.

The bacterial wilt of *Pogostemon patchouli* was characterized by yellowing of the leaves followed by wilting and defoliation. The entire plant dried up within two to three weeks time and vascular tissues of stem and roots showed brown discolouration (James *et al.,* 1994).

Smith (1896) was the first to describe the shape and size of the bacterium as rod shaped measuring 0.5 x 1.5 µm in size. Khan (1974) reported three isolates of *P. solanacerum* obtained from tomato, brinjal and potato were gram negative, non capsulated, non-acid fast, non spore forming and cells were small rods.

Khan *et al.* (1979) succeeded in inducing infection in tomato, chillies and brinjal plants within 7-25 days of inoculation by root injury inoculation technique.

2.1.1.5 *Meloidogyne incognita*

Plant parasitic nematodes are found in all agricultural regions of the world and any crop is likely to suffer damage from these parasites. Their effect may also be additive to other stress factors and can induce predisposition of their host to attack by other pathogens such as fungi and bacteria. There is considerable evidence that the combination of plant parasitic nematodes with fungal pathogens is sufficient to induce heavy crop losses (Zacheo, 1993).

The root-knot caused by *Meloidogyne incognita* on coleus was reported by Patel *et al.* (1989). Sharma (1990) isolated a significant number of root knot nematode larvae along

with *Fusarium* from wilted plants of soybean in microplot of Botany Department, Lucknow University.

Haseeb *et al.* (2000) reported root knot incidence in coleus and confirmed the species as *M. incognita*, which causes stunting, yellowing and wilting of the plants showing irregular galls in the root system.

2.1.1.6 Symptomatology

Rajendra and Vadivelu (1991) showed that the newly introduced *C. forskohlii* plant with medicinal value exhibited stunted growth and yellow patches and severe galling of roots due to *Meloidogyne incognita*. Tuber formation was found commensurate with the degree of galling and yield reduction was 65 per cent under field condition. The pathogenic study which was carried out in controlled condition revealed that the effective damage to the plant was caused by 1000 larvae/plant.

2.1.2. Ashwagandha

2.1.2.1 *Fusarium solani*

The genus *Fusarium* was identified for the first time by Link (1809) and was accepted in an amended form by Fries (1849) for species with fusiform, septate spores described and clearly illustrated by Martins and Von (1842). Later *Fusisporum solani* transferred to *Fusarium solani* by Saccardo (1881).

Siddaramaiah *et al.* (1982) reported *F. solani* on tulsi for the first time from Dharwad. Kishore *et al.* (1985) observed damping off of seedling of opium poppy (*Papaver somniferum* L.) caused by *F. solani* reported from balarampur.

Gupta *et al.* (2004) reported occurrence of severe form of root rot and wilt disease in ashwagandha (*Withania somnifera* Dunal) nurseries and the regular crop under Lucknow conditions. The disease was found to cause 30-50 per cent plant mortality. Further, investigations to characterize the infecting fungus led to identification of *Fusarium solani* as the causative organism and it was a new report of root rot and wilt disease in *Withania somnifera* from UP.

2.1.2.2 *Meloidogyne incognita*

Pandey (1994) recorded occurrence of plant parasitic nematodes in the roots and rhizosphere of *Withania somnifera* and also reported that no work has been done on damage potential and management of *Meloidogyne incognita* on *Withania somnifera*.

Plant growth retardation and gall formation in the root system indicated the presence of root-knot nematode, which was confirmed as *Meloidogyne incognita* race-2. Greenhouse trials were conducted to determine the influence of different inoculum levels of *Meloidogyne incognita* growth and yield of *W. somnifera* (Pandey and Kalra, 2003).

2.2 THE IMPORTANCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

The first clear indication of improved plant growth and biological control of root pathogens due to seed bacterization with rhizobacteria came from the works of Burr *et al*. (1978) and Kloepper *et al*. (1980) who reported the plant growth promoting effects of *Pseudomonas* strains which were antagonistic to a wide range of plant pathogens *in vitro*. These studies also provided the first evidence that the rhizosphere microbiota could be modified significantly with microorganisms introduced with the planting material. Kloepper *et al*. (1989) coined the term plant growth promoting rhizobacteria (PGPR) to include bacteria inhabiting the root and rhizosphere soil which have the ability to increase plant growth.

2.3 SCREENING AND SELECTION OF PGPR STRAINS

It has been suggested that microorganisms isolated from the root or rhizosphere of a specific crop adopted better to that crop and provide effective control of diseases than organisms originally isolated from other plant species. Such plant associated microorganisms serve as better biocontrol agents because they are already closely associated and adopted to the plant or plant part as well as to the particular environmental condition in which they are supposed to function. The screening of such locally adopted strains has yielded improved biocontrol strains in some cases (Cook and Baker, 1983). However, now-a-days microbial biodiversity studies have enhanced the identification of potential bioagents suited to varied environmental conditions.

Identification of effective antagonists strains represents only the first step towards the development of effective biological control. In order for biocontrol to be implemented on a practical level, the antagonists must be ecologically fit to survive, become established and function within the particular conditions of the ecosystem.

After identification of several organisms as potential antagonists it is advisable to continue to work with selected strains to determine the specific mechanisms, interactions, conditions and requirements responsible for effective biological control. Consummate understanding of these characteristics makes it possible to establish the limitations as well as the full potential of biocontrol to develop strategies for management and implementation.

Slininger *et al.* (2003) developed such indices, *viz.* relative performance index based on bioagents growth and their antagonistic activity under different conditions. In addition, use of different markers, *viz.* antibiotic production and other regulatory mechanisms by bioagents gained the importance in rapid identification of biocontrol agents. This kind of screening and selection offer the system for successful development and commercialization of potential biocontrol agents.

Surjit Sen *et al.* (2006) reported that in dual culture, significant growth inhibition of *Sclerotium rolfsii* by *Pseuomonas* BRL-1 was observed. Mycelial growth was restricted near bacterial streak. Increase in incubaton period was proportionate to growth inhibition of *S. rolfsii* upto six days. Microscopic study of mycelia from interacting zone showed hyphal shrivelling mycelial deformities like swelling, fragmentation, short branching, and finally, lysis.

2.4 RHIZOBACTERIA AS BIOCONTROL AGENTS

Rhizobacteria are ideal for use as biocontrol agents since they inhabit the rhizosphere that provides the front line defense for roots against attack by pathogens. Pathogens encounter antagonism from rhizobacteria before and during their primary infection of roots. Rhizobacteria are reported to provide protection against diverse plant pathogens.

2.4.1 Effect of PGPR on fungal pathogens

Sedra and Malouhy (1994) studied six antagonists from 420 samples obtained from conducive and suppressive soils, for their inhibitory activity against *F. oxysporum* f.sp. *albedinis*. These antagonists suppressed the growth of *F. oxysporum* f.sp. *albedinis in vitro* by 24-47 per cent and its sporulation by 70-99 per cent. Gupta *et al.* (1999) isolated *P. aeruginosa* from potato rhizosphere that displayed a strong antagonistic activity against important fungal pathogens, *viz. Macrophomina phaseolina* and *Fusarium oxysporum*.

Tripathi and Johri (2002) studied the biocontrol potential of fluorescent pseudomonas isolated from rhizosphere of pea and wheat *in vitro* and *in vivo* against maize sheath blight caused by *Rhizoctonia solani*. They found some isolates to possess multiple disease control potential, while some others exhibited biocontrol potential against specific pathogens, which indicated that fluorescent pseudomonads are diverse with respect to their biocontrol potential. Ahmadzadeh *et al.* (2004) reported that antagonistic rhizobacteria, more specifically fluorescent pseudomonads and certain *Bacillus* species possessed the ability to inhibit fungal and bacterial root diseases of agricultural crops.

Plant growth-promoting rhizobacterial strain belonging to fluorescent pseudomonads were isolated from the rhizosphere of rice and sugarcane. Among 40 strains that were confirmed as fluorescent psueodmonads, 18 exhibited strong antifungal activity against *Fusarium oxysporum* and *Rhizoctonia bataticola*, mainly through production of antifungal metabolites (Ramesh Kumar *et al.*, 2002).

Tiwari and Thrimurthy (2007) reported that twenty-one isolates of *Pseudomonas fluorescens* were isolated from the rhizosphere of rice, maize, wheat, chickpea, mung, urd, soybean and sunflower from Raipur and Bastar regions. Among these seven isolates which showed bright fluorescence under UV light were further tested. The isolates showed positive response of siderophore production and plant growth promoting activity on rice cv. Bamleshwari. Among the isolates PFR 1 and PFR 2 were found significantly superior to control in increasing the shoot length and root length. *In vitro* evaluation of the *P. fluorescens* isolates also confirmed their antagonistic ability against both *Pyricularia grisea* and *Rhizoctonia solani* in dual culture tests.

Pure culture of *P. aeruginosa* was obtained from the soil and studied for siderophore production. The antifungal activity of the strain against three phytopathogenic fungi, *viz. F. moniliformae*, *Altenaria solani* and *Helminthosporoum halodes* was assayed by poison food technique. Inhibition of these fungal pathogens appeared to be due to production of antifungal secondary metabolites by *P. aeruginosa* (Kanika Sharma *et al.*, 2007). A list of fungal pathogens suppressed by rhizobacteria are presented below.

Rhizobacteria suppressing phytopathogens

Sl. No.	Bacteria	Pathogen suppressed	Reference
1	*Alcaligenes*	*Fusarium oxysporum* f.sp. *dianthi*	Yuen and Schroth (1986)
2	*Alcaligenes odorans*	*Sclerotium rolfsii*	Rangeswaran and Prasad (1998)
3	*Arthrobacter*	*Fusarium oxysporum* f.sp. *dianthi*	Sneh *et al.* (1984)
4	*Azotobacter chroococcum*	*Rhizoctonia solani*	Meshram and Jager (1983)
5	*Bacillus coagulans* MRI	*F. moniliformae*	Pal *et al.* (1996)
6	*Bacillus subtilis*	*R. solani*	Merriman *et al.* (1974)
7	*B. subtilis* MR 112	*R. solani*	Rosales *et al.* (1995)
8	*B. subtilis*	*Macrophomina phaseolina*	Pal *et al.* (1996)
9	*Burkholderia cepacia*	*S. rolfsii*	Jayaswal *et al.* (1992)
10	*B. cepacia*	*F. moniliformae*	Hebbar *et al.* (1992)
11	*Enterobacter* sp.	*Pythium*	Hadar *et al.* (1983)
12	*E. herbicola*	*R. solani*	Rosales *et al.* (1995)
13	*Flavobacterium*	*Pythium*	Chen *et al.* (1987)
14	*Psedomonas aeruginosa*	*R. solani*	Rosales *et al.* (1995)
15	*P. fluorescens*	*Thielaviopsis basicola*	Ahl *et al.* (1986)

16	*P. fluorescens*	*S. rolfsii*	Jagadeesh *et al.* (1998)
17	*P. fluorescens* 7-14	*Pyricularia, R. solani*	Chatterjee *et al.* (1996)
18	*P. glumae* EM85	*P. ultimum*	Pal *et al.* (1996)
19	*P. putida* A12	*F. oxysporum* f.sp. *cucumerinum*	Simeoni *et al.* (1987)
20	*Rhizobium*	*M. phaseolina*	Chakraborty and Purkayastha (1984)
21	*P. cepacia* RB-425	*P. ultimum, Pyricularia oryzae, Verticillium dahliae*	Homma *et al.* (1989)

2.4.2 Efficacy of PGPR strains against *Rolstonia solanacearum*

Various antagonistic rhizobacteria have been reported to be active in the rhizosphere and shown to play a significant role in suppressing the population and activity of *R. solanacearum.* The mechanisms by which they bring about the beneficial effect vary. Jagadeesh (2000) reported that bacterial wilt of tomato caused by *R. solanacearum* was controlled by the rhizobacteria to an extent of 16.66 to 83.33 per cent. Inoculation of three strains (fluorescent pseudomonas strain RJA112 and RBG 114 and Arthrobacter RBE 201) and the reference strain (*P. fluorescens* CHAO) resulted in 83.33 per cent disease control.

Anith *et al.* (2004) reported that when PGPR (*Pseudomonas putida*, *Bacillus pumilus*) and Actigard (acibenzolar-S-methyl) application were combined, the bacterial wilt incidence caused by *R. solanacearum* was reduced when compared to the untreated control. A list of microbes and their mode of action in inhibiting *R. solanacearum* has been presented.

Microorganisms inhibiting *Ralstonia solanacearum*

Sl. No.	Antagonist	Mechanism of biocontrol	Reference
1	*Bacillus* spp.	ND	Kulkarni (1978)
2	*Bacillus* spp.	Antibiotics	Anuratha and Gnanamanickyam (1990)
3	*Bacillus* spp.	Antibiotics	Perez *et al.* (1997)
4	*B. subtilis*	ND	Shekhawat *et al.* (1992)
5	*B. subtilis*	ND	Karuna and Khan (1993)
6	*B. subtilis*	ND	Anon. (1999)
7	*Colpoda* (Protozoa)	Predation	Kulkarni (1978)
8	*Enterobacter* sp.	ND	Shekhawat *et al.* (1992)
9	*Erwinia* sp.	Anibiotics	Fucikowsky *et al.* (1990)
10	*Pseudomonas* sp.	Anibiotics	Perez *et al.* (1997)
11	*Pseudomonas aeruginosa*	ND	Karuna (1993)
12	*P. cepacia* B5	2-keto gluconic acid	Aoki *et al.* (1991)

13	*P. fluorescens*	Systemic resistance	Kempe and Sequiera (1983)
14	*P. fluorescens*	Siderophores	Anuratha nad Gnanamanickyam (1990)
15	*P. fluorescens* PF59	Siderophores	Hartmann *et al.* (1992)
16	*P. fluorescens*	ND	Karuna and Khan (1993)
17	*P. fluorescens* 90B-422	Antibiotics	Wei *et al.* (1994)
18	*P. fluorescens* P.f G32	Antibiotics, Siderophores	Mulya *et al.* (1996)
19	*P. fluorescens* BC-8	Siderophores	Ciampi *et al.* (1996)
20	*P. fluorescens* FPP5	Siderophores	Chao *et al.* (1997)
21	*P. gladioli*	Siderophores	Hartman *et al.* (1992)
22	*P. glumae*	Systemic resistance	Furuya *et al.* (1991)
23	*P. glumae*	Antibiotics	Furuya *et al.* (1991)
24	*Streptomyces* sp.	ND	Kulkarni (1978)
25	*Streptomyces corchorusii*	Antibiotics	El-Abyad *et al.* (1996)
26	*Penicillium purpurescens*	Antibiotics	Suresh and Rai (1992)
27	*P. solanacearum* B1	Bacteriocin	Cuppels *et al.* (1978)
28	*P. solanacearum* ABPS 121	Bacteriocin	Chen and Echandi (1984)
29	*P. solanacearum* B82	Systemic resistance	McLaughlin and Sequeira (1988)
30	*P. solanacearum*	Bacteriocin	Arwiyanto *et al.* (1994)

2.4.3 Biocontrol potential of PGPR against nematodes

Sikora (1990) found that *P. fluorescens* exhibited an *in vitro* repellent effect towards *R. similis* and *Meloidogyne* spp.

Jonathan *et al.* (2000) studied the efficacy of plant growth promoting uncharacterized actinomycetes (strain 29 and 45) and the nematode parasitic bacterium *Pasteuria penetrans* (isolate 100) against *M. incognita* race 1 on tomato and banana. Seed treatment with *P. fluorescens* and *P. chlororaphis* significantly reduced the root gall of *M. incognita* race 1 in tomato cv. Rutgers (Johnathan *et al.,* 2000).

Pseudomonas fluorescens, Bacillus spp. and arbuscular mycorrhizae were tested against *M. incognita* and *Tylechulus semipenetrans* in horticultural crops such as citrus, tomato, potato and chilli. The results showed that these organisms could be used as successful biocontrol agents for the management of plant parasitic nematode (Rajendran *et al.,* 2001). Seenivasan and Lakshmanan (2001) studied the nematoxic effects of culture filtrates of *P. fluorescens* strain Pf1 on *Hirschmanniela gracilis* at 25, 50, 75 and 100 per cent concentration *in vitro*. Application of *P. fluorescens* or *B. subtilis* increased the growth and yield of chickpea and reduced the infection by *M. incognita* by minimizing the number of galls/root system, egg mass production and soil population (Khan *et al*., 2001). Mortality of *M. incognita* juveniles was observed to be similar both in unheated and heated culture filtrates of *P. fluorescens* and the mortality increased with increase in concentration (Sirohi *et al*., 2000).

P. fluorescens and *B. thuringiensis* showed nematicidal activity against juveniles and adults of *M. incognita* infesting tomato plants. The mortality levels of *M. incognita* increased with increase in the concentration of bacterial cells (5 x 10^8 cfu/ml) (Hanna *et al*., 1999). Soil application of *P. fluorescens* @ 2.5 kg/ha significantly reduced *M. incognita* population in sugarbeet and increased the plant growth and yield (Kavitha, 2006).

Devi and Dutta (2002) reported that bhendi seeds treated with *P. fluorescens* for 12 hours exhibited significant increase in growth and reduced root galling of *M. incognita*. Culture filtrates of *Pseudomonas fluorescens* caused significant reduction in egg hatching of *M. incognita* and resulted in considerable reduction in nematode population densities in soil and subsequent root knot development in tomato (Khan and Akram, 2000).

Jonathan *et al.* (2006) reported that there was highest reduction in nematode egg hatch and the greatest mortality of *M. incognita* juveniles in the culture filtrate of *P. fluorescens* strain Pfb22 at 100 per cent concentration. The nematode infestation was reduced both in soil and roots, with the least number of adult females, number of egg masses, number of eggs per egg mass and gall index of *M. incognita* in banana plants treated with the local isolate of *P. fluorescens* Pfb22 under glasshouse conditions. Cultures and cell free culture filtrates (CFC) of 133 bacterial strains at three different dilutions (S-4, S-16, S-64) were tested *in vitro* against *Meloidogyne javanica* J2 mortality. Sixteen isolates showing 50 per cent or more mortality at lowest dilution were short listed. Four isolates, identified as *Providencia rettgeri*, *Vibrios* sp. and *Pseudomonas putida* reduced egg hatching of *M. javanica* from 20 to 30 per cent (Ashima Kapoor *et al*., 2007).

2.5 BIOCHEMICAL CHARACTERIZATION OF PGPR

Various phenotypic and biochemical methods have been developed and used for characterizing fluorescent pseudomonad isolates. The genus *Pseudomonas* is characterized by gram-negative rod shaped aerobic cells and are associated with plants. The important species include *P. fluorescens, P. putida*, *P. aeruginosa* and *P. aureofaciens*. Most of the tests conducted for identification of fluorescent pseudomonads have been based on physiological and nutritional tests (Krieg and Holt, 1984). Among the *Pseudomonads* group, *P. aeruginosa* forms a light cluster and grows above 41°C (Hildebrand *et al*., 1992). Most of the associated *Pseudomonas* sp. belong to *P. fluorescens* and *P. putida* complex. There was no clear distinction between *P. fluorescens* and *P. putida* (Sheath *et al.,* 1981). However, these two species are identified based on trehalose utilization and gelatin liquefaction. In this, *P. fluorescens* exhibits positive for both the tests whereas *P. putida* show negative response (Hildebrand *et al.*¸1992). The species of fluorescent pseudomonas are again grouped in different biovars and subgroups based on similarity in biochemical tests (Champion *et al*., 1980; Barrett *et al*., 1986). Thus, rapid identification of potentially and economically viable bioagents is possible through various methods of biochemical characterization (Weller *et al*., 2002; Ongena *et al*., 1999; Zehnder *et al*., 2000; Singh *et al*., 2000).

2.6 BIOCONTROL MECHANISMS OF PGPR

PGPR exhibit multiple number of mechanisms to promote plant growth and to serve as potential biocontrol agents. Generally, PGPR traits associated with the biocontrol of plant pathogens include :

(1) Atmospheric nitrogen fixation and its supply to plants

(2) Synthesizing various phytohormones including auxins and cytokinins

(3) Providing mechanisms for the solubilization of minerals such as phosphorus

(4) Antibiotic synthesis (Haas and Defago, 2005),

(5) Secretion of iron binding siderophores to obtain soluble iron from the soil and provide it to a plant thereby deprive fungal pathogens in the vicinity, of soluble iron (Neilands and Leong, 1986; Dowling *et al*., 1996).

(6) Production of low molecular weight metabolites such as hydrogen cyanide with antifungal activity (Dowling and O'Gara, 1994).

(7) Production of enzymes including chitinase, β-1-3-glucanase, protease and lipase which can lyse some fungal cells (Chet and Inbar, 1994).

(8) Production of oxidative stress enzymes such as catalases, superoxide dismutases, peroxidase and polyphenol oxidases for scavenging active oxygen species.

(9) Out-competing phytopathogens for nutrients and occyping niches on the root surface (Kloepper *et al*., 1988, O'Sullivan and O'Gara, 1992; Loper *et al*., 1997).

(10) Lowering the production of stress ethylene in plants with the enzyme ACC deaminase (Hyodo, 1991; Glick *et al*., 1998; Penrose *et al*., 2001).

2.7 PLANT GROWTH PROMOTION

Rhizobacterial strains were found to increase plant growth after inoculation in seeds and therefore called "Plant growth promoting rhizobacteria" (Kloepper *et al*., 1980). The mechanisms of growth promortion by these PGPR are complex and appear to comprise both changes in the microbial balance in the rhizosphere and alterations in host plant physiology (Glick *et al*., 1999). Plant growth promoting rhizobacteria, including fluorescent pseudomonads are capable of surviving and colonizing the rhizosphere of all field crops. They promote plant growth by secreting auxins gibberellins and cytokinins (Vidyasekaran, 1998).

PGPR has a significant impact on plant growth and development in both indirect or direct ways. Indirect promotion of plant growth occurs when bacteria or prevent some of the deleterious effects of a phytopathogenic organism by one or more mechanisms. On the other hand, the direct promotion of plant growth by PGPR generally entails providing the plant with compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment (Glick, 1995; Glick *et al.*¸1999). Plant growth benefits due to the addition of PGPR include increase in germination rates, root growth, yield including grain, leaf area, chlorophyll content, magnesium, nitrogen and protein content, hydraulic activity, tolerance to drought and salt stress, shoot and root weights and delayed leaf senesence (Lucy *et al*., 2004). Seed treatment with PGPR resulted in increased yield and growth in potato under field conditions (Kloepper *et al.,* 1980). Van Peer and Schippers (1988) documented the increased root and shoot fresh weight of tomato, cucumber, lettuce and potato as a result of bacterization with *Pseudomonas* strains.

Mashooda Begum *et al.* (2003) studied the effectiveness of plant growth promoting rhizobacterial isolates against some seed borne fungal diseases. Among them *B. pumilus* (SE-34), *B. pasteurii* (T_4), *B. subtilis* (IN 937-6) and *B. subtilis* (GB-03) strains stood first in the improvement of crop, both in greenhouse and field condition. Potential strains increased the biomass of plants, total number of leaves, fruits, length, girth, biomass of the fruit. The colonization of these bacterial strains reduced the incidence of seed mycoflora which indirectly enhanced the per cent seed germination and vigour index of seedlings. Minakshi *et al.* (2005) isolated a total of 113 rhizobacteria from different rhizotic zones of pigeonpea. Seed treatment using four isolates, *viz.* RS29, RS39, RS41 and RP3 resulted in 90 per cent seed germination in contrast with 50 per cent obtained in untreated control after 72 h of incubation and the isolates RS34, ER17, RP7 and RS41 increased shoot height and shoot dry biomass as compared to uninoculated control whereas isolates RS45, RS36, RS37, ER23, RP24 influenced root dry biomass significantly.

2.8 SIDEROPHORE PRODUCTION

Siderophores are low molecular weight, extracellular compounds with a high affinity for ferric iron, that are secreted by microorganisms to take up iron from the environment (Hofte, 1993) and their mode of action in suppression of disease were thought to be solely based on competition for iron with the pathogen (Bakker *et al*., 1993; Duijff *et al*., 1997). Fluorescent pseudomonas are characterized by the production of yellow-green pigments termed pyoverdines which fluoresce under UV light and function as siderophores (Demange

et al., 1987). The role of siderophores produced by fluorescent pseudomonads in plant growth promotion was first reported by Kloepper *et al.* (1981). The siderophores of fluorescent psduomonads were later reported to be implicated in the suppression of plant pathogens (Bakker *et al.,* 1986; Becker and Cook, 1988) competition for iron between pathogens and siderophores of fluorescents pseudomonads has been implicated in the biocontrol of wilt diseases caused by *Fusarium oxysporum* (Kloepper *et al.*, 1980; Scher and Bakker, 1982), damping off cotton caused by *Pythium ultimum* (Loper, 1988)) and pythium root rot of wheat (Becker and Cook, 1988). Pyoverdines chelate iron in the rhizosphere and deprive pathogens of iron which is required for their growth and pathogenesis (Leong, 1986). Rhizobacteria produce various types of siderophores (Pseudobactin and ferrooxamine B) that chelate the scarcely available iron and thereby prevent pathogens from acquiring iron (Loper and Buyer, 1991).

The involvement of siderophore production in disease suppression by *Pseudomonas* strain WCS 358 was studied on carnation, radish and flax using *Fusarium oxysporum* f.sp. *dianthi*, *F. oxysporum* f.sp. *raphani* and *F. oxysporum* f.sp. *lini* respectively. Siderophore production by a plant growth promoting fluorescent *Pseudomonas* sp. RBT 13 effective against several fungal and bacterial pathogens has been demonstrated (Dileep Kumar and Dubey, 1993). Five strains of fluorescent pseudomonads exhibited growth promotion of lentil and biocontrol of wilt caused by *F. oxsporum* f.sp. *lini* with siderophore production as the mechanism (Rao *et al*., 1999). Several strains of siderophore producing *P. fluorescens* have been shown to inhibit *F. oxysporum* f.sp. *cubense*, *F. oxysporum* f.sp. *vasinfectum*, *Rhizoctonia solani* and *Acrocylindrium oryzae* (Sakthivel *et al*., 1986).

Pseudomonas culture and purified siderophores showed good antifungal activity against the plant deleterious fungi, *viz. Aspergillus niger. A. flavus*, *A. oryzae, F. oxysporum* and *Sclerotium rolfsii* (Manwar *et al*., 2004). Though siderophores are part of primary metabolism (iron is an essential element), on occasions they also behave as antibiotics which are commonly considered to be secondary metabolites (Haas and Defago, 2005). Suryakala *et al.* (2004) have reported that siderophores exerted maximum impact on *Fusarium oxysporum* than on *Alternaria* sp. and *Colletotrichum capsici*.

Leong (1986) reported that the fluorescent pseudomonads had the property to form ferric siderophores complex which prevent the availability of iron to the microorganisms. Ultimately this led to iron starvation and prevented the survival of the microorganisms including nematodes. *Pseudomonas aeruginosa* strain IE-6 and its streptomycin resistant strain IE-6^+ markedly suppressed nematode population densities in root and subsequent root-knot development. The iron concentration in soil was lowered by the addition of an iron chelator (Siddiqui and Shaukat, 2003).

2.9 HYDROGEN CYANIDE (HCN) PRODUCTION

The cyanide ion is exhaled as HCN and metabolized to a lesser degree in to other compounds. HCN first inhibits the electron transport and the energy supply to the cell is disrupted leading to the death of the organisms. It inhibits proper functioning of enzymes and natural receptors reversible mechanism of inhibition (Corbett, 1974) and it also known to inhibit the action of cytochrome oxidase (Gehring *et al*., 1993). HCN is produced by many rhizobacteria and is postulated to lay a role in biological control of pathogens (Defago *et al.,* 1990). Production of HCN by certain strains of fluorescent pseudomonads has been involved in the suppression of soil borne pathogens (Voisard *et al*., 1989). Suppression of black root rot of tobacco (Stutz *et al*., 1986) and take-all of wheat (Defago *et al*., 1990) by *P. fluorescens* strain CHAO was attributed to the production of HCN. *Pseudomonas fluorescens* HCN inhibited the mycelial growth of *Pythium in vitro* (Westsleijn, 1990). The cyanide producing strain CHAO stimulated root hair formation, indicating that the strain induced and altered plant physiological activities (Voisard *et al*., 1989). Four of the six PGPR strains that induced systemic resistance in cucumber against *Colletotrichum orbiculare* produced HCN (Wei *et al*., 1991). Fluorescent *pseudomonas* strain RRS1 isolated from Rajanigandha (tuberose) produced HCN and the strain improved seed germination and root length (Saxena *et al*., 1996). Pessi and Haas (2000) reported that low oxygen concentrations are a pre-requisite for the activity of the transcription factor *ANR* which positively regulates HCN biosynthesis.

HCN from *P. fluorescens* strain CHAO not repressed by fusaric acid played a significant role in disease suppression of *F. oxysporum* f.sp. *radicis- lycopersici* in tomato (Duffy *et al*., 2003). Ramettee *et al.* (2003) reported that hydrogen cyanide is a broadspectrum antimicrobial compound involved in biological control of root disease by many plant associated fluorescent pseudomonads. Further, they noted that the enzyme HCN synthase is encoded by three biosynthetic genes (*henA, henB* and *henC*).

2.10 INDOLE-3-ACETIC ACID (IAA) PRODUCTION

IAA is phytohormone which is known to be involved in root initiation, cell division and cell enlargement (Salisbury, 1994). This horne is very commonly produced by PGPR (Barazani and Friedman, 1999). Vessey (2003) has reviewed the production of this hormone and implicated it in the growth promotion by PGPR. However, the effect of IAA on plants depends on the plant sensitivity to IAA and the amount of IAA produced from plant associated bacteria and induction of other phytohormones (Peck and Kende, 1995). Patten and Glick (2002) demonstrated that bacterial IAA from *P. putida* played a major role in the development of host plant root system. Similarly, IAA production in *P. fluorescens* HP 72 correlated with suppressing of creeping bent grass brown patch (Suzuki *et al*., 2003).

2.11 ANTIBIOSIS

Antibiotics are generally considered to be organic compounds of low molecular weight produced by microbes. Antibiosis plays an active role in the biocontrol of plant disease and it often acts in concert with competition and parasitism. Antibiosis has been postulated to play an important role in disease suppression by rhizobacteria (Gutterson *et al*., 1986). A variety of secondary metabolites listed in below are produced by fluorescent pseudomonads that exhibit antimicrobial properties.

Secondary metabolites produced by fluorescent pseudomonads effective in biocontrol of pathogens

Meatbolite	*Pseudomonas* sp.	Pathogen controlled	Reference
Pyoluteorin	*P. fluorescens* Pf-5	*Pythium*	Howell and Stipanovic (1980)
	P. fluorescens Pf-5	*Pythium*	Kraus and Loper (1992)
Pyrrolnitrin	*P. cepacia*	*Rhizoctonia solani*	Yoshihima *et al.* (1989)
	P. fluorescens PEM-2	*R. solani*	Carmi *et al.* (1994)
	P. fluorescens BL915	*R. solani*	Hill *et al.* (1994)
Phenazine-1-carboxylic acid (PCA)	*P. fluorescens* 2-79	*Gaeumannomyces*	Brisbane and Rovira (1988)
	P. aeruginosa	*R. solani*	Rosales *et al.* (1995)
2,4-Diacetyl-phloroglucinol (DAPG)	*P. aureofasciens*	*Gaeumannomyces*	Vincent *et al.* (1991)
	P. cepacia	*R. solani*	Rosales *et al.* (1995)
	P. fluorescens Q2-87	*Gaeumannomyces*	Bangera and Thomashow (1996)
Oomycin A	*P. fluorescens*	*Pythium ultimum*	Gutterson *et al.* (1986)

Fluorescent pseudomonad strains are known to reduce fungal growth *in vitro* by the production of one or more antifungal antibiotics that may also have activity *in vivo* (Carruthers *et al*., 1994; Mazzola *et al*., 1995; Pal, 1995; Whipps, 2001). Several strains of *Pseudomonas*

spp. have been shown to produce wide array of antibiotics which include 2,4-diacetyl phloroglucinol, hydrogen cyanide, kanosamine, phenazine-1-carboxylic acid, pyoluteorin, oomycin A, pyrrolnitrin, pyocyanin and viscosinamide as well as several other uncharacterized moieties (Michereff *et al.*, 1994; Milner *et al.*, 1996; Keel and Defago, 1997; Whipps, 1997; Tharne *et al.*, 1999). Phloroglucinols phenazines, pyoluteorin, pyrrolnitrin and cyclic lipopeptides all of which are diffusible and hydrogen cyanide is volatile in nature (Haas and Defago, 2005).

Root associated fluorescent pseudomonads produce and excrete secondary metabolites which are inhibitory to plant pathogenic organisms including fungi, bacteria and nematodes (Thomashow and Weller, 1995; Haas and Keel, 2003). Among these metabolites the polyketide compound, DAPG has received particular attention because of its broad spectrum antifungal, antibacterial and antihelminthic activity (Keel *et a.*, 1992; Keel and Defago, 1997). Phenazines (PHZ) are N containing heterocylic pigments synthesized by species of *Pseudomonas, Streptomyces, Burkholderia* and *Brevibacterium* (Budzikiewicz, 1993; Stevans *et al.*, 1994). Pyrrolnitrin (PRN) is a broad spectrum antifungal metabolite produced by many fluorescent and non-fluorescent strains of the genus *Pseudomonas* (Howell and Stipanovic, 1979). A phenyl pyrrol derivative of PRN has been developed as an agricultural fungicide. Pyrrolnitrin persists actively in the soil for at least 30 days.

Pyoluteorin (PLT) is an aromatic polyketide antibiotic consisting of a resorcinol ring derived through polyketide biosynthesis. PLT is produced by several *Pseudomonas* sp. including strains that suppress plant diseases caused by phytopathogenic fungi (Murhofer *et al.*, 1994). PLT mainly inhibits the oomycetous fungi including *Pythium ultimum* against which it is strongly active when applied to seeds. PLT-producing Pseudomonads decrease the severity of *Pythium* damping off (Nowak-Thompson *et al.*, 1999). *Pseudomonas fluorescens* strain CHAO and its antibiotic over-producing derivative CHAO/PME 3424, repeatedly reduced *M. incognita* galling in tomato, brinjal, mung and soybean in early growth stage. A strong negative correlation existed between rhizobaceria colonization and nematode invasion (Siddiqui and Shaukat, 2003).

2.12 INDUCED SYSTEMIC RESISTANCE (ISR)

Induced resistance is defined as an enhancement of the plants defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimulation. The resulting elevated resistance due to an inducing agent upon infection by pathogen is called induced systemic resistance (ISR) or systemic acquired resistance (SAR) (Hammerschmidt and Kuc, 1995). The induction of systemic resistance by rhizobacteria is referred to as ISR, whereas that by other agencies is called SAR (Van Loon *et al.*, 1998). Once resistance is induced, it will afford non-specific protection against pathogenic fungi, bacteria, nematodes and viruses as well as against insect pests.

A large number of defense enzymes that have been associated with ISR include phenylalanine ammonia lyase (PAL), chitinase, β-1,3-glucanase, peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD), catalase (CAT), lipoxygenase (LOX), ascorate peroxidase (APX) and proteinase inhibitors (Koch *et al.*, 1992; Schneider and Ullrich, 1994; Van Loon, 1997). These enzymes also bring about liberation of molecules that elicit the initial steps in induction of resistance, phytoalexins and phenolic compounds (Keen and Yoshikawa, 1993; Van Loon *et al.*, 1998).

Induced systemic resistance by PGPR has been achieved in large number of crops including *Arabidopsis* (Pieterse *et al.*, 1996), cucumber (Wei *et al.*, 1996), tomato (Duijff *et al.*, 1997), potato (Doke *et al.*, 1987), radish (Leeman *et al.*, 1996), carnation (Van Peer *et al.*, 1991), sugarcane (Viswanathan and Samiyappan, 1999), chilli brinjal (Ramamoorthy and Samiyappan, 2001; Bharathi *et al.*, 2004), rice (Vidhyasekaran *et al.*, 1997; Nandakumar *et al.*, 2001) and mango (Vivekananthan *et al.*, 2004) against broad spectrum of pathogens including fungi (Leeman *et al.*, 1995; Doke *et al.*, 1987), bacteria (Liu *et al.*, 1995), nematodes (Paul and Kumar, 2003) and viruses (Murhofer *et al.*, 1994; Kandan *et al.*, 2005).

Seed treatment and seedling root dipping induced early and enhanced levels of PO in rice plants (Nayar, 1996). Two peroxidase isoforms were induced in the PGPR-treated rice

plants inoculated with the sheath blight pathogen, *R. solani* (Nandakumar *et al*., 2001). High level expression of PO was reported in *P. fluorescens* Pf1 treated chilli plants challenged with *C. capsici* (Bharathi *et al*., 2004).

Similarly, increased activity of PPO was observed in PGPR treated tomato plants challenged with *F. oxysporum* f.sp*. lycopersici* (Ramamoorthy *et al*., 2002).

Plants treated with *Pseudomonas* strains initially showed higher level of PAL compared to control (Chen *et al*., 2000). Radjacommare *et al.* (2004) reported that seedling dip with talc based formulation of *P. fluorescens* induced the activity of PAL in finger millet leaves against blast disease. The inoculation of PGPR strains *P. putida* 89B-27 and *Serratia marcescens* 90-166 and the pathogen, *F. oxysporum* f.sp. *cucumerinum* on separate halves of roots of cucumber seedlings exhibited that both PGPR strains induced systemic resistance against the *Fusarium* wilt as expressed by delayed disease symptom development and reduced number of dead plants (Liu *et al.,* 1995). The same PGPR strains also induced systemic resistance in cucumber against bacterial angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans* (Liu *et al*., 1995).

Maize plants raised from *P. fluorescens* treated seeds showed higher activity of peroxidase, polyphenol oxidase and PAL, when leaf sheaths were inoculated with the pathogen, *R. solani*. The bacterized seeds with *P. fluorescens* lead to accumulation of higher phenolic compounds and higher activity of PO, PPO and PAL that may play a role in defense mechanism in plants against pathogen (Sivakumar and Sharma, 2003). Kloepper *et al.* (2004) also observed, control of nematode diseases in tomato and bell pepper by treatments with PGPR strains through induction of systemic resistance. Siddiqui and Shaukat (2002) observed that the application of PGPR strains to one half of the split root system of tomato caused a significant reduction (42%) in nematode penetration in the other half of the split root system and this was attributed to ISR activity of the strain.

Hariprasad and Umesh (2007) reported that PGPR application were made by seed, root and foliar spray treatments separately in combinations in field. Among the PGPR strains *Bacillus subtilis* strain GB_3 was the most effective in providing significant suppression of bacterial spot and was well correlated with increased activity of defense related enzymes, *viz.* peroxidase and PAL. PGPR that were effective in greenhouse were also able to induce resistance in tomato against bacterial spot under field conditions.

2.13 BIOFORMULATIONS OF PGPR STRAINS

An important area of biological control is the development of formulations that would case for viable microbial activity for long period of time. Mass multiplication of PGPR in a suitable medium and development of a powder formulation was first carried out in 1980. A dried powder formulation of PGPR, especially is important for seed treatment and soil application. The survival of PGPR in a dried formulation and the effectiveness of methyl cellulose in a powder formulation for coating sugarbeet seed has been well documented (Suslow, 1980). A talc-based formulation of PGPR has been developed for inoculation of potato seed pieces (Kloepper and Schroth, 1981). Talc based formulation of *P. fluorescens* isolated from the rhizosphere of different crops has been developed (Kloepper *et al*., 1980; Vidhyasekaran and Muthamilan, 1995).

Root dip with *P. fluorescens* formulated in talc was found to be effective in reducing *M. incognita* and caused 40 per cent reduction in root galls under glasshouse conditions in grape vine (Mani, 1996). Glasshouse and microplot experiment with *P. fluorescens* strain Pf1 at 1, 2 or 4 g/plant in grape vine reduced the severity of infection by *M. incognita* (race 3) and enhanced root colonization by the rhizobacterium. Colonization was observed in the roots produced during second season (Shanthi *et al*., 1998). Biopriming of plants with some PGPB can also provide systemic resistance against a broad spectrum of plant pathogens. Diseases of fungal bacterial and viral origin and in some instances, even damage caused by insects and nematodes can be reduced after application of PGPB (Compant *et al*., 2005).

2.14 RHIZOBACTERIA IN THE MANAGEMENT OF PLANT DISEASES

PGPR are having the ability to protect above ground plant parts against fungal, bacterial and viral diseases by induced systemic resistance (ISR). Kloepper *et al.* (1992) reported that among the PGPR, fluorescent pseudomonads are the most exploited bacteria for biological control of soil borne and foliar plant pathogens. In the past three decades numerous strains of fluorescent pseudomonads have been isolated from the soil and plant roots by several workers and their biocontrol activity against soil borne and foliar pathogens have been reported (Austin *et al*., 1997; Mew and Rosales 1986; Rabindran and Vidyasekaran, 1996; Viswanathan and Samiyappan, 2001; Ramamoorthy *et al*., 2002).

Pseudomonas fluorescens isolates are effective bacterial antagonists for the management of soil borne and foliar diseases. Among the various isolates tested, *P. fluorescens* isolate Pf_1 effectively inhibited mycelial growth of the pathogen *in vitro* conditions and decreased the fruit rot incidence under greenhouse conditions (Ramamoorthy and Samiyappan, 2001).

The application of biocontrol PGPR strains has given promising results in cereals, vegetables, fruit and ornamental plant production under glass house and field conditions (Raupach and Kloepper, 1998). In greenhouse and field experiments, PGPR strain *B. pumilus* INR-7 effectively protected pearl millet against downy mildew (Niranjan Raj *et al*., 2003).

PGPR mediated resistance in mango trees infected with *Colletotrichum gloesporiodes* significantly reduced the anthracnose infection besides enhancing fruit yield under field conditions (Vivekananthan *et al*., 2004). These studies clearly indicate the PGPR have diverse mechanism to operate to combact the pests and pathogens and work efficiently in both greenhouse and field conditions.

3. MATERIAL AND METHODS

A systematic study was undertaken to isolate native strains of rhizobacteria from major coleus and ashwagandha growing areas of Karnataka, in order to test their efficacy against different pathogens affecting these crops, their mechanisms of biocontrol and plant growth promotion was assessed. The experiments were carried out from 2005-08 in the Department of Plant Pathology, Saidapur Farm and Institute of Agricultural biotechnology (IABT), College of Agriculture, University of Agricultural Sciences, Dharwad. Details of the materials used and methods followed for conducting the experiments are described hereunder

Experimental site

Dharwad is located in Northern Transitional tract of Karnataka state at 15°26'N, latitude and 75°7'E longitude at an altitude of 678 m above mean sea level. Dharwad is considered to be mild tropical rainy region.

The monthly weather data during the period of investigation at the meteorological observatory of Main Agricultural Research Station (MARS), Dharwad is presented in Appendix II. The soil of the experimental site was sandy loam.

3.1 SURVEY TO ASSESS THE INCIDENCE OF WILT COMPLEX IN COLEUS AND ASHWAGANDHA IN KARNATAKA

A random survey was undertaken in the major medicinal plant growing districts of Karnataka (places/locations surveyed are presented in the Table 1). Samples of soil and roots were collected from sufficiently wet fields from the rhizospheres of coleus and ashwagandha crops. In the similar manner, totally 10 spots were selected randomly for taking infected soil and plant samples representing the whole field. Later from this, a composite sample of 200 g of soil and 5 g of root were formed. Randomly 100 plants were selected in different locations in a field and number of plants wilted were counted and the mean wilt incidence was expressed in percentage. Completely wilted plants were also collected. The per cent disease incidence was calculated by using the formula.

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Each sample was taken separately in polythene bags and tied with a rubber band and labeled immediately. Information pertaining to the locality, crop history, stage of the crop, *etc.* was also noted along with the samples. Samples of soil and roots were analyzed on the day of collection or after keeping for a few days under refrigerated conditions. The soil was mixed thoroughly and 200 cc of soil was processed following Cobb's sieving and decanting method (Cobb, 1918) followed by modified Baermann's funnel method (Schindler, 1961).

3.2 ISOLATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) STRAINS

Samples of soil and root system from healthy rhizosphere of coleus and ashwagandha were collected from ten plants each at random from each field. Each sample consisted of 100 g soil and 10 g roots. Rhizobacteria were isolated from rhizospheres of coleus and ashwagandha crops according to the method of Weller and Cook (1983).

3.2.1 Processing of roots

The soil adhering to the healthy root systems of crops like coleus and ashwagandha were washed off thoroughly. Roots were cut from the tip into 2-3 cm bits. The root segments

were further washed in distilled water and blotted to remove the moisture. One gram of the root material was transferred to 100 ml sterile water blank in a 250 ml conical flask and shaken for 20 minutes at 250 rpm in a rotary shaker to dislodge bacteria adhering to the root surface. Similarly, one gram of rhizosphere soil was mixed thoroughly in 100 ml sterile water and was processed following serial dilution agar plate technique (Aneja, 2002). Suitable dilutions (10^{-5} and 10^{-6}) of both rhizosphere and rhizoplane solutions were plated on King's B medium (King *et al.*, 1954) and nutrient agar medium to isolate fluorescent pseudomonads and total bacteria respectively. The plates were incubated at room temperature (28±2℃) for 24-48 h (Aneja, 2002).

Colonies that came up on KB plates were observed under UV light on a transilluminator. The colonies fluorescing under UV light were picked up, purified and preserved. Representative colonies on the NA plates were picked up, purified and preserved in nutrient broth. These strains were maintained at -80℃ with 50 per cent glycerol.

3.3 ISOLATION OF CAUSAL AGENT(S)

3.3.1 Isolation of fungi

Infected specimens were washed in tap water. Roots and stems were cut into small pieces. The pieces were surface sterilized in 1 per cent sodium hypochlorite ($NaOCl_2$) solution for 20-30 sec. from root and stem portions. Ten pieces each were placed on potato dextrose agar (PDA). Plates were incubated at 28±2℃ for 5-7 days. Hyphal tips growing from infected bits were transferred to PDA slants. The fungus was purified by using hyphal tip technique (Rangaswami, 1972), such pure culture tubes were preserved in a refrigerator at 4℃ and used for further studies.

3.3.1.1 Identification

The fungi belonging to the genus *Fusarium* was identified based on the spore morphology and colony characters of the fungus by referring to the illustrated genera of imperfect fungi (Barnett and Hunter, 1972). The characters were compared with those described by Booth (1971) and the fungi were identified as *F. chlamydosporum* and *F. solani*.

3.3.1.2 Mass multiplication of isolated fungi

Sand corn meal medium was prepared in the proportion of 95:5 in order to get maximum inoculum of the fungus. About 400 g of sand-maize meal medium was taken in 1000 ml flasks and watered to 20 per cent of its weight and sterilized at 1.33 kg/cm² pressure for one hour. The pure cultures of *Fusarium chlamydosporum* and *F. solani* were inoculated separately to different flasks under aseptic conditions and incubated at 28±2℃ for 20 days. The flasks were shaken on alternate days to get uniform growth. The gaint cultures so obtained were used for preparing sick pot in nethouse of Department of Plant Pathology.

3.3.2 Isolation of bacterium

Four pieces of stem cut at six cm away from the collar were surface sterilized in 0.1 per cent mercuric chloride solution for 30 sec, washed in sterile distilled water thrice and placed in 15 ml of sterile distilled water in screw-capped tubes for 30 minutes. If the water became turbid, it was used for isolation of bacterium. Four such pieces were used for isolation from infected coleus plant.

A loopful of turbid solution was streaked on sucrose peptone agar in petridishes and the plates were incubated at 28℃. *Ralstonia solanacearum* colonies appeared after 36 h of incubation at 28℃. The colonies were typically white fluidal with spiral pink center. A loopful of bacterial growth was suspended in sterile distilled water and kept at 4℃ for short term storage and at -20℃ in 50 per cent glycerol for long term storage. Discrete colonies were purified by subculturing successively for four times on nutrient agar. From the last plate individual colonies were picked up and transferred to sucrose peptone agar medium slants.

3.3.2.1 Identification and preservation of the bacteria

The identification of the bacterium, isolated from coleus plants was made based on its morphological physiological, cultural and biochemical characteristics besides pathogenicity studies, based on reports of Kelman (1954) and Schaad (1992).

Well isolated typical virulent colonies of *R. solanacearum* on TZC medium were picked and streaked separately on TZC medium in sterilized Petriplates. The plates were incubated at 32°C for 48 h. The well-separated virulent colonies of *R. solanacearum* were picked up with sterile inoculation loop and suspended in sterile distilled water in sterile propylene culture collection tubes and stored at 4°C in refrigerator for further use as stock culture.

3.3.3 Collection of cultures and maintenance of root-knot nematodes

Root-knot infected coleus and ashwagandha plants were collected from the farmer's field during survey in Karnataka in polythene bags and kept in the freezer. Root portion was carefully removed from the soil and washed gently under running tap water. Eggmasses were picked and kept for hatching in water in a petridish. After 24-36 hours, hatched juveniles were used to inoculate tomato, coleus and ashwaganda grown in sterilized soil:sand (1:1) mixture in greenhouse. These plants served as culture plants. After giving sufficient time so as to complete 3-4 generations of the nematode, the plants were depotted carefully. The root system were washed free of soil, the galls containing eggmasses were used to get inoculum of the nematode for further studies throughout.

3.4 EXTRACTION OF NEMATODES

Soil samples of 200 g were washed thoroughly and processed using combined Cobb's and sieving and Baermann's funnel method as given below.

(i) 200 g of soil was taken in 1000 ml beaker and sufficient quantity of water was added to make a soil solution.

(ii) This was stirred thoroughly and the heavier particles were allowed to settle down.

(iii) Hard particles and stones, if any, were removed and then the soil solution was passed through a set of sieves of 60, 250, 325 and 400 mesh sieves.

(iv) The sievates from 325 and 400 mesh sieves were collected and poured over tissue paper spread over a coarse mesh placed in a petridish (Baermann's funnel).

(v) The level of water in the Baermann's funnel was maintained to keep the tissue paper wet and kept undisturbed for 48 hours.

(vi) The contents from the petridish were emptied into a beaker, diluted to a suitable volume and population counts were taken with help of Fenwick's multichamber counting dish, using research stereobinocular microscope.

The root knot nematode and other plant parasitic nematodes present in the suspension were identified to genus level by observing different morpho-anatomical characters. Their number present in the suspension were determined by taking the average number of nematodes present in five different one millitre aliquots of nematode suspension.

3.5 IDENTIFICATION OF ROOT KNOT NEMATODE SPECIES

The galled root system was immersed in a beaker containing boiling 0.1 per cent cotton blue in lactophenol and left overnight for clearing (Southey, 1986). The roots infected by root knot nematode were washed. The females were dissected out from the well developed galls of the roots under the stereobinocular microscope and were transferred to a drop of lactophenol taken on a clean glass slide. The posterior portion of the females was cleaned. The perinal region was trimmed and mounted for observation under oil immersion

objective of a compound microscope. The identification of the species was made on the basis of characters of perineal pattern described by Eisenback *et al.* (1981).

3.6 PROVING THE PATHOGENICITY OF WILT COMPLEX DISEASE OF COLEUS AND ASHWAGANDHA

Rooted cuttings of coleus and ashwagandha plants raised in polythene bags were removed carefully and transplanted to earthern pots containing steam sterilized standard potting media consisting of soil:sand:farm yard manure in 3:1:1 ratio. After 10 days of transplanting the coleus and ashwagandh plants were used for inoculation. A control treatment was maintained without inoculum.

(A) Coleus	(B) Ashwagandha
1. *Fusarium chlamydosporum* alone	(i) *Fusarium solani*
2. *Ralstonia solancearum* alone	(ii) *Meloidogyne incognita*
3. *Meloidogyne incognita* alone	(iii) Control
4. Control	

Gaint cultures of inocula of *F. chlamydosporum* and *F. solani* were inoculated to coleus and ashwagandha respectively. Pathogenicity tests of *Ralstonia solanacearum* was conducted by preparing aqueous suspension of the bacteria grown on sucrose peptone broth medium for 48 h (10^8 cfu per ml) (Anon., 1998). Twenty millilitre of the suspension was poured to the base of coleus plants in earthen pots.

The eggmasses of *M. incognita* from culture plants were transferred carefully to a wire gauze sieve containing two layers of facial tissue paper and kept in a petridish holding sufficient water to remain in contact with the bottom of petridish. After 24 h the contents of a petridish were emptied into a beaker, diluted to a suitable volume and population counts were made with the help of Fenwick's multi chamber counting dish. Based on the requirement, the suspension was diluted with sterile water and known number of nematodes were inoculated to well established coleus and ashwagandha plants.

The pots were maintained at 25 per cent moisture holding capacity and the moisture loss was maintained by adding water on weight basis. Observations were made every alternate day regarding development of wilt symptoms when the plants showed wilt symptoms such plants were carefully uprooted and the pathogens were reisolated by standard tissue isolation method. The pathogens reisolated were compared with original cultures.

3.7 EFFECT OF DIFFERENT INOCULUM DENSITY OF *M. incognita* ON COLEUS AND ASHWAGANDHA

Two separate pot culture experiments were conducted in the nethouse, Department of Plant Pathology, College of Agriculture, Dharwad to evaluate the effect of different levels of inoculum of *M. incognita* on the growth parameter and root-knot disease of coleus and ashwagandha plants and to determine the pathogenic levels of the nematode.

The rooted cuttings of coleus and ashwagandha seedling were carefully transplanted to the earthen pots as described earlier. After 10 days of transplanting, suspensions containing desirable number of infective juveniles of the nematode were inoculated on to the exposed roots of the seedlings and later covered by sterilized soil. The number of juveniles present in a millilitre of the suspension was determined by taking the average number of juveniles present in five different, one millilitre aliquots of the suspension. Uniform quantity of this suspension containing desired number of juveniles was used for inoculation.

The inoculum levels maintained for the pathogenicily test were : A check; Associated check (suspension without juveniles); 10; 100; 1000 and 5000 infective juveniles per

treatment. Four replications were maintained for each treatment. The pots were placed on the nethouse benches and suitably randomized.

Just before the completion of the experiment, plant height, number of branches, fresh weight, dry weight of roots and shoot number of galls and root-knot index, number of juveniles per 200 cc of soil collected from the inoculated plants were recorded.

3.8 SCREENING OF RHIZOBACTERIA FOR ANTAGONISM AGAINST SPECIES OF *Fusarium, Ralstonia* AND *Meloidogyne*

3.8.1 *In vitro* screening of rhizobacteria against *Fusarium chlamydosporum* sp.

Fifty rhizobacterial strains isolated from healthy rhizosphere and rhizoplane of coleus and ashwagandha from different locations of Karnataka were tested for their inhibitory activity against mycelial growth of *Fusarium* by following the dual culture technique (Dennis and Webster, 1971). Mycelial discs of 5 mm diameter of seven days-old culture of *Fusarium* was placed in the middle of the Petri plate containing 20 ml PDA medium. Twenty four hour old culture of each rhizobacterial strains were streaked parallelly on either side of the fungal disc (3 cm away from the disc). The plates were incubated at room temperature (28±2°C) for 8-10 days, until the plate covered completely by the fungus in control. The plates with only fungal disc without bacterial streaks served as control. Each treatment was replicated three time. After incubation, *i.e.* when control plate reached 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was calculated by using the formula of Vincent (1947) as follows;

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

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

3.8.2 *In vitro* screening of rhizobacteria against *Rolstonia solanacearum*

The same fifty rhizobacterial strains were screened for their ability to inhibit the growth of *Ralstonia solanacearum* by following the dual culture assay (Ganesan and Gnanamanickyam, 1987). A luxuriant lawn of *R. solanacearum* was prepared on nutrient agar plates by spreading 1000 µl of 24h old *R. solanacearum* multiplied in Kelman's broth. Ten µl of the rhizobacterial isolates grown in nutrient broth overnight were spotted on the lawn. The inoculated plates were incubated at 30ºC for 48 hours.

Observations were recorded for the production of zone of inhibition around the rhizobacterium and the diameter of the inhibition zone (DIZ) was measured after 48h of incubation.

3.8.3 *In vitro* screening of rhizobacteria against nematode

3.8.3.1 Preparation of cell free extract

A single colony of each rhizobacterial strains was cultured in a screw-capped test tubes containing 10 ml of sterilized nutrient broth, incubated at 28ºC and mechanical shaker at 150 rpm for 48 h. The culture was subsequently passed through sterilized Whatman filter papers no.1 and 42, concentrated by centrifugation at 10,000 rpm for 10 min. and the

supernatant was collected and finally passed through Millipore filter of 0.22 µm. This was designated as undiluted standard cell free filtrate of cent per cent concentration. The cell free extract was further diluted to 75, 50 and 25 per cent respectively and these dilutions were used to study their effect on nematodes (Niknam and Dhawan, 2001). *In vitro* evaluation of PGPR strains against root-knot nematode was carried out on egg hatching and juveniles mortality.

3.8.3.2 *In vitro* egg hatching test

Egg masses were collected from culture plants maintained in Nematology nethouse, Department of Plant Pathology, Agricultural College, Dharwad. Eggmasses were picked up and treated with NaOCl (1%) to dissolve the egg matrix and to separate the individual eggs. A known number of eggs (50) were carefully transferred to each vials containing 5 ml of cell free culture filtrate of PGPR strains of 100, 75, 50 and 25 per cent concentrations. Inoculated vials are incubated at room temperature (28±2ºC) for 36h. Two controls were maintained by transforming 50 eggs to a vial containing sterilized nutrient broth and water. After 48h, the number of juveniles hatched was counted under stereo binocular microscope and per cent inhibition of egg hatching in different dilutions in each vials was calculated.

3.8.3.3 *In vitro* mortality test of juveniles

Freshly hatched, 50 active juveniles were counted in a counting dish using a stereo binocular microscope and were carefully transferred to individual vials containing 5 ml of each of the bacterial cell free filtrates of different concentrations (100, 75, 50 and 25 per cent). Each treatment was replicated three times and arranged in completely randomized design and incubated at 28±2ºC. Observations were recorded at 12h, 24h and 48h after exposure period and per cent mortality was calculated.

3.9 MORPHOLOGICAL AND BIOCHEMICAL HARACTERIZATION OF PGPR STRAINS

3.9.1 Morphological characterization

Seven selected efficient PGPR strains were examined for the colony morphology, growth, pigmentation, cell shape and gram reaction as per the standard procedures given by Anon. (1957) and Barthalomew and Mittewer (1950).

3.9.2 Biochemical characterization of PGPR strains

The biochemical characterization of selected efficient PGPR strains was essentially done as per the procedures outlined by Cappuccino and Sherman (1992). The tests conducted are detailed below and the chemical composition of media used for the tests are listed in Appendix I.

3.9.2.1 Catalase activity

Young cultures (24 h) of PGPR strains grown in the nutrient agar medium were added with 3 per cent hydrogen peroxide (H_2O_2). Observations made for the production of gas bubbles (positive reaction) (Schaad, 1992).

3.9.2.2 Gelatin liquefaction

To the pre-sterilized nutrient gelatin (Appendix I) deep tubes, the test PGPR cultures were inoculated and tubes were incubated at 28±2°C for 24 h. The tubes were later kept in a refrigerator at 4°C for 30 minutes. The tubes with cultures that remained liquefied were taken as positive and those that solidified on refrigeration were taken as negative for the test (Blazevic and Ederer, 1975).

3.9.2.3 Lipid hydrolysis

Overnight cultures of test PGPRs were spotted on tributryn agar (Appendix I) plates and were incubated at 28±2°C for 24 to 48 h. Formation of clear zone around the colony was taken as positive for the test.

3.9.2.4 Starch hydrolysis

PGPR strains of 24 hour old were spotted on the starch agar (Appendix I) plates and incubated at 28±2°C for 24 h. After incubation, the plates were flooded with Lugols iodine solution. Formation of clear zone around the colony was taken as positive for the test (Eckford, 1927).

3.9.2.5 Casein hydrolysis

PGPR strains were (Young cultures) spotted on the skimmed milk agar (Appendix I) plates and incubated at 28±2°C for 24 h. The production of clear halo around the colony was taken as positive for the test (Seeley and Vandemark, 1970).

3.9.2.6 Indole production

The test cultures were inoculated to the pre-sterilized SIM agar (Appendix I) tubes. The tubes were then incubated for 48 h at 28±2°C. After inoculations, each tube was added with 10 drops of Kovac's reagent. The production of cherry red colour was taken as positive for the indole production.

3.9.2.7 Hydrogen sulfide (H_2S) production

Pre-sterilized tubes containing SIM agar (Appendix I) were stabbed with the test cultures all along the walls of the test tubes. Inoculated tubes were incubated for 48 h at 28±2°C. After incubation, the development of black colour along the line of the stab was noted as positive for the test (Cappuccino and Sherman, 1992).

3.9.2.8 Urease test

The overnight cultures were inoculated to the test tubes containing sterilized urea broth (Appendix I) and incubated for 24-48 h at 28±2°C. The development of pink colour was taken as positive for the test.

3.9.2.9 Acid and gas production

Test tubes containing glucose broth (Appendix I) and Durham's tube (inserted in inverted position) and bromocresol purple (0.04% solution added @ 15 ml/l) were sterilized. The test cultures were inoculated to each tube. After three days of incubation at 28±2°C, the colour change of the broth from purple to yellow was taken as positive for acid production. Accumulation of gas in Durham's tube was taken as positive for the gas production (Seeley and Vandemark, 1970).

3.10 EFFICACY OF PGPR STRAINS ON PLANT GROWTH UNDER *IN VITRO* CONDITIONS

3.10.1 Ashwagandha seed bacterization

Seeds of ashwagandha were surface sterilized with one per cent sodium hypochlorite for 30 seconds rinsed in sterile distilled water and dried overnight under sterile stream of air in a laminar air flow. PGPR strains grown in NB were taken in screw-capped tubes. Carboxymethylcellulose (CMC) was added as an adhesive material @ 100 mg to this. Known number of seeds (100) was soaked in 10 ml rhizobacterial suspension (10^8 cfu/ml) for 12h. Then the seeds were dried under sterile stream of air.

3.10.1.1 Roll towel method: Plant growth promoting activity of PGPR strains were assessed based on the seedling vigour index using the standard roll towel method (ISTA, 1993). Fifty

bacterized seeds were kept over the presoaked germination paper. The seeds were held in position by placing another presoaked germination paper strip and gently pressed. The polythene sheet along with seeds were then rolled and incubated in growth chamber for 15 days. Three replications were maintained for each treatment. Seeds soaked in sterile water served as control. Root length and shoot length of individual seedlings were measured and the germination percentage of seeds was recorded.

The vigour index was calculated by using the formula as described by Abdul Baki and Anderson (1973).

Vigour index (VI) = Seedling length (Mean root length x mean shoot length) x Germination %

Five seedlings were taken randomly from each rhizobacterial treatment and their fresh weight was recorded. Later, the seedlings were kept in the hot air oven for 4 days at 60ºC for complete desication and dry weight of the seedlings was recorded.

3.10.2 Pot culture method

The growth promotion ability of selected rhizobacterial strains was also evaluated under pot culture conditions. Surface sterilized seeds of ashwagandha were steeped in suspension of PGPR strains overnight and a sterile water treated control was also maintained. The treated seeds were sown in pots containing 1 kg of sterilized soil: sand. The germination percentage, shoot length, and root length as well as fresh weight and dry weight of seedlings and vigour index was calculated. Seeds treated in sterile water and later were sown in a pot. This served as a control.

3.10.3 Bacterization of coleus cuttings

Uniform sized, pencil-thick coleus cuttings were selected and dipped in rhizobacterial strains for 12 h and planted in pots containing 1 kg of 1:1 sterilized soil and sand. The cuttings dipped in sterile water serves as control. The pots were kept on benches in glasshouse and suitably watered. Observations on shoot length, root length, no. of tubers, no of branches, fresh and dry weight of the coleus plants were recorded at 150 days after planting.

3.11 PREPARATION OF TALC BASED FORMULATIONS OF PGPR STRAINS

A loopful of different PGPR strains was inoculated to nutrient broth separately and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (28±2ºC). After 48 h of incubation, the broth containing 9×10^8 cfu/ml was used for the preparation of talc based formulation.

To 400 ml of bacterial suspension, one kg of the purified talc powder (sterilized at 105ºC for 12h) 15 g calcium carbonate (to adjust the pH to neutral) and 10 g of carboxy methyl cellulose (CMC) as an adhesive were mixed under aseptic conditions following the method described by Vidhyasekaran and Muthamilan (1995). The product was shade dried to reduce the moisture content below 20 per cent and then packed in polythene bags and sealed. At the time of application, the population of the bacteria in talc formulation was checked to 2.5 to 3×10^8 cfu/g (Plate 20).

3.12 METHOD OF APPLICATION OF BIOFORMULATIONS

(a) Seed treatment: Ashwagandha seeds were soaked in double the volume of sterile distilled water containing talc-based formulation (10 g/kg of seed). After 24h, the suspension was dried off and the seeds were dried under sterile air and used for sowing (Vidhyasekaran *et al.*, 1997) in pots under glasshouse and field conditions.

(b) Seedling dip method: Fresh cuttings of coleus raised in polyhouse were removed carefully after one month of planting and their roots were dipped in water containing talc formulation (50 g l^{-1}) for 3h. Care was taken to ensure that only roots were immersed in the inoculum. The seedlings were transplanted in earthen pots (5 kg capacity) containing sterilized soil: sand:

FYM (3:1:1) at the rate of single seedling per pot and also in plot. 30 per cent moisture maintained in pots.

After establishment of seedlings (30 DAP) in pot and field experiments a booster dose of talc based bioformulations of PGPR strains was also given.

3.13 EVALUATION OF PGPR STRAINS AGAINST INDIVIDUAL AND COMBINATION OF DIFFERENT PATHOGENS OF COLEUS AND ASHWAGANDHA UNDER GLASSHOUSE CONDITIONS

3.13.1 Coleus

A glasshouse study was conducted to test the efficacy of selected PGPR strains bioformulations against *F. chlamydosporum*, *R. solanacearum* and *M. incognita* individually and against combinations of pathogens on coleus.

Seedlings treated with different PGPR bioformulations were planted in glasshouse. After 10 days of their establishment, the above said pathogens were inoculated into the individual pots into the root zone (@ 50 g sand corn meal medium of *F. chlamydosporum*, 50 ml NB of 48 h *R. solanaceanum* and 100 *Meloidogyne* juveniles/pot), by making three holes around the plant at root zone and covered with sterilized soil.

The following treatments were maintained with three replications and the pots were arranged in completely randomized design.

Treatment no.	Treatment details
T_1	RB1 + *Fusarium*
T_2	RB10 + *Fusarium*
T_3	RB13 + *Fusarium*
T_4	RB22 + *Fusarium*
T_5	RB31 + *Fusarium*
T_6	RB43 + *Fusarium*
T_7	RB50 + *Fusarium*
T_8	*Fusarium* (control)
T_9	RB1 + *Ralstonia*
T_{10}	RB10 + *Ralstonia*
T_{11}	RB13 + *Ralstonia*
T_{12}	RB22 + *Ralstonia*
T_{13}	RB31 + *Ralstonia*
T_{14}	RB43 + *Ralstonia*
T_{15}	RB50 + *Ralstonia*
T_{16}	*Ralstonia* (control)
T_{17}	RB1 + *Fusarium* + *Ralstonia*
T_{18}	RB10 + *Fusarium* + *Ralstonia*
T_{19}	RB13 + *Fusarium* + *Ralstonia*
T_{20}	RB22 + *Fusarium* + *Ralstonia*
T_{21}	RB31 + *Fusarium* + *Ralstonia*
T_{22}	RB43 + *Fusarium* + *Ralstonia*

Treatment no.	Treatment details
T_{23}	RB50 + *Fusarium* + *Ralstonia*
T_{24}	*Fusarium* + *Ralstonia* (control)
T_{25}	RB1 + *Meloidogyne*
T_{26}	RB10 + *Meloidogyne*
T_{27}	RB13 + *Meloidogyne*
T_{28}	RB22 + *Meloidogyne*
T_{29}	RB31 + *Meloidogyne*
T_{30}	RB43 + *Meloidogyne*
T_{31}	RB50 + *Meloidogyne*
T_{32}	*Meloidogyne* (control)
T_{33}	RB1 + *Fusarium* + *Meloidogyne*
T_{34}	RB10 + *Fusarium* + *Meloidogyne*
T_{35}	RB13 + *Fusarium* + *Meloidogyne*
T_{36}	RB22 + *Fusarium* + *Meloidogyne*
T_{37}	RB31 + *Fusarium* + *Meloidogyne*
T_{38}	RB43 + *Fusarium* + *Meloidogyne*
T_{39}	RB50 + *Fusarium* + *Meloidogyne*
T_{40}	*Fusarium* + *Meloidogyne* (control)
T_{41}	RB1 + *Ralstonia* + *Meloidogyne*
T_{42}	RB10 + *Ralstonia* + *Meloidogyne*
T_{43}	RB13 + *Ralstonia* + *Meloidogyne*
T_{44}	RB22 + *Ralstonia* + *Meloidogyne*
T_{45}	RB31 + *Ralstonia* + *Meloidogyne*
T_{46}	RB43 + *Ralstonia* + *Meloidogyne*
T_{47}	RB50 + *Ralstonia* + *Meloidogyne*
T_{48}	*Ralstonia* + *Meloidogyne* (control)
T_{49}	RB1 + *Fusarium* + *Ralstonia* + *Meloidogyne*
T_{50}	RB10 + *Fusarium* + *Ralstonia*+ *Meloidogyne*
T_{51}	RB13 + *Fusarium* + *Ralstonia*+ *Meloidogyne*
T_{52}	RB22 + *Fusarium* + *Ralstonia*+ *Meloidogyne*
T_{53}	RB31 + *Fusarium* + *Ralstonia*+ *Meloidogyne*
T_{54}	RB43 + *Fusarium* + *Ralstonia*+ *Meloidogyne*
T_{55}	RB50 + *Fusarium* + *Ralstonia*+ *Meloidogyne*
T_{56}	*Fusarium* + *Ralstonia* + *Meloidogyne* (control)
T_{57}	No RB, No pathogens

3.13.2 Ashwagandha

Seeds treated with different PGPR bioformulations were sown in pots. After 30 days of their establishment, different pathogen, *viz. F. solani* and *M. incognita* were inoculated into the individual pots into the root zone (@ 50 g sand corn meal medium of *F. solani* and 100 infective juveniles of *M. incognita* per pot), by making three holes around the plant and covered with sterilized soil.

The following treatments were maintained with three replications and the pots were arranged in completely randomized design.

Treatment no.	Treatment details
T_1	RB1 + *Fusarium*
T_2	RB10 + *Fusarium*
T_3	RB13 + *Fusarium*
T_4	RB22 + *Fusarium*
T_5	RB31 + *Fusarium*
T_6	RB43 + *Fusarium*
T_7	RB50 + *Fusarium*
T_8	*Fusarium* (control)
T_9	RB1 + *Meloidogyne*
T_{10}	RB10 + *Meloidogyne*
T_{11}	RB13 + *Meloidogyne*
T_{12}	RB22 + *Meloidogyne*
T_{13}	RB31 + *Meloidogyne*
T_{14}	RB43 + *Meloidogyne*
T_{15}	RB50 + *Meloidogyne*
T_{16}	*Meloidogyne* (control)
T_{17}	RB1 + *Fusarium* + *Meloidogyne*
T_{18}	RB10 + *Fusarium* + *Meloidogyne*
T_{19}	RB13 + *Fusarium* + *Meloidogyne*
T_{20}	RB22 + *Fusarium* + *Meloidogyne*
T_{21}	RB31 + *Fusarium* + *Meloidogyne*
T_{22}	RB43 + *Fusarium* + *Meloidogyne*
T_{23}	RB50 + *Fusarium* + *Meloidogyne*
T_{24}	RB0 + *Fusarium* + *Meloidogyne* (control)
T_{25}	No RB, No pathogen

The pot culture experiments were maintained in Nematology nethouse, Department of Plant Pathology during December-2007 to May-2008. Observation on disease expression was recorded from the day symptoms appeared and continued until fresh infections ceased to

appear and the disease severity was scored based on symptoms appeared and days taken for expression of symptoms as given below.
$\leq$ 25 days : ++++, 26-50 days: +++, 51-75: ++, $\geq$ 76 days: +, no symptoms: -.
Root rot index and gall index were calculated by following the scale suggested by Chidananda Prabhu (1987) and Taylor and Sasser (1978) as given below.

Root rot index (0-5 scale) (Chidananda Prabhu, 1987)

0 – Healthy (No symptoms at all)

1 – Slight discolouration at the collar region, roots are healthy

2 – Discolouration upto 1 to 1.5 inch from ground level, tap roots discoloured and secondary roots still healthy.

3 – Complete discolouration of tap roots 2 to 2.5 inch of the stem and root started discoloured.

4 – Majority of secondary roots discoloured and root tips infected.

5 – All roots rotting and complete discolouration.

Gall index (Taylor and Sasser, 1978)

No galls or eggmasses	0
1 to 2 galls or eggmasses	1
3 to 10 galls or eggmasses	2
11 to 30 galls or eggmasses	3
31 to 100 galls or eggmasses	4
More than 100 galls or eggmasses	5

3.14 ELUCIDATION OF MECHANISMS OF BIOCONTORL OF PGPR STRAINS

3.14.1 Siderophore production

Production of siderophore by PGPR strains was assayed by plate assay method as described by Schwyn and Neilands (1987). In order to study the involvement of siderophores in the inhibition of the pathogen, the different rhizobacteria were spotted on the lawn of the pathogen prepared on KB medium amended with 1 mM iron as $FeCl_3$ (Dileep Kumar and Dubey, 1993). Siderophore production by the rhizobacterial strains was tested following the method of Schwyn and Neilands (1987).

3.14.1.1 Deferration: Removal of contaminating Iron from glasswares

Glass is a good ion exchange surface and hence could get contaminated with iron on the surface. In the siderophore studies, contaminating iron therefore was avoided. Treating the glasswares for the removal of iron became essential. All the glasswares used in the siderophore assays and in the preparation of Chrome Azurol S blue agar medium (CAS) were soaked in 2N HCl solution for at least 24h. After removing from acid solution, the glasswares were invariably washed with double distilled water. The acid washing of glasswares was found to be crucial for obtaining consistent and reliable results.

3.14.1.2 Preparation of CAS for the detection of siderophores

CAS medium is used to detect siderophore production by PGPR strains (Schwyn and Neilands, 1987). To prepare one litre of blue agar CAS medium, 60.5 mg Dehydrated Chromo Azurol S (Himedia) was dissolved in 50 ml water and mixed with 10 ml of iron solution (1mM

$FeCl_3.6H_2O$ in 10 mM Hcl). While stirring, this solution was slowly added with 40 ml aqueous solution containing 72.9 mg cetyl trimethyl ammonium bromide with continuous stirring and the final solution was autoclaved.

King's B agar medium was prepared with PIPES (30.2g) and Difco agar (18.0g). The pH of the medium was adjusted to 6.8 by addition of 50 per cent (w/w) sodium hydroxide (NaOH) solution and autoclaved. Cooled CAS dye was added along the glass wall with gentle agitation to achieve mixing without formation of foam. To each plate 20 ml of CAS agar dye was added. The plates were stored in a refrigerator (4ºC) for 24 h before use.

3.14.1.3 Spotting of rhizobacterial strains

PGPR strains (10µl) grown overnight were spotted on CAS plates and incubated at (28±2ºC) for 48h. Yellow to orange coloured clear zone around the spotted colony was taken as positive indication of siderophore production. The extent of siderophore biosynthesis was measured in terms of the diameter of the zone developed.

3.14.2 HCN production

Ability of the efficient PGPR strains to produce HCN was assessed as per the method of Wei *et al.* (1991). Whatman no.1 filter paper pads were placed on the lids of the petriplates and the plates were sterilized. Tryptic soya agar medium (TSA) (Himedia, Mumbai) amended with glycine (4.4 g/l) was sterilized and poured into the sterile plates. Twenty four hours old rhizobacterial strains were streaked on to the medium. The filter paper padding in each plate was soaked with two ml sterile picric acid solution (Picric acid, 2.5g; Na_2Co_3, 12.5 g and distilled water, 1 litre) (Miller and Higgins, 1970). Inoculated plates were sealed with parafilm in order to contain the gaseous metabolite produced by the antagonistic rhizobacteria and allow for a chemical reaction with picric acid on the top.

After incubation for a week at 28±2ºC, the colour changes of the filter paper was noticed and the HCN production potential of the antagonistic rhizobacteria was assessed as per the following scoring.

No colour change	:	No HCN production
Brownish colouration	:	Weak HCN production
Brownish to orange	:	Moderate HCN production
Orange to reddish brown	:	Strong HCN production

3.14.3 Indole-3-acetic acid (IAA) production

PGPR strains were inoculated into 10 ml of minimal salt medium supplemented with 100 µg/ml of tryptophan and incubated at 30°C under shaking for 48 h. Broth cultures were centrifuged at 7500 rpm for 10 minutes. To one ml of aliquot of the supernatant of the cultures 2 ml of Salkowskis reagent (Gordon and Weber, 1951) was added and incubated at 30°C for 25 minutes. Absorption was read at 530 nm and the concentration of IAA in each PGPR strains was determined and quantified by comparison with a standard curve of IAA (Surjit Sen *et al.*, 2006).

3.14.4 Detection of fluorescin and pyocyanin

Pseudomonas agar (Himedia, Mumbai) favours the formation of fluorescein whereas Pseudomonas agar P (Himedia, Mumbai). Stimulate the pyocyanin production and reduces fluorescein formation (King *et al.*, 1954). All the isolates of PGPR strains were tested for production of fluorescein and pyocyanin.

3.14.5 Antimicrobial metabolite (antibiotics) production

The antibiotic activity of selected efficient PGPR strains was determined and assessed by extracting and testing the toxicity of metabolites produced by them following the method of Kraus and Loper (1992).

The strains were grown for 48h in five ml of nutrient broth amended with glucose (2% w/v). The growth was spun at 10,000 rpm for 10 minutes and the filterate was collected. The metabolites in the filtrates were extracted with an equal volume of chloroform. The metabolites were also extracted from the pellet and pooled. The upper aqueous layer was discarded and to the remaining chloroform phase, a pinch of sodium sulphate was added to dry off the water. It was again spun at 8,000 rpm for 8 minutes and sodium sulphate was pelleted. The clear layer was decanted and chloroform was removed by flushing in air. The residue was redissolved in 200 µl of acetone and 70 µl was spotted on to a Thin Layer Chromatography (TLC) plate (Silica gel 60 F_{254}, 20 x 20 cm, 0.2 mm thickness, Merck). The plate was chromatogrammed using chloroform: acetone (9:1) as the solvent system. Later, the plate was observed under UV light at 254 nm. The metabolites were marked and the Retention factor (Rf) value were calculated.

The metabolites were eluted and redissolved in acetone: water (1:10). One hundred µl of the eluted portions were centrifuged to pellet the silica gel and the clear suspension was further analysed for the toxicity against the test pathogen. The per cent inhibition of the individual antibiotics produced by each PGPR strains was calculated and results were tabulated.

3.14.6 Production of volatile compounds

Volatile compound production of PGPR strains was assayed by inverted plate technique (paired plate technique) (Dennis and Webster, 1971), where two lids of separate Petriplates were taken and poured with 20 ml of PDA for one plate and 20 ml of NA on another plate. PDA plates was inoculated with the test pathogen *F. chlamydosporum* (5 mm disc) and NA amended plates were streaked with a loopful of 48 h old PGPR cultures. The petriplates were sealed mouth to mouth with parafilm. Control set consisted of only *Fusarium* on PDA inverted over unstreaked NA plate. Paired plates were incubated at 30℃ for 7 days as triplicate. After incubation period colony diameter of the fungal pathogen was measured and compared with the control set. Inhibition percentage was calculated and volatile compounds production was detected as nil, low, medium and high.

3.15 INDUCED SYSTEMIC RESISTANCE (ISR)

3.15.1 Assay of defense related enzymes and compounds

3.15.1.1 Sample collection

Samples were collected from individual treatments to study the induction of defense enzymes in response to pathogen attack in coleus and ashwagandha plants under glasshouse conditions. Leaves, stems and roots from different treatments were collected and used for analysis.

3.15.1.2 Enzyme extract

The leaf, stem and root sample, collected from bacterized and pathogen inoculated coleus and ashwagandha plants were immediately homogenized with liquid nitrogen. One g of powdered sample was extracted with 2 ml of sodium phosphate buffer, 0.1M (pH 7.0) at 4ºC. The homogenate was centrifuged for 20 min at 10,000 rpm. Protein extracts prepared from coleus and ashwaganda tissues were used for estimation of defense enzymes like peroxidase (PO) polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and total phenols.

3.15.1.2.1 Assay of peroxidase (PO)

Assay of PO activity was carried out as per the procedure described by Hammerschmidt *et al*. (1982). The reaction mixture consisted of 2.5 ml of a mixture containing 0.25 per cent (v/v) guaiacol in 0.01M. Sodium phosphate buffer, pH 6.0 and 0.1 M hydrogen peroxide. Enzyme extract (0.1 ml) was added to initiate the reaction which was followed colorimetrically at 470 nm. Crude enzyme preparations were diluted to give changes in absorbance at 470 nm of 0.1 to 0.2 absorbance units/min. Boiled enzyme was used as blank. Activity was expressed as the increase in absorbance at 470 nm min^{-1} mg^{-1} of protein.

3.15.1.2.2 Assay of polyphenoloxidase (PPO)

A sample of one g was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) at 4ºC. The homogenate was centrifuged at 20,000 rpm for 15 min at 4ºC. The supernatant served as enzyme source and polyphenoloxidase activity was determined as per the procedure given by Mayer *et al*. (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.1M catechol was added and the activity was expressed as change in absorbance min^{-1} mg^{-1} of protein.

3.15.1.2.3 Assay of phenylalanine ammonia lyase (PAL)

A plant sample of one g was homogenized in 3ml of ice cold 0.1 M sodium borate buffer, pH 7.0, containing 1.4 mM of 2-mercaptoethanol and 50 mg of insoluble polyvinylpyrrolidene (PVP). The resulting extract was filtered through cheese cloth and the filtrate was centrifuged at 20,000 rpm for 15 min at 4ºC and the supernatant was used as the enzyme source. PAL activity (EC 4.3.1.5) was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Sample containing 0.5 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer pH 8.8 and 9.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30ºC. The amount of trans-cinnamic acid synthesized was calculated (Dickerson *et al*, 1984). Enzyme activity was expressed in fresh weight basis as n mol transcinnamic acid min^{-1} mg^{-1} of sample.

3.15.1.2.4 Total phenolic content

Phenol content was estimated as per the procedure given by Zieslin and Ben-Zaken (1993). One g of sample was homogenized in 10 ml of 80% methanol with pestle and mortar and agitated for 15 min at 70ºC. One ml of the methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1N) and the solution was kept at 25ºC. After 3 min one ml of saturated solution of sodium carbonate and one ml of distilled water was added and the reaction mixture was incubated for 1 h. at 25ºC. The absorption of the developed blue colour was measured using UV-visible spectrophotometer at 725 nm. The content of the total soluble phenols was calculated according to a standard curve obtained from a Folin-Ciocalteau reagent with a phenol solution (C_6H_6OH) and expressed as catechol-equivalents mg^{-1} tissue weight.

3.16 GENETIC VARIABILITY AMONG THE PGPR STRAINS

Random amplified polymorphic DNA (RAPD) analysis was used to detect the variations among the efficient PGPR strains. Standard protocols were used for the isolation of template DNA and RAPD analysis.

Requirements:

- 1% SDS: one g of sodium dodecyl sulphate was dissolved in 100 ml of distilled water.
- Tris 0.1 mol l^{-1}
- Lysis solution (0.2N NaOH and 1% SDS)
- Phenol: chloroform: Isoamyl alcohol (25:24:1 v/v/v)

- 70% ethanol
- 100 μM random primer
- 95% Ice cold isopropynol
- 25 ng $μl^{-1}$ template DNA
- 6.0 U $μl^{-1}$ Taq DNA polymerase
- dNTP's and Taq Buffer A

3.16.1 Isolation of DNA from rhizobacteria

Procedure

(i) PGPR cultures were grown in 5ml NB with 10% glycerol (v/v) for 72 h at 28±2ºC.

(ii) Eppendorf tube of 1.5 ml was used to centrifuge the cells at 13,000 rpm for 5 minutes.

(iii) The pellet was suspended in 200 μl Tris 0.1 mol L^{-1} and added with 200 μl of lysis solution (NaOH 0.2 N and 1% SDS).

(iv) Above solution was mixed and deproteinized with 700 μl of phenol: chloroform: isoamyl alcohol (25:24:1 v/v/v) and centrifuged at 13000 rpm for 10 min.

(v) Top layer containing bacterial DNA was taken without disturbing the bottom layer and it was transferred to fresh 1.5 ml microcentrifuge tube.

(vi) To this, 700 μl of ice cold 95% ethanol/isoproponol was added to precipitate the DNA and spinned.

(vii) Final washing was given with 70% ethanol and centrifuged at 8000 rpm for 5 min.

(viii) Precipitated DNA was dried at room temperature and resuspended in 100 μl of water.

(ix) The DNA obtained was further quantified and electrophoresed on 0.8% agarose gel stained with ethidium bromide and photographed under UV light.

3.16.2 Polymerase chain reaction

Requirements for PCR

(1) Random primers: commercial kits OPA, OPB and OPF of random decamer DNA primers were obtained from M/S Genei Bangalore. A total of 30 random primers were used for the assay.

(2) dNTPs: The four individual dNTPs such as dATP, dGTP, dCTP and dTTP were obtained from m/s Bangalore Genei, Pvt. Ltd. Bangalore.

(3) Taq DNA polymerare: Taq DNA polymerase and 10x Taq assay buffer A were obtained from M/S Bangalore Genei, Pvt. Ltd., Bangalore.

(4) Thermo cycler: Eppendorf thermocycler was used for amplification

Sequence details of random primers used in RAPD analysis for different PGPR strains

OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC

OPA-04	AATCGGGCTG
OPA-05	AGGGGTCTTG
OPA-06	GGTCCCTGAC
OPA-07	GAAACGGGTG
OPA-08	GTGACGTAGG
OPA-09	GGGTAACGCC
OPA-10	GTGATCGCAG
OPB-01	GTTTCGCTCC
OPB-02	TGATCCCTGG
OPB-03	CATCCCCCTG
OPB-04	GGACTGGAGT
OPB-05	TGCGCCCTTC
OPB-06	TGCTCTGCCC
OPB-07	GGTGACGCAG
OPB-08	GTCCACACGG
OPB-09	TGGGGGACTC
OPB-10	CTGCTGGGAC
OPF-01	ACGGATCCTG
OPF-02	GAGGATCCCT
OPF-03	CCTGATCACC
OPF-04	GGTGATCAGG
OPF-05	CCGAATTCCC
OPF-06	GGGAATTCGG
OPF-07	CCGATATCCC
OPF-08	GGGATATCGG
OPF-09	CCAAGCTTCC
OPF-10	GGAAGCTTGG

Thermoprofile for PCR

The PCR amplification for RAPD analysis was performed according to Williams *et al.* (1990) with certain modifications. The optimum conditions for DNA amplifications used were as follows.

Profile	Step	Temperature	Duration (mix)	Number of cycles
1	Initial denaturation	94°C	5	
2	Denaturation	94°C	1	40
3	Annealing	36°C	1	
4	Extension	72°C	2	
5	Final extension	72°C	10	
6	Hold temperature	4°C	Forever	

Master mix for PCR

DNA from different PGPR strains was used for RAPD analysis following the method recommended by Bhat and Jarret (1995) with required modification.

Amplification reaction mixture was prepared in 0.2 ml thin walled PCR tubes containing following components. The total volume of each reaction mixture was 20µl. The cocktail for the PCR amplification was found to be optimum were

(1) Template DNA (25 ng/µl) – 1.0 µl

(2) 10x assay buffer with 15mM $Mgcl_2$ – 2.00 µl

(3) dNTP's mix (2.5 mM each) – 2.00 µl

(4) Primer (5pM/µl) – 2.00 µl

(5) Taq DNA polymerase (3 units/µl) – 0.2 µl

(6) Sterile distilled water – 12.8 µl

Except template DNA the master mix was distributed to PCR tubes (19µl/tube) and later 1 µl of template DNA from the respective PGPR strains was added making the final volume of 20 µl.

3.16.3 Separation of amplified products by agarose gel electrophoresis

Requirements

(1) Electrophoretic unit: Gel casting trough, gel combs, power-pack and UV-Transilluminator

(2) Agarose (1.2%)

(3) Bromophenol blue

(4) Ethidium bromide (0.5 µg/ml)

(5) 50 x TAE (stock): Tris – free base – 60.5 g

Glacial acetic acid – 14.25 ml

0.5 M EDTA – 25 ml

Make up the volume to 250 ml pH 8.0

(6) Working solution (1 x TAE): 20 ml of 50 x TAE was made upto 1000 ml by using distilled water.

Procedure

- One gram of agarose was weighed and added to a 250 ml conical flask containing 100 ml of 1 x TAE buffer.
- The agarose was melted by heating the solution on a microoven and the solution was stirred to ensure even mixing and complete dissolution of agarose.
- The solution was then cooled to about 50ºC.
- Two to three drops of ethidium bromide (0.5 µg ml^{-1}) was added.
- The solution was mixed and poured into the gel casting plat form after inserting the comb in the trough.
- While pouring sufficient care was taken for not allowing the air bubbles to trap in the gel.
- The gel was allowed to solidify and the comb was removed after placing the solidified gel into the electrophoretic apparatus containing sufficient buffer (1 x TAE) so as to cover the wells completely.
- The amplified products (20 µl) to be analysed were carefully loaded into the sample wells, after adding bromophenol blue with the help of micropipette.
- Electrophoresis was carried out at 60 volts until the tracking dye migrated to the end of the gel.
- Ethidium bromide stained DNA bands were viewed under UV – transilluminator and photographed for documentation.

3.16.4 Scoring the amplified fragments

The amplified profiles for all the primers were compared with each other and the bands of DNA fragment were scored as '1' for presence and '0' for the absence of a band generating the '0' and '1' matrix and per cent polymorphism was calculated by using the formula.

$$\text{Per cent polymorphism} = \frac{\text{No. of polymorphic bands}}{\text{Total number of bands}} \times 100$$

3.16.5 Analysis of RAPD-PCR amplified fragments

Pair wise genetic similarities between PGPR strains were estimated by DICE similarity coefficient, clustering was done using the symmetric matrix of similarity coefficient and cluster obtained based on unweighted pair group arithmetic mean (UPGMA) using sequential agglomerative hierarical nested (SAHN) cluster analysis of NTSYS-PC program version 2.0 (Exeter software, New York, USA) described by Rohlf (1998).

3.17 EVALUATION OF PGPR STRAINS UNDER FIELD CONDITIONS

An experiment was conducted with eight treatments and three replications for coleus and ashwagandha during July-2007 to March-2008 at MARS, Saidapur farm, Agricultural College, UAS, Dharwad. A randomized block design was used. The soil was naturally infested with *Fusarium* and *Meloidogyne* with a population density of more than one (1.5) nematode per CC of soil. The treatment details are as follows.

T_1 – RB1

T_2 – RB10

T_3 – RB13

T_4 – RB22

T_5 – RB31

T_6 – RB43

T_7 – RB50

T_8 – Control

Selected efficient PGPR strains were mass multiplied on talc based bio formulations and the seedlings and seeds of coleus and ashwagandha were treated as described in 3.11 and 3.12.

Observations like plant growth parameter at different growth stages (60, 90, 150 DAP and at harvest) plant height, number of branches and per cent wilt incidence and at harvest yield parameters like weight of tubers, length and number of tubers, fresh and dry weight total biomass and yield were recorded.

3.18 STATISTICAL ANALYSIS

The data obtained in the present investigations for various parameters in the experiments were subjected to ANOVA for a completely randomized design for *in vitro* studies and randomized complete block design for *in vivo* studies by using M-STAT C programme (Gomez and Gomez, 1984).

4. EXPERIMENTAL RESULTS

The results on the survey for diseases incidence in coleus and ashwagandha, isolation of participating pathogens, their pathogenicity: screening and selection of efficient rhizobacterial strains effective against species of *Fusarium* spp., *Ralstonia* sp. and *Meloidogyne* was carried out. An attempt was also made to elucidate the mechanism of biocontrol and plant growth promotion of potent antagonistic strains. In the present investigation, the laboratory and pot culture experiments were conducted in the Department of Plant Pathology, IABT and Nematology net house, College of Agriculture, University of Agricultural Sciences, Dharwad and field experiments were conducted in naturally affected field in Saidapur farm, Main Horticultural Research Station, UAS, Dharwad. The results of the experiments conducted during 2005-08 are presented under the following headings.

4.1 SURVEY FOR WILT COMPLEX IN MAJOR COLEUS AND ASHWAGANDHA GROWING AREAS OF KARNATAKA

A survey was conducted in six districts of Karnataka. The survey data indicated that the disease was present throughout the state (Table1), wherever coleus and ashwagandha was grown. In coleus, the wilt complex was noticed in all the locations surveyed with a range from 19.00 to 44.66 per cent. Maximum wilt complex incidence was observed in Gokak of Belgaum district (44.66%) and least incidence in Muddebihal of Bijapur district (19.00%).

In Bijapur district, maximum disease incidence was observed in Nalvathwada (35.66) followed by Areshankara (26.66%) and lowest was in Muddebihal (19.00%). In Belgaum district, maximum disease incidence was observed in Gokak (44.66%) and least was in Chikkodi (36.66%). In Dharwad district, 30 per cent of disease incidence was noticed from Dharwad and 32.50 per cent disease incidence in Tadas. Disease incidence of 36.33 per cent in GKVK, 40.66 in Hesaragatta of Bangalore and in Tumkur district with a maximum incidence 36.00 per cent was recorded in Kaggere followed by Amruthur (33.33%) respectively.

Survey data revealed that the cropping area of ashwagandha was less and the disease incidence was also meager. However, maximum disease incidence of 30.33 was recorded in Dharwad district followed by Bijapur district (21.55%).

4.2 SURVEY AND ISOLATION OF NATIVE PGPR STRAINS FROM MAJOR COLEUS AND ASHWAGANDHA GROWING AREAS OF KARNATAKA

A random survey was undertaken for the isolation of PGPR strains in major coleus and ashwagandha growing districts of Karnataka. Fifty PGPR strains were isolated from six districts of Karnataka, *viz.* Bijapur, Belgaum, Dharwad, Uttara Kannada, Tumkur and Bangalore. Isolated strains were designated as RB series 1, 2, 3 and so on. Out of six districts surveyed, maximum (11) strains were obtained from Bijapur district followed by Belgaum (9 strains) (Table 2).

4.3. SYMPTOMATOLOGY

4.3.1 Coleus

In coleus wilt complex, the symptoms depended on the pathogen(s) involved. The major diseases identified during survey were wilt and collar rot caused by *Fusarium chlamydosporum*, root rot caused by *Rhizoctonia bataticola*, bacterial wilt caused by *Ralstonia solanacerum* and root-knot caused by *Meloidogyne incognita*. The individual pathogen infection may have lead to wilt/root rot or the combination of pathogens also lead to wilt complex. All these pathogens are known to form complexes with nematode, leading to synergistic effect on severity of the disease.

Table 1. Incidence of disease in major coleus and ashwagandha growing areas of Karnataka during 2005-2006

District	Location/ Place	Per cent disease incidence	
		Coleus	Ashwagandha
Bijapur	Muddebihal	19.00	-
	Nalvathwada	35.66	23.00
	Areshankara	26.66	19.33
	Nagarabettaa	20.33	22.33
Mean		25.41	21.55
Belgaum	Gokak	44.66	-
	Arabhavi	38.00	18.50
	Chikkodi	36.00	-
Mean		39.55	18.50
Dharwad	Dharwad	30.00	32.66
	Tadasa	32.50	28.00
Mean		31.25	30.33
Uttara Kannada	Mundagod	30.00	23.33
	Sirsi	20.33	16. 66
	Hulekal	29.33	-
Mean		26.55	19.99
Tumkur	Amruthuru	33.33	-
	Kaggere	36.00	22.00
	Kodagihalli	31.66	-
Mean		33.66	22.00
Bangalore	Hesaragatta	40.66	19.66
	G.K.V.K	36.33	21.00
Mean		38.49	20.33

Table 2. Isolation of PGPR strains from coleus and aswagandha rhizospheres in different parts of Karnataka

District	Location	Crop	No. of isolates	Total No. of isolates	
Bijapur	Muddebihal	Coleus	2	11	RB01, RB02, RB03, RB04, RB05, RB06 RB07, RB08, RB09, RB10, RB11
	Nalvathwada	Coleus	1		
		Ashwagandha	2		
	Areshankara	Coleus	2		
		Ashwagandha	1		
	Nagarabettaa	Coleus	2		
		Ashwagandha	1		
Belgaum	Gokak	Coleus	2	9	RB12, RB13, RB14, RB15, RB16, RB17 RB18, RB19, RB20
	Arabhavi	Coleus	3		
		Ashwagandha	2		
	Chikkodi	Coleus	2		
Dharwad	Dharwad	Coleus	2	7	RB21,RB22, RB23, RB24, RB25, RB26 RB27
		Ashwagandha	2		
	Tadasa	Coleus	2		
		Ashwagandha	1		
Uttara Kannada	Mundagod	Coleus	2	8	RB28, RB29, RB30, RB31 RB32,RB33 RB34, RB35
		Ashwagandha	1		
	Sirsi	Coleus	2		
		Ashwagandha	1		
	Hulekal	Coleus	2		
Tumkur	Amruthuru	Coleus	2	7	RB36, RB37, RB38, RB39 RB40,RB41 RB42,
	Kaggere	Coleus	2		
		Ashwagandha	1		
	Kodagihalli	Coleus	2		
Bangalore	Hesaragatta	Coleus	2	8	RB43, RB44, RB45, RB46 RB47, RB48 RB49, RB50
		Ashwagandha	2		
	G.K.V.K	Coleus	2		
		Ashwagandha	2		

4.3.1.1 *Fusarium* wilt

Fusarium chlamydosporum infected plants exhibited gradual yellowing, marginal necrosis and drying of leaves followed by loss in vigour and premature death. Such plants showed discolouration of roots and complete decaying of tap and lateral root system. The bark of such plants easily peeled off extensive sloughing and shredding of affected bark. Infected plants were killed finally. The infected roots and tubers showed rotting and emitted bad odour (Plate 1).

4.3.1.2 Bacterial wilt

Water soaked patches with linear streaks on collar region of the infected plants were observed. Leaves became flaccid and drooped quickly, wilting and drying of the plants. The leaves showed roll up symptoms and whole plant dried up. Wilted plants came off easily with a gentle pull and vascular discolorations were observed. Such tubers when pressed exhibited oozing of bacterial exudates (Plate 2).

4.3.1.3 Root-knot of coleus

Root-knot caused by *Meloidogyne incognita* showed stunting, yellowing of plants, drying and defoliation of the leaves. Such plants showed irregular galls on the root system, later presence of several such galls produced a knoted appearance of tubers (Plate 3).

4.3.2 Ashwagandha

4.3.2.1 Wilt/root rot of ashwagandha

Infected plants exhibited loss of turgidity withering and drooping of the leaves and wilting of plants. Death and decaying of roots, brown to black discolouration of roots with pulpiness were observed. White cottony growth of the fungus was observed near collar region (Plate 5).

4.3.2.2 Root-knot of ashwagandha

Infected plants in the field showed stunting and chlorotic symptoms, loss of vigour. Foliage showed tip drying. The galls resembled bead like structures. The galls were seen alternatively on the roots (Plate 6).

4.4 ISOLATION OF DIFFERENT PATHOGENS OF COLEUS AND ASHWAGANDHA FROM DIFFERENT PARTS OF KARNATAKA

4.4.1 Coleus

During survey, diseased samples were collected from different places and the pathogen(s) were isolated. *Fusarium chlamydosporum* was isolated from all most all locations collected and proved to be the most predominant pathogen among the fungi causing wilt complex in coleus. It was collected from 15 locations listed in the Table 3. *Rhizoctonia* and *Sclerotium* were also isolated from few places but were not predominant. Next predominant pathogen isolated was *R. solanacearum*. The soil samples collected were used for extraction of nematodes where *Meloidogyne* were found to be predominant.

In coleus *F. chlamydosporum*, *R. solanacearum* and *M. incognita* were found to be predominant pathogens. Hence only these three were used for further studies (Plates 8 and 9).

4.4.2 Ashwagandha

It was seen during the survey that the cropped area of ashwagandha was very less and the disease (wilt complex) incidence was meager when compared to coleus. The pathogens, *viz.* *F. solani* and *M. incognita* were isolated (Table 3, Plates 10 and 11).

Yellowing and marginal necrosis

Drooping and drying

Wilting and death of plants

Plate 1. Symptoms of *Eusatium* Wilt of coleus

Water soaked lesions

Roll up of leaves

Quick drooping and flaccid leaves

Drying, wilting and collapsing of plant

Plate 2. Symptoms of *Ralstonia* wilt coleus

Stunting and yellowing

Galled roots and knots

Plate3. Symptoms of root knot of coleus

Rotting and vascular discoloration

Disintegration of roots

Plate 4. Symptoms of wilt complex of coleus

Withering and drooping Wilting and Drying Death and decaying

Plate 5. Symptoms of *Fusarium* wilt/root of ashwagandha

Stunting and tip drying of foliage Bead like galls on roots

Plate 6. Symptoms of root knot of ashwagangha

Rotting, decaying and Disintegration of roots

Plate 7. Symptoms of wilt/root knot complex of ashwagandha

4.5 ESTIMATION OF NEMATODE POPULATION IN COLEUS RHIZOSPHERE

A random survey was conducted in six districts of Karnataka to find out the pathogenic nematode species associated with coleus rhizosphere. Seventeen soil samples were collected from different locations. The plant pathogenic nematode encountered were : *Aphelenchus* spp., *Criconema* spp., *Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Rotylenchulus* spp. *Tylenchus*-like PPN, *Xiphinema* spp. and other Dorylaimid PPN. The nematode distribution and their respective densities in different locations are presented in Table 5. Muddebihal, Arabhavi and Hesaragatta samples showed a high diversity of plant pathogenic nematodes. *Meloidogyne* spp., *Pratylenchus* spp. and dorylamid PPN occurred more frequently followed by *Helicotylenchus* and *Tylenchus*-like PPN with absolute frequency of 100, 94.11 and 88.23 respectively. *Criconema* spp. *Rotylenchulus* spp., *Aphelenchus* spp. and *Xiphinema* spp. were less frequently encountered in the samples with absolute frequencies of 17.64, 35.29, 35.29 and 58.82 respectively.

From the data represented in Table 5 it is clear that in the area surveyed, *Meloidogyne* spp. was the most predominant nematode associated with coleus rhizosphere inducing numerous galls and knots on coleus, followed by *Pratylenchus* and other dorylaimid PPN.

4.6 IDENTIFICATION OF PREVAILING ROOT-KNOT NEMATODE SPECIES INFECTING COLEUS AND ASHWAGANDHA IN KARNATAKA

The affected root samples and soils of coleus and ashwagandha were used for extraction of *M. incognita* as described in 'Material and Methods'. The morphology of perineal pattern was used for species identification. Important diagnostic characters of the perineal pattern of thus identified species as *M. incognita* are summarized in Table 4. The observed characters were compared with the descriptions given by Eisenback *et al.* (1981) (Plate 10).

4.7 PATHOGENICITY TESTS

Pathogenicity tests for *F. chlamydosporum*, *R. solanacerum* and *M. incognita* on coleus and *F. solani* and *M. incognita* on ashwagandha were carried out as described in 'Material and Methods'. Coleus and ashwagandha plants inoculated with different pathogens were observed for development of symptoms. The earliest, typical wilt symptoms were observed in coleus inoculated with *R. solanacerum* (15 days of incubation) followed by *F. chlamydosporum*, 20 days after inoculation. Whereas *M. incognita* took more days for infection and for gall formation.

In case of ashwagandha, pots treated with *F. solani* took 25 days for expression of typical wilt/root rot symptoms and *M. incognita* took still more days for infection and for gall formation (Plate 12).

The symptoms produced in each treatment in coleus and ashwagandha were similar to the one described in the symptomatology. The pathogens upon reisolation from the infected plants were similar to the cultures initially/originally isolated.

4.8 EFFECT OF DIFFERENT INOCULUM LEVELS OF *M. incognita* ON PLANT GROWTH PARAMETERS AND ROOT-KNOT INCIDENCE IN COLEUS AND ASHWAGANDHA

Different initial inoculum levels of *M. incognita* on coleus and ashwagandha caused reduction in growth parameters, which increased with the corresponding increase in initial inoculum levels (Tables 7 and 8). A significant ($P \leq 0.01$) reduction in plant height, weight and total biomass was observed in inoculated plants compared to uninoculated control. Highest reduction in plant height and plant weight was recorded in both coleus and ashwagandha

Table 3. Isolation of major soil-borne and wilt complex pathogens of coleus and ashwagandha

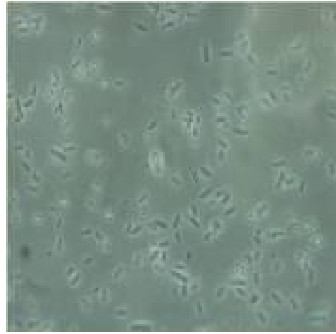
District	Location	Coleus					Ashwagandha	
		Fusarium	*Rhizoctonia*	*Sclerotium*	*Ralstonia*	*Meloidogyne*	*Fusarium*	*Meloidogyne*
Bijapur	Muddebihal	+	-	-	-	+	-	
	Nalvathwada	+	+	+	-	+	+	+
	Areshankara	+	-	-	-	+	+	-
	Nagarabettaa	-	+	-	-	+	+	+
Belgaum	Gokak	+	+	-	-	+	-	
	Arabhavi	+	+	-	-	+	+	+
	Chikkodi	+	-	+	-	+	-	
Dharwad	Dharwad	+	-		-	+	+	+
	Tadasa	+	-		-	+	+	+
Uttara Kannada	Mundagod	+	+		-	+	+	+
	Sirsi	+	-		-	+	-	+
	Hulekal	-	-	+	-	+	-	
Tumkur	Amruthuru	+	-		+	+	-	
	Kaggere	+	-		+	+	+	+
	Kodagihalli	+	+		-	+	-	
Bangalore	Hesaraghatta	+	+		+	+	-	+
	G.K.V.K	+	-		+	+	+	+

+ Present
- Absent

Table 4. Important characters of perineal pattern of prevailing root-knot nematode species of coleus and ashwagandha in Karnataka

Feature	Characters observed	Original description (Eisenback *et al.,* 1981)
Dorsal arch	High squarish	High squarish
Lateral field	Lateral ridges absent, marked by breaks and forks in striae	Lateral ridges absent, marked by breaks and forks in striae
Striae	Coarse, smooth to wavy	Coarse, smooth to wavy & zigzag
Tail terminus	Often with distinct whorl	Often with distinct whorl

Pure culture


Micro and Macroconidia

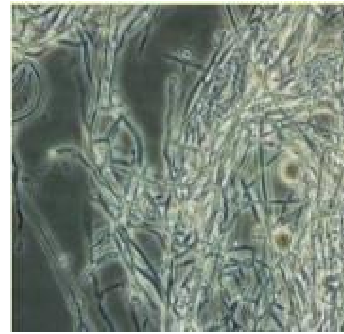
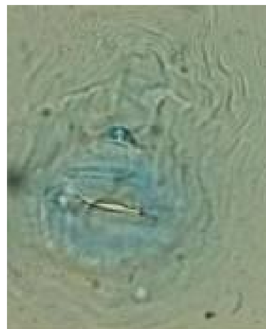

Chlamydospores

Plate 8. Culture of *Fusarium Chlamydosporum*

Colonies of Ralstonia on sucrose peptone medium

Plate 9. Culture of *Ralstonia* solanacearum



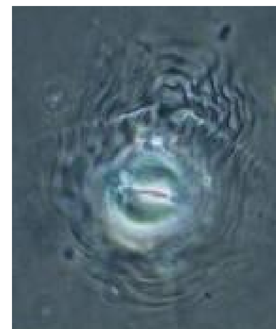

Phase contrast

Plate 10. Perineal pattern of *Meloidoyne incognita*

Pure culture

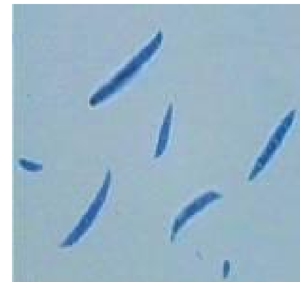

Micro and Macroconidia

Plate 11. Culture of Fusarium solani

Table 5. Plant parasitic nematodes associated with coleus and their absolute densities

District	Location	*Aphelenchus* spp.	*Criconema* spp.	*Helicotylenchus* spp.	*Meloidogyne* spp.	*Pratylenchus* spp.	*Rotylenculus* spp.	*Tylenchus* spp.	*Xiphinema* spp.	Other Dorylaimid PPN
Bijapur	Muddebihal	8.25	8.25	16.5	108.25	16.5	-	16.5	8.25	16.25
	Nalvathwada	-	9.99	30	169.9	30	-	30	19.99	39.99
	Areshankara	-	-	10.66	117.33	32	-	10.66	-	53.33
	Nagarabettaa	9.99	-	19.99	139.99	30	-	-	9.9	30
Belgaum	Gokak	-	-	66.66	150	83.33	25	33.33	8.33	58.33
	Arabhavi	-	9.32	74.66	205.33	102.66	65.33	74.66	28	84
	Chikkodi	-	-	25	108.33	66.66	25	16.66	-	41.66
Dharwad	Dharwad	-	-	49.99	230.00	30.90	30.00	49.99	19.88	39.99
	Tadasa	-	-	25	121.24	65.33	46.66	37.33	9.33	56
Uttara Kannada	Mundagod	-	-	-	210	49.99	-	69.99	-	30
	Sirsi	-	81.66	35	186.66	-	-	-	23.33	93.33
	Hulekal	-	56	-	121.33	37.33	-	28	-	37.33
Tumkur	Amruthur	-	-	30	120	9.9	-	-	-	-
	Kaggere	-	-	-	228	25.33	-	-	12.54	38
	Kodagihalli	-	-	21.33	128	53.33	-	21.33	-	74.66
Bangalore	Hesaragatta	11.55	46.55	93.11	233.1	105	-	35	-	81.66
	G.K.V.K.	-	-	93.33	256.66	70	35	58.33	46.66	-

Table 6. Community analysis of plant parasitic nematodes associated with coleus rhizospheres

Nematode species	Absolute Frequency	Relative frequency
Aphelenchus spp	17.64	0.48
Criconema spp.	35.29	3.44
Helicotylenchus spp.	82.35	9.60
Meloidogyne spp.	100	46.04
Pratylenchus spp.	94.11	13.28
Rotylenculus spp.	35.29	3.68
Tylenchus spp	76.47	7.83
Xiphinema spp.	58.82	3.02
Other Dorylaimid PPN	88.23	12.58

plants receiving an inoculum level of 5000 juveniles/plant (T_6) (Plates 13 and 14). Whereas increased plant height, weight and total biomass was observed in coleus and ashwagandha plants receiving an inoculum level of 10 juveniles/plant (T_3).

No significant difference was observed between T_5 and T_6 for root-knot index in coleus and ashwagandha. Significant differences occurred between $T_{3,}$ T_4 and T_6.

The final nematode population and root-knot index increased with the corresponding increase in initial nematode population. An inoculum level of 100 J2/plant was found to cause more damage in coleus and ashwagandha plants. Maximum nematode population and root-knot index was observed in coleus and ashwagandha in T6 (5000 J2/plant) (Tables 7 and 8, Fig. 1 and 2, Plates 13 and 14).

4.9 *IN VITRO* SCREENING OF PGPR STRAINS AGAINST *Fusarium chlamydosporum*

PGPR strains isolated from different parts of Karnataka were maintained and screened against *Fusarium* for mycelial inhibition by dual culture technique *in vitro* as explained in 'Material and Methods'. Inhibition zone (in mm) was recorded and the per cent inhibition was calculated: the results thus obtained are presented in the Table 9 and Plate 15.

The per cent inhibition varied from 7.77 to 86.11, with a mean of 55.96. The results obtained were highly significant between the different PGPR strains tested and also over control. Maximum per cent inhibition of 86.11 was observed in RB50 followed by RB13 (83.33) and RB 43 (82.22). Least inhibition of 7.77 pre cent was observed in RB49 followed by RB27 (28.55). Among the fifty PGPR strains tested, 19 inhibited the mycelial growth to an extent of 75.00 per cent or more (Table 9).

4.10 *IN VITRO* SCREENING OF RHIZOBACTERIA AGAINST *Ralstonia solanacearum*

The same fifty rhizobacterial strains were tested for inhibition of *Ralstonia solanacearum* on NA medium. In dual culture tests, the isolates showed great variation in the inhibition of the pathogen, with the zone of inhibition (ZOI) varying from 5mm to 22 mm dia. The strain RB31 and RB50 produced the highest ZOI of 22 mm dia. followed by RB43, RB22 and RB01 (Table 10 and Plate 16). Out of 50 PGPR strains tested 17 strains produced a ZOI of $\geq$ 20 mm dia. and remaining 33 strains showed a ZOI of $<$20 mm dia.

4.11 EFFECT OF CELL FREE CULTURE FILTRATES OF PGPR STRAINS ON JUVENILE MORTALITY OF *M. incognita IN VITRO*

Cell free culture filtrates of fifty PGPR strains were tested *in vitro* for their nematicidal action on *M. incognita*. Data indicated that various PGPR strains and their different concentrations were highly deleterious to the nematode (Table 11). In general, juvenile mortality increased with increase in exposure period and increase in concentration of PGPR strains. No nematode mortality was recorded in control (distilled water).

A maximum nematode mortality (95% and above) were observed in cent per cent concentration of strains RB1, RB2, RB6, RB7, RB9, RB10, RB13, RB15, RB18, RB22, RB24, RB29, RB31, RB35, RB39, RB43 and RB50. Similar results were observed with 75, 50 and 25 per cent concentration of the culture filtrates of the same strains. The lowest juvenile mortality was recorded in RB 49 (14.00) at 25 per cent concentration.

Interaction between concentration and time at 100 per cent concentration with 12, 24 and 48 h exposure time recorded the 100 per cent mortality of juveniles in strains RB50 and RB43 followed by 24 and 48 h exposure time in RB31 and RB10. The lowest mortality of

Table 7. Effect of different inoculum levels of *M. incognita* on plant parameters and root knot incidence in coleus

Inoculum level	Shoot length (cm)	Root length (cm)	Shoot weight (g)		Tuber yield (g)		Total biomass (g)		Root knot index	Nematode population/ 200 cc of soil
			Fresh	Dry	Fresh	Dry	Fresh	Dry		
T1 Check	34.66	20.00	88.82	22.50	15.20	3.68	104.02	26.18	0.00	0
T2 Associated Check	33.66	19.06	86.88	21.75	15.70	3.25	102.58	25.40	0.00	0
T3 10	35.33	21.16	108.62	25.72	18.50	4.61	127.45	30.33	2.50	780
T4 100	33.00	15.66	58.64	12.65	9.70	2.50	68.34	15.15	3.25	3600
T5 1000	27.66	12.50	54.62	11.37	6.61	1.75	61.23	13.12	4.00	5030
T6 5000	21.33	9.26	50.17	9.75	5.63	0.93	55.80	10.68	4.75	6440
SEm±	0.71	0.64	0.76	0.47	0.40	0.26	0.87	0.50	0.25	11.90
CD@ 1%	2.88	2.59	3.11	1.89	1.62	1.04	3.53	2.04	1.02	48.45

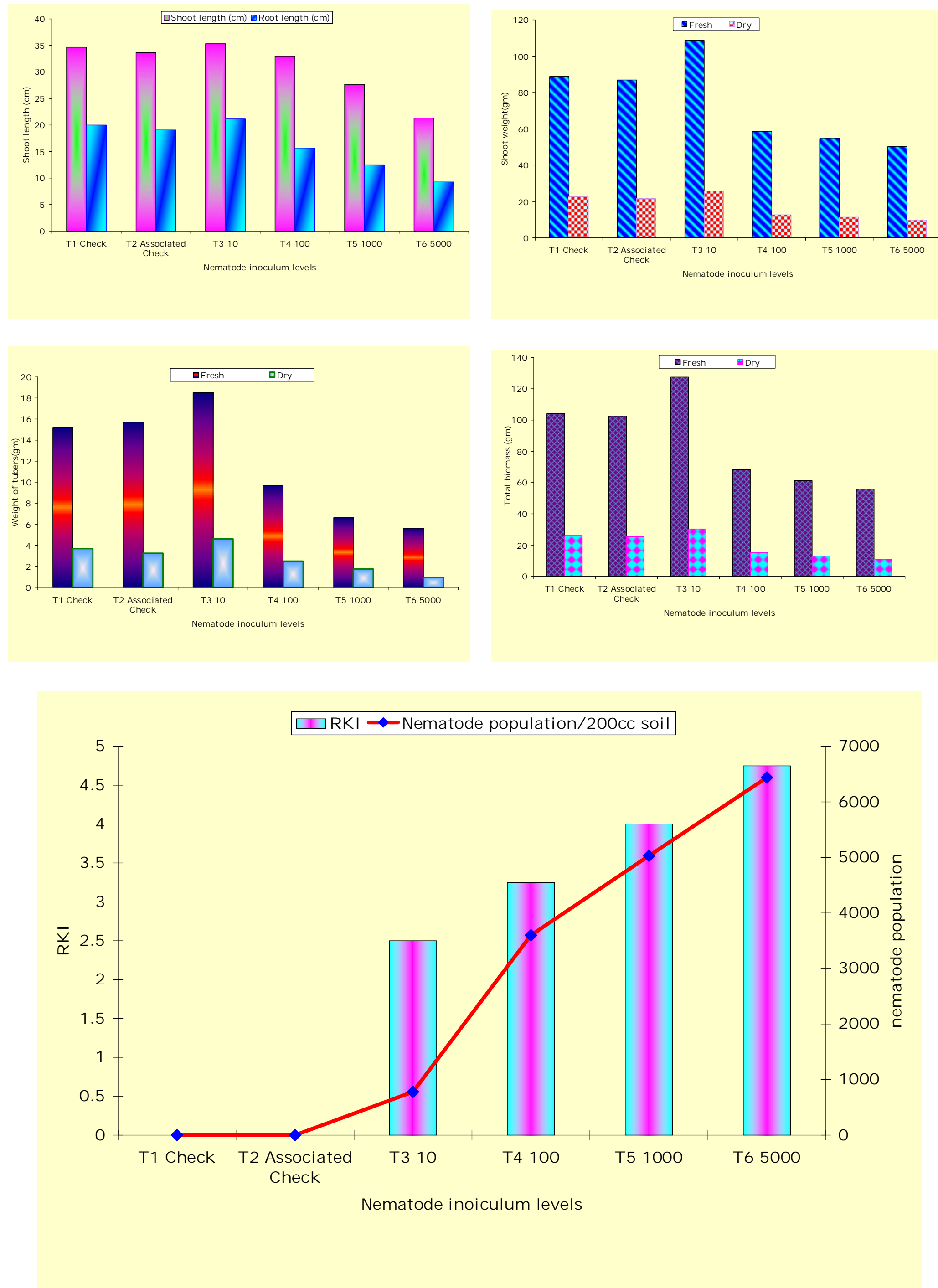


Fig 1e. Effect of different inoculum levels *of M. incognita* on plant parameters root knot incidence in Coleus

Table 8. Effect of different inoculum levels of *M. incognita* on plant parameters and root knot incidence in ashwagandha

Inoculum level	Shoot length (cm)	Root length (cm)	Shoot weight (g)		Root weight (g)		Total biomass (g)		Root knot index	Nematode population/ 200 cc of soil
			Fresh	Dry	Fresh	Dry	Fresh	Dry		
T1 Check	37.13	7.94	22.63	5.31	2.28	0.43	24.91	5.74	0.00	0
T2 Associated Check	36.88	7.61	25.00	5.50	2.59	0.38	27.59	5.95	0.00	0
T3 10	38.33	8.08	25.88	5.60	3.13	0.65	30.78	6.25	2.25	630
T4 100	29.44	6.20	19.63	4.38	2.06	0.35	21.69	4.73	3.00	2400
T5 1000	23.81	4.51	12.25	2.97	1.88	0.23	13.88	3.95	4.25	4050
T6 5000	17.75	3.19	10.25	1.51	1.65	0.19	11.90	1.70	5.00	5100
SEm±	0.99	0.29	1.12	0.29	0.10	0.03	0.93	0.42	0.14	12.28
CD@ 1%	4.02	1.18	4.55	1.18	0.40	0.13	3.80	1.71	0.59	49.97

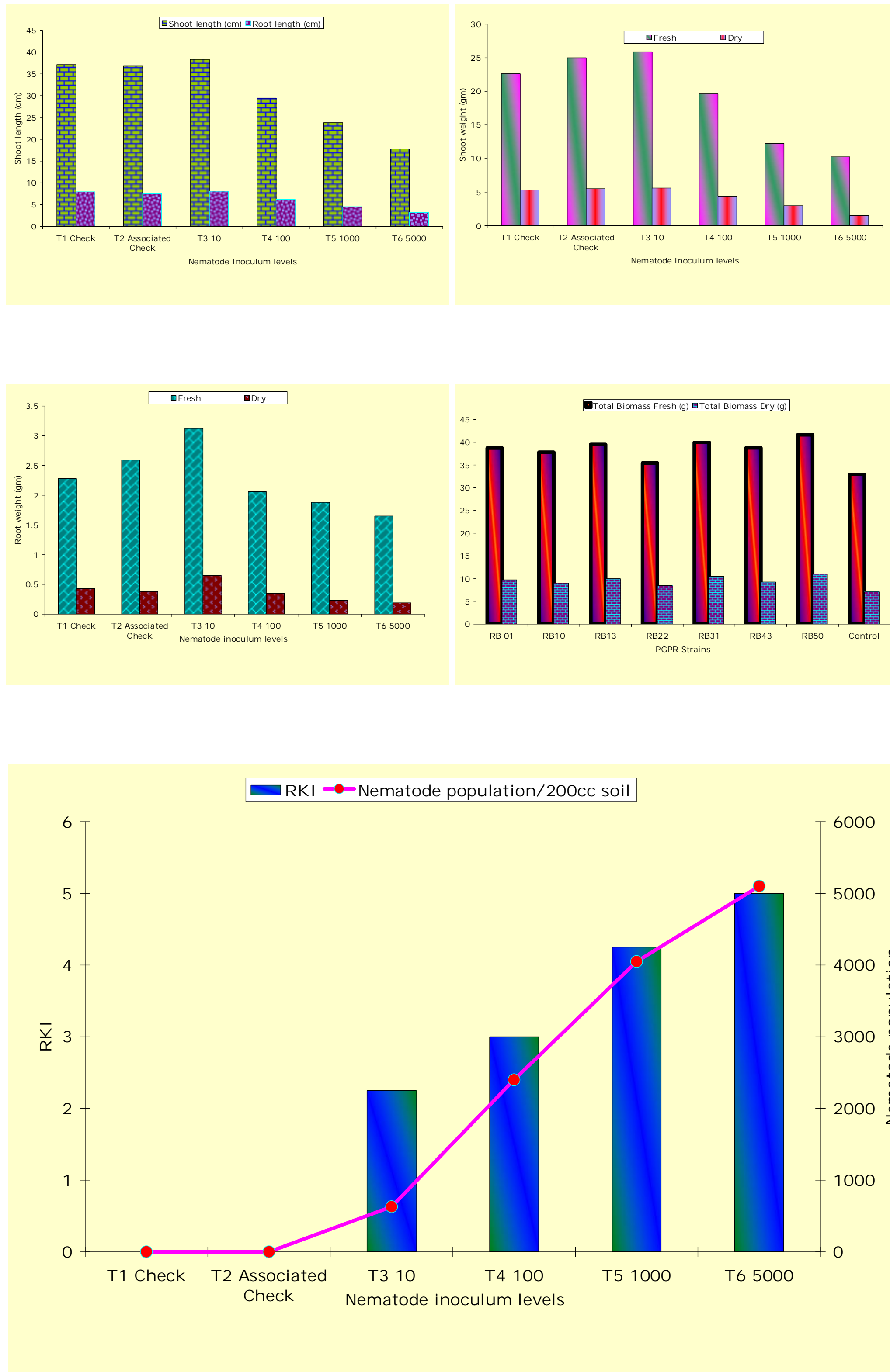


Fig 2a. Effect of different inoculum levels of M. incognita on plant parameters in aswagandha

Control *Fusarium Ralstonia Meloidogyne*

Control *Fusarium Meloidogyne*

Plate 12. Pathogenicity of different pathogens of coleus and ashwangandha

T1 T2 T3 T4 T5 T6

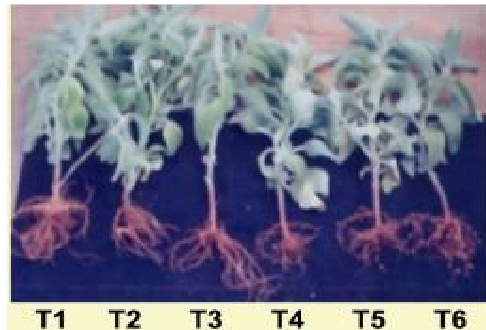


Plate13. Different inoculum leves of *Meloidogyne incognita* on coleus

Plate 14. different inoculum leves of *Meloidogyne incognita* on ashwagandha

10.66 and 12.66 were observed in 25 per cent concentration with 12 and 24 h exposure period in the strain RB49 (Table 11).

The immobile/inactive nematodes were randomly picked and placed in sterile water. None of the juveniles regained their activity, indicates that the nematicidal action of the PGPR strains was long lasting.

4.12 EFFECT OF CULTURE FILTRATES OF PGPR STRAINS ON HATCHING OF *M. incognita*

Hatching of *M. incognita* juveniles increased with decrease in concentration of PGPR strains. However, hatching was significantly reduced at all concentrations of PGPR strains. The interaction between treatment and concentration was significant which indicated that an increase in concentration tended to modify the effect of other in a significant manner.

The culture filtrates of PGPR at 100, 75, 50 and 25 per cent significantly inhibited the hatching of eggs ($P \leq 0.001$; Table 12). The greatest decrease in egg hatching was recorded (1.50) with the PGPR strains RB50, RB31 and RB1 in treatment concentration interaction (97 per cent inhibition over control).

In case of PGPR strains RB1, RB2, RB6, RB7, RB9, RB10, RB13, RB15, RB18, RB22, RB24, RB29, RB31, RB35, RB39, RB43 and RB50 only ≤ 4 eggs hatched into juveniles (Table 12). Egg hatching was completely inhibited in strains RB1, RB31 and RB50 at cent per cent concentration. Maximum number of juveniles were hatched in control (50.00) followed by 35.91 in RB49 at 25 per cent concentration.

4.13 SELECTION OF EFFICIENT PGPR STRAINS EFFECTIVE AGAINST DIFFERENT PATHOGENS OF COLEUS AND ASHWAGANDHA

Fifty rhizobacterial strains isolated from different ecosystem of Karnataka in coleus and ashwagandha were subjected to *in vitro* screening against soil borne and wilt complex pathogens: *Fusarium*, *Ralstonia* and *Meloidogyne* of coleus and ashwagandha under *in vitro*.

Of fifty rhizobacterial strains screened against *Fusarium* in dual cultural techniques, the results revealed that nineteen strains were highly effective in inhibiting the mycelial growth of the test pathogen to an extent of 75.00 per cent and above were selected and listed in Table 13.

In vitro inhibition test conducted on luxuriant lawn of *R. solanacearum* revealed that out of 50 strains, seventeen inhibited the target pathogen by producing an inhibition zone of 20 mm dia. or more. There were selected and listed in Table 13.

Effect of culture filtrates of PGPR strains on juvenile mortality and hatching inhibition in *M. incognita* was tested, seventeen strains were selected which proved to be highly larvicidal and ovicidal. This selected PGPR strain shows 80.00 per cent or more juvenile mortality and less hatching of juveniles (≤4) these are listed in Table 13.

Based on the *in vitro* performance of the strains selected for their efficiency on individual pathogens seven strains showing a high efficacy against species of *Fusarium*, *Ralstonia* and *Meloidogyne* were finally selected. These selected efficient strains were used for further studies.

4.14 IDENTIFICATION OF THE PGPR STRAINS

Seven efficient PGPR strains that were selected based on *in vitro,* efficacy against *Fusarium, Ralstonia* and *Meloidogyne* were subjected to morphological and biochemical tests for identification. Various tests that were conducted and their results are represented in Table 14. Morphological and biochemical characterization of the PGPR strains revealed that, they belonged to the genus *Pseudomonas*. All these were gram negative, rod shaped and had the

Table 9. ***In vitro*** **screening of PGPR strains against** ***Fusarium chlanydosporum***

PGPR strains	Radial mycelial growth (mm)	Per cent mycelial inhibition
RB01	17.00	81.11 (64.52)*
RB02	43.60	51.48 (45.83)
RB03	22.30	75.22 (60.12)
RB04	21.00	76.66 (61.08)
RB05	17.50	80.55 (63.81)
RB06	18.00	80.00 (63.41)
RB07	16.30	81.88 (64.54)
RB08	26.30	70.77 (57.25)
RB09	28.00	68.88 (56.07)
RB10	18.00	80.00 (63.41)
RB11	29.30	67.44 (55.18)
RB12	49.00	45.55 (42.36)
RB13	15.00	83.33 (66.13)
RB14	55.00	38.88 (38.55)
RB15	57.60	36.00 (36.85)
RB16	29.60	67.11 (54.98)
RB17	32.00	64.44 (55.37)
RB18	21.60	76.00 (60.65)
RB19	52.00	42.22 (40.50)
RB20	62.60	30.44 (33.47)
RB21	55.60	38.22 (38.17)
RB22	17.50	80.55 (63.89)
RB23	51.60	42.66 (41.34)
RB24	20.60	77.11 (61.39)
RB25	53.00	41.11 (39.21)
RB26	18.00	80.00 (63.41)
RB27	64.30	28.55 (32.25)
RB28	69.00	23.33 (28.86)
RB29	54.00	40.00 (39.79)
RB30	61.30	31.55 (34.12)
RB31	17.00	81.11 (64.52)
RB32	55.00	38.88 (38.23)
RB33	17.60	80.44 (63.72)
RB34	49.30	45.22 (42.40)
RB35	20.00	77.77 (61.84)
RB36	27.00	70.00 (57.18)
RB37	18.30	79.66 (63.05)
RB38	51.00	43.33 (41.15)
RB39	61.00	32.22 (34.61)
RB40	59.00	34.44 (35.71)
RB41	55.60	38.22 (38.17)
RB42	60.00	33.33 (35.23)
RB43	16.00	82.22 (65.29)
RB44	62.00	31.11 (33.88)
RB45	47.00	47.77 (43.70)
RB46	21.60	76.00 (60.65)
RB47	60.00	33.33 (35.24)
RB48	41.00	54.44 (47.52)
RB49	83.00	7.77 (16.10)
RB50	12.50	86.11 (68.09)
Control	90.00	-
Mean		55.96 (49.45)
SEm±		0.36
CD@ 1%		1.33

*Figures in the parenthesis are arc sine transformed values

Table 10. *In vitro* inhibition of *Ralstonia solanacearum* by hizobacterial strains on nutrient agar

PGPR strains	Zone of inhibition (mm, dia.)
RB01	21.00
RB02	14.00
RB03	21.00
RB04	20.50
RB05	20.00
RB06	16.00
RB07	17.00
RB08	18.50
RB09	20.00
RB10	20.00
RB11	12.00
RB12	15.00
RB13	21.50
RB14	21.00
RB15	20.00
RB16	18.00
RB17	18.50
RB18	17.00
RB19	11.00
RB20	09.00
RB21	20.50
RB22	21.50
RB23	10.50
RB24	11.50
RB25	16.50
RB26	06.50
RB27	05.00
RB28	10.00
RB29	21.00
RB30	17.50
RB31	22.00
RB32	16.30
RB33	20.00
RB34	14.00
RB35	18.00
RB36	15.50
RB37	12.00
RB38	14.00
RB39	10.00
RB40	13.50
RB41	18.00
RB42	10.00
RB43	21.60
RB44	09.50
RB45	17.00
RB46	13.00
RB47	16.50
RB48	20.50
RB49	15.00
RB50	22.00
Mean	16.50

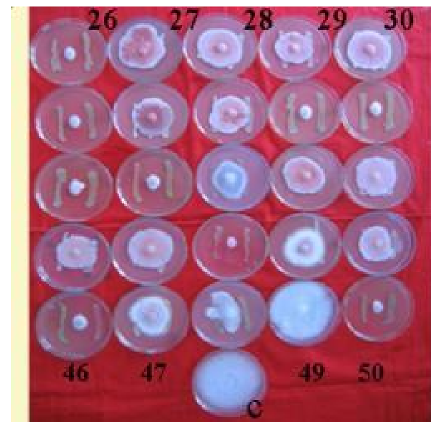


Plate 15. ***In vitro*** **screening of PFPR strains against** ***Fusarium chlamydosporum***

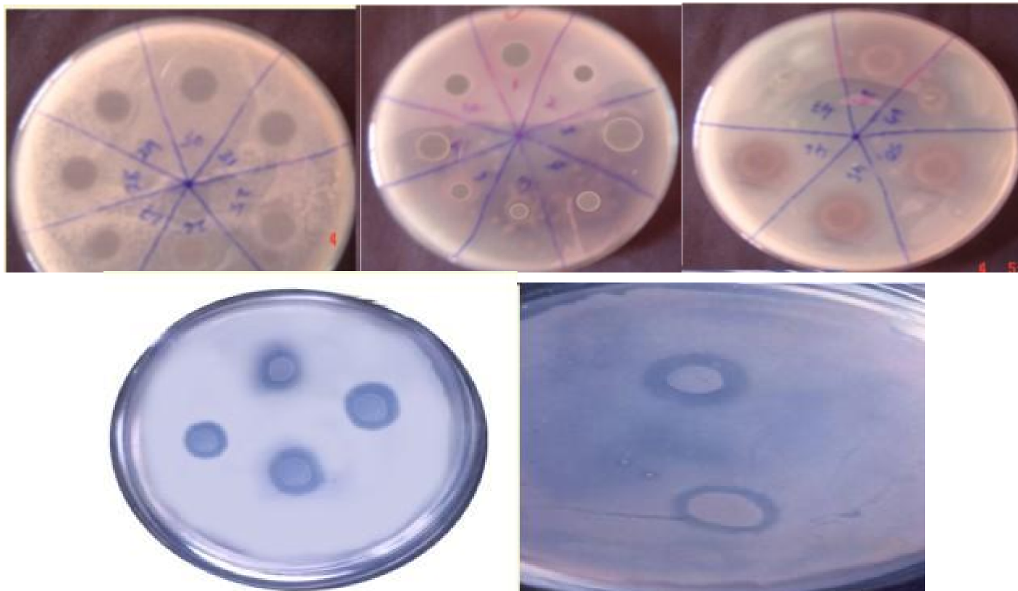


Plate 16. ***In vitro*** **inhibition** ***Ralstonia solanacearum*** **from PGPR strains**

Table 11. Effect of culture filtrates of PGPR strains on juvenile mortality of *Meloidogyne incognita*

PGPR Strains	Mortality of *M. incognita*																
	100 % concentration				75 % concentration				50 % concentration				25 % concentration				Mean
	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	
RB01	99.33 (87.25)*	99.33 (87.25)	99.33 (87.25)	99.33 (87.27)	88.66 (70.31)	92.66 (74.29)	96.66 (79.56)	92.66 (74.72)	77.33 (61.54)	81.33 (64.38)	90.66 (72.20)	83.11 (66.04)	60.66 (51.13)	76.66 (61.09)	84.66 (66.93)	74.00 (59.72)	87.27 (71.93)
RB02	94.66 (76.67)	97.33 (80.70)	100.00 (89.96)	97.33 (82.44)	85.33 (67.46)	89.33 (70.92)	91.33 (72.87)	88.66 (70.42)	70.00 (56.77)	77.33 (61.54)	84.66 (66.93)	77.33 (61.75)	61.33 (51.53)	74.00 (59.31)	78.66 (62.47)	71.33 (57.77)`	83.66 (68.09)
RB03	80.66 (63.89)	88.66 (70.31)	92.66 (74.29)	87.33 (69.50)	67.33 (55.12)	75.33 (60.20)	84.66 (66.93)	75.77 (60.75)	59.33 (50.36)	72.66 (58.45)	80.66 (63.89)	70.88 (57.57)	54.66 (47.65)	67.33 (55.12)	71.33 (57.60)	64.44 (53.46)	74.61 (60.32)
RB04	59.33 (50.36)	77.33 (61.54)	83.33 (65.88)	73.33 (59.26)	54.66 (47.65)	65.33 (53.90)	76.66 (61.09)	65.55 (54.22)	47.33 (43.45)	51.33 (45.74)	59.33 (50.36)	52.66 (46.52)	36.66 (37.25)	46.66 (43.07)	56.66 (48.81)	46.66 (43.04)	59.55 (50.76)
RB05	71.33 (57.60)	83.33 (65.88)	89.33 (70.92)	81.33 (64.80)	63.33 (52.71)	69.33 (56.35)	87.33 (69.13)	73.33 (59.40)	51.33 (45.74)	67.33 (55.12)	81.33 (64.38)	66.66 (55.08)	44.00 (41.53)	58.66 (49.97)	62.66 (52.31)	55.11 (47.94)	69.11 (56.80)
RB06	92.66 (74.29)	94.66 (76.67)	100.00 (89.96)	95.77 (80.30)	82.66 (65.37)	89.33 (70.92)	95.33 (77.55)	89.11 (71.28)	77.33 (61.54)	84.66 (66.93)	92.66 (74.29)	84.88 (67.59)	59.33 (50.36)	73.33 (58.88)	82.66 (65.37)	71.77 (58.20)	85.38 (69.34)
RB07	91.33 (72.87)	95.33 (77.55)	100.00 (89.96)	95.55 (80.12)	76.66 (61.09)	88.66 (70.31)	91.33 (72.87)	85.55 (68.09)	65.33 (53.90)	74.66 (59.76)	85.33 (67.46)	75.11 (60.37)	57.33 (49.19)	70.66 (57.18)	80.66 (63.89)	69.55 (56.76)	81.44 (66.34)
RB08	54.66 (47.65)	67.33 (55.12)	75.33 (60.20)	65.77 (54.32)	50.66 (45.36)	60.66 (51.13)	66.00 (54.31)	59.11 (50.27)	37.33 (37.64)	54.66 (47.65)	62.00 (51.92)	51.33 (45.74)	25.33 (30.20)	44.66 (41.92)	52.00 (46.12)	40.66 (39.41)	54.22 (47.44)
RB09	90.00 (71.59)	93.33 (75.04)	100.00 (89.96)	94.44 (78.86)	87.33 (69.13)	90.00 (71.59)	96.00 (78.68)	91.11 (73.13)	82.66 (65.37)	88.66 (70.31)	91.33 (72.87)	87.55 (69.52)	76.66 (61.09)	81.33 (64.38)	80.00 (63.42)	79.33 (62.96)	88.11 (71.12)
RB10	96.66 (79.56)	100.00 (89.96)	100.00 (89.96)	98.66 (85.59)	90.66 (72.20)	91.33 (72.87)	94.66 (76.67)	92.22 (73.91)	85.33 (67.46)	87.33 (69.13)	90.66 (72.20)	87.77 (69.60)	80.66 (63.89)	82.66 (65.37)	84.66 (66.93)	82.66 (65.41)	90.33 (73.62)
RB11	51.33 (45.76)	63.33 (52.71)	71.33 (57.60)	62.00 (52.02)	47.33 (43.45)	55.33 (48.04)	60.00 (50.75)	54.22 (47.41)	36.66 (37.25)	52.66 (46.51)	58.66 (49.97)	49.33 (44.57)	30.66 (33.61)	48.66 (44.21)	51.33 (45.74)	43.55 (41.19)	52.27 (46.30)
RB12	46.66 (43.07)	50.66 (45.36)	62.66 (52.31)	53.33 (46.91)	39.33 (38.82)	46.66 (43.07)	57.33 (49.19)	47.77 (43.69)	34.66 (36.05)	42.66 (40.76)	50.66 (45.36)	42.66 (40.72)	24.66 (29.76)	32.66 (34.84)	40.66 (39.60)	32.66 (34.73)	44.11 (41.52)
RB13	94.66 (76.67)	97.33 (80.72)	100.00 (89.96)	97.33 (82.44)	90.66 (72.20)	94.66 (76.67)	96.66 (79.56)	94.00 (76.14)	86.66 (68.56)	90.66 (72.20)	92.66 (74.29)	90.00 (71.68)	78.66 (62.47)	82.66 (65.37)	80.66 (63.89)	80.66 (63.91)	90.50 (73.54)
RB14	62.66 (52.31)	76.66 (61.09)	80.66 (63.89)	73.33 (59.10)	56.66 (48.81)	70.66 (57.18)	78.66 (62.47)	68.66 (56.15)	48.66 (44.21)	50.66 (45.36)	62.66 (52.31)	54.00 (47.30)	36.66 (37.25)	40.66 (39.60)	48.66 (44.21)	42.00 (40.35)	59.50 (50.72)

Table 11. Contd…..

PGPR Strains	Mortality of *M. incognita*																
	100 % concentration				75 % concentration				50 % concentration				25 % concentration				Mean
	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	
RB15	92.66 (74.29)	96.66 (79.56)	100.00 (89.96)	96.44 (81.27)	86.66 (68.56)	92.66 (74.29)	94.66 (76.67)	91.33 (73.17)	84.66 (66.93)	88.66 (70.31)	90.66 (72.20)	88.00 (69.81)	72.66 (58.45)	82.66 (65.37)	86.66 (68.56)	80.66 (64.13)	89.11 (72.10)
RB16	48.66 (44.21)	54.66 (47.65)	60.66 (51.13)	54.66 (47.67)	36.66 (37.25)	48.66 (44.21)	52.66 (46.51)	46.00 (42.66)	32.66 (34.84)	44.66 (41.92)	48.66 (44.21)	42.00 (40.32)	28.66 (32.35)	36.66 (37.25)	40.66 (39.60)	35.33 (36.40)	44.50 (41.76)
RB17	60.66 (51.13)	68.66 (55.94)	82.66 (65.37)	70.66 (57.48)	54.66 (47.65)	62.66 (52.31)	76.66 (61.09)	64.66 (53.69)	48.66 (44.21)	58.66 (49.97)	70.66 (57.18)	59.33 (50.45)	36.33 (37.25)	50.66 (47.36)	62.66 (52.31)	50.00 (44.97)	61.16 (51.65)
RB18	94.66 (76.67)	96.66 (79.56)	100.00 (89.96)	97.11 (82.06)	90.66 (72.20)	92.66 (74.29)	94.66 (76.67)	92.66 (74.38)	86.66 (68.56)	88.66 (70.31)	90.66 (72.20)	88.66 (70.36)	74.66 (59.76)	78.66 (62.47)	80.66 (63.89)	78.00 (62.04)	89.11 (72.21)
RB19	74.66 (59.76)	78.66 (62.47)	84.66 (65.37)	78.66 (62.53)	66.66 (54.71)	70.66 (57.18)	78.66 (62.47)	72.00 (58.12)	52.66 (46.51)	64.66 (53.50)	68.66 (55.94)	62.00 (51.98)	44.66 (41.92)	50.66 (45.36)	54.66 (47.65)	54.66 (47.67)	65.66 (54.40)
RB20	66.66 (54.71)	72.66 (58.45)	78.66 (62.47)	72.66 (58.54)	54.66 (47.65)	68.66 (55.94)	74.66 (59.76)	66.00 (54.45)	46.66 (43.07)	50.66 (45.36)	62.66 (52.31)	53.33 (46.91)	34.66 (36.05)	46.66 (43.07)	48.66 (44.21)	50.00 (44.98)	58.83 (50.25)
RB21	86.66 (68.56)	92.66 (74.29)	94.66 (76.67)	91.33 (73.17)	80.66 (63.89)	88.66 (70.31)	92.66 (74.29)	87.33 (69.50)	68.66 (55.94)	74.66 (59.76)	84.66 (68.56)	76.66 (61.42)	54.66 (47.65)	68.66 (55.94)	72.66 (58.45)	43.33 (41.11)	80.16 (64.53)
RB22	94.66 (76.67)	100.00 (89.96)	100.00 (89.96)	98.22 (85.53)	90.66 (72.20)	96.66 (79.56)	96.66 (79.56)	94.66 (77.11)	86.66 (68.56)	90.66 (72.20)	90.66 (72.20)	89.33 (70.99)	78.66 (62.47)	84.66 (66.93)	86.66 (68.56)	65.33 (54.01)	91.38 (74.90)
RB23	70.66 (57.18)	78.66 (62.47)	84.66 (66.93)	78.00 (62.19)	64.66 (53.50)	70.66 (57.18)	78.66 (62.47)	71.33 (57.72)	58.66 (49.97)	66.66 (54.71)	70.66 (57.18)	65.33 (53.95)	48.66 (44.21)	52.66 (46.51)	62.66 (54.71)	83.33 (65.99)	67.66 (55.59)
RB24	92.66 (74.29)	94.66 (76.67)	100.00 (89.96)	95.77 (80.30)	90.66 (72.20)	92.66 (74.29)	96.66 (79.56)	93.33 (75.35)	88.66 (70.31)	90.66 (72.20)	92.66 (74.29)	90.66 (72.27)	80.66 (63.89)	84.66 (66.93)	86.66 (68.56)	56.00 (48.48)	90.94 (73.59)
RB25	64.66 (53.50)	72.66 (58.45)	80.66 (63.89)	72.66 (58.62)	56.66 (48.81)	68.66 (55.94)	76.66 (61.09)	67.33 (55.28)	48.66 (44.21)	54.66 (47.65)	60.66 (51.13)	54.66 (47.67)	36.66 (37.25)	48.66 (44.21)	56.66 (48.81)	84.00 (66.46)	60.50 (51.25)
RB26	50.66 (45.36)	62.66 (52.31)	74.66 (59.76)	62.66 (52.48)	46.66 (43.07)	58.66 (49.97)	70.66 (57.18)	58.66 (50.07)	36.66 (37.25)	44.66 (41.92)	58.66 (49.97)	46.66 (43.04)	30.66 (33.61)	38.66 (38.43)	44.66 (41.92)	47.33 (43.42)	51.50 (45.89)
RB27	46.66 (43.07)	54.66 (47.65)	62.66 (52.31)	54.66 (47.68)	40.66 (39.60)	50.66 (45.36)	58.66 (49.97)	50.00 (44.98)	34.66 (36.05)	42.66 (40.76)	50.66 (45.36)	42.66 (40.72)	28.66 (32.35)	36.66 (37.25)	38.66 (38.43)	38.00 (37.98)	45.50 (42.35)

Table 11. Contd…..

PGPR Strains	Mortality of *M. incognita*																
	100 % concentration				75 % concentration				50 % concentration				25 % concentration				Mean
	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	
RB28	48.66 (44.21)	54.66 (47.65)	60.66 (51.13)	54.66 (47.67)	42.66 (40.76)	48.66 (44.21)	56.66 (48.81)	49.33 (44.59)	38.66 (38.43)	40.66 (39.60)	48.66 (44.21)	42.66 (40.75)	30.66 (33.61)	34.66 (36.05)	36.66 (37.25)	34.00 (35.63)	45.16 (42.16)
RB29	90.66 (72.20)	96.66 (79.56)	100.00 (89.96)	95.77 (80.57)	86.66 (68.56)	90.66 (72.20)	92.66 (74.29)	90.00 (71.68)	80.66 (63.89)	86.66 (68.56)	88.66 (70.31)	85.33 (67.59)	72.66 (58.45)	78.66 (62.47)	80.66 (63.89)	77.33 (61.60)	87.11 (70.36)
RB30	28.66 (32.35)	36.66 (37.25)	42.66 (40.76)	36.00 (36.79)	24.66 (29.76)	30.66 (33.61)	40.66 (39.60)	32.00 (34.32)	20.66 (27.02)	24.66 (29.76)	36.66 (37.25)	27.33 (31.34)	16.66 (24.07)	20.66 (27.02)	30.66 (33.61)	22.66 (28.23)	29.50 (32.67)
RB31	99.33 (87.25)	100.00 (89.96)	100.00 (89.96)	99.33 (87.25)	93.33 (75.04)	95.33 (77.55)	96.66 (79.56)	95.11 (77.38)	86.66 (68.56)	90.66 (72.20)	92.66 (74.29)	90.00 (71.68)	78.66 (62.47)	84.66 (66.93)	85.33 (67.46)	82.88 (65.62)	91.83 (75.48)
RB32	82.66 (65.37)	90.66 (72.20)	94.66 (76.67)	89.33 (71.41)	74.66 (59.76)	80.66 (63.89)	88.66 (70.31)	81.33 (64.65)	68.66 (55.94)	74.66 (59.76)	80.66 (63.89)	74.66 (59.86)	56.66 (48.81)	62.66 (52.31)	68.66 (55.94)	62.66 (52.35)	77.00 (62.07)
RB33	58.66 (49.97)	70.66 (57.18)	82.66 (65.37)	70.66 (57.51)	54.66 (47.65)	62.66 (52.31)	76.66 (61.09)	64.66 (53.69)	50.66 (45.36)	58.66 (49.97)	64.66 (53.50)	58.00 (49.61)	42.66 (40.76)	46.66 (43.07)	50.66 (45.36)	46.66 (43.06)	60.00 (50.97)
RB34	76.66 (61.05)	84.66 (66.93)	88.66 (70.31)	83.33 (66.11)	62.66 (52.31)	68.66 (55.94)	80.66 (63.89)	70.66 (57.38)	48.66 (44.21)	56.66 (48.81)	62.66 (52.31)	56.00 (48.44)	36.66 (37.25)	40.66 (39.60)	42.66 (40.76)	40.00 (39.20)	62.50 (52.78)
RB35	90.66 (72.20)	96.66 (79.56)	100.00 (89.96)	95.77 (80.57)	86.66 (68.56)	92.66 (74.29)	94.66 (76.67)	91.33 (73.17)	80.66 (63.89)	88.66 (70.31)	90.66 (72.20)	86.66 (68.80)	72.66 (58.45)	82.66 (63.89)	80.66 (65.37)	78.66 (62.57)	88.11 (71.28)
RB36	48.66 (44.21)	54.66 (47.65)	60.66 (51.13)	54.66 (47.67)	42.66 (40.76)	46.66 (43.07)	50.66 (45.36)	46.66 (43.06)	36.66 (37.25)	40.00 (39.60)	46.66 (43.07)	41.33 (39.97)	28.66 (32.35)	32.66 (34.84)	36.66 (37.25)	32.66 (34.81)	43.83 (41.38)
RB37	70.66 (57.18)	86.66 (68.56)	88.66 (70.31)	82.00 (65.35)	62.66 (52.31)	70.66 (57.18)	74.66 (59.76)	69.33 (56.42)	50.66 (45.36)	56.66 (48.81)	60.66 (51.13)	56.00 (48.43)	36.66 (37.25)	42.66 (40.76)	46.66 (43.07)	42.00 (40.36)	62.33 (52.64)
RB38	76.66 (61.09)	82.66 (65.37)	90.66 (72.20)	83.33 (66.22)	68.66 (55.94)	74.66 (59.76)	76.66 (61.09)	73.33 (58.93)	62.66 (52.31)	70.66 (57.18)	72.66 (58.45)	68.66 (55.98)	50.66 (45.36)	54.66 (47.65)	60.66 (51.13)	55.33 (48.05)	70.16 (57.30)
RB39	92.66 (74.29)	94.66 (76.67)	100.00 (89.96)	95.77 (80.30)	86.66 (68.56)	88.66 (70.31)	92.66 (74.29)	89.33 (71.05)	80.66 (63.89)	84.66 (66.93)	88.66 (70.31)	84.66 (67.04)	74.66 (59.76)	78.66 (62.47)	80.66 (63.89)	78.00 (62.04)	86.94 (70.11)
RB40	38.66 (38.43)	42.66 (40.76)	50.66 (45.36)	44.00 (41.52)	34.66 (36.05)	40.66 (39.60)	48.66 (44.21)	41.33 (39.95)	30.66 (33.61)	36.66 (37.25)	42.66 (40.76)	36.66 (37.20)	26.66 (31.07)	30.66 (33.61)	36.66 (37.25)	31.33 (33.97)	38.33 (38.16)
RB41	54.66 (47.65)	68.66 (55.94)	74.66 (59.76)	66.00 (54.45)	48.66 (44.21)	52.66 (46.51)	60.66 (51.13)	54.00 (47.28)	40.66 (39.60)	46.66 (43.07)	50.66 (45.36)	46.00 (42.68)	28.66 (32.35)	36.66 (37.25)	40.66 (39.60)	35.33 (36.40)	50.33 (45.20)
RB42	46.66 (43.07)	54.66 (47.65)	60.66 (51.13)	54.00 (47.29)	40.66 (39.60)	48.66 (44.21)	52.66 (46.51)	47.33 (43.44)	34.66 (36.05)	40.66 (39.60)	46.66 (43.07)	40.66 (39.57)	24.66 (29.76)	30.66 (33.61)	32.00 (34.85)	29.33 (32.73)	42.83 (40.76)

Table 11. Contd…..

PGPR Strains	Mortality of *M. incognita*																
	100 % concentration				75 % concentration				50 % concentration				25 % concentration				Mean
	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	12 h	24 h	48 h	Mean	
RB43	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	92.66 (74.29)	94.66 (76.67)	100.00 (89.96)	95.77 (80.30)	88.66 (70.31)	90.66 (72.20)	96.66 (79.56)	92.00 (74.02)	80.66 (63.89)	84.66 (66.93)	86.66 (68.56)	84.00 (66.46)	92.94 (77.69)
RB44	30.66 (33.61)	34.66 (36.05)	36.66 (37.25)	34.00 (35.63)	38.66 (32.35)	30.66 (33.61)	34.66 (36.05)	31.33 (34.00)	22.66 (28.41)	26.66 (31.07)	30.66 (33.61)	26.66 (31.03)	18.66 (25.58)	20.66 (27.02)	22.66 (28.41)	20.66 (27.00)	28.16 (31.92)
RB45	36.66 (37.25)	40.66 (39.60)	42.66 (40.76)	40.00 (39.20)	32.66 (34.84)	38.66 (38.43)	40.66 (39.60)	37.33 (37.62)	26.66 (31.07)	34.66 (36.05)	38.66 (38.43)	33.33 (35.18)	20.66 (27.02)	28.66 (32.35)	30.66 (33.61)	26.66 (30.99)	34.33 (35.75)
RB46	26.66 (31.07)	30.66 (33.61)	34.66 (36.05)	30.66 (33.58)	22.66 (28.41)	26.66 (31.07)	30.66 (33.61)	26.66 (31.03)	20.66 (27.02)	24.66 (29.76)	28.66 (32.35)	24.66 (29.71)	16.66 (24.07)	18.66 (25.58)	20.66 (27.02)	18.66 (25.56)	25.16 (29.97)
RB47	60.66 (51.13)	72.66 (58.45)	78.66 (62.47)	70.66 (57.35)	56.66 (48.81)	64.66 (53.50)	70.66 (57.18)	64.00 (53.16)	44.66 (41.92)	52.66 (46.51)	58.66 (49.97)	52.00 (46.13)	40.66 (39.60)	48.66 (44.21)	52.66 (46.51)	47.33 (43.44)	58.50 (50.02)
RB48	82.66 (65.37)	86.66 (68.56)	88.66 (70.31)	86.00 (68.08)	74.66 (59.76)	80.66 (63.89)	84.66 (66.93)	80.00 (63.52)	60.66 (51.13)	70.66 (57.18)	76.66 (61.09)	69.33 (56.47)	48.66 (44.21)	54.66 (47.65)	60.66 (51.13)	54.66 (47.67)	72.50 (58.94)
RB49	30.66 (33.61)	34.66 (36.05)	36.66 (37.25)	34.00 (35.63)	24.66 (29.76)	28.66 (32.35)	30.66 (33.61)	28.00 (31.91)	12.66 (20.82)	16.66 (24.07)	22.66 (28.41)	17.33 (24.44)	10.66 (19.03)	12.66 (20.82)	18.66 (25.58)	14.00 (21.81)	23.33 (28.45)
RB50	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	94.66 (76.67)	96.66 (79.56)	96.66 (79.56)	96.00 (78.60)	90.66 (72.20)	92.66 (74.29)	94.66 (76.67)	92.66 (74.38)	80.66 (63.89)	84.66 (66.93)	86.66 (68.56)	84.00 (66.46)	93.16 (77.35)
NB	4.66 (22.49)	7.66 (24.07)	9.66 (26.06)	7.11 (15.28)	5.66 (14.92)	9.66 (17.75)	10.66 (19.03)	8.44 (16.69)	6.66 (13.29)	11.66 (17.75)	13.66 (19.03)	10.44 (24.21)	14.66 (12.41)	16.66 (15.67)	19.66 (17.75)	16.88 (15.69)	10.72 (18.71)
DW	00.00 (0)	00.00 (0)	00.00 (0)	0.00 (0.00)	00.00 (0)	00.00 (0)	00.00 (0)	0.00 (0.00)	00.00 (0)	00.00 (0)	00.00 (0)	0.00 (0.00)	00.00 (0)	00.00 (0)	00.00 (0)	0.00 (0.00)	0.00 (0.00)

*Figures in the parenthesis are arc sine transformed values

	SEm ±	CD (p=0.01)
Treatment (T)	0.168	0.615
Concentration (C)	0.044	0.170
TXC	0.337	1.229
Hour (H)	0.040	0.148
TXH	0.292	1.065
CXH	0.081	0.295
TXCXH	0.584	2.129

Table 12. Effect of culture filtrates of PGPR strains on egg hatching of *Meloidogyne incognita*

PGPR Strains	100 % concentration		75 % concentration		50 % concentration		25 % concentration		Mean (No. of J2 hatched)
	No. of J2 hatched	% Inhibition over control	No. of J2 hatched	% Inhibition over control	No. of J2 hatched	% Inhibition over control	No. of J2 hatched	% Inhibition over control	
RB01	00.00(0.7)*	100.00	00.00(0.70)	100.00	01.33(1.34)	97.34	02.66(1.77)	94.68	1.50(1.30)
RB02	00.00(0.7)	100.00	01.33(1.34)	97.34	02.66(1.77)	94.68	05.33(2.41)	89.34	2.33(1.56)
RB03	04.33(2.19)	91.34	06.33(2.61)	87.34	07.66(2.85)	84.68	08.33(2.97)	83.34	6.66(2.65)
RB04	07.33(2.79)	85.34	11.33(3.43)	77.34	14.33(3.85)	71.34	18.33(4.33)	63.34	12.83(3.60)
RB05	05.33(2.41)	89.34	08.33(2.97)	83.34	10.33(3.29)	79.34	13.33(3.71)	73.34	9.33(3.09)
RB06	00.00(0.7)	100.00	01.33(1.34)	97.34	02.33(1.67)	95.34	04.33(2.19)	91.34	2.00(1.48)
RB07	00.00(0.7)	100.00	02.33(1.67)	95.34	03.66(2.03)	92.68	04.66(2.27)	90.68	2.66(1.67)
RB08	06.33(2.16)	87.34	09.33(3.13)	81.34	11.33(3.43)	77.34	15.33(3.97)	69.34	10.58(3.29)
RB09	00.00(0.7)	100.00	01.33(1.34)	97.34	03.33(1.95)	93.34	05.33(2.41)	89.34	2.50(1.60)
RB10	00.00(0.7)	100.00	00.66(1.05)	98.68	02.33(1.68)	95.34	03.66(2.03)	92.68	1.66(1.36)
RB11	07.33(2.79)	85.34	10.00(3.23)	80.00	13.66(3.76)	72.68	17.66(4.26)	64.68	12.16(3.51)
RB12	04.33(2.19)	91.34	11.33(3.13)	81.34	15.33(3.97)	69.34	17.33(4.22)	65.34	11.58(3.38)
RB13	00.33(0.88)	99.34	00.66(1.05)	98.68	02.33(1.67)	95.34	04.33(2.19)	91.34	1.91(1.45)
RB14	10.33(3.29)	79.34	13.33(3.71)	73.34	19.00(4.41)	62.00	20.33(4.56)	59.34	15.75(3.99)
RB15	00.33(0.88)	99.34	02.33(1.67)	95.34	03.33(1.95)	93.34	05.33(2.41)	89.34	2.83(1.73)
RB16	08.33(2.97)	83.34	13.33(3.71)	73.34	23.00(4.84)	54.00	24.00(4.94)	52.00	17.16(4.12)
RB17	11.33(3.43)	77.34	17.33(4.22)	65.34	27.00(5.24)	46.00	32.66(5.75)	34.68	22.08(4.66)
RB18	00.66(1.05)	98.68	02.33(1.67)	95.34	04.33(2.19)	91.34	04.66(2.27)	90.68	3.00(1.79)
RB19	15.00(3.93)	70.00	18.33(4.33)	63.34	22.66(4.81)	54.68	25.66(5.11)	48.68	20.41(4.55)
RB20	12.33(3.58)	75.34	16.66(4.14)	66.68	19.66(4.49)	60.68	22.66(4.81)	54.68	17.83(4.25)
RB21	07.33(2.79)	85.34	12.66(3.62)	75.68	18.66(4.37)	62.68	27.33(5.27)	45.34	16.50(4.02)
RB22	00.33(0.88)	99.34	00.66(1.05)	98.68	01.66(1.46)	96.68	03.66(2.03)	92.68	1.58(1.35)
RB23	14.00(3.8)	72.00	19.33(4.45)	61.34	21.66(4.70)	56.68	29.33(5.46)	41.34	21.08(4.60)
RB24	00.66(1.05)	98.68	01.33(1.34)	97.34	03.33(1.95)	93.34	04.66(2.27)	90.68	2.50(1.65)
RB25	06.33(2.61)	87.34	10.33(3.29)	79.34	11.33(3.43)	77.34	15.33(3.97)	69.34	10.83(3.33)
RB26	09.33(3.13)	81.34	13.33(3.71)	73.34	20.33(4.56)	59.34	26.66(5.21)	46.68	17.41(4.15)
RB27	12.66(3.62)	74.68	15.66(4.02)	68.68	21.33(4.67)	57.34	29.33(5.46)	49.34	19.75(4.44)
RB28	05.33(2.41)	89.34	10.33(3.29)	79.34	13.33(3.71)	73.34	20.33(4.56)	59.34	12.33(3.49)
RB29	00.66(1.05)	98.68	02.33(1.67)	95.34	03.33(1.95)	93.34	04.66(2.27)	90.68	2.75(1.73)
RB30	10.33(3.29)	79.64	15.00(3.93)	70.00	18.66(4.37)	62.68	26.66(5.21)	46.68	17.66(4.20)

Table 12. Contd……

PGPR Strains	100 % concentration		75 % concentration		50 % concentration		25 % concentration		Mean (No. of J2 hatched)
	No. of J2 hatched	% Inhibition over control	No. of J2 hatched	% Inhibition over control	No. of J2 hatched	% Inhibition over control	No. of J2 hatched	% Inhibition over control	
RB31	00.00(0.70)	100.00	00.66(1.05)	98.68	01.66(1.46)	96.68	03.66(2.03)	92.68	1.50(1.31)
RB32	20.33(4.56)	59.34	22.66(4.81)	54.68	27.66(5.30)	44.68	31.66(5.67)	36.68	25.58(5.08)
RB33	16.33(4.10)	67.34	20.33(4.56)	59.34	27.33(5.27)	45.34	32.33(5.73)	34.34	24.08(4.91)
RB34	16.33(4.10)	67.34	22.00(4.74)	56.00	30.00(5.52)	40.00	34.66(5.93)	30.68	25.75(5.07)
RB35	00.33(0.88)	99.34	02.33(1.67)	95.34	04.33(2.19)	91.34	05.33(2.41)	89.34	3.08(1.79)
RB36	09.66(3.18)	80.68	12.66(3.62)	74.68	17.66(4.26)	64.68	22.33(4.77)	55.34	15.58(3.96)
RB37	16.66(4.14)	66.68	18.66(4.37)	62.68	23.66(4.91)	52.68	29.33(5.46)	49.34	22.08(4.72)
RB38	11.33(3.43)	79.34	14.66(3.89)	70.68	20.33(4.56)	59.34	24.33(4.98)	51.34	17.66(4.22)
RB39	00.33(0.88)	99.34	02.33(1.67)	95.34	04.33(2.19)	91.34	05.33(2.41)	89.34	3.08(1.79)
RB40	05.00(2.33)	90.00	09.66(3.18)	80.68	17.33(4.22)	65.34	21.66(4.70)	56.68	13.41(3.61)
RB41	14.66(3.89)	70.68	16.33(4.10)	67.34	20.33(4.56)	59.34	25.33(5.08)	49.34	19.16(4.41)
RB42	07.66(2.85)	88.68	12.33(3.58)	75.34	15.33(3.97)	69.34	18.33(4.33)	63.34	13.41(3.68)
RB43	00.33(0.88)	99.34	00.66(1.05)	98.68	02.33(1.67)	95.34	04.33(2.19)	91.34	1.91(1.45)
RB44	08.33(2.97)	83.34	11.33(3.43)	77.34	15.33(3.97)	69.34	19.66(4.49)	60.68	13.66(3.72)
RB45	12.33(3.58)	75.34	15.66(4.02)	68.68	24.66(5.01)	50.68	35.33(5.98)	29.34	22.00(4.65)
RB46	19.33 (4.45)	61.34	25.66(5.11)	48.68	37.33(6.15)	25.34	40.33 (6.39)	19.34	30.66(5.52)
RB47	17.66(4.26)	64.68	22.33(4.77)	55.34	13.33(5.81)	33.34	37.33(6.15)	25.34	27.66(5.25)
RB48	12.66(3.62)	74.68	17.66(4.26)	64.68	20.66(4.60)	58.64	25.66(5.11)	48.68	19.16(4.40)
RB49	25.33(5.08)	49.34	34.33(5.90)	31.34	39.66(6.33)	20.68	44.33(6.69)	11.34	35.91(6.00)
RB50	00.00(0.70)	100.00	00.33(0.88)	99.34	02.33(1.67)	95.34	03.33(1.95)	93.34	1.50(1.30)
NB	44.00(6.67)	12.00	46.33(6.84)	7.34	47.66(6.94)	4.68	49.66(7.08)	0.68	46.91(6.88)
DW	50.00(7.10)	0.00	50.00(7.10)	0.00	50.00(7.10)	0.00	50.00 (7.10)	0.00	50.00(7.10)
Mean		9.02(2.66)		12.03(3.18)		15.89(3.73)		19.39(4.17)	

*Figures in the parenthesis are √X+0.5 transformations

	SEm ±	CD (p= 0.01)
Treatment (T)	0.036	0.133
Concentration (C)	0.010	0.036
Tx C	0.073	0.269

Table 13. Efficient PGPR strains effective against different pathogens of coleus and ashwagandha

Fusarium (F)	*Ralstonia* (R)	*Meloidogyne* (M)	F&R	F&M	R& M	F,R&M
RB1	RB1	RB1	RB1	RB1	RB1	RB1
RB3	RB3	RB2	RB3	RB7	RB9	RB10
RB4	RB4	RB6	RB4	RB10	RB10	RB13
RB5	RB5	RB7	RB5	RB13	RB13	RB22
RB6	RB9	RB9	RB10	RB18	RB15	RB31
RB7	RB10	RB10	RB13	RB22	RB22	RB43
RB10	RB13	RB13	RB22	RB24	RB29	RB50
RB13	RB14	RB15	RB31	RB31	RB31	
RB18	RB15	RB18	RB33	RB35	RB43	
RB22	RB21	RB22	RB43	RB43	RB50	
RB24	RB22	RB24	RB50	RB50		
RB26	RB29	RB29				
RB31	RB31	RB31				
RB33	RB33	RB35				
RB35	RB43	RB39				
RB37	RB48	RB43				
RB43	RB50	RB50				
RB46						
RB50						

Table 14. Morphological, biochemical characterization and identification of the selected PGPR strains

PGPR strains	Morphological					Biochemical										Assigned genus
	YPKB	FLUV	GR	CS	SF	GL	SH	CA	CH	LH	AGP	HSP	UP	G4^{0}C	G41^{0}C	
RB1	+	+	-	zLong rod	-	+	-	+	+	+	+	+	+	-	+	*Pseudomonas*
RB10	-	+	-	Rod	-	+	-	+	+	+	+	+	+	-	+	*Pseudomonas*
RB13	+	+	-	Rod	-	+	-	+	+	+	+	+	+	-	+	*Pseudomonas*
RB22	-	+	-	Rod	-	+	-	+	+	+	+	+	+	-	+	*Pseudomonas*
RB31	+	+	-	Short Rod	-	+	-	+	+	+	+	+	+	-	+	*Pseudomonas*
RB43	+	+	-	Long Rod	-	+	-	+	+	+	+	+	+	-	+	*Pseudomonas*
RB50	+	+	-	Short Rod	-	+	-	+	+	+	+	+	+	-	+	*Pseudomonas*

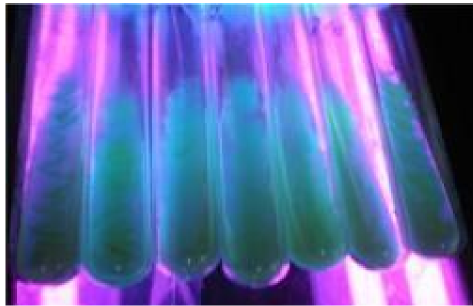
+ Positive
- Negative

YPKB- Yellow pigmentation on KB
FLUV- Fluorescence under UV
GR- Gram Reaction
CS- Cell Shape
SF – Spore formation
GL- Gelatin Liquefaction
SH- Starch Hydrolysis

CA- Catalase Activity
CH- Casein Hydrolysis
LH- Lipid Hydrolysis
AGP- Acid and Gas Production
HSP- Hydrogen Sulphide Production
UP- Urease Production
G 4°&41°C- Growth at 4 and 41°C

PGPR strains on KB medium

PGPR on NA slants



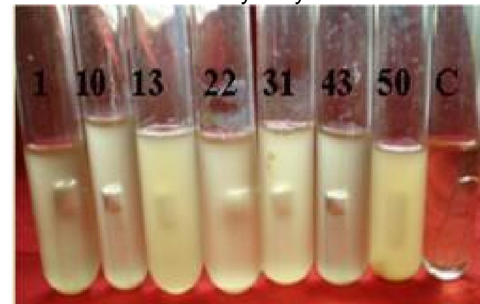
Fluorescence under UV (+)

Gelatin liquefaction

Starch hydrolysis (-)

Casein hydrolysis

Lipid hydrolysis (+)



Acid and gas production (+)

Hydrogen sulphide production (+)

Urease production (+)

Plate 17. Morphological and Biochemical Characterization of PGPR strains

ability to produce water soluble yellow green pigment. Further, these showed production of fluorescence under UV tested, positive for gelatin liquefaction, casein hydrolysis, lipid hydrolysis, catalase activity, acid and gas production, hydrogen sulphide production, urease activity and grew at 41°C. They treated negative for spore formation, starch hydrolysis and growth at 4°C (Table 14 and Plate 17).

4.15 PLANT GROWTH PROMOTING ACTIVITY OF PGPR STRAINS *IN VITRO*

The growth promoting activity by seven selected PGPR strains were tested for seed germination and seedling vigour by treating ashwagandha seeds both in roll towel and pot culture method. Ashwagandha seeds treated with the different PGPR strains showed improved plant growth parameters than untreated seeds.

4.15.1 Roll towel method

In roll towel method, the PGPR strains RB50, RB31 and RB1 showed more than 85 per cent seed germination and produced higher shoot and root length as well as fresh and dry weight of ashwagandha seedlings with enhanced vigour index after 15 days. The maximum vigour index of 976.70 was recorded in RB50 treated seedlings followed by 911.66 and 872.54 vigour index in RB31 and RB1 treated seedlings respectively. Least germination (68.33%), less shoot and root length as well as less fresh and dry weight of seedlings with less vigour index of 489.92 was observed in untreated control (Table 15 and Fig. 3).

4.15.2 Pot culture method

In pot culture studies, the PGPR strains RB50, RB31 and RB1 showed maximum germination (>85%) and produced more shoot and root length with enhanced fresh and dry weight of seedlings compared to other strains and control.

The highest vigour index 753.87, maximum shoot and root length (4.67 and 3.93 cm) was recorded in RB50 treated seedlings. Least vigour index (315.16), shoot and root length (2.60 and 2.2 cm) was registered in untreated control (Table 16 and Fig. 4).

Plant growth promoting activity in ashwagandha through seed bacterization in roll towel and pot culture method revealed that the vigour index and plant growth parameters (shoot and root length as well fresh and dry weight) of the seedlings tested with different PGPR strains were more in roll towel compared to pot culture.

4.16 INFLUENCE OF ANTAGONISTIC PGPR STRAINS ON PLANT GROWTH PROMOTION IN COLEUS UNDER GLASSHOUSE CONDITIONS

The data on shoot length, number of branches, number of tubers, tuber length, fresh and dry weight of shoot and root as well as total biomass of coleus as influenced by bacterization with different PGPR strains are given in Table 17 and depicted in Fig. 5 and Plate 18.

4.16.1 Shoot length

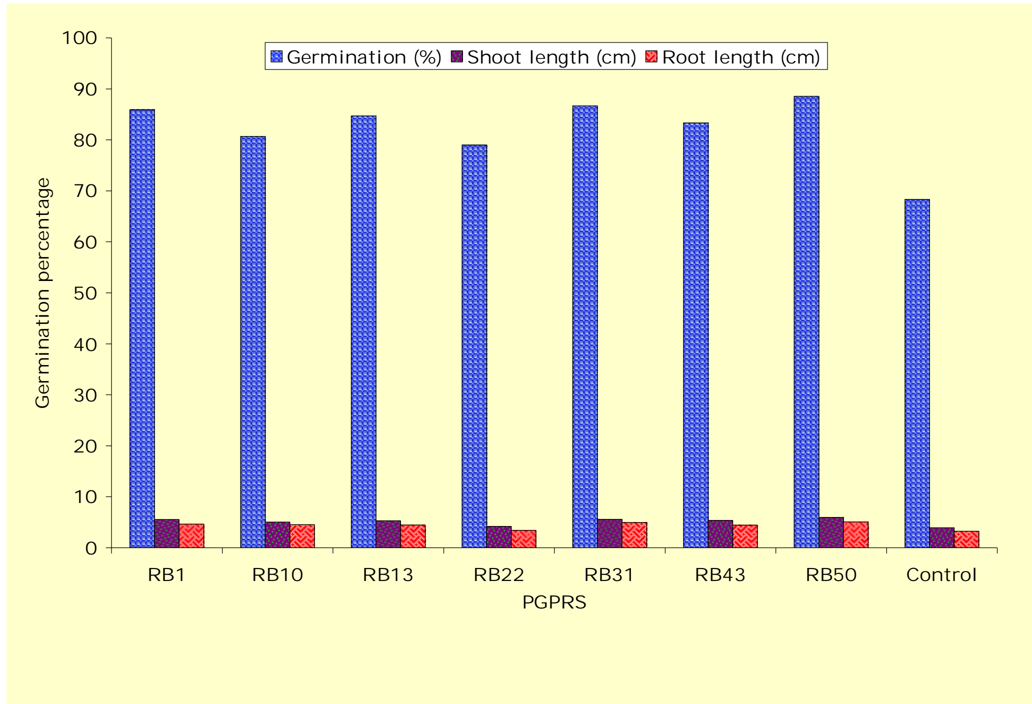
The study revealed highest shoot length of 32.37, 41.74, 59.30 and 65.33 cm at 60, 90, 150 and 180 days after planting, respectively in the treatment RB50. The efficacy of the treatment in enhancing the shoot length was on par with the treatment RB31, RB13 and RB1 at 60, 90, 150 and 180 days after planting. However at 180 days after planting significant difference in shoot length was noticed in RB50 (65.33 cm). The strains RB10, RB22 and untreated control plants respectively recorded the least shoot length of 57.84, 56.71 and 34.95 cm at 180 days after planting (Table 17).

Table 15. Plant growth promoting activity of selected PGPR strains in ashwagandha seedlings under *in vitro* conditions (Roll towel method)

PGPR strains	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	SVI
RB1	85.88 (67.90)*	5.53	4.63	0.14	0.02	872.54
RB10	80.66 (63.88)	5.03	4.53	0.13	0.02	771.10
RB13	84.70 (66.94)	5.31	4.46	0.14	0.04	827.51
RB22	79.00 (62.33)	4.17	3.43	0.11	0.02	600.40
RB31	86.66 (68.55)	5.57	4.95	0.16	0.05	911.66
RB43	83.33 (65.88)	5.35	4.43	0.13	0.03	814.97
RB50	88.55 (70.19)	5.95	5.08	0.18	0.08	976.70
Control	68.33 (55.73)	3.92	3.25	0.10	0.01	489.92
SEm±	0.33	0.13	0.18	0.01	0.01	
CD@1%	1.39	0.55	0.75	0.03	0.02	

SVI- Seedling Vigour Index

*Figures in the parenthesis are arc sine transformed values



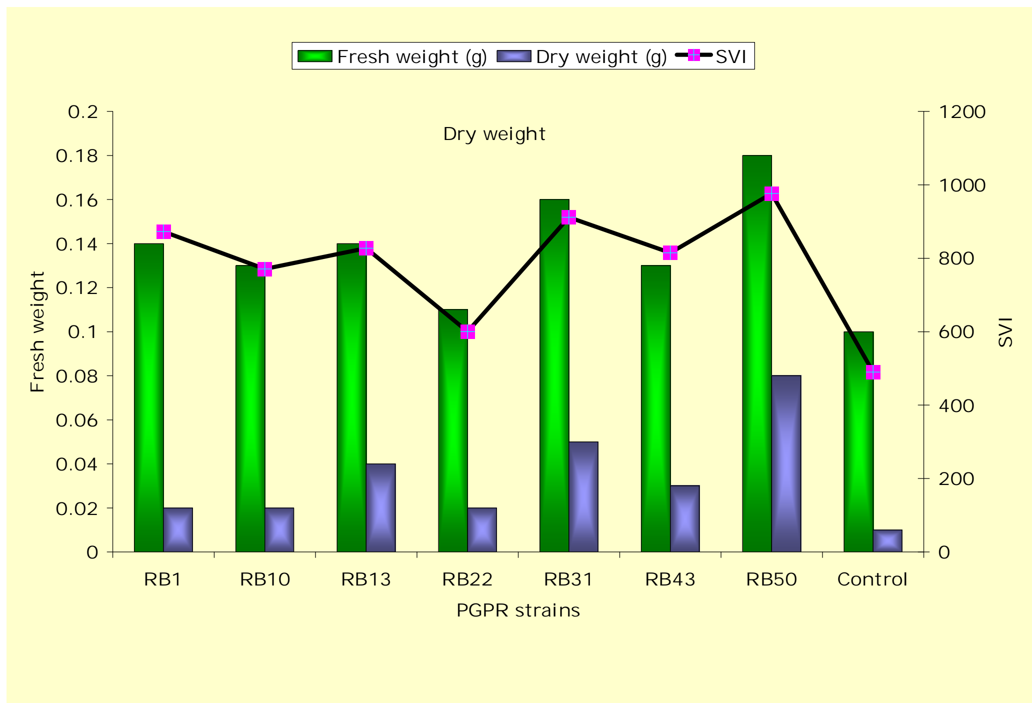


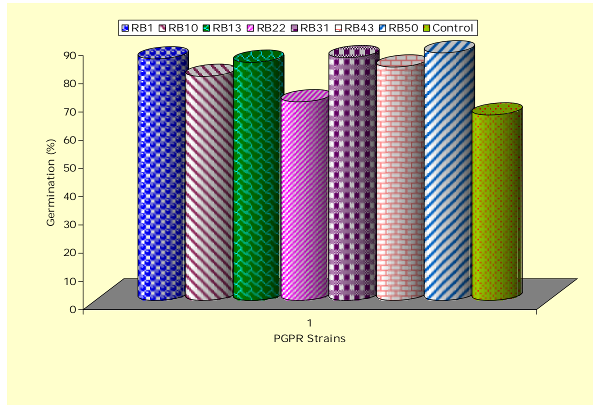
Fig. 3: Plant growth promoting activity of selected PGPR strains in ashwagandha seedlings under in vitro conditions

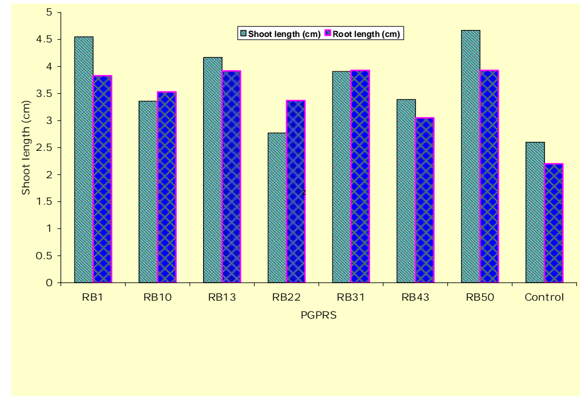
Table 16. Plant growth promoting activity of selected PGPR strains in ashwagandha seedlings in pot culture

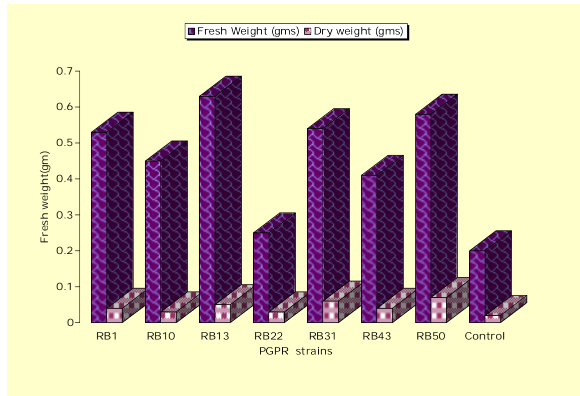
PGPR strains	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	SVI
RB1	85.55 (67.63)*	4.55	3.83	0.53	0.04	691.24
RB10	79.33 (62.93)	3.36	3.53	0.45	0.03	525.95
RB13	84.33 (66.65)	4.17	3.92	0.63	0.05	682.22
RB22	70.33 (58.24)	2.77	3.37	0.25	0.03	4.31.82
RB31	86.00 (68.00)	3.91	3.93	0.54	0.06	679.41
RB43	82.66 (65.37)	3.39	3.05	0.41	0.04	532.33
RB50	87.66 (69.41)	4.67	3.93	0.58	0.07	753.87
Control	65.66 (54.10)	2.60	2.20	0.20	0.02	315.16
SEm±	0.40	0.16	0.14	0.02	0.00	
CD1%	1.67	0.64	0.60	0.10	0.01	

SVI- Seedling Vigour Index

*Figures in the parenthesis are arc sine transformed values







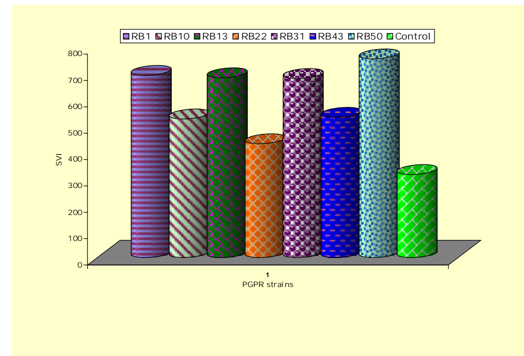


Fig 4: Plant growth promoting activity of selected PGPR strains in ashwagandha seedling in pot culture

4.16.2 Number of branches and tubers

The highest number of branches and tubers per plant was observed in RB50 (13.33 and 10.33) followed by RB31 (11.00 and 8.00) respectively. Number of branches and tubers per plant recorded were on par with each other in RB50, RB31 and RB1. In untreated control plants, the number of branches and number of tubers/plant recorded was only 6.67 and 5.33 respectively (Table 17).

4.16.3 Tuber length

Significant increase in tuber length in case of strains RB50, RB31, RB1 and RB13 was recorded when compared to other strains and untreated control. Strain RB50, RB31, RB1, RB13, RB43 and RB10 were on par with each other. The lowest tuber length was recorded in case of RB22 and untreated control which were on par with each other (Table 17).

4.16.4 Plant weight

A significant increase in shoot and root weight as well as total biomass was observed in the PGPR treated plants.

The highest shoot and root fresh weight (115.00 & 26.83 g) as well as accumulation of dry matter (16.50 &10.83 g) respectively in coleus plants was observed in RB50. Lowest shoot and root fresh weight (60.33 & 10.83 g) as well as dry matter production (7.33 & 2.08 g) was recorded in untreated (Table 17).

4.16.5 Total biomass

The data on total biomass of coleus plants revealed that all the plants treated with PGPR strains yielded high biomass on fresh and dry weight basis compared to control. Maximum of 141.83 (fresh) and 27.33 g (dry) biomass was recorded from RB50. Lowest biomass (103.66 and 16.1 g) was observed in RB22 on fresh and dry weight basis followed by untreated (Table 17).

4.17 INFLUENCE OF ANTAGONISTIC PGPR STRAINS ON PLANT GROWTH PROMOTION IN ASHWAGANDHA UNDER GLASSHOUSE CONDITION

4.17.1 Shoot length

The study revealed highest shoot length of 23.75, 27.83, 33.75 and 36.33 cm at 60, 90, 150 and 180 days after planting respectively in RB50. The efficacy of the strains RB1, RB13, RB43 in enhancing the plant height was on par with each other at 90, 150 and 180 days after planting. The untreated plants recorded the least plant height of 17.0, 19.5, 23.58 and 26.33 cm at 60, 90, 150 and 180 days after planting respectively (Table 18).

4.17.2 Number of branches

The highest number of branches was observed in ashwagandha plants treated with RB50 and RB31 (6.00 and 4.67) at 180 DAP. Significant difference in increase in number of branches per plant was observed between untreated control, RB22 and RB50, remaining all treatments are on par with each other. Lowest number of branches (3.33) was observed in untreated (Table 18).

4.17.3 Root length

The highest root length of 17.50 cm was recorded in RB50. However, other treatments like RB1, RB13 and RB31 were also equally effective in enhancing the root length which recorded 14.00, 14.42 and 15.31 cm respectively (Table 18). Lowest root length of 10.92 and 8.58 were observed in RB22 and untreated respectively.

4.17.4 Plant weight

Significant increase in shoot and root fresh weight was observed in all the plants treated with PGPR strains. However, highest shoot and root fresh weight 37.16 and 4.50 g were recorded in RB50 followed by RB31 (35.50 and 4.50 g). Lowest fresh weight of 30.00 and 3.00 g of shoot and root were observed in untreated (Table 18 and Plate 19).

Higher dry matter accumulation of shoot and root was noticed in PGPR treated plants. Highest dry matter of shoot and root (9.50 and 1.50 g) was recorded in RB50. Least dry matter production was observed in untreated control.

4.17.5 Total biomass

Significantly higher total biomass was produced due to inoculation of PGPR strains. The data revealed that greater biomass accumulated in RB50 (41.66 and 11.00 g) on fresh and dry weight basis followed by RB31 (40 and 10.50 g) respectively (Table 18, Fig. 6 and Plate 19). Lowest biomass of 33.00 and 7.09 g was recorded on fresh and dry weight basis in untreated control.

4.18 BIOCONTROL POTENTIALITY OF EFFICIENT PGPR STRAINS IN COLEUS AND ASHWAGANDHA AGAINST DIFFERENT PATHOGENS (ALONE AND IN COMBINATIONS)

The pathogenic potential of *F. chlamydosporum, R. solanacearum* and *M. incognita*, on coleus and also *F. solani* and *M. incognita* on ashwagandha was determined. In glasshouse, pot culture experiments were conducted to investigate the efficacy of talc based PGPR strains in suppression of different pathogens and their interactions on coleus and ashwagandha pathogens as described in 'Material and Methods'. The results are presented in Tables 19, 20 and Plate 21.

Significant reduction in plant growth parameters such as plant height, number of branches, fresh and dry weight whereas more disease severity, maximum root rot and root knot index was observed in control treatments where only individual pathogens and their combinations without PGPR strains were inoculated in both the crops.

Application of PGPR strains (RB50 and RB31) recorded maximum plant height, number of branches, fresh and dry weight of the plants with less disease severity, root rot and root knot index in individual as well as interactions of different pathogens in coleus and ashwagandha.

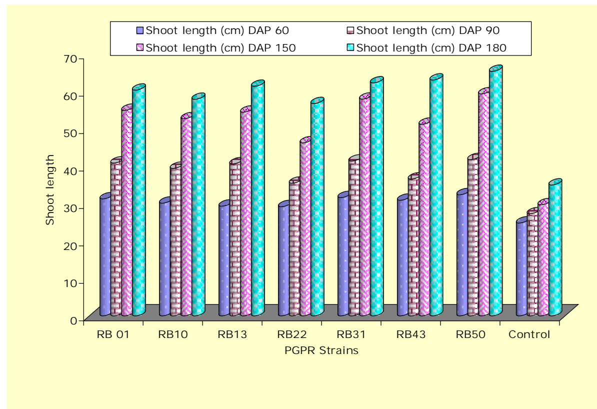
The perusal of data (Table 19 and 20) indicates that root rot appeared only in those treatments where *F. chlamydosporum*, *R.solanacearum* were inoculated either singly or in combinations with root- knot nematodes. Inoculation of root-knot nematode alone did not produce root-rot and less disease severity in coleus and ashwagandha. Maximum root-rot index (5.0) and more disease severity (++++) was observed in T56 (*Meloidogyne* + *Fusarium* + *Ralstonia*) and T40 (*Meloidogyne* + *Fusarium*) followed by T48 (*Meloidogyne* + *Ralstonia*) and T24 (*Fusarium* + *Ralstonia*), in coleus whereas in case of ashwagandha T24 (*Meloidogyne* + *Fusarium*) followed by T8 (*Fusarium* alone) *i.e.*, in all control treatments without PGPR applications, the expression of symptoms was early and disease severity, root-rot index was more.

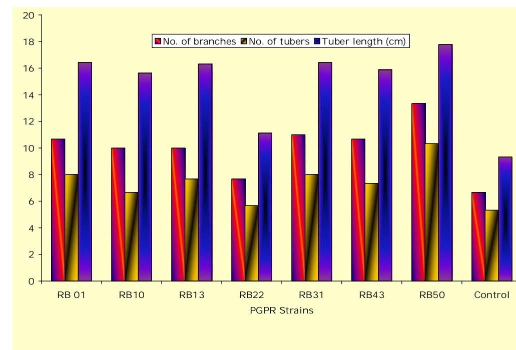
There was significant increase in root knot index in plants inoculated with nematode alone (Table 19 and 20). Less number of galls, with no root rot index was observed in plants treated with nematode and different PGPR strains.

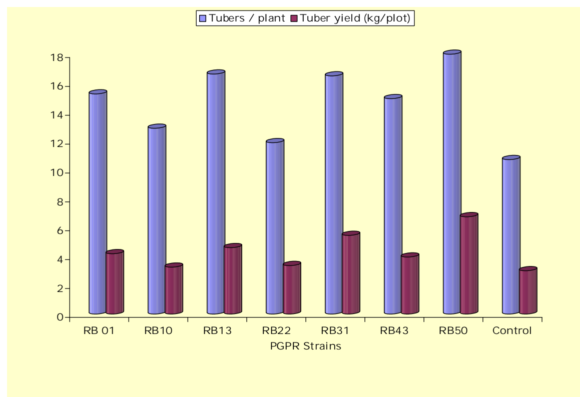
In the present study, increase in plant height, number of branches, fresh and dry weight of the plant as well as less disease severity, less root-rot and root-knot index were observed due to PGPR treatments compare to control (only pathogen). The expression of symptoms and protection of the plants from infection of coleus and ashwagandha depended

Table 17. Effect of PGPR strains on plant growth promoting activity in coleus under glasshouse conditions

PGPR strains	Shoot length (cm) DAP				No. of branches	No. of tubers	Tuber length (cm)	Shoot weight (g)		Root weight (g)		Total biomass (g)	
	60	90	150	180				Fresh	Dry.	Fresh	Dry	Fresh	Dry
RB 01	31.25	40.83	54.90	60.31	10.67	8.00	16.43	104.67	14.83	25.00	10.50	129.34	25.33
RB10	30.10	39.40	52.65	57.84	10.00	6.67	15.63	97.00	14.25	24.50	9.17	121.50	23.42
RB13	29.30	40.60	54.43	61.32	10.00	7.67	16.31	112.67	14.50	23.00	8.10	135.67	22.60
RB22	29.17	35.45	46.23	56.71	7.67	5.67	11.12	88.33	11.00	15.33	5.10	103.66	16.10
RB31	31.60	41.34	57.92	62.25	11.00	8.00	16.42	107.17	14.42	25.17	8.52	132.34	22.94
RB43	30.87	36.55	51.17	63.00	10.67	7.33	15.87	97.33	13.00	16.67	7.25	114.00	20.25
RB50	32.37	41.74	59.30	65.33	13.33	10.33	17.77	115.00	16.50	26.83	10.83	141.83	27.33
Control	24.80	27.30	29.67	34.95	6.67	5.33	9.33	60.33	7.33	10.83	2.08	71.16	9.41
SEm±	0.81	0.99	1.66	1.87	0.75	0.53	0.53	0.45	0.66	1.32	0.59	1.26	0.54
CD@ 1%	3.33	4.11	5.04	5.89	3.12	2.18	2.20	1.86	2.72	5.46	2.45	5.22	2.23







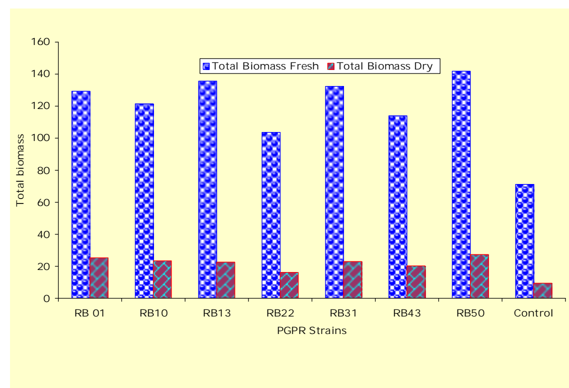
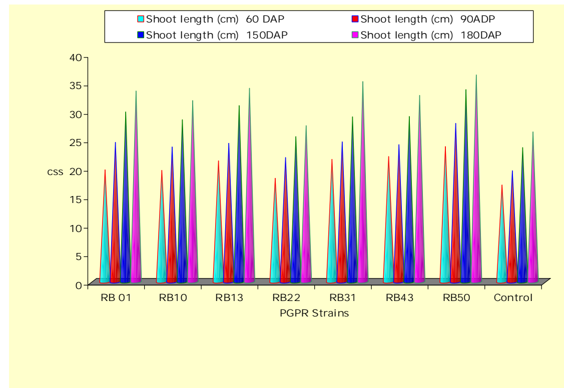
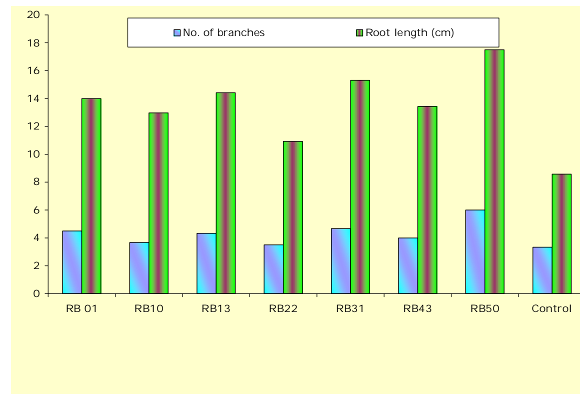


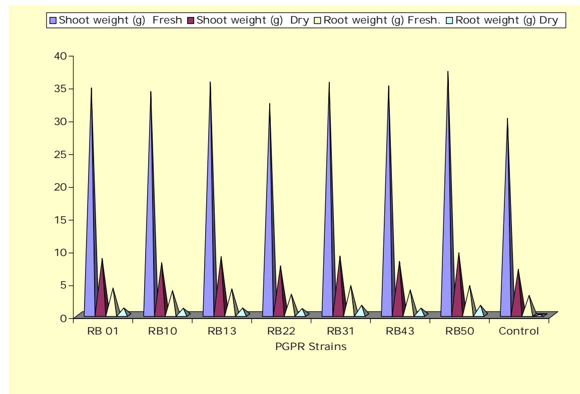
Fig. 5: Effect of PGPR strains on plant growth promoting activity in coleus under glasshouse conditions

Table 18. Effect of PGPR strains on plant growth promoting activity in ashwagandha under glasshouse conditions

PGPR strains	Shoot length (cm) DAP				No. of branches	Root length (cm)	Shoot weight (g)		Root weight (g)		Total Biomass (g)	
	60	90	150	180			Fresh	Dry	Fresh	Dry	Fresh	Dry
RB 01	19.67	24.50	29.83	33.50	4.50	14.00	34.63	8.65	4.12	1.09	38.75	9.74
RB10	19.58	23.67	28.47	31.83	3.67	12.97	34.08	8.00	3.73	1.05	37.81	9.05
RB13	21.25	24.33	30.95	34.00	4.33	14.42	35.53	8.92	4.00	1.08	39.53	10.00
RB22	18.17	21.83	25.50	27.42	3.50	10.92	32.25	7.50	3.20	1.00	35.45	8.50
RB31	21.50	24.58	28.98	35.17	4.67	15.31	35.50	9.00	4.50	1.50	40.00	10.50
RB43	22.00	24.08	29.03	32.75	4.00	13.42	34.95	8.20	3.84	1.06	38.79	9.26
RB50	23.75	27.83	33.75	36.33	6.00	17.50	37.16	9.50	4.50	1.50	41.66	11.0
Control	17.00	19.50	23.58	26.33	3.33	8.58	30.00	7.00	3.00	0.09	33.00	7.09
SEm±	0.58	0.58	0.58	0.82	0.40	0.40	0.54	0.52	0.12	0.04	0.58	0.54
CD@ 1%	2.38	2.38	2.38	3.41	1.65	1.66	2.23	2.17	0.50	0.17	2.38	2.23







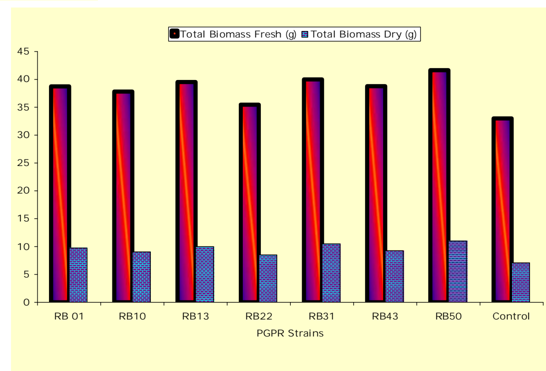


Fig 6: Effect of PGPR strains on plant growth promoting activity in ashwagandha under glasshouse conditions

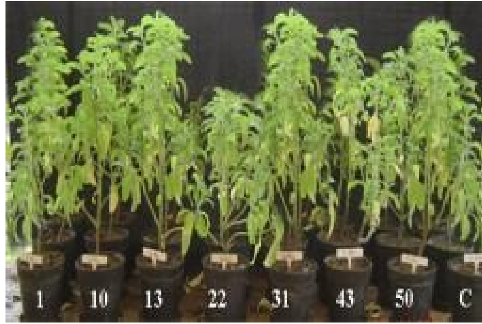


Plate 18. Efficacy of PGPR strains on plant growth promotion in coleus under glasshouse condition

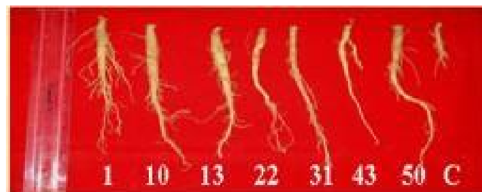


Plate 19. Efficacy o PGPR strains on plant growth promotion in ashwagandha under glasshouse condition

Table 19. Biocontrol potentiality of PGPR strains in coleus against different pathogens (alone and in combinations)

Treatment	Plant height (cm)	No. of branches	Fresh weight (g)	Dry weight (g)	Disease severity	Root rot index (0-5 scale)	Gall index (0-5 scale)
T1-RB01+F	32.5	5.0	97.5	15.60	+	1.50	-
T2-RB10+F	29.5	4.5	93.5	14.50	++	3.00	-
T3- RB13+F	34.0	6.0	107.5	17.00	+	1.50	-
T4 –RB22+F	28.0	4.0	94.75	15.00	++	2.50	-
T5 –RB31+F	36.5	6.5	116.5	18.50	+	1.00	-
T6 –RB43+F	30.0	5.5	95.50	15.28	++	2.00	-
T7 –RB50+F	37.0	7.0	120.0	18.90	+	1.00	-
T8 - F	25.0	3.5	80.0	13.00	+++	3.50	-
T9-RB01+R	31.5	4.5	94.00	15.00	++	2.00	-
T10-RB10+R	27.5	4.0	92.50	14.25	+++	3.50	-
T11-RB13+R	32.0	5.0	101.00	16.50	++	2.00	-
T12-RB22+R	26.0	4.0	93.00	14.25	+++	3.00	-
T13-RB31+R	34.0	5.5	107.00	16.90	++	1.50	-
T14-RB43+R	28.0	4.5	93.50	14.50	++	2.50	-
T15-RB50+R	35.0	6.0	110.5	17.50	+	1.50	-
T16 -R	22.0	3.5	69.50	11.12	++++	4.00	-
T17-RB01+F+R	29.0	4.0	92.50	14.25	++	3.00	-
T18-RB10+F+R	26.0	3.0	88.75	13.90	+++	4.00	-
T19- RB13+F+R	31.5	4.5	94.50	15.00	++	3.00	-
T20-RB22+F+R	25.5	3.0	89.00	14.00	+++	4.00	-
T21 -RB31++RF	33.0	4.5	99.50	15.80	++	2.50	-
T22-RB43+R+F	27.0	3.5	90.50	14.10	+++	3.50	-
T23-RB5+R0+F	33.5	5.0	108.50	17.10	+	2.00	-
T24 -F+R	20.5	3.0	52.75	8.50	++++	4.50	-
T25-RB01+M	37.0	6.0	100.00	16.00	-	-	3.0
T26-RB10+M	35.5	5.0	97.50	15.25	-	-	3.5
T27-RB13+M	37.0	6.0	109.0	17.40	-	-	2.5
T28-RB22+M	35.0	5.0	98.50	15.25	-	-	3.5
T29-RB31+M	37.50	7.0	118.50	18.50	-	-	2.5
T30-RB43+M	36.00	5.5	99.00	15.70	-	-	3.0
T31-RB50+M	38.00	7.0	122.75	19.50	-	-	2.0
T32 - M	26.50	4.5	87.50	14.00	+	-	5.0
T33-RB01+F+M	31.00	4.5	93.00	14.55	++	2.50	2.5
T34-RB10+F+M	30.50	3.0	90.50	14.50	++	3.50	3.5
T35- RB13+F+M	32.00	5.0	103.00	16.50	++	2.50	2.0
T36-RB22+F+M	30.00	3.5	90.00	14.50	+++	3.50	3.5
T37-RB31+F+M	33.50	5.5	109.50	17.25	++	2.00	2.5
T38-RB43+F+M	30.50	4.0	92.50	14.50	++	3.00	2.5
T39-RB50+F+M	35.00	6.0	112.75	18.00	++	1.50	2.0
T40-F+M	24.00	3.0	75.00	12.50	++++	5.00	4.5
T41-RB01+R+M	31.00	4.0	90.00	14.30	+++	3.00	2.5
T42-RB10+R+M	26.00	3.5	86.50	13.80	+++	4.00	3.5
T43-RB13+R+M	32.00	4.0	94.50	15.00	++	3.00	2.5
T44-RB22+R+M	25.00	3.5	88.00	14.00	+++	4.00	3.0
T45-RB31+R+M	33.00	5.0	100.00	16.00	++	2.50	2.5
T46-RB43+R+M	28.00	4.0	89.75	14.30	+++	3.50	3.0
T47-RB50+R+M	33.00	5.0	108.50	17.35	+++	2.00	2.0
T48-R+M	22.00	3.0	68.00	11.50	++++	4.50	4.00
T49-RB01+F+R+M	28.00	3.0	89.00	14.25	+++	3.50	2.5
T50-RB10+ F+R+M	25.50	3.0	85.00	13.60	+++	4.00	3.0
T51-RB13+ F+R+M	29.50	3.0	93.50	14.50	++	3.00	2.5
T52-RB22+ F+R+M	25.00	2.0	86.50	13.84	+++	4.00	3.0
T53-RB31+ F+R+M	30.50	3.5	97.50	15.50	++	3.00	2.0
T54-RB43+ F+R+M	27.00	2.5	87.00	13.75	+++	3.50	3.00
T55-RB50+ F+R+M	33.00	4.00	103.75	16.50	++	2.50	1.50
T56-F+R+M	20.00	1.00	48.50	7.75	++++	5.00	3.50
T57-Healthy	41.50	10.00	135.50	21.80	-	-	-

Table 20. Biocontrol potentiality of PGPR strains in ashwagandha against different pathogens (alone and in combinations)

Treatment	Plant height (cm)	No. of branches	Fresh weight (g)	Dry weight (g)	Disease severity	Root rot index (0-5 scale)	Gall index (0-5 scale)
T1-RB01+F	35.75	4.0	34.50	6.75	++	2.5	-
T2+RB10+F	29.50	3.5	32.00	6.25	+++	4.0	-
T3-RB13+F	38.50	4.0	35.50	6.90	++	2.0	-
T4-RB22+F	28.50	3.0	31.50	6.00	+++	3.5	-
T5-RB31+F	40.25	4.5	36.00	7.00	+	2.0	-
T6-RB43+F	34.50	3.5	33.50	6.50	+++	3.0	-
T7-RB50+F	41.50	5.0	36.50	7.50	+	1.5	-
T8-F	25.50	2.5	20.00	5.00	++++	4.50	-
T9-RB01+M	35.00	4.0	36.50	7.20	-	-	3.0
T10-RB10+M	29.00	4.0	35.80	6.95	+	-	4.0
T11-RB13+M	37.00	4.5	37.00	7.50	-	-	2.5
T12-RB22+M	26.50	3.5	33.50	6.00	-	-	4.0
T13-RB31+M	38.75	5.0	37.50	7.50	-	-	2.5
T14-RB43+M	33.25	4.0	35.00	6.50	-	-	3.5
T15-RB50+M	39.50	5.0	38.00	7.70	-	-	2.0
T16-M	23.75	3.0	32.00	6.30	+	-	5.0
T17-RB01+F+M	30.50	3.0	33.00	6.50	+++	3.00	3.0
T18-RB10+F+M	28.00	3.0	30.00	6.00	+++	4.50	3.5
T19-RB13+F+M	31.90	3.5	33.50	6.50	+++	3.00	2.5
T20-RB22+F+M	25.10	2.5	31.50	6.00	+++	4.00	3.5
T21-RB31+F+M	32.50	3.5	35.00	6.75	++	2.50	2.0
T22-RB43+F+M	30.00	3.0	32.00	6.20	+++	3.50	3.0
T23-RB50+F+M	35.00	4.0	35.50	6.75	++	2.50	2.0
T24-F+M	21.50	2.0	18.00	4.00	++++	5.00	4.0
T25-Healthy	45.00	6.5	41.00	8.50	-	-	-

F- *Fusarium*
R- *Ralstonia*
M- *Meloidogyne*

Plate 20. Talc based bioformulations of PGPR strains

Plate 21. General view of the pot experiments of coleus and ashwagandha

on PGPR strains. Even in individual or combinations of pathogens treated with PGPR strains RB50 and RB31 recorded maximum plant height, number of branches, more fresh and dry weight with least disease severity, as well as root-rot and root-knot indices of coleus and ashwagandha.

From the present investigation it is clear that the expression of symptoms, disease severity and root-rot indices was more when the nematode, *M. incognita* interacted with other pathogens and in combination.

4.19 ELUCIDATION OF MECHANISMS OF BIOCONTROL

4.19.1 Siderophore production

PGPR strains formed yellow coloured halo zone around the rhizobacterial colony on dark blue coloured agar plates indicating the production of siderophore. The diameter of the yellow halo varied with the strains. The siderophore producing strains chelated the iron from the dye chromazurol/Fe^{3+}/hexadecyltrimethyl ammonium bromide and turned dark blue to yellowish fluorescent colour by all most all strains. The results of siderophore production indicated that the PGPR strain RB50, RB31 and RB43 showed a higher activity when compared to other strains measured by zone of colouration of 28, 27 and 23 mm surrounding the colonies on dark blue CAS medium respectively (Table 21 and Plate 22).

4.19.2 HCN production

All the strains tested were positive for hydrogen cynide (HCN) production. Among these HCN producing strains, RB13 and RB50 changed the yellow colour of the filter paper to dark brown to red when compared to other strains and were scored as strong (+++) indicating higher level of HCN production. The PGPR strains RB1 and RB31 were scored as moderate (++) and strains RB10 and RB43 were graded as weak (+) whereas RB22 scored as nil (–) since there is no production of HCN based on the colour development (Table 21 and Plate 22).

4.19.3 Fluorescein and Pyocaynin production

PGPR strains was surrounded with yellow to greenish yellow zone on *Pseudomonas* agar F, whereas PGPR strains grown on *Pseudomonas* agar P formed a colony that was surrounded by a blue to green zone or with red to dark brown zone due to fluorescein and pyocyanin production respectively. It was found that all the PGPR strains tested were known to produce fluorescein and pyacyanin in vaired proportion after 48 h of incubation. The strain RB 13 showed strong production of fluorescein and strains RB31 and RB50 showed strong production of pyocyanin in the specific *Pseudomonas* agar medium (Table 21 and Plate 22).

4.19.4 Indole acetic acid (IAA) production

Among the seven PGPR strains grown in culture medium amended with tryptophan, the strains produced IAA as detected by the Salkowski reagent under spectrophotometer, it ranged between 13 to 19 μg/ml are given in Table 21. The highest concentration of IAA was observed from PGPR strain RB50 (19 μg/ml) followed by RB31 (18 μg/ml). Lowest concentration of IAA was produced in RB22 (13 μg/ml) (Table 21).

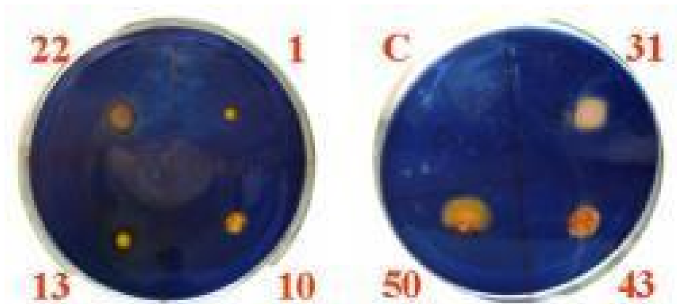
4.19.5 Effect of volatile metabolites of PGPR strains

An experiment was conducted to evaluate the effect of volatile components produced by the PGPR strains, in paired plate technique. The results revealed that the strains produces considerable amount of volatile metabolites which varied with the strains. Higher concentration of volatile metabolites were produced in strains RB50, RB31 and RB1 (+++), which inhibited the test pathogen *Fusarium* to an extent of > 80 per cent *i.e.* 87.77, 86.66 and 82.22 per cent respectively (Table 22). The strain RB43 and RB13 produced volatile metabolites moderately (++), which inhibited *Fusarium* to an extent of 72.22 and 66.66. Lowest activity of volatile metabolites was observed in RB22 and RB10 (+) which inhibited the

Table 21. Detection of Siderophore, HCN, Fluorescein, Pyocyanin and IAA production by the PGPR strains

PGPR strains	Siderophore production		HCN production	Fluorescein production	Pyocyanin production	IAA production(µg/ml)
		Zone of coloration (mm dia.)				
RB1	+	05	++	++	+	17.00
RB10	++	19	+	+	++	14.00
RB13	+	06	+++	+++	++	16.00
RB22	++	20	+	+	+	13.00
RB31	+++	27	++	++	+++	18.00
RB43	++	23	+	+	+	15.00
RB50	+++	28	+++	++	+++	19.00
Control	-	-	-	-	-	-

- No production, + Low, ++ Medium and +++Strong production



Siderophore production

Hydrogen Cyanide (HCN) production

Fluorescein production

Pyocyanin production

Plate 22.Elucidation of biocontrol mechanisms of efficient PGPR strains

Table 22. Effect of volatile metabolites released from the PGPR strains on mycelial growth of *Fusarium chlamydosporum* by paired plate technique

PGPR strains	Volatile metabolites	Radial mycelial growth (mm)	Per cent inhibition
RB1	+++	16.00	82.22 (65.05)*
RB10	+	55.00	38.88 (38.55)
RB13	++	30.00	66.66 (54.71)
RB22	+	40.00	55.55 (48.16)
RB31	+++	12.00	86.66 (66.44)
RB43	++	25.00	72.22 (58.17)
RB50	+++	11.00	87.77 (68.55)
Control		90.00	-
		SEm±	0.47
		CD@1%	1.96

*Figures in the parenthesis are arc sine transformed values

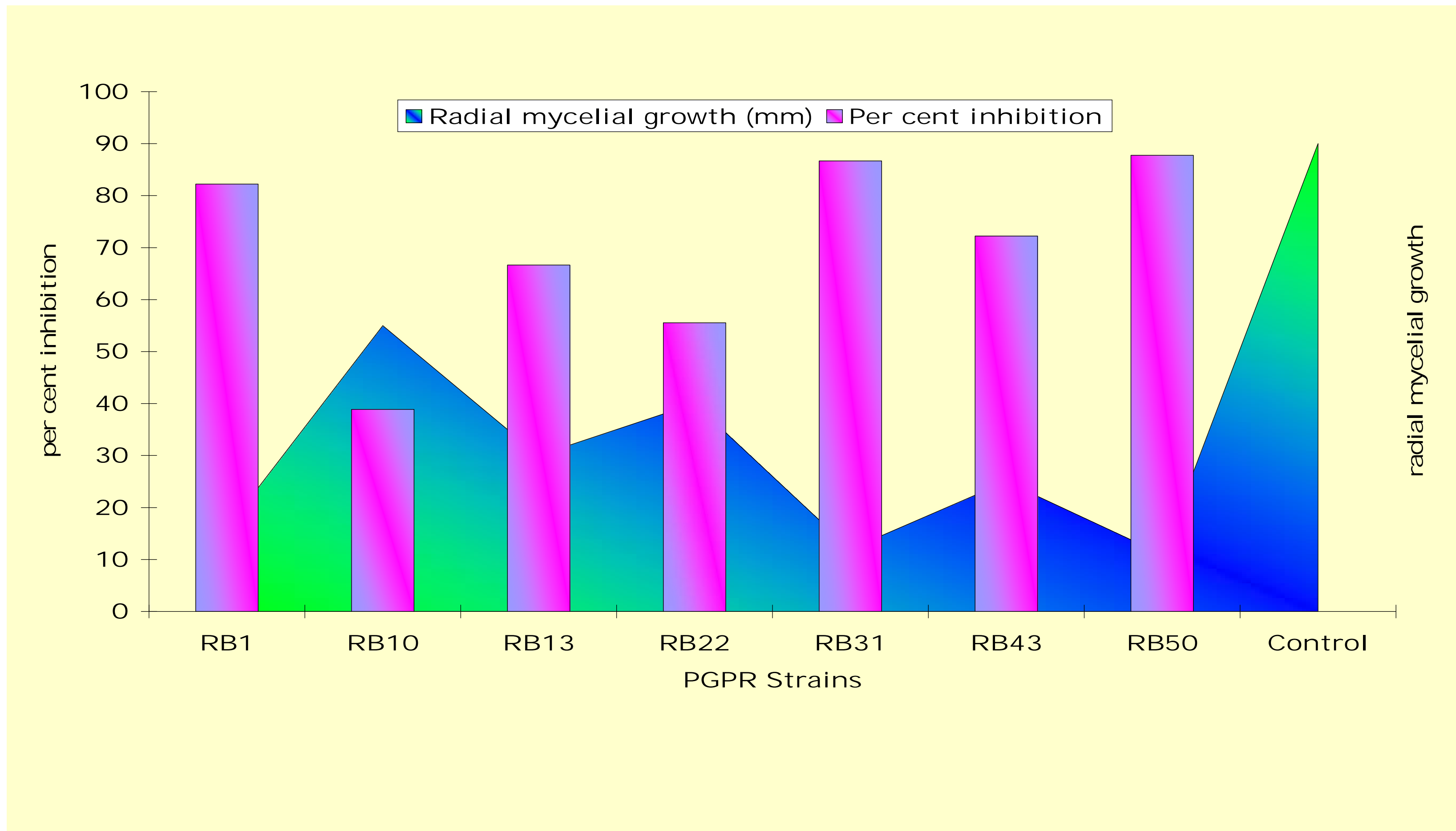


Fig. 7: Effect of volatile metabolites released from the PGPR strains on mycelial growth of Fusarium by paired plate technique

target pathogen only to a lesser extent of 55.55 and 38.88 per cent respectively (Table 22, Fig. 7 and Plate 23).

4.19.6 Antimicrobial antibiotic production

Based on *in vitro* performance of PGPR strains against *Fusraim*, *Ralstonia* and *Meloidogyne*, seven efficient PGPR strains were selected to elucidate their mechanisms. To ascertain the antimicrobial metabolite production by the strains, metabolic products of each strains were extracted and separated by TLC plates as described in 'Material and Methods'.

All the seven strains produced more than one metabolite, which appeared as dark blue spots under UV light (254 nm). The Rf values of the metabolites varied from 0.12 to 0.94 (Table 23 and Plate 24).

The metabolites with Rf values of 0.69 and 0.94 were commonly produced in all the strains tested. Similarly, the metabolites with an Rf value of 0.25 and 0.37 were present only in RB1, RB10, RB13 and RB22. Strain RB31 produced only one metabolite with an Rf value of 0.94. Maximum of 5 metabolites were produced in RB1 and RB10, four metabolites in RB13 and RB22 whereas two metabolites with an Rf value of 0.69 and 0.94 were produced in RB43 and RB50 respectively (Plate 24).

When all the unidentified metabolites on TLC plates were scraped, eluted and redissolved in acetone and tested for *in vitro* inhibition by streaking parallel to the disc of *Fusarium* placed at the center of the petriplate as described in 'Material and Methods', the data revealed that, all the metabolites tested inhibited *Fusarium* significantly. The per cent inhibition varied from 17.77 to 52.22. Maximum per cent inhibition (52.22%) was observed in metabolite with Rf value of 0.37 produced by RB10 followed by 44.44 per cent inhibition in metabolite with Rf value of 0.25 in strain RB1. Lowest inhibition of *Fusarium* (17.77) was observed in RB13 (Rf 0.25) (Table 23 and Plate 24).

4.20 INDUCED SYSTEMIC RESISTANCE

Induced systemic resistance through as inferred biochemical analysis revealed the increased activities of the enzymes, *viz.* peroxidase (PO), polyphenol oxidase (PPO), phenylalamine ammonia lyase (PAL) and also phenolic compounds in the PGPR treated plants in coleus and ashwagandha challenged with individual and combinations of their pathogens (at 180 DAP). In general, the expression of defense enzymes and other compounds in PGPR treated plants upon inoculation with different pathogens and their interaction in coleus and ashwagandha was comparatively more when compared to uninoculated control.

4.20.1 Peroxidase (PO)

Assay of peroxidase activity in coleus and ashwagandha plants inoculated with different pathogens showed differences among the various treatments. Increased activity of PO was observed with the treatment RB50 and RB31 upon challenge inoculation with individual and combination of pathogens, when compared to untreated control and it was significantly different from all other treatments (Table 24).

In coleus PO activity varied from 8.24 per cent to 55.38 per cent. The plants treated with RB50 followed by RB31, RB13 and RB1 showed higher PO activity irrespective of the pathogens and their interactions. Lowest PO activity was observed in untreated plants followed by RB22.

In ashwagandha PO activity varied from 13.15 per cent to 95 per cent. The plants treated with RB50 and RB31 showed higher PO activity in treatment received *Fusarium* (F) alone, *Meloidogyne* (M) alone or F+M. Least PO activity was recorded in RB22 treated plants (Table 25).

Table 23. Antibiotic(s) production of PGPR strains by TLC and testing their efficacy against *Fusarium chlamydosporum*

PGPR strains	Antibiotics/ metabolites	RF value	Per cent inhibition
RB1	A1	0.12	30.00 (33.19)*
	A2	0.25	44.44 (41.79)
	A3	0.37	31.11 (33.88)
	A4	0.69	27.77 (31.78)
	A5	0.94	31.11 (33.88)
RB10	A1	0.12	33.33 (35.24)
	A2	0.25	23.33 (28.87)
	A3	0.37	52.22 (46.25)
	A4	0.69	32.22 (34.56)
	A5	0.94	22.22 (28.10)
RB13	A1	0.25	17.77 (24.92)
	A2	0.37	34.44 (35.90)
	A3	0.69	33.37 (35.24)
	A4	0.94	28.88 (32.49)
RB22	A1	0.25	31.11 (33.88)
	A2	0.37	27.22 (31.43)
	A3	0.69	29.33 (32.84)
	A4	0.94	18.33 (25.33)
RB31	A1	0.94	26.66 (31.07)
RB43	A2	0.69	26.11 (30.71)
	A3	0.94	25.55 (30.31)
RB50	A1	0.69	24.44 (29.65)
	A2	0.94	23.33 (28.87)
Control	0	0	0
		SEm±	0.28
		CD@ 1%	1.07

*Figures in the parenthesis are arc sine transformed values

Plate 23. Effect of volatile metabolites of PGPR on mycelial inhibition of *Fusarium chlamydisporum*

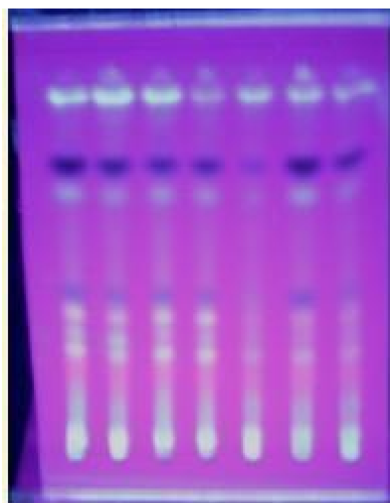


Plate 24. Production of Antibiotics from PGPR strains by TLC and testing their efficacy against *Fusarium chlamydosporum*

4.20.2 Polyphenol oxidase (PPO)

All the PGPR strains tested indicated significant increase in the activity of PPO in coleus and ashwagandha in different treatments when compared to control plants. However, accumulation of polyphenol oxidase was higher in RB50 followed by RB31 and RB1 treated plants challenged with *Fusarium*, *Ralstonia* and *Meloidogyne* individually and also in combinations.

PPO activity varied from 0.011 to 0.032. Maximum PPO activity was noticed in T_{55}, *i.e.* RB50 challenged with *Fusarium+Ralstonia+ Meloidogyne* (0.032) which showed 77.77 per cent increase over control followed by T_{39} *i.e.* RB50 challenged with *Ralstonia + Meloidgyne*, 76.47 per cent showed increase over control. Lowest PPO activity was observed in T_{12} *i.e.* RB22 when challenged with *Ralstonia* alone (0.111) which registered only 10 per cent increase over untreated control (Table 24).

In case of ashwagandha, PPO activity varied from 0.058 to 0.098. Higher PPO activity was noticed in T_{23} RB50 treated plants challenged with *Fusarium+Meloidogyne* (0.098), 63.33 per cent increase over control followed by $T_{7,}$ i.e. RB50 challenged with *Fusarium* alone showed 60.00 per cent increase over control (Table 25).

4.20.3 Phenyalanine ammonialyase (PAL)

Increase in the activity of PAL was observed in the PGPR treated strains in coleus and ashwagandha plants.

PAL activity in coleus upon treatment with PGPR strains challenged with pathogens individually and in combinations varied from 0.051 to 0.112. Maximum PAL activity was observed in T_{23}, *i.e.* RB50 treated plants challenged with *Fusarium* and *Ralstonia* (0.112), registering 86.66 per cent increase over control, followed by T_{21} *i.e.* RB3 plants challenged with *Fusarium* and *Ralstonia* (0.108) with 80.00 per cent increase over control (Table 24).

In ashwagandha, PAL activity varied from 0.069 to 0.094. Highest PAL activity was noticed in T_7 *i.e.* RB50 plants challenged with *Fusarium* alone which showed 61.81 per cent increase over control followed by T_{15} *i.e.* RB50 challenged with *Meloidogyne* alone (0.091) with 56.89 per cent increase over control (Table 25).

4.20.4 Total phenols

Increase in accumulation of total phenols was observed in all the PGPR treated coleus and ashwagandha plants.

In coleus, phenol accumulation varied from 0.60 to 0.92. Highest phenol accumulation was noticed in T_{55} *i.e.* RB 50 plants challenged with *Fusarium + Ralstonia + Meloidogyne* (0.92) followed by T_{23} (i.e. RB50 and challenged with *Fusarium + Ralstonia* (0.91), respectively (Table 24).

Phenol accumulation varied from 0.60 to 0.99 in ashwagandha plants treated with different PGPR strains. Highest accumulation of phenols was recorded in T_{23}, *i.e.* RB50 challenged with *Fusarium* and *Meloidogyne* (0.98), showed 80.00 per cent increase over control (0.55) followed by T_7 RB50 challenged with *Fusarium* alone (0.80) (77.77% increase over control). Lowest phenol accumulation was noticed in T_4 which received RB22 challenged with *Fusarium* alone (0.60) and recorded only 33.33 per cent increase over control (Table 25).

4.21 GENETIC VARIABILITY OF PGPR STRAINS

Random amplified polymorphic DNA (RAPD) was used to detect the variations among the PGPR strains. OPA, OPB and OPF series of primers obtained from M/s Bangalore Genie, were used to determine genetic distance between them and to construct a dendrogram.

Table 24. Effect of PGPR strains on induced systemic resistance (ISR) in coleus plants, inoculated with different pathogens in pot culture

Treatment	PO		PPO		PAL		Total phenols	
	*Δ in absorbance	% IOC**	Δ in absorbance	% IOC	Δ in absorbance	% IOC	Δ in absorbance	% IOC
T1-RB01+F	0.098	30.66	0.017	54.54	0.061	35.55	0.66	50.00
T2-RB10+F	0.096	28.00	0.013	18.18	0.600	33.33	0.63	43.18
T3- RB13+F	0.098	30.66	0.017	54.54	0.065	44.44	0.69	56.81
T4 –RB22+F	0.095	26.66	0.012	9.09	0.051	11.76	0.60	36.36
T5 –RB31+F	0.100	33.33	0.018	63.63	0.075	66.66	0.79	79.50
T6 –RB43+F	0.097	29.33	0.016	45.55	0.052	15.55	0.65	47.70
T7 –RB50+F	0.102	36.00	0.019	72.72	0.080	77.77	0.87	97.72
T8 - F	0.075	-	0.010	-	0.045	-	0.44	-
T9-RB01+R	0.099	26.92	0.014	40.00	0.082	36.66	0.79	51.90
T10-RB10+R	0.094	20.51	0.012	20.00	0.076	26.66	0.70	34.61
T11- RB13+R	0.097	24.35	0.014	30.00	0.088	46.66	0.80	53.80
T12–RB22+R	0.093	19.23	0.011	10.00	0.066	10.00	0.77	48.07
T13 –RB31+R	0.101	29.48	0.015	50.00	0.092	53.33	0.88	69.23
T14 –RB43+R	0.098	25.64	0.013	30.00	0.078	30.00	0.75	44.23
T15 –RB50+R	0.103	32.05	0.016	60.00	0.096	60.00	0.90	73.07
T16 - R	0.078	-	0.010	-	0.060	-	0.52	-
T17-RB01+F+R	0.100	47.05	0.018	38.46	0.098	63.66	0.80	33.33
T18-RB10+F+R	0.098	44.11	0.016	23.07	0.082	33.33	0.75	25.00
T19- RB13+F+R	0.099	45.58	0.017	30.76	0.092	53.30	0.81	35.00
T20-RB22+F+R	0.096	41.17	0.014	7.96	0.068	13.33	0.70	16.66
T21 -RB31++RF	0.102	50.00	0.019	46.15	0.108	80.00	0.82	36.66
T22-RB43+R+F	0.100	47.05	0.015	15.38	0.089	48.33	0.79	31.66
T23-RB5+R0+F	0.104	52.94	0.020	53.84	0.112	86.66	0.91	51.66
T24 - F+R	0.068	-	0.013	-	0.060	-	0.60	-
T25-RB01+M	0.099	41.42	0.020	33.33	0.088	51.72	0.81	62.00
T26-RB10+M	0.098	40.00	0.018	20.00	0.078	34.48	0.75	50.00
T27 RB13+M	0.100	42.85	0.020	33.33	0.085	46.55	0.82	64.00
T28–RB22+M	0.095	35.71	0.017	13.33	0.072	24.13	0.66	32.00
T29 –RB31+M	0.101	44.28	0.021	40.00	0.090	55.17	0.84	68.00
T30–RB43+M	0.097	38.57	0.019	26.66	0.082	41.37	0.79	58.00
T31-RB50+M	0.103	47.14	0.023	53.33	0.095	63.79	0.88	76.00
T32 - M	0.070	-	0.015	-	0.050	-	0.50	-
T33-RB01+F+M	0.098	28.94	0.025	47.05	0.091	44.44	0.72	30.00
T34-RB10+F+M	0.093	22.36	0.023	35.29	0.088	39.68	0.72	30.90
T35- RB13+F+M	0.099	30.26	0.026	52.94	0.093	47.61	0.73	32.72
T36-RB22+F+M	0.090	18.42	0.020	17.64	0.082	30.15	0.68	23.63
T37-RB31+F+M	0.100	31.57	0.028	64.70	0.095	50.79	0.75	36.36
T38-RB43+F+M	0.095	25.00	0.022	29.41	0.090	42.85	0.70	27.27
T39-RB50+F+M	0.101	32.89	0.030	76.47	0.098	55.55	0.80	45.45
T40-F+M	0.076	-	0.017	-	0.063	-	0.55	-
T41-RB01+R+M	0.099	52.30	0.023	43.150	0.101	48.52	0.78	39.28
T42-RB10+R+M	0.096	47.69	0.019	18.75	0.095	39.70	0.71	26.78
T43- RB13+R+M	0.093	50.76	0.022	37.50	0.102	50.00	0.80	49.85
T44-RB22+R+M	0.095	46.15	0.018	12.50	0.088	29.41	0.68	21.42
T45-RB31+R+M	0.100	53.84	0.025	56.25	0.105	54.41	0.83	48.21
T46-RB43+R+M	0.097	49.23	0.021	31.25	0.098	44.11	0.74	32.14
T47-RB50+R+M	0.101	55.38	0.028	75.00	0.108	58.82	0.87	55.35
T48-R+M	0.065	-	0.016	-	0.068	-	0.56	-
T49-RB01+F+R+M	0.109	12.37	0.029	61.11	0.105	40.00	0.86	43.33
T50-RB10+ F+R+M	0.106	9.27	0.024	33.33	0.100	33.33	0.80	33.33
T51-RB13+ F+R+M	0.103	11.34	0.028	55.55	0.108	44.00	0.87	45.00
T52-RB22+ F+R+M	0.105	8.24	0.020	11.11	0.092	22.66	0.72	20.00
T53-RB31+ F+R+M	0.113	16.49	0.030	66.66	0.109	45.33	0.90	50.00
T54 –RB43+ F+R+M	0.107	10.30	0.026	44.44	0.102	36.00	0.82	36.66
T55 –RB50+ F+R+M	0.124	27.83	0.032	77.77	0.110	46.66	0.92	53.33
T56- F+R+M	0.097	-	0.018	-	0.075	-	0.60	-

* Changes in absorbance /min/mg of protein ** Per cent increase over control

F- *Fusarium*, R- *Ralstonia*, M- *Melodogyne*

Table 25. Effect of PGPR strains on induced systemic resistance (ISR) in ashwagandha plants, inoculated with different pathogens in pot culture

Treatment	PO		PPO		PAL		Total phenols	
	Δ in absorbance*	% IOC**	Δ in absorbance	% IOC	Δ in absorbance	% IOC	Δ in absorbance	% IOC
T1-RB01+F	0.053	39.47	0.067	34.00	0.079	43.63	0.75	66.66
T2+RB10+F	0.048	26.31	0.065	30.00	0.073	32.72	0.70	55.55
T3-RB13+F	0.055	44.73	0.070	40.00	0.081	47.27	0.78	73.33
T4-RB22+F	0.045	13.15	0.061	22.00	0.069	25.45	0.60	33.33
T5-RB31+F	0.058	52.63	0.072	44.00	0.085	54.54	0.79	75.55
T6-RB43+F	0.050	31.57	0.064	28.00	0.075	36.36	0.73	62.22
T7-RB50+F	0.060	57.89	0.080	60.00	0.089	61.81	0.80	77.77
T8-F	0.038	-	0.050	-	0.055	-	0.45	-
T9-RB01+M	0.046	31.42	0.068	28.30	0.080	37.93	0.78	56.00
T10-RB10+M	0.042	20.00	0.062	16.98	0.075	29.31	0.75	50.00
T11-RB13+M	0.048	37.14	0.070	32.07	0.082	41.37	0.82	64.00
T12-RB22+M	0.040	14.28	0.058	9.43	0.070	20.68	0.71	42.00
T13-RB31+M	0.051	45.71	0.074	39.62	0.086	48.27	0.84	68.00
T14-RB43+M	0.044	25.71	0.065	22.64	0.078	34.48	0.76	52.00
T15-RB50+M	0.054	54.28	0.080	50.94	0.091	56.89	0.88	76.00
T16-M	0.035	-	0.053	-	0.058	-	0.50	-
T17-RB01+F+M	0.058	45.00	0.085	41.66	0.085	37.09	0.84	52.72
T18-RB10+F+M	0.051	27.50	0.076	26.66	0.079	27.41	0.76	38.18
T19-RB13+F+M	0.066	65.00	0.088	46.66	0.088	41.-93	0.85	54.54
T20-RB22+F+M	0.049	22.50	0.072	20.00	0.076	22.58	0.71	29.09
T21-RB31+F+M	0.072	80.00	0.091	51.66	0.090	45.16	0.89	61.81
T22-RB43+F+M	0.050	25.00	0.081	35.00	0.080	29.03	0.80	45.45
T23-RB50+F+M	0.078	95.00	0.098	63.33	0.094	51.61	0.99	80.00
T24-F+M	0.40	-	0.060	-	0.062	-	0.555	-

* Changes in absorbance /min/mg of protein ** Per cent increase over control

F- *Fusarium*, M- *Melodogyne*

The profile of amplicons obtained using different primers for seven strains of PGPR is given in Table 26. A total of 227 DNA bands were detected using 30 random primers. Of the 227 level bands, 198 bands were polymorphic (87.22%). Out of 30 primers used for amplification OPA-01, OPA-10, OPB-02, OPB-03, OPB-05 and OPB-06 showed 100 per cent polymorphism (Table 26 and Plate 25). The per cent polymorphism varied from 50 to 100. Two primers did not show any amplification. The banding profile per primer also varied from minimum of 3 bands (OPB-6) to maximum of 13 bands (OPF-4). From the RAPD analysis, the results revealed that a total of 87.22 per cent polymorphism was found between the PGPR strains, indicating there is a molecular variability among the strains. Information on banding pattern for all the primers was used to determine genetic distance between the strains and to construct a dendrogram by using unweighted pair group method (UPGMA).

Based on simple matching coefficient, a genetic similarity matrix was constructed to assess the genetic relatedness among the PGPR strains. The genetic similarity coefficient of seven strains based on RAPD analysis is given in the Table 27. The similarity coefficient ranged from 0.40 to 0.84. The maximum genetic similarity of 84.00 per cent was between RB31 and RB50. There was 75.00 per cent similarity between RB1 and RB10, whereas least similarity (40 per cent) between RB13 and RB31 (Table 27).

Further, the dendrogram constructed by UPGMA from the pooled data clearly showed two major clusters A and B at similarity coefficient of 0.52 (Fig. 8). Cluster A was classified upto sub-sub cluster A4 and cluster B was classified upto two minor clusters comprising RB31 and RB50 strains (Uttara Kannada and Bangalore strains) which showed 84 per cent similarity.

Cluster A sub-grouped into A1, A2, A3 and A4. A4 comprising two strains RB1 and RB10 (Bijapur strains). A1, A2 and A3 each comprising only one strains from Tumkur, Belgaum and Dharwad districts (Fig. 8 and Plate 25).

FIELD EXPERIMENTS

4.22 EFFECT OF TALC FORMATIONS OF PGPR STRAINS ON THE PLANT GROWTH CHARACTERS IN COLEUS AND ASHWAGANDHA UNDER FIELD CONDITIONS

A field experiment was conducted in Saidapur farm to test the efficacy of selected PGPR strains, which performed well in *in vitro* and glasshouse experiments. The soil was infested with *Fusarium chlamydosporum*, *F. solani* and *Meloidogyne incognita*. Therefore it was considered as 'Hot Spot' for experiment purpose, where the crops have the history of 30.00and 32.66 per cent wilt incidence and also *M. incognita* population was more than one per cc of soil. Hence, the experiment was carried out in Saidapur farm, Main Horticulture Research Station (MHRS), University of Agricultural Sciences, Dharwad. Experiment was laid out in Randomized Block Design (RBD) for coleus and ashwagandha as described in 'Material and Methods'. Observations on plant growth parameters and disease incidence are presented in the Tables 28 to 31and depicted in Fig. 9 to 11 and Plates 26 and 27.

4.22.1 Plant height

The data from coleus field experiment revealed that due to PGPR strain treatment enhanced the plant height. Significant difference in plant height was noticed in the strain RB50 and RB31 at 60, 150 and 180 days after planting. Highest plant height of 33.57, 40.50, 52.17 and 60.33 were observed at 60, 90, 150 and 180 days after planting respectively in RB50 and per cent increase over control in plant height in the strain RB50 was 86.50, 78.02 and 54.90 at 60, 90 and 150 days after planting. Lowest plant height was recorded in RB22 followed by untreated. At 180 days after planting the plant height in RB22 is on par with untreated (Table 28).

Table 26. DNA banding profile of 30 primers in seven efficient PGPR strains

Primer	Total bands	Polymorphic bands	Per cent polymorphism
OPA-01	7	7	100.00
OPA-02	10	9	90.00
OPA-03	8	6	75.00
OPA-04	8	4	50.00
OPA-05	7	4	57.00
OPA-06	3	2	66.66
OPA-07	9	8	88.88
OPA-08	-	-	-
OPA-09	8	5	62.50
OPA-10	6	6	100.00
OPB-01	5	3	60.00
OPB-02	7	7	100.00
OPB-03	5	5	100.00
OPB-04	13	12	92.30
OPB-05	9	9	100.00
OPB-06	3	3	100.00
OPB-07	9	9	100.00
OPB-08	8	7	87.50
OPB-09	7	6	85.71
OPB-10	8	7	87.50
OPF-01	9	7	77.77
OPF-02	4	3	75.00
OPF-03	8	5	62.50
OPF-04	13	11	84.61
OPF-05	12	10	83.33
OPF-06	9	8	88.88
OPF-07	11	10	90.90
OPF-08	-	-	-
OPF-09	11	8	72.72
OPF-10	10	7	70.00
Total	227	198	87.22

Table 27. Similarity co-efficient based on RAPD pooled over 30 primers in seven PGPR strains

PGPR strains	RB1	RB10	RB13	RB 22	RB 31	RB 43	RB 50
RB1	1.00						
RB10	0.75	1.00					
RB13	0.73	0.66	1.00				
RB22	0.75	0.66	0.69	1.00			
RB31	0.49	0.54	0.40	0.48	1.00		
RB43	0.58	0.58	0.61	0.60	0.55	1.00	
RB50	0.56	0.55	0.44	0.54	0.84	0.60	1.00

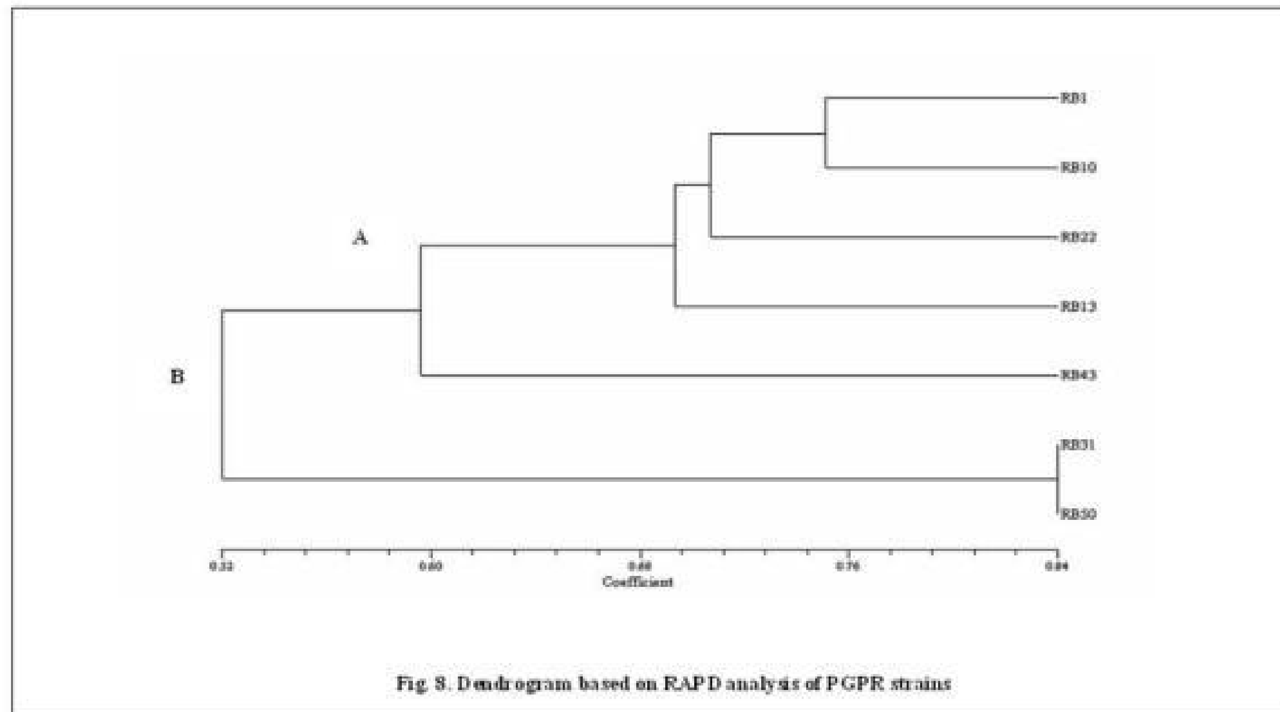
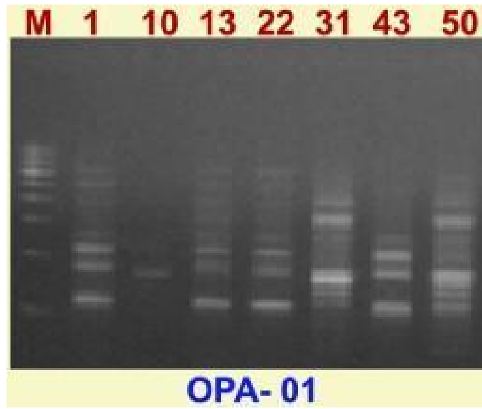
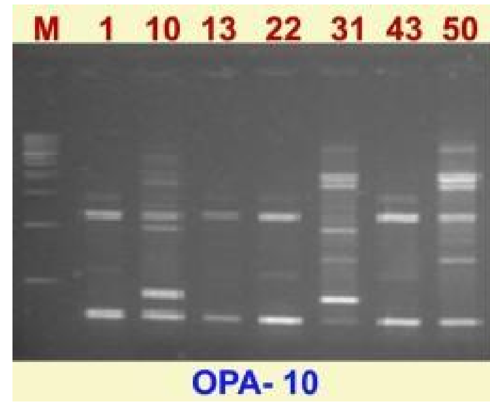
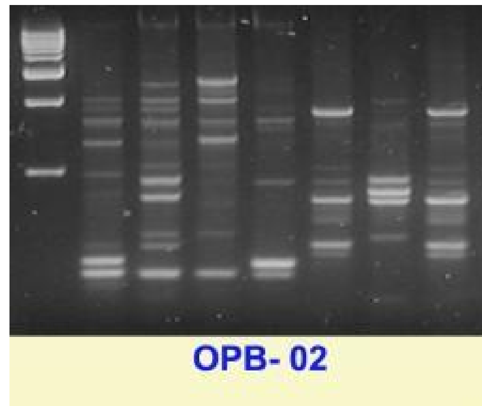


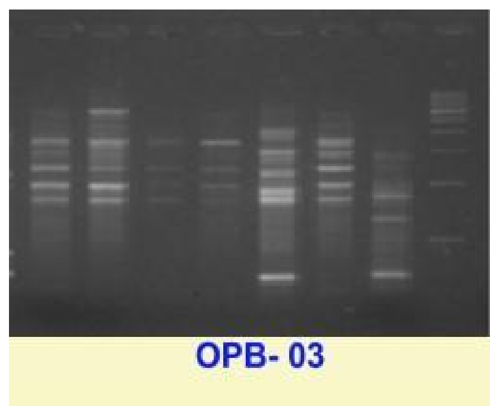
Fig. 8. Dendrogram based on RAPD analysis of PGPR strains

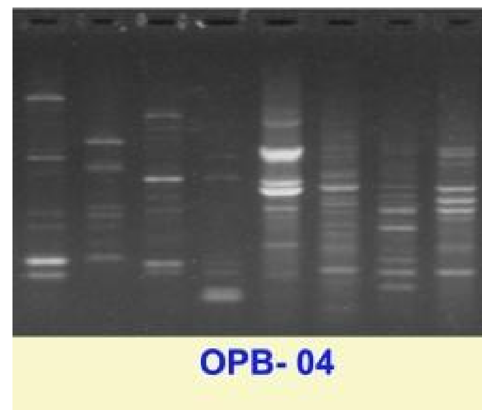
Fig 8. Dendrogram based on RAPD analysis of PGPR strains

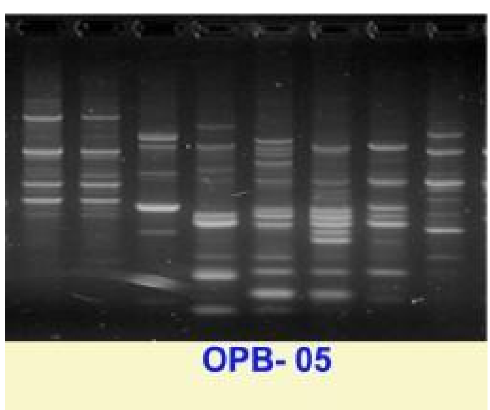


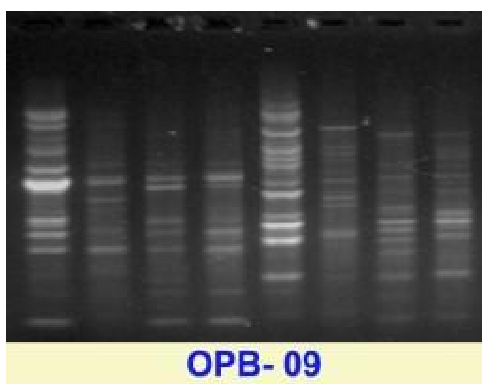












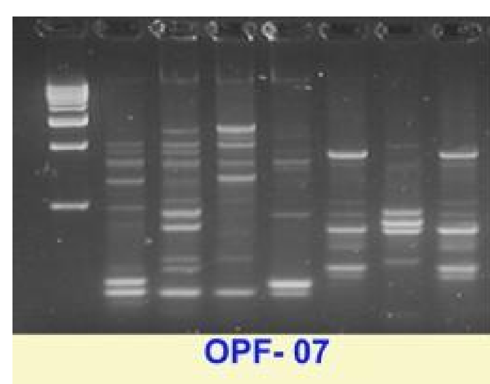


Plate 25. Genetic variability of PGPR strains

In ashwagandha, increased plant height was recorded in PGPR treated plants. Highest plant height was observed in RB50, followed by RB31, RB13 and RB1. However, no significant difference was noticed among these treatments. They are on par with each other at 90,150 and 180 days after planting respectively. Lowest height was recorded in RB22 which was on par with untreated plant at 60, 90, 150 and 180 days after planting. Highest per cent increase over control in plant height was recorded in RB50 at 180 days after planting (83.56) (Table 29).

4.22.2 Number of branches

In coleus, regarding number of branches highest value was recorded in RB50 followed by RB31, RB13 and RB1 at 60, 90, 150 and 180 days after planting. RB50, RB31, RB13 were on par with each other at 60 DAP. There was no significant difference in number of branches in RB50 and RB31 at 60 and 150 days after planting. Highest number of branches (32.17) were observed in RB50 at 180 days after planting. Lowest number of branches were observed in RB22 followed by uninoculated control. However, no significant difference was observed between these two treatments at 180 days after planting.

In case of ashwagandha, number of branches per plant varied from 3.50 to 13.80. Significant difference in number of branches was observed only in RB50 and RB31 at 60 and 90 days after planting. No significant difference was observed between other treatments, they are on par with each other. RB50 significantly superior to RB31 at 60DAP. Least number of branches were noticed in RB22 and untreated control, they are also on par with each other at 60, 90, 150 and 180 days after planting (Table 29).

4.23 EFFICACY OF TALC FORMULATIONS OF PGPR STRAINS ON YIELD PARAMETERS AND DISEASE INCIDENCE IN COLEUS AND ASHWAGANDHA UNDER FIELD CONDITIONS

4.23.1 Coleus

4.23.1.1 Number of tubers

The highest number of tubers in coleus was observed in RB50 treated plants (18.00). This was followed by RB13 and RB31 (16.63 and 16.50) respectively. The treatments RB1, RB13, RB31, RB43 and RB50 were on par with each other. RB22 treated plants registered only 11.87, whereas untreated plant registered only 10.70 tubers per plant (Table 30).

4.23.1.2 Tuber length

RB 50 produced the highest tuber length of 31.68 cm in coleus. However, the treatments RB31, RB13 and RB1 were also effective in enhancing the tuber length, which registered 29.28, 23.17 and 22.45 cm respectively. No significant differences were observed between the treatment RB10, RB 22 and RB 43, they are statistically on par with each other. Lowest tuber length of 17.59 was recorded in untreated control (Table 30).

4.23.1.3 Fresh weight of tubers

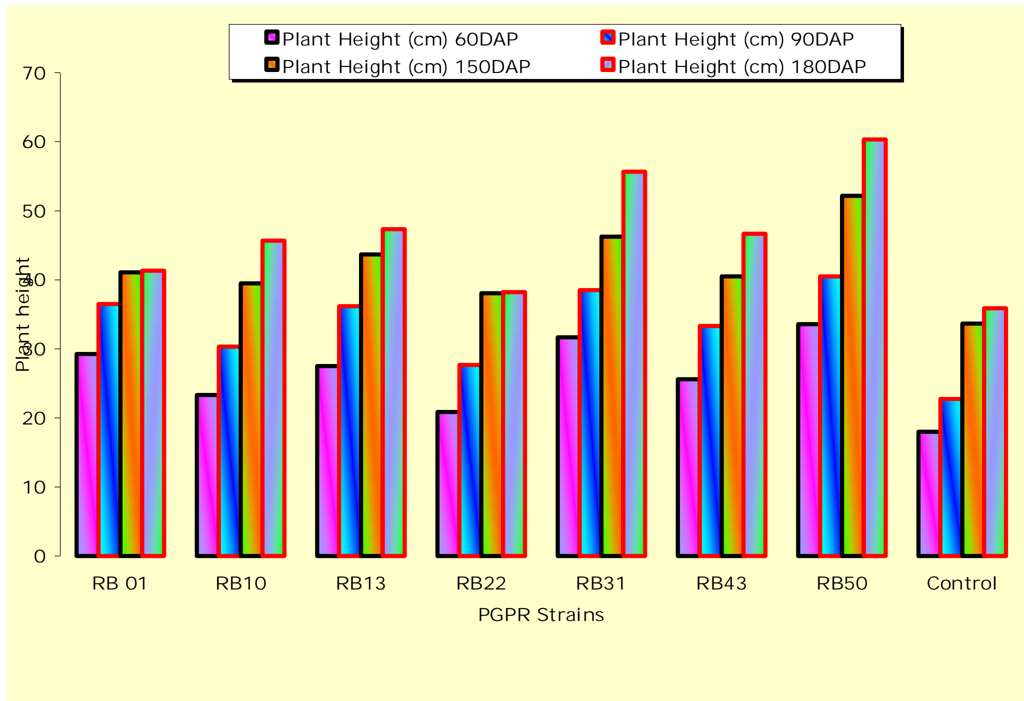
Increased plant weight through enhanced tuber weight and shoot weight as well as total biomass was recorded due to PGPR treatments in coleus. Highest fresh weight of tubers was noticed in RB 50 (342.02 g) followed by RB 31 (272.46 g) and RB 13 (230.01 g) respectively. All the treatments were statistically significant in increasing tuber weight. Lowest tuber weight of 145.77 was recorded in untreated control (Table 30).

4.23.1.4 Dry weight of tubers

In coleus, plants dry matter accumulation was maximum in RB50, RB31 and RB13 (74.17, 62.29 and 50.02). Least dry matter accumulation was observed in RB22 (31.17) followed by untreated control (30.00), which were on par with each other.

Table 28. Effect of talc formulations of PGPR strains on the plant growth characters in coleus under field condition

PGPR strains	60 DAP			90 DAP			150 DAP			180 DAP		
	Plant height (cm)	% IOC	No. of branches	Plant height (cm)	% IOC	No. of branches	Plant height (cm)	% IOC	No. of branches	Plant height (cm)	% IOC	No. of branches
RB 01	29.25	62.22	18.23	36.50	60.43	22.93	41.08	22.75	22.17	41.33	14.42	25.33
RB10	23.33	29.67	12.30	30.33	33.31	17.30	39.50	17.35	20.22	45.67	27.21	21.17
RB13	27.50	52.77	18.67	36.17	58.98	23.00	43.67	29.70	24.17	47.33	31.83	27.00
RB22	20.87	15.94	11.67	27.67	21.62	16.33	38.07	13.06	19.17	38.23	6.49	19.83
RB31	31.67	75.94	19.33	38.50	69.23	23.00	46.25	37.36	28.03	55.67	55.06	28.67
RB43	25.60	42.22	12.67	33.33	46.50	18.50	40.50	20.28	20.27	46.67	30.00	22.00
RB50	33.57	86.5	19.43	40.50	78.02	26.20	52.17	54.90	29.33	60.33	68.05	32.17
Control	18.00	-	10.03	22.75	-	13.50	33.67	-	16.83	35.90	-	19.50
SEm±	0.57		0.60	0.84		0.69	0.90		0.45	1.09		0.43
CD@ 5%	1.72		1.81	2.55		2.10	2.74		1.38	3.29		1.32



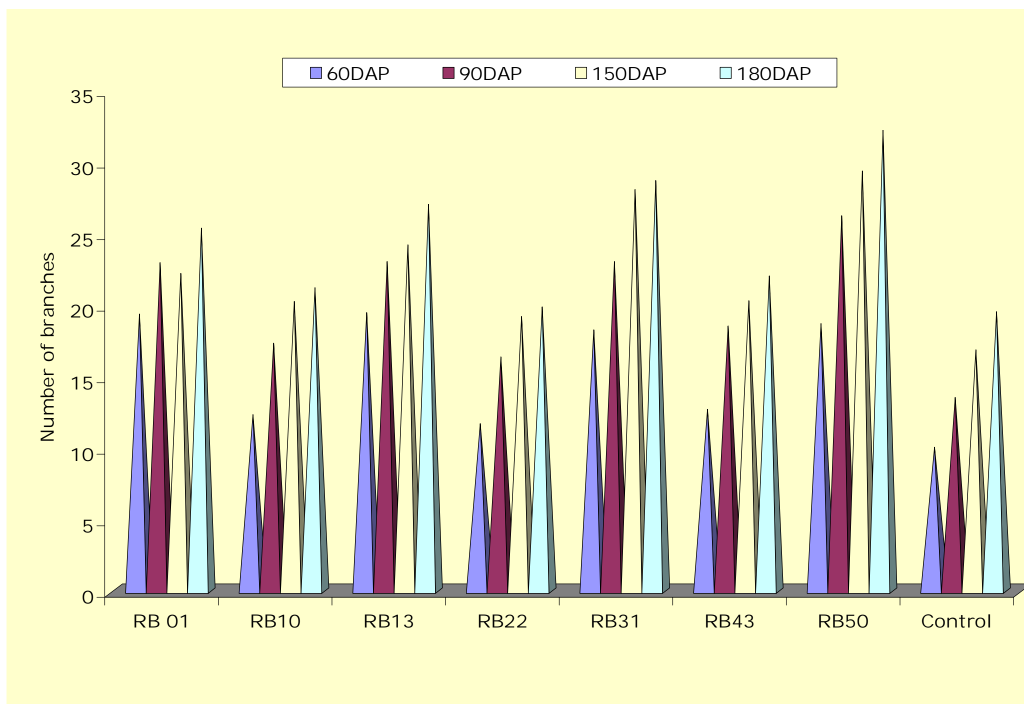
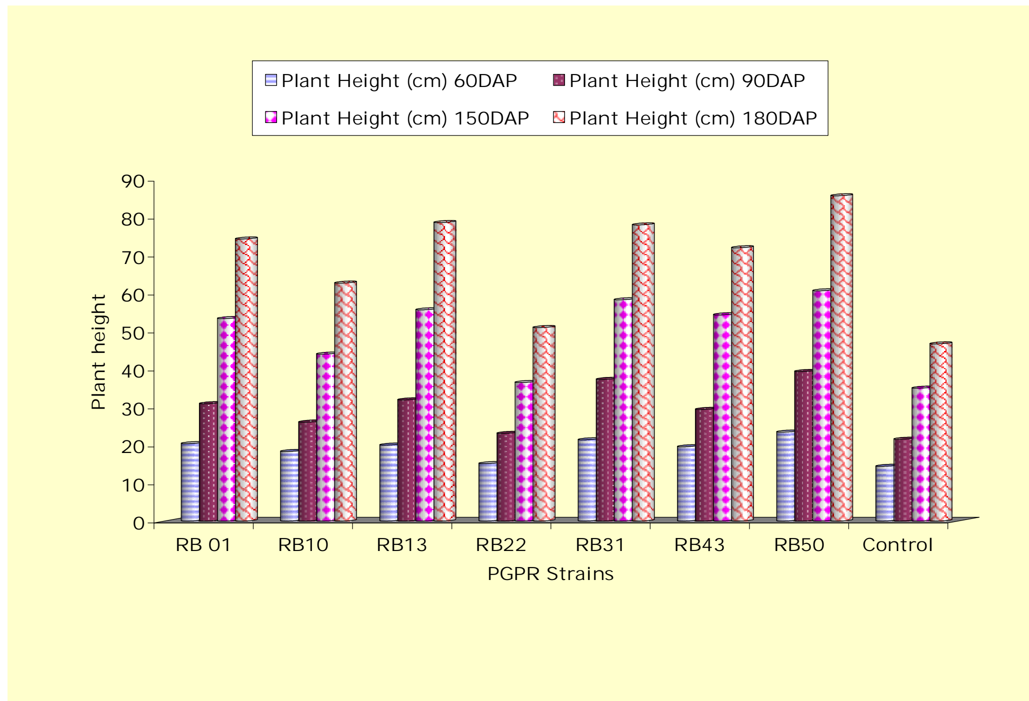


Fig 9. Effect of talc formulations of PGPR strains on the plant growth characters in coleus under field condition

Table 29. Effect of talc formulations of PGPR strains on the plant growth characters in ashwagandha under field condition

PGPR strains	60 DAP			90 DAP			150 DAP			180 DAP		
	Plant height (cm)	% IOC	No. of branches	Plant height (cm)	% IOC	No. of branches	Plant height (cm)	% IOC	No. of branches	Plant height (cm)	% IOC	No. of branches
RB 01	20.42	41.60	4.33	30.92	43.25	6.40	53.33	52.37	10.33	74.33	59.26	11.07
RB10	18.33	27.11	4.00	26.08	20.85	5.73	44.00	25.71	8.27	62.67	34.28	10.20
RB13	20.05	39.04	4.53	31.92	47.91	6.73	55.67	59.05	9.80	78.67	68.56	11.93
RB22	15.17	5.20	3.53	23.13	7.18	4.80	36.50	4.28	7.73	51.00	9.27	9.60
RB31	21.42	48.54	5.20	37.32	72.93	7.40	58.33	66.65	10.53	78.00	67.13	12.40
RB43	19.58	35.78	4.53	29.43	36.37	6.87	54.33	55.22	9.33	72.00	54.27	11.33
RB50	23.45	62.62	6.60	39.40	82.57	9.53	60.67	73.34	12.20	85.67	83.56	13.80
Control	14.42	-	3.50	21.58	-	4.87	35.00	-	7.13	46.67	-	8.80
SEm±	0.48	-	0.40	1.01	-	0.44	3.27	-	0.77	4.26	-	0.60
CD@ 5%	1.44	-	1.22	3.07	-	1.34	9.92	-	2.32	12.92	-	1.81



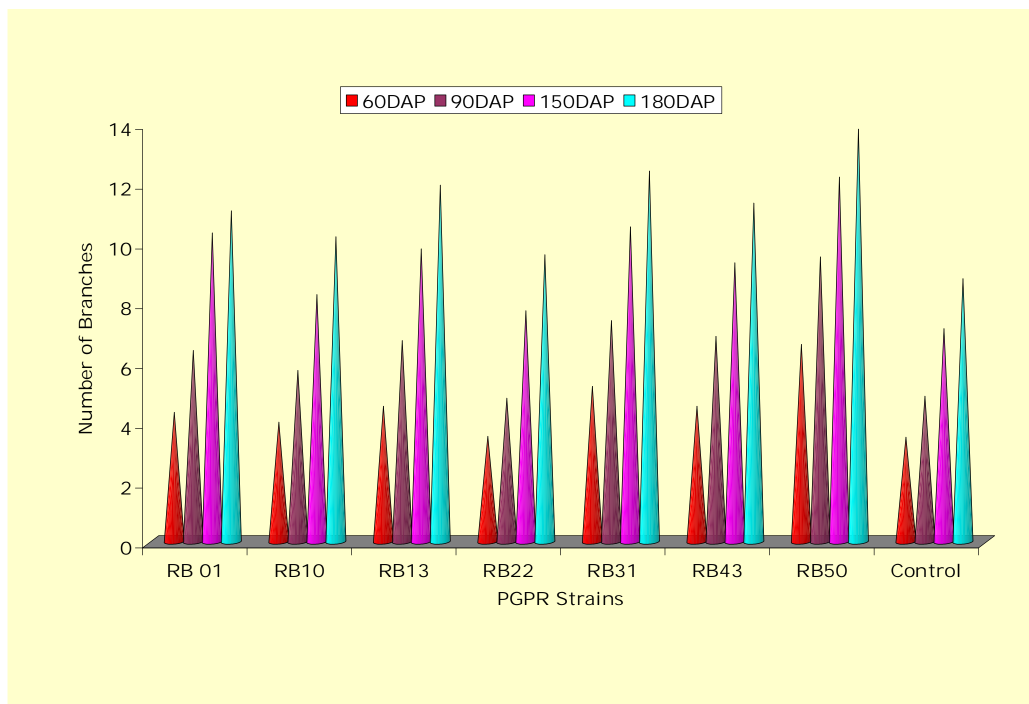


Fig. 10: Effect of talc formulations of PGPR strains on the plant growth characters in ashwagandha under field condition

4.23.1.5 Fresh weight of shoot

Highest shoot fresh weight of 901.00 was recorded in RB50 followed by 822.08 in RB31 treated coleus plants. Significant differences between all the PGPR treated plants were observed. Lowest shoot fresh weight was recorded in untreated control (584.67) (Table 30).

4.23.1.6 Dry weight of shoot

In coleus, highest dry matter accumulation of shoot was recorded in RB50 (138.10 g) followed by RB 31 (128.60) and RB 13 (119.83 g). All the treatments are significant in dry matter accumulation. Lowest dry matter production was registered in untreated control (48.00 g) (Table 30).

4.23.1.7 Total biomass

Significant higher biomass was observed in coleus plant treated with PGPR treatments, both on fresh weight as well as dry weight basis. Highest biomass of 1243.02 g and 212.27 g were recorded on fresh weight and dry weight basis respectively in RB50, followed by 1094.54 and 190.89 in RB31 (Table 30).

4.23.1.8 Root-knot index

In coleus, highest root knot index (RKI) 2.20 was recorded in untreated control followed by RB10 (1.60). Lowest RKI was noticed in RB 50 (0.66), RB31 (0.73) and RB13 (0.80) which showed 70.00, 66.81 and 63.63 per cent reduction in root-knot index respectively. No significant difference was observed among different strains.

4.23.1.9 Disease incidence

In coleus, lowest disease incidence of 10.00 per cent (64.70% reduction over control) was recorded in RB50 followed by 13.33 per cent of RB31 (52.94% reduction over control) Highest disease incidence of 28.33 per cent was recorded in untreated control followed by 25.00 per cent in RB10 treated plants (Table 30, Fig. 11 and Plate 26). In general due to PGPR treatment there was reduction of disease incidence in coleus which varied from 64.70 to 11.75 per cent reduction over control respectively.

4.23.1.10 Yield

In coleus, significant increase in tuber yield was recorded in all the plants treated with PGPR strains. However, highest tuber yield was observed in the treatment RB50 (6.74 kg) followed by RB31 and RB13 (5.45 and 4.60 kg) respectively. RB01 treated plants recorded 418 kg and RB22 treated plants recorded 3.36 kg, whereas RB10 treated plants recorded only 3.25 kg/plot. Lowest tuber fresh weight of 3.00 kg was registered in untreated control (Table 30).

4.23.2 Ashwagandha

4.23.2.1 Root length

Increased root length of 16.83, 16.20, 15.73, 15.57, 15.10 and 14.67 were respectively recorded in RB50, RB31, RB13, RB43 RB1 and RB10 treated plants. There was no significant difference among these treatments. Lowest root length of 11.50 in RB22 treated plant was observed which is on par with untreated control (11.00) (Table 31).

4.23.2.2 Fresh weight of roots

In ashwagandha, highest root weight of 18.65 g was registered in RB50 followed by RB31 (17.13 g), RB13 and RB1: they were statistically on par. Lowest root weight was noticed in RB22 treated plants (10.43) followed by untreated control (Table 31).

4.23.2.3 Dry weight of roots

Highest dry matter accumulation was encountered in RB50 (6.57) followed by RB31, RB13 and RB1 (6.00, 5.8 and 5.67) which are statistically on par. Lowest dry matter accumulation was observed in untreated control (3.25).

4.23.2.4 Fresh weight of shoot

Highest shoot fresh weight was registered in RB50 (206.67) followed by RB31 and RB13 (184.47 and 170.17) respectively. Untreated control recorded lowest shoot fresh weight of 103.33 (Table 31).

4.23.2.5 Dry weight of shoot

Dry matter accumulation of shoot was 85.95 g in RB50 followed by 84.17 g in RB31 which are statistically on par with each other. Lowest dry matter accumulation was registered in untreated control (Table 31).

4.23.2.6 Total biomass

Total biomass of all the treatments were significant. Highest biomass of 225.32 and 87.45 g were recorded in RB50 on fresh weight and dry weight basis respectively. Lowest biomass of 11.86 and 46.92 was recorded in untreated control (Table 31).

4.23.2.7 Root-knot index

In ashwagandha, lowest root-knot index of 0.75 followed by 0.80 was recorded in RB50 and RB31. This two treatments were on par with each other. Highest RKI was recorded in untreated control (3.06) followed by RB10 treated plants (1.93). In general, there was a drastic reduction in RKI due to PGPR treatments compared to untreated.

4.23.2.8 Disease incidence

In ashwagandha, there was significant reduction in disease incidence in PGPR treated plots when compared to untreated plots. In PGPR treatments RB50 recorded the lowest disease incidence of 8.33 per cent, (73.69 per cent reduction over control) followed by RB31 (11.67: 63.15% reduction over control). Highest disease incidence of 31.66 was recorded in untreated control (Table 31, Fig. 12 and Plate 27).

4.23.2.9 Yield

In case of ashwagandha, fresh berry weight and root yield were taken into account. Highest berry weight of 97.00 g/plant was recorded in RB50 followed by 87.38 g/plant on RB31. Lowest berry weight of 35.13 g/plant was recorded in untreated control.

Highest fresh weight of ashwagandha roots was recorded in RB50 (373 g/plot) followed by RB31 (354.6 g/plot) and 319.6 g/plot in RB13 treated plots respectively. Lowest root yield of 185.6 g/plot was registered in untreated control (Table 31).

Table 30. Efficacy of talc formulations of PGPR strains on yield parameters and disease incidence in coleus under field conditions

PGPR strains	Tubers/ plant	Tubers			Shoot weight (g)		Total Biomass (g)		Root knot index	Per cent disease incidence	Per cent decrease over control	Tuber Yield (fresh wt. kg/ plot)
		Length (cm)	Fresh wt. (g)	Dry wt. (g)	Fresh	Dry	Fresh	Dry				
RB 01	15.25	22.45	209.27	36.78	755.17	109.83	9.64.44	127.61	1.13	16.67 (24.03)*	41.05	4.18
RB10	12.87	19.33	173.30	31.50	649.83	68.05	823.13	99.55	1.60	25.00 (29.91)	11.75	3.25
RB13	16.63	23.17	230.01	50.02	786.73	119.83	1016.74	169.85	0.80	20.00 (26.44)	29.40	4.60
RB22	11.87	18.57	168.17	31.17	613.67	57.43	811.84	88.6	1.53	23.33 (28.65)	17.64	3.36
RB31	16.50	29.28	272.46	62.29	822.08	128.60	1094.54	190.89	0.73	13.33 (21.32)	52.94	5.45
RB43	14.92	19.58	198.00	36.33	643.83	76.17	841.83	112.50	1.33	18.33 (25.29)	41.05	3.95
RB50	18.00	31.68	342.02	74.17	901.00	138.10	1243.02	212.27	0.66	10.00 (18.42)	64.70	6.74
Control	10.70	17.59	145.77	30.00	584.67	48.00	730.44	80.00	2.20	28.33 (32.12)	-	3.00
SEm±	0.50	0.58	0.58	1.19	3.72	2.17	4.83	2.16	0.21	1.65		0.26
CD@ 5%	1.52	1.75	1.75	3.59	11.32	6.59	14.66	6.55	0.65	5.02		0.79

*Figures in the parenthesis are arc sine transformed values

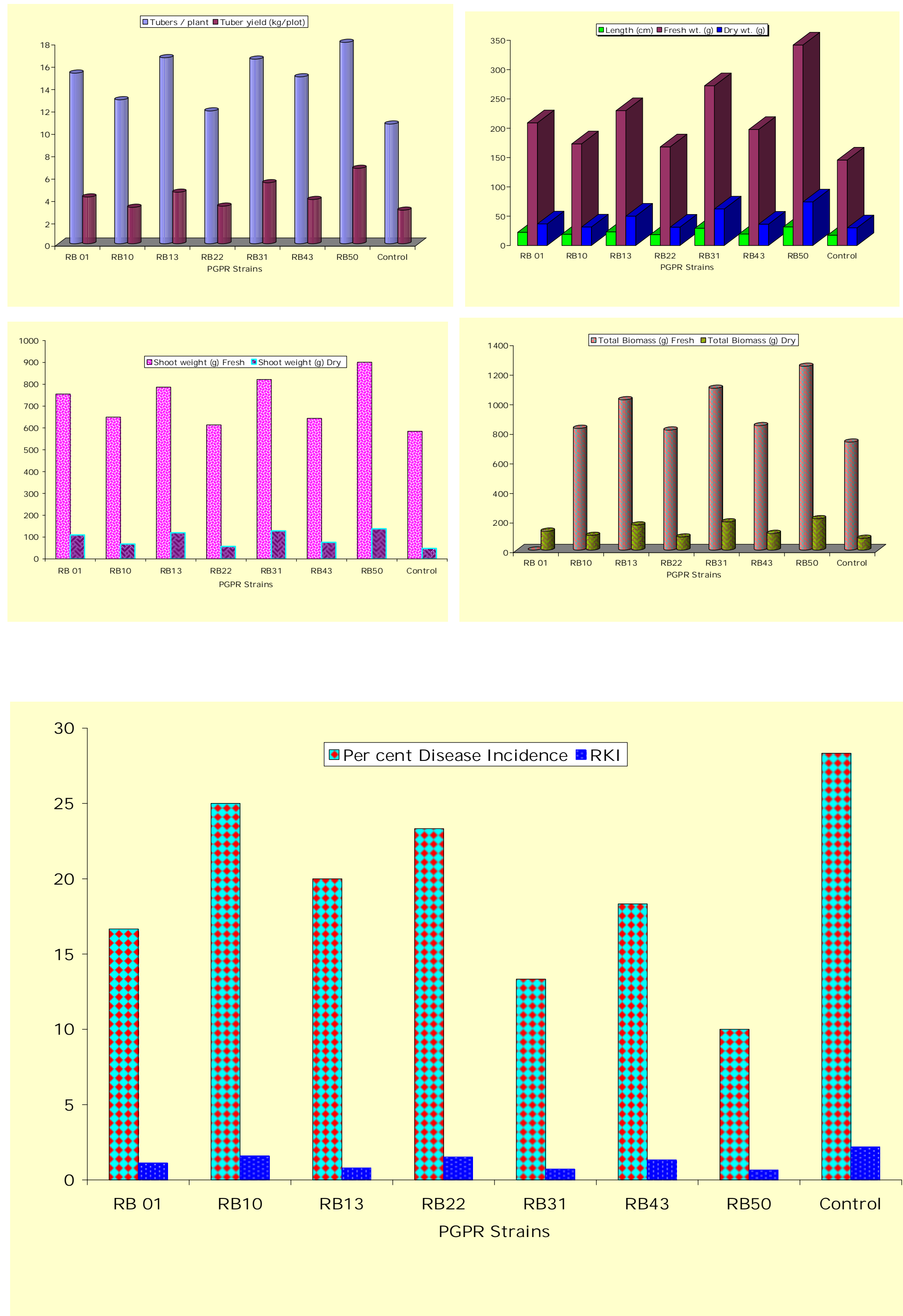
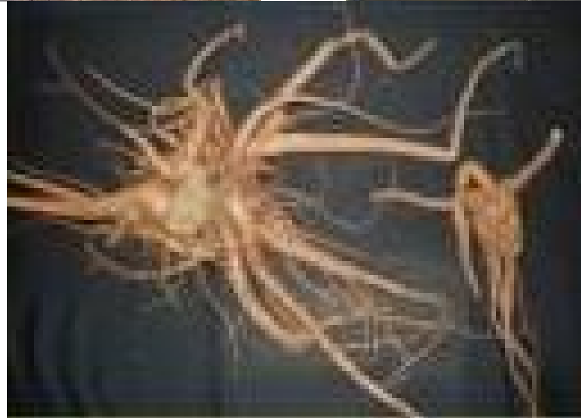


Fig. 11: Efficacy of talc formulations of PGPR strains on disease incidence in coleus under field conditions

General view of field experiment



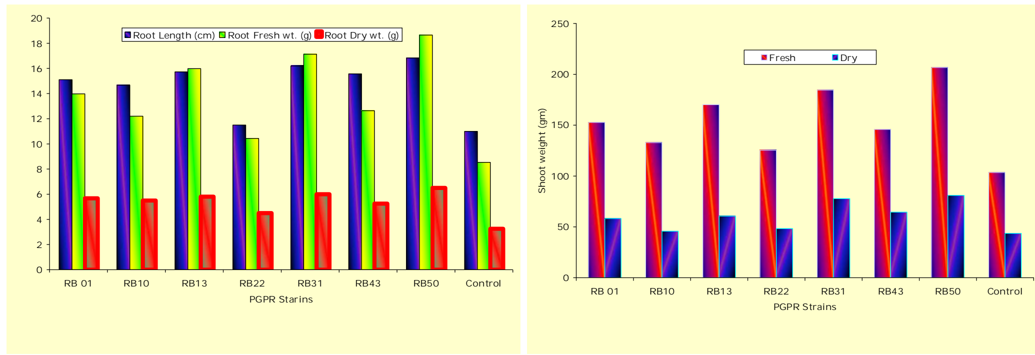
RB50 **Control**

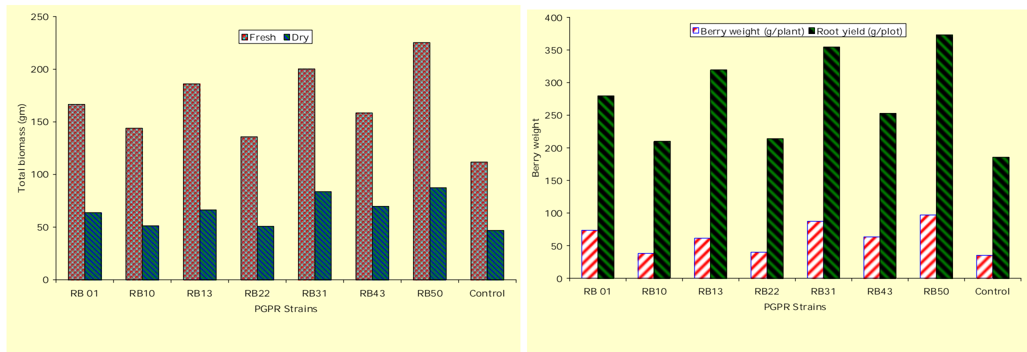
Plate 26. Efficacy of talc formulation of PGPR strains in coleus under field conditions

Table 31. Efficacy of talc formulations of PGPR strains on yield parameters and disease incidence in ashwagandha under field conditions

PGPR strains	Root weight (g)			Shoot weight (g)		Total Biomass (g)		Root knot index	Per cent disease incidence	Per cent decrease over control	Berry wt. (fresh wt. g/ plant)	Root yield (fresh wt. g/ plot)
	Length (cm)	Fresh (g)	Dry	Fresh wt.	Dry wt.	Fresh	Dry					
RB 01	15.10	13.98	5.67	152.67	58.17	166.65	63.84	1.33	13.13 (21.32)*	58.54	73.56	279.67
RB10	14.67	12.20	5.50	132.80	45.80	144.00	51.30	1.93	26.67 (31.05)	15.78	38.19	210.00
RB13	15.73	15.98	5.80	170.17	60.60	186.15	66.40	1.00	16.67 (24.03)	47.36	61.32	319.60
RB22	11.50	10.43	4.50	125.33	48.33	135.83	50.83	1.46	23.33 (28.84)	26.33	40.00	214.00
RB31	16.20	17.13	6.00	184.47	77.78	200.20	83.78	0.80	11.67 (19.87)	63.15	87.38	354.60
RB43	15.57	12.64	5.25	145.83	64.50	158.47	69.75	1.40	18.33 (25.29)	42.12	63.44	252.80
RB50	16.83	18.65	6.50	206.67	80.95	225.32	87.45	0.75	08.33 (16.59)	73.69	97.00	373.00
Control	11.00	8.53	3.25	103.33	43.57	111.86	46.92	3.06	31.67 (34.21)	-	35.13	185.60
SEm ±	0.79	0.80	0.35	2.67	1.88	6.47	2.94	0.32	1.38		3.01	5.91
CD@5%	2.39	2.43	1.07	8.11	5.71	19.62	8.91	0.97	4.19		9.14	17.92

*Figures in the parenthesis are arc sine transformed values





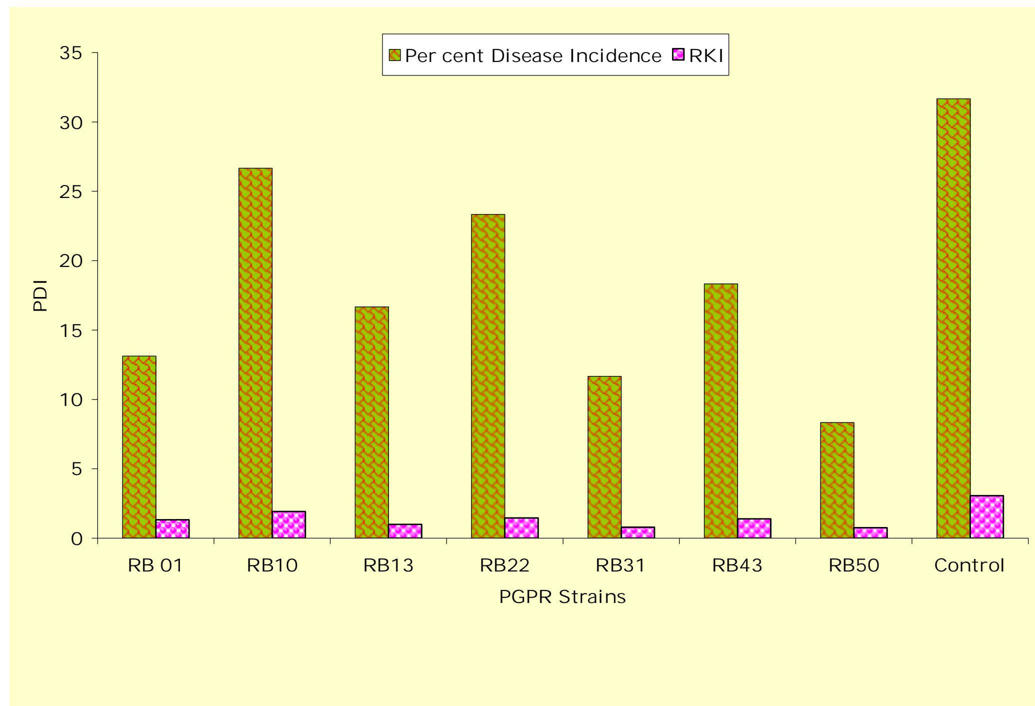


Fig. 12 : Efficacy of talc formulations of PGPR strains on disease incidence in ashwagandha under field conditions.

General view of field experiment

Plate 27. Efficacy of talc formulation of PGPR strains in ashwagandha under field conditions

5. DISCUSSION

Coleus and ashwagandha are widely cultivated for their tuberous roots as major sources of forskolin and withaferin which are extensively used in ayurvedic medicines. Soil borne pathogens causing wilt complex are the major constraints in the cultivation of these two important medicinal plants. The complex occurs in several parts of India wherever these crops are grown. According to Fawcett (1931), “nature does not work with pure cultures” and several plant diseases are manifested by associated organisms which result in disease syndromes of complex nature. Many plant parasitic nematodes involve themselves in diseases caused by fungi, bacteria and viruses and contribute to the losses attributed to the better known pathogens. The combination of nematodes and other soil-borne plant pathogens often results in synergistic interaction wherein the crop loss is greater than expected from either of the pathogens acting alone or due to an additive effect of the two or more together (Francl and Wheeler, 1993).

Among several nematodes of economic importance, root-knot nematodes are most widely studied and are commonly found involved in synergistic interactions with wilt inducing fungi and bacteria. Association of *Fusarium chlamydosporum*, *R. bataticola*, *R. solanacearum* with *M. incognita* in coleus and *F. solani* with *M. incognita* in respect of ashwagandha have been recorded in literature.

Bacterial wilt caused by *R. solanacearum* is a serious disease resulting in losses upto 90.6 per cent in extreme cases (Kishun, 1987). There are no effective control measures for these diseases especially complexes. Microbial control of soil borne disease is economically viable and ecofriendly, aimed at sustainable and organic agriculture. At this juncture, recent quests for effective, safe and lasting disease management programmes have been targeted primarily towards the development of new and better products to replace conventional toxic pesticides. Amongst the microorganisms, plant growth promoting rhizobacteria (PGPR) hold a great promise with the dual advantage of plant growth promotion and disease suppression. Among PGPR, *Pseudomonas* sp. have received a greater attention. They have the ability to utilize a wide assay of compounds as their carbon and energy sources. And, they are known to possess diverse mechanisms of biocontrol. For effective control of soil borne pathogens in coleus and ashwagandha especially nematodes, there is a need to isolate efficient rhizobacteria, preferably from the same environmental niche, in which they will be used. Such isolates will be more ecologically fit than the introduced strains. Hence in the present study, an attempt was made to isolate, screen and select *native* rhizobacteria antagonistic to *Fusarium, Ralstonia* and *Meloidogyne* to study their plant growth effects and to elucidate their mechanism of biocontrol. The results obtained during the experimentation are discussed hereunder.

5.1 SURVEY FOR MAJOR COLEUS AND ASHWAGANDHA GROWING AREAS FOR THE INCIDENCE OF WILT COMPLEX IN KARNATAKA

In general, root-knot disease caused by *Meloidogyne* spp. is one of the major constraints to agriculture. Very little information is available with respect to the incidence and severity of the disease on coleus and ashwagandha in Karnataka. Hence, a survey was undertaken to assess the wilt complex incidence in coleus and ashwagandha in Bijapur, Belgaum, Dharwad, Uttara Kannada, Tumkur and Bangalore districts.

The findings of the present study revealed that the wilt complex of coleus and ashwagandha was observed in all the districts surveyed. But overall disease incidence was more in Belgaum district (39.55%). However, high root knot nematode population was recorded in GKVK followed by Hesaragatta (Bangalore).

The present survey also indicated that the root knot disease caused by *M. incognita* was observed in all the districts surveyed as well as its the association with *F. chlamydosporum* in most of the locations surveyed with a high frequency of both the pathogens (*M. incognita* + *F. chlamydosporum*) from soil and root samples collected.

During survey, diseased samples were collected from different locations and pathogen(s) were isolated. *F. chlamydosporum* and *M. incognita* were isolated from all most all the locations collected and thus they proved to be the most predominant pathogens in coleus and ashwagandha. *Ralstonia solanacerum* was noticed only from the southern districts of Karnataka *i.e.* Tumkur and Bangalore. This indicates that there is a slow spread of bacterial wilt in Karnataka on coleus.

In ashwagandha the highest disease incidence (30.33%) was observed in Dharwad district. Only *M. incognita* and *F. solani* were found to be predominant pathogens. Similar survey carried out by Ramaprasad (2005) in north Karnataka revealed that *F. chlamydosporum* and *M. incognita* were more predominant in coleus whereas Vijayakumari (2004) surveyed in south Karnataka and reported the incidence of *R. solanacerum* in coleus. Verma and Sharma (2008), conducted a survey in Safed musli growing areas of Rajasthan. Both reported a severe root rot caused by *Fusarium solani* and *Rhizoctonia solani.*

5.2 ISOLATION OF PGPR STRAINS

Isolation of PGPR strains from different locations help to identify the organisms that are suited to varied nutrient and environmental conditions. In the present study, fifty rhizobacterial strains were isolated from six districts of Karnataka in coleus and ashwagandha rhizosphere, *viz.* Bijapur, Belgaum, Dharwad, Uttara Kannada, Tumkur and Bangalore. Among the places surveyed¸ highest rhizobacterial population was observed in Bijapur and Belgaum districts. Similarly, most of the research workers have isolated fluorescent pseudomonads from varying ecosystems. Among many of the fluorescent pseudomonads, *P. fluorescens* was isolated predominantly from natural soils for the management of soil borne and foliar diseases (Weller *et al*., 2002; Zehnder *et al*., 2000; Singh *et al*., 2000). A similar study conducted by Jonathan *et al.* (2005) to isolate rhizobacteria in the rhizosphere of betelvine in Tamil Nadu, revealed the presence of fluorescent pseudomonads and other beneficial rhizobacteria.

Karthikeyan *et al.* (2007) isolated rhizosphere, non-rhizosphere and diazotrophic microbial populations from commercially grown *Coleus forskohlii*, *Aloe vera, Ocimum sanctum* and *Cartharanthus roseus* in Tamil Nadu.

5.3 SYMPTOMATOLOGY

F. chamydosporum inoculated coleus plants exhibited symptoms after 30 days. The infected plants were well characterized by gradual yellowing and drying of leaves followed by loss of vigour and pre-mature death. Such plants showed discolouration of roots and complete decaying of root system. Such affected plants were killed finally due to severe rot. The infected tubers showed rotting and emitted bad odour. The symptoms produced by the pathogen were found to be in agreement with Shyla (1998), Sachidananda (2005) and Ramaprasad (2005).

Bacterial wilt caused by *R. solanacerum* exhibited wilting symptoms within 20 days after inoculation in coleus. Water soaked patches with linear streaks on collar region of the infected plants were observed. Leaves became flaccid and drooped quickly, some leaves rolled up and whole plant dried. Wilted plant came of easily with a gentle pull and vascular discolourations were observed. Similar symptoms were observed by Vijayakumari (2004) in coleus infected by *R. solanacearum*.

Root-knot caused by *M. incognita* showed stunted and chlorotic symptoms. Irregular galls were seen on the roots. Similar symptoms due to *M. incognita* on coleus and ashwagandha were also observed by Haseeb *et al.* (2000), Pandey and Kalra (2003).

5.4 PATHOGENICITY TEST

Pathogenicity tests for *F. chlamydosporum*, *R. solanacearum* and *M. incognita* for coleus and *F. solani* and *M. incognita* for ashwagandha were carried out. The symptoms were similar to that described in 5.3.

5.5 ISOLATION OF DIFFERENT PATHOGENS

The predominant pathogens involved in coleus wilt complex are *F. chamydosporum*, *R. solanacearum* and *M. incognita* in coleus and *F. solani* and *M. incognita* in ashwagandha. These were isolated and further identified based on morphological characters. Shyla (1998), Boby and Bagyaraj (2003), Sachidananda (2005), Ramaprasad (2005) also isolated *F. chlamydosporum* by following standard tissue isolation method. The fungus produced abundant macroconidia which are curved, three to five septate, microconidia were fusiform to clavate with rounded apex, with single septum. Chlamydospores were produced terminaly or intercalary, globose to oval smooth or rough walled. Fungus produced whitish to pink colonies with wooly and abundant mycelium on potato dextrose agar. Growth rate was rapid. The description of the fungus agreed with the description given for *F. chlamydosporum* by Commonwealth Mycological Institute (CMI), Kew, Surrey, England (Booth, 1971), Shyla (1998) and Boby Bagyaraj (2003) in coleus. The fungus isolated from ashwagandha was striate, dense and white to light brown in colour. Macroconidia were hyaline, cylindrical to falcate with 2-4 septa, apex macroconidia were pointed and beaked. Conidiophores were elongated and sparsely branched. The chlamydospores were globose to oval, smooth and rough walled and the fungus was identified as *F. solani*.

5.6 ESTIMATION OF PLANT PARASITIC NEMATODES ASSOCIATED WITH COLEUS

Soil and root samples collected from 17 different locations of Karnataka revealed the presence of *Aphelenchus* spp., *Criconema* spp. *Helicotylenchus* spp. *Meloidogyne* spp. *Pratylenchus* spp., *Rotylenchulus* spp., *Tylenchus*-like PPN, *Xiphinema* spp. and other dorylaimid PPN.

The root-knot nematode *M. incognita* was found to be the most predominant species in the samples collected followed by *Pratylenchus* spp. The higher density of nematodes especially *M. incognita* may predispose the coleus roots for the infection by other soil borne plant pathogens, which may lead to the aggravated wilt syndrome. The results are in confirmation with the result obtained by Sivakumar and Marimuthu (1984) and Parameshwari (2003) who reported similar nematode genera in their survey of nematode parasites associated with betelvine in Tamil Nadu and Karnataka, respectively.

5.7 IDENTIFICATION OF PREVAILING ROOT-KNOT NEMATODE SPECIES IN KARNATAKA

The perennial pattern of the prevailing root-knot nematode species in coleus and ashwagandha from Karnataka characteristically showed high squarish dorsal arch, absence of lateral ridges in lateral field, marked by breaks and forks in striae. Striae were coarse, smooth to wavy and the tail terminus contained a distinct whorl (Table 4 and Plate 10). These findings are in confirmation with those mentioned by Eisenback *et al.* (1981) for the root knot nematode *M. incognita*. By studying perineal patterns of these isolated populations which showed high squarish dorsal arch, zig-zag striae around the vulva, it was seen that these characters were very similar to *M. incognita*. Thus, the prevailing pathogen was identified as *Meloidogyne incognita*.

5.8 EFFECT OF DIFFERENT INOCULUM DENSITIES OF *M. incognita* ON COLEUS AND ASHWAGANDHA

Inoculation experiments in glasshouse was conducted to study the host suitability for nematode reproduction and host parasite interactions. In the present study, a progressive decrease in plant height was observed with increasing inoculum levels. An inoculum level of 100 or more J_2/plant was pathogenic on coleus and ashwagandha, causing significant reduction in shoot length, root length and plant weight (fresh and dry) (Tables 7, 8, Plates 13 and 14). Similar results were recorded in blackgram (Kalita and Phukan, 1993). Plants inoculated with 10 J_2 showed significantly increased shoot and root height, weight and total biomass compared to uninoculated control. This was in accordance with the results obtained

by Patnaik and Das (1986) in edible coleus and Pankaj and Siyanand (1990) in bitter gourd and round melon. Rajendran and Vadivelu (1991), Senthamarai *et al.* (2006) observed that the lower inoculum levels increased the shoot and root weight when compared to uninouclated check on *C. forskohlii* infected with *M. incognita*. In general, it has been found that lower levels of inoculum cause a spurt in plant growth as evidenced by Lingaraju and Goswami (1993). Increase in root weight was partly due to presence of undeteriorated galling and partly due to root proliferation as two or more lateral roots were observed to emerge at the gall sites and accumulation of nutrients (Pankaj and Siyanand, 1990).

An inoculum level of 10 J_2/plant was sufficient to induce gall formation in *C. forskohlii* and *W. somnifera*, but 100 J_2 or more per plant produced more galls. Similar results were previously obtained by Patnaik and Das (1986) in edible coleus. Highest RKI was observed at 1000 and 5000 J_2/plant but nematode population per 200 cc soil at maximum inoculum level was less compared to low inoculum level which might be attributed to the competition among the nematode juveniles for food and space. This was in conformity with the results of Senthamanarai *et al.* (2006).

5.9 *IN VITRO* SCREENING OF PGPR STRAINS AGAINST *Fusarium chlamydosporum*

Fifty rhizobacterial strains isolated were screened by dual culture method against *Fusarium* sp. All the isolates significantly inhibited the growth of the fungus. The per cent inhibition varied from 7.77 to 86.11, however 19 strains were highly efficacious in inhibiting the pathogen to an extent of 75 per cent or more. Similar findings have been reported by Kanika Sharma *et al.* (2007) who reported mycelial inhibition of *F. moniliformae*, *Alternaria solani* and *Helminthosporium halodes* due to production of antifungal secondary metabolites by *P. aeruginosa*. Due to the presence of iron chelating ability, siderophore producing bacteria inhibit harmful microorganisms by competing for iron and thus reduces the levels of freely available ferric ions (Kloepper *et al*., 1980; Davision, 1986). Furthermore, chemically, siderophore are phenolic compounds, which are antimicrobial in nature and may be responsible for antifungal activity of the test strain. The mycoparasitic potential of *Pseudomonas* spp. is well documented (Keel and Defago, 1997). Thus, this phenomenon has often been used as means for *in vitro* screening of biocontrol agents (Elad *et al*., 1980). In the present study, RB50 and RB13 showed maximum mycelial inhibition of *Fusarium* spp. *in vitro*.

5.10 *IN VITRO* SCREENING OF ANTAGONISTIC RHIZOBACTERIA AGAINST *R. solanacearum*

A total of fifty rhizobacterial strains were screened against *R. solanacearum* under *in vitro*. Seventeen strains were found to be potent antagonists. Various research workers also demonstrated the role of rhizobacteria in the inhibition of *R. solanacearum* and the mechanisms with which they bring about the beneficial effect. Wei *et al.* (1994); Mulya *et al.* (1996); Chao *et al.* (1997) reported the inhibition of *R. solanacearum* from *P. fluorescens* was due to antibiotics and siderophore production.

5.11 EFFECT OF CULTURE FILTRATES OF PGPR STRAINS AGAINST *M. incognita*

In the present study, *in vitro* bioassay with cell free culture filtrates of fifty PGPR strains at different concentrations revealed an increased juvenile mortality and egg hatching inhibition with increase in exposure period as well as concentration. *M. incognita* juveniles and eggs were highly vulnerable to the native PGPR strains. Among the fifty PGPR strains tested, seventeen strains showed significantly higher larvicidal and ovicidal action on *M. incognita* juveniles and eggs respectively.

Reduction in mobility and viability of juveniles and eggs of *M. incognita* are induced by secondary metabolites such as 2, 4-diacetylphlorglucinol (DAPG), pyrolnitrin, tropolone, pyocyanin, phenazines and lytic enzymes which are produced in culture filtrates of *P. fluorescens* (Elsherif and Grossmann, 1996; Dunne *et al*., 1998). Similar toxic property of *P.*

fluorescens culture filterates was also reported on the juvenile mortality of *M. incognita* and *Heterodera cajani* (Gokte and Swarup, 1988). The juvenile mortality and egg hatching inhibition of *M. incognita* observed in the present study might be due to antibiosis.

5.12 EFFECT OF PGPR STRAINS ON SEED GERMINATION AND SEEDLING VIGOUR *IN VITRO*

In the present study, RB50 and RB31 treated seeds were found to increase the seed germination and the vigour index of ashwagandha significantly when compared to untreated control both in roll towel and pot culture method. The observation made in the present study corroborate the observations made by Ramamoorthy *et al.* (2002) and Kavitha (2005) with other native strains, where enhanced germination and seedling vigour were recorded in tomato and hot pepper respectively due to seed treatment with *P. fluorescens* and *B. subtilis*.

5.13 GROWTH PROMOTION BY PGPR STRAINS IN COLEUS AND ASHWAGANDHA PLANTS IN GLASSHOUSE AND FIELD CONDITIONS

The pot and field experiments conducted in the present investigation on growth promotion in coleus and ashwagandha revealed significant increase in plant growth parameters, *viz.* plant height, shoot and root weight, tuber length, number of branches and total biomass on fresh weight and dry weight basis in plants treated with RB50 and RB31.

The mechanisms of growth promotion by fluorescent pseudomonads are complex and appear to compare both changes in the microbial balance in the rhizosphere and alteration in the host plant physiology (Glick *et al*., 1999).

Significant increases in plant growth parameters in the present study may be attributed to the production of plant growth regulators such as auxins, gibberellins, cytokinins and ethylene (Frankenberger and Arshad, 1995). It has often been inferred that rhizobacterially produced auxins are responsible for growth promotion. Indole acetic acid promotes ethylene production by stimulating the enzyme in the ethylene biosynthetic pathway (Kende, 1993).

5.14 BIOCONTROL POTENTIALITY OF PGPR STRAINS IN COLEUS AND ASHWAGANDHA

Pot culture tests were conducted to know the interaction among the pathogens. *F. chlamydosporum, R. solanacearum* and *M. incognita* on coleus and *F. solani* and *M. incognita* on ashwagandha are potential pathogens. Due to application of efficient PGPR strains the activity in expressing the symptoms as well as severity of the disease of fore mentioned pathogens was reduced.

Individual pathogens inoculated to coleus plants showed varying results. The results indicated that when plants inoculated with *R. solanacearum* showed early symptoms within 20 days. Plants inoculated with *F. chlamdosporum* exhibited yellowing and drying symptoms within 30 days of inoculation whereas in ashwagandha, *F. solani* inoculated plants exhibited the symptoms of wilting and root rot within 25 days after inoculation. The symptoms developed late in case of inoculation of *M. incognita* alone in coleus and ashwagandha.

In the present investigation, it is inferred that *R. solanacearum* alone inoculated plants showed early symptoms and caused maximum disease severity followed by *F. chlamydosporum.* These results are in accordance with the findings of Vijayakumari (2004), Shyla (1998), Ramaprasad (2005) who conducted a pathogenicity study with *R. solanacearum* and *F. chlamydosporum* on coleus and proved that they caused early symptom and maximum disease incidence. The plants inoculated simultaneously with the pathogens *F. chlamydosporum* + *R. solanacearum* + *M. incognita* exhibited the most early symptoms within 15 days and recorded more disease severity and root rot when compared with other combinations and PGPR treatments. Interaction of *Meloidogne* with *Fusarium* and *Ralstonia*

lead to synergistic effect. All the organisms were pathogenic in independent inoculations. But combined infection resulted in rapid drying of the shoot followed by root in control, which was further delayed and protected for long time in PGPR treated plants.

In artificial inoculation tests, it has been demonstrated that maximum rotting occurred only when *R. solanacearum* was inoculated followed by *F. chlamydosporum* and *M. incognita*. Since environmental conditions required for the fore mentioned pathogens are different, it is possible that a succession of the two/more pathogens occur in the same field. This possibility is particularly interesting since agroclimatic conditions are not uniform throughout the crop season. Periodic isolation of pathogens from the same field is necessary to test this hypothesis.

The reduction in number of root-galls and decrease in soil nematode population in treatment where *Fusarium* and *Ralstonia* were also present along with nematode, suggests that *Fusarium* and *Ralstonia* were inhibitory to nematode multiplication. Similar type of reduced root-knot index in the presence of wilt fungi had been reported by Powell (1971) and Pathak *et al*. (1999). This is probably due to the adverse effect on nematode penetration and direct invasion of giant cells by the fungi and bacteria disrupting nematode feeding and subsequent reproduction. Root rot incidence was significantly higher in simultaneous inoculation with nematode and fungi or bacteria. This might be due to the predisposition of plants by the nematode to the fungus and bacterium. It was evident that the nematode acted as a predisposing factor for entry of the pathogen by causing injury on the root surface as well as weakened the root tissues by causing rotting or lesions. Thereby the soil-borne pathogens had easy access for causing greater damage as reported by Schindler *et al.* (1960).

Aggravation of disease was due to preinfection by nematode which predisposes the crop for secondary infection. This is in conformity with the findings of Mani and Sethi (1987) who suggested that the nematode provide a congenial atmosphere either by facilitating the entry of fungi or bacteria, into roots through the openings or modifying the substrate or by producing stimulants in the form of secretions which help the multiplication of fungi. Sumer Jan and Khan (2002) reported that the high incidence in the presence of nematode might be due to the physiological changes in the host induced by the nematode infection and resulting in accumulation of carbohydrates and amino acids. Such an environment would be favourable to fungal and bacterial growth (Borchers and Wyss, 1981).

Application of rhizobacteria caused reduction in hatching, interference with host recognition and invasion as the bacteria envelops or binds the root surface with carbohydrate – lectin (Oostendorp and Sikora, 1990), Mortality of infective juveniles (J2) by producing toxic metabolites and nematicidal components (Spiegel *et al.,* 1991) and alteration of specific root exudates which control nematode behaviour (Racke and Sikora, 1992).

5.15 ELUCIDATION OF MECHANISMS OF PGPR STRAINS

It is necessary to prove the antimicrobial mechanisms of each strains, because the production and especially the quantity of secondary metabolites are often specific for a strain (Fravel, 1988). Hence, seven efficient PGPR strains were tested for the siderophore, HCN, pyocyanin, fluorescein, IAA, volatile metabolites, antimicrobial antibiotics and induced systemic resistance.

5.15.1 Siderophores in biological control

All the tested PGPR strains invariably produced siderophores as evidenced by the formation of yellowish orange halo zone on CAS plates. The strains RB50, RB31 and RB43 were strong producer of siderophores. RB1 and RB13 strains produced small halo zones indicating the low efficiency in siderophore production.

Siderophore-mediated competition for iron by *Pseudomonas* sp. as well as induced resistance are primary mechanisms shown to be responsible for suppression of *Fusarium* wilt (Lynch, 1990). Manwar *et al*. (2004) also demonstrated siderophore based maximum inhibition by *Pseudomonas* sp. against *A. niger*. Similarly, Jagadeesh *et al.* (2001) reported the role of fluorescent siderophore in the biological control of bacterial wilt of tomato. Though

siderophores are part of primary metabolism (iron is an essential element), on occasions they also behave as antibiotics which are commonly considered to be secondary metabolites (Haas and Defago, 2005). Thus, siderophore producing PGPR strains can serve as one of the effective biocontrol agents against plant pathogens compared to non-producing strains.

5.15.2 Hydrogen cyanide (HCN)

HCN production by rhizobacteria has been postulated to play an important role in the biological control of pathogens (Defago *et al*., 1990). All the tested strains were positive for HCN production, which acts as an inducer of plant resistance. RB13 and RB50 were found to be strong HCN producers by changing yellow colour of the filter paper to dark brown to red. HCN production by antagonistic rhizobacteria has been reported by Saxena *et al.* (1996) and Rangeswaran and Prasad (1998). The ability to produce HCN and siderophores has been associated with their role as growth promoters (Schippers, 1993). HCN is known to inhibit the electron transport, disrupting the energy supply to the cells, ultimately leading to death of the pathogen (Knowles, 1976).

5.15.3 Fluorescein and pyocyanin production by PGPR strains

In the present study, most of the PGPR strains were found to produce fluorescein and pyocyanin in the specific *Pseudomonas* agar medium. RB13 showed strong production of fluorescein and strain RB31 and RB50 showed strong pyocyanin production. Similar results were obtained by Rachid and Ahmed (2005) who reported the specific production of fluorescein and pyocyanin by the *Pseudomonas*. Manwar *et al.* (2004) reported that the specific fluorescin, pyocyanin and pyoverdine inhibited the growth of *Aspergillus niger* under *in vitro* conditions. This showed the importance of fluorescein and pyocyanin production in the control of deleterious soil-borne pathogens by fluorescent pseudomonads.

5.15.4 Production of volatile metabolites

The mechanism of antibiosis by the antagonistic rhizobacteria and inhibition of pathogens was more pronounced through volatile metabolites. Volatile toxic substances produced by antagonists could diffuse easily and inhibited the growth of the pathogen in *in vitro* and even in soil they easily diffuse through the air filled pores of the soil and inhibited the soil-borne pathogens.

The present study revealed that production of volatile metabolite, components and inhibition of the test pathogen by volatile metabolites varied among different antagonistic rhizobacteria. The strains RB50, RB31 and RB1 produces more volatile metabolites which inhibited the mycelial growth of *Fusarium* to an extent of 85 per cent or more. Antagonistic potential through volatile metabolites and direct parasitism on pathogen among different isolates of an antagonist PGPR strains also varied.

Hutchinson and Cawan (1972) reported that differences in the amount of growth inhibition of test fungi by gaseous metabolites from different species of *Trichoderma* could be accounted for differences in the rate of production of CO_2 and concentration of acetaldehyde and ethanol. Dennis and Webster (1971); Bandopadhyay *et al.* (2008) also reported acetaldehyde as inhibitory metabolite of *T. viride.*

5.15.5 Antimicrobial antibiotics

In the present study, the cell free extract of seven efficient strains yielded two to five metabolites separated on a TLC plate with different Rf values. The eluted portions of the metabolites showed inhibitory activity in *in vitro* against *Fusarium* sp. This indicates that the antagonistic property of these rhizobacteria is due to the antimicrobial metabolites.

The Rf values of some metabolites were comparable to those reported for other antibiotic producing strains of Pseudomonads (Gurusiddaiah *et al*., 1986; Reddy *et al.,* 2007). The ability of antibiotic positive biocontrol strains to protect plants against a range of soil borne pathogens is well documented. Antibiotic production has been recognized as an

important factor in the suppression of a variety of soil borne pathogens by PGPR (Thomashow and Weller, 1988).

However, in the absence of the standard metabolites and based on the Rf values in the same solvent system, it can be inferred that our strains also probably produced 2, 4-diacetylphloroglucinol (DAPG), pyrrolnitrin, pyoluteorin, oomycin, *etc.*

5.16 INDUCED SYSTEMIC RESISTANCE (ISR)

Induced resistance is a state of enhanced defensive capacity against broad spectrum of pests and pathogens developed by a plant when appropriately stimulated (Van Loon *et al.*, 1998). The resulting elevated resistance due to biotic agents is referred to as ISR whereas that by other than biological control agents is called systemic acquired resistance (SAR) (Zhu-Salzaman *et al.*, 2005).

In our study, we concentrated on biotic (*Pseudomonas*) inducers for inducing the defense molecules challenged with *Fusarium, Ralstonia* and *Meloidogyne* in coleus and only *Fusarium* and *Meloidogyne* in ashwagandha. The ISR in this study was primarily focused for the defense related proteins, *viz.* PO, PPO, PAL and phenols.

The results of the present study revealed that there was significant increase in the activity of PO, PPO, PAL and total phenolic contents in coleus and ashwagandha plants treated with PGPR strains RB50 and RB31. Similar studies, which showed an increase in PO, PPO and PAL activity were reported by Sandeep (2004) and Krishnaveni (2005) in *P. fluorescens* treated banana plants infested with *M. incognita* and *H. multicinctus*.

PO, PPO and PAL are linked to the ISR pathway regulated by jasmonates and ethylene and that is activated by saprophytic microorganisms including rhizobacteria (Van Loon *et al.*, 1998). PAL is the first enzyme in phenylpropanoid metabolism involved in the production of phenolics and phytoalexins that prevent establishment of the pathogens (Daayf *et al.*, 1997). The present study also indicated enhanced activity of PO, PPO, PAL enzymes due to PGPR treatment with RB 50 and RB31, which might have prevented the establishment of nematodes, fungi and bacteria within the coleus and ashwagandha roots.

Jonathan *et al.* (2006) also observed similar increase in plant growth and reduction in *M. incognita* population in banana plants treated with native isolates of *P. fluorescens* and also observed increased activity of PO, PPO and PAL enzymes.

5.17 GENETIC VARIABILITY AMONG THE EFFICIENT STRAINS OF PGPR THROUGH RAPD-PCR

PGPR strains isolated, screened and tested *in vitro* and *in vivo* biocontrol as well as plant growth promotion by diverse mechanisms were difficult to distinguish these species using traditional morphological differences. To understand more variation among the strains a PCR based technique *i.e.* RAPD was used in the present investigation.

In the present investigation, OPA, OPB and OPF series of primers were used to determine genetic distance between trains and to construct a dendrogram. Of the 30 primers tested for amplification, seven primers, *viz.* OPA-01, OPA-10, OPB-02, OPB-03, OPB-05, OPB-06 an OPB-07 showed cent per cent polymorphism.

This information helps us to identify efficient strains with diverse mechanisms directly from the soil, but needs to be conducted several times to get repetitive results. Similarity coefficient ranged from 40 to 84 per cent. The highest similarity was observed between the strains RB31 and RB50.

The dendrogram obtained from the RAPD profiles revealed that the seven efficacious strains were differentiated into two clusters A and B. In cluster A, sub-cluster A4 comprised two strains RB1 and RB10 obtained from Bijapur district. Remaining strains belonged to different clusters obtained from different districts of Karnataka. But the strains RB31 and

RB50, though geographically isolated (coming from a far-off locations - Uttara Kannada and Bangalore) showed 84 per cent similarity.

The results revealed that, the same strains shows morphologically similar (short rods) with similar mechanism of strong siderophore, pyocyanin, IAA and volatile metabolites production. Wiedmann *et al.* (2000); Stafford *et al.* (2005) also demonstrated various molecular methods for subtyping rhizobacterial strains, including pulsed field gel electrophoresis (PEGE), PCR based typing methods (RAPD-PCR), DNA sequence based typing and ribotyping. Similarly, Ramesh Kumar *et al.* (2002) and Barriuso *et al.* (2005) also used PCR-RAPD analysis to PGPR strains which exhibited plant growth promotion and strong antifungal activity. Since differentiation by morphological, biochemical methods was limited.

In the present study also, the results obtained here showed the possibility of using RAPD technique to distinguish variability among the isolates of PGPR strains. The information could then be used to determine specific primers that would allow identification of PGPR strains directly from the soil.

The cell shape and strong siderophores, fluorescein, pyocyanin, IAA and volatile metabolites producing strains *viz.* RB31 and RB50 belong to the group differentiated by RAPD test which have 84 per cent similarity than other isolates. This indicates that the genetic variability revealed through RAPD test is authentic (Khan and Anwar, 2008).

5.18 EFFECT OF BIOFORMULATIONS OF PGPR STRAINS ON DISEASE INCIDENCE AND YIELD IN COLEUS AND ASHWAGANDHA UNDER GLASSHOUSE AND FIELD CONDITIONS

The bioefficacy studies conducted in pot culture and field experiment indicated the efficacy of the talc formulations of PGPR strains in enhancing the plant growth parameters as they exhibited higher antagonistic activity against different pathogens and their combinations. Bioformulations containing RB50 and RB31 effectively reduced the disease incidence and root-knot infestation in coleus and ashwagandha than other treatments and untreated control in glasshouse and field conditions.

The field experiments conducted in the present investigation also revealed a significant increase in plant growth parameters, *viz.* plant height, shoot and root weight, tuber length, number of branches as well as tuber/root/berry yield per plant in coleus and ashwagandha treated with PGPR strains.

Management of insect pests and plant diseases by different *Pseudomonas* strains through different formulations have been reported by many workers (Zehnder *et al*., 1997; Raupach and Kloepper, 1998), Vivekananthan *et al*., 2004). A similar study conducted by Jonathan *et al.* (2000 and 2004) also indicated that the rhizobacteira, *viz. P. fluorescens* and *Bacillus* spp. were reported to induce profuse root development in banana, tomato and betelvine and reduced *M. incognita* population.

Increase in yield under field conditions in RB50 and RB31 treated plants was significantly higher than the untreated control. Similar results were recorded in the studies conducted by Dunne *et al.* (1998), Duffy *et al.* (1996) in rice and Oostendorp and Sikora (1989) in sugarbeet.

5.19 MULTIPLE MODES OF ACTION BY PGPR STRAINS AGAINST MULTIPLE SOIL BORNE PLANT PATHOGENS

The present study revealed that seven strains were highly effective against species of *Fusarium, Ralstonia* and *Meloidogyne in vitro*. They enhanced seed germination and vigour index, plant growth promotion in ashwagandha and coleus. Further they reduced the incidence of the disease and population of pathogen in glasshouse and in field conditions.

The elucidated mechanisms included production of siderophore, HCN, antibiotics, volatile metabolites and ISR activity.

In conclusion, exploitation of some biochemical and molecular tools in this study helped to track the biologically, metabolically and ecologicaly competent seven *Pseudomonas* strains out of 50 strains of PGPR isolated from different parts of Karnataka. The strains, *viz.* RB50 and RB31 performed well against *Fusarium Ralstonia* and *Meloidogyne in vitro* and *in vivo* and also in enhancing the plant growth, reducing the disease incidence, inducing systemic resistance as evidenced by enhanced production of defense enzymes. The chemical pesticides which are used for the different pathogens for the management of diseases are very expensive besides being hazardarous to environment and human health. Hence, the isolates of PGPR obtained in the present study can be further tested for toxicological aspects and mass produced, which might be useful for development of ecologically sustainable biocontrol strategy for the management of several plant pathogens and insect pests, simultaneously in a sustainable manner, so that it can reach the end users.

FUTURE LINE OF INVESTIGATION

1. Biochemical characterization and role of the antibiotics/secondary metabolites derived from the PGPR strains

2. Identifying different mechanisms of action facilitate the combination of strains (consortia), to hit insect pests and pathogens with a broader spectrum of microbial weapons

3. Easy formulations and determine stability, suited to plant colonization

4. Development of transgenic plants that having multiple mechanisms of action of PGPR strains

6. SUMMARY AND CONCLUSIONS

The present investigation was undertaken during the period from 2005-08 at the Department of Plant Pathology, College of Agriculture and Saidapur Farm, University of Agricultural Sciences, Dharwad, which included surveys and surveillance to identify endemic areas or hot spots for wilt complex of coleus and ashwagandha in Karnataka: The specific objectives were to isolate and identify of native PGPR strains from coleus and ashwagandha rhizospheres, screen them against species of *Fusarium, Ralstonia* and *Meloidogyne* causing wilt complex in coleus and ashwagandha and to elucidate their mechanisms of biocontrol. The ability of the strains to promote plant growth and to reduce disease incidence was also assessed in glasshouse and field conditions. The salient features of the findings are summarized below.

1. A random survey conducted in major coleus and ashwagandha growing areas of Karnataka revealed the association of *Fusarium* and *Meloidogyne* throughout the state and *Ralstonia solanacearum* on coleus restricted to only southern districts.

2. A total of fifty PGPR strains were isolated from coleus and ashwagandha rhizospheres in different districts of Karnataka, Bijapur district yielded maximum strains (11) followed by Belgaum (9) district.

3. *In vitro* screening of different PGPR strains against species of *Fusarium, Ralstonia* and *Meloidogyne* resulted in 19, 17 and 17 efficient strains, respectively. Among these strains, seven highly effective strains, commonly efficaceous against all the pathogens were selected and used further.

4. Of the seven efficient strains tested, RB50 and RB31 enhanced the plant growth parameters and recorded higher vigour index in roll towel and pot culture methods in ashwagandha. Seedling bacterization of coleus and ashwagandha also enhanced plant growth parameters in glasshouse condition.

5. Morphological and biochemical characterization of efficient PGPR strains revealed the strains collected from different regions differed. They were identified as *Pseudomonas* spp.

6. Talc-based bioformulations were prepared for the promising strains and their efficacy was tested under pot and field conditions.

7. Mechanisms involved in biocontrol were studied: The results revealed the production of siderphore, HCN, IAA, antimicrobial antibiotics and volatile metabolites from the efficient PGPR strains.

8. The efficient PGPR strains which performed well *in vitro* and showed enhanced plant growth promotion were taken for further studies. Bioformulations containing PGPR strains were evaluated against species of *Fusarium, Ralstonia and Meloidogyne* either individually or in combination for their efficacy to reduce the disease incidence of coleus and ashwagandha under glasshouse condition. Among the various bioformulations, RB50 and RB31 significantly reduced the disease incidence and enhanced the plant growth compared to non-bacterized plants.

9. Induced systemic resistance was assessed through higher activity of enzymes like peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and total phenol in coleus and ashwagandha plants treated with PGPR bioformulations, challenged with *Fusarium, Ralstonia* and *Meloidogyne* individually and in their combinations was inferred to be taking place.

10. Molecular variability of efficient PGPR strains using random primers in RAPD-PCR showed the variability among the antagonistic PGPR strains isolated from different locations. Strain RB50 and RB31 were found to be closely related with 84 per cent similarity obtained from Bangalore and Uttara Kannada. These results are in agreement with the morphological and biochemical characterization.

11. In field conditions, strains RB50 and RB31 were found to be effective in increasing plant growth parameters, *viz.* plant height, number of branches, fresh and dry weight of shoot and root, length of root, yield of coleus and ashwagandha plants and also in decreasing number of galls (RKI) and disease incidence.

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*Original not seen

APPENDIX I

Composition of different growth media/reagents/indicators used

A) Potato dextrose agar (Okon *et al.*, 1977)	
Peeled potato	200.00 g
Dextrose	20.00 g
Agar	18.00 g
Distilled water	1000 ml
B) Nutrient agar (Anon, 1957)	
Peptone	5.00 g
Beef extract	3.00 g
Sodium chloride	5.00 g
Agar	18.00 g
Distilled water	1000 ml
Adjust pH to 6.8-7.2 before adding agar.	
C) King's B agar (King *et al.*, 1954)	
Peptone	16.00 g
$MgSO_4$	1.60 g
K_2HPO_4	1.60 g
Glycerol	10 ml
Agar	18.00 g
Distilled water	1000 ml
Adjust pH to 7.0	
D) Starch agar	
Peptone	5.00 g
Beef extract	3.00 g
Starch solution	10.00 ml
Agar	18.00 g
Distilled water	1000 ml
Adjust pH to 7.2	
E) Iodine solution (For starch hydrolysis)	
Iodine	0.50 g
Potassium iodide	1.00 g
Distilled water	1000 ml
F) Glucose broth	
Peptone	5.00 g
Beef extract	3.00 g
Glucose	5.00 g
Distilled water	1000 ml
BCP solution	15 ml
G) Nutrient gelatin (Cappuccino and Sherman, 1992)	
Peptone	5.00 g
Beef extract	3.00 g
Gelatin	120.00 g
Distilled water	1000 ml
Adjust pH to 6.8-7.0	

H) SIM agar (Cappuccino and Sherman, 1992)	
Peptone	30.00 g
Beef extract	3.00 g
Ferrous ammonium Sulphate	0.20 g
Sodium thiosulphate	0.025 g
Distilled water	1000 ml
Agar	18.00 g
Adjust pH to 7.3 before adding agar	
I) Skimmed milk agar	
Skim milk powder	10.00 g
Peptone	5.00 g
Distilled water	1000 ml
Agar	18.00 g
Adjust pH to 7.2 before adding agar	
J) Tributryn agar	
Peptone	5.00 g
Beef extract	3.00 g
Tributryn	10.00 ml
Agar	18.00 g
Distilled water	1000 ml
Adjust pH to 7.2	
K) Urea broth	
Yeast extract	0.10 g
KH_2PO_4	9.10 g
Na_2HPO_4	9.50 g
Urea	20.00 g
Phenol red	0.010 g
Distilled water	1000 ml
Adjust pH to 6.8	
L) Trypticase soy agar	
Tryptone	17.00 g
Soyatone	3.00 g
Glucose	2.50 g
Agar	18.00 g
Distilled water	1000 ml
M) Sucrose peptone agar (Mehan, 1995)	
Sucrose	
Dipotassium hydrogen phosphate	
Peptone	
Magnesium chloride	
Distilled water	
Agar	
Adjust pH to 7.2	
Just before pouring in to the plates 500 µl of filter sterilized 2, 3, 5- Triphenyl Tetrazolium Chloride (TTC) (10 mg/ml).	

APPENDIX II

Mean monthly meteorological data for the experimental year (2007-08) and the mean of past 57 years (1950 – 2006) of Main Agricultural Research Station, University of Agricultural Sciences, Dharwad

Month	Rainfall (mm)		Temperature (℃)				Relative humidity (%)	
			Mean maximum		Mean minimum			
	2007-08	1950-2006	2007-08	1950-2006	2007-08	1950-2006	2007-08	1950-2006
April	86.40	48.50	36.70	37.35	21.40	19.86	55	75.52
May	65.00	81.06	34.60	33.73	21.30	21.39	61	65.91
June	220.10	112.38	29.70	28.88	21.30	21.47	80	80.95
July	211.20	151.30	27.00	29.09	21.10	20.99	85	87.00
August	176.00	97.17	27.10	26.99	20.50	20.29	85	85.98
September	180.80	103.63	27.20	28.57	20.30	19.89	83	81.91
October	74.80	127.83	29.70	30.07	19.40	18.43	68	75.84
November	54.00	32.62	29.50	30.08	15.10	15.90	53	68.03
December	Trace	5.32	29.00	29.37	14.60	12.52	65	62.96
January	Trace	0.06	30.40	29.62	14.00	14.62	72	62.81
February	00.00	1.09	31.90	32.52	15.70	16.42	67	51.19
March	12.80	0.45	35.30	36.41	19.70	19.56	49	55.81

PLANT GROWTH PROMOTING RHIZOBACTERIA, THEIR CHARACTERIZATION AND MECHANISMS IN THE SUPPRESSION OF SOIL BORNE PATHOGENS OF COLEUS AND ASHWAGANDHA

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ABSTRACT

Major coleus and ashwagandha growing districts of Karnataka were surveyed for the prevalence of wilt/root-knot complex in these crops. Survey revealed the association of *Fusarium chlamydosporum, Ralstonia solanacearum* and *Meloidogyne incognita* with coleus and ashwagandha. As many as fifty native plant growth promoting rhizobacterial (PGPR) strains from healthy coleus and ashwagandha rhizospheres were isolated, maintained and screened *in vitro* against *F. chlamydosporum, R. solanacearum* and *M. incognita* causing wilt/root knot complex. Of them 19, 17 and 17 strains were respectively found to be highly potent antagonists: Among these strains seven highly effective strains commonly inhibitory to all the pathogens were selected and characterized as *Pseudomonas* spp. and used further. When assessed for their mechanism of biocontrol, these potent antagonists produced at least one antimicrobial antibiotic, siderophore, HCN, IAA, fluorescien, pyocyanin and volatile metabolites. Molecular variability through RAPD-PCR showed highest (84 per cent) similarity between rhizobacterial strains RB31 and RB50, though these were isolated from geographically diverse locations.

A greenhouse experiment was conducted to assess the plant growth promotion and vigour index by potent antagonists in coleus and ashwagandha. Talc-based bioformulations were prepared for the promising strains and their efficacy was tested under pot and field conditions. Bioformulations containing PGPR strains were evaluated against species of *Fusarium, Ralstonia* and *Meloidogyne* (either individually or in combination) for their efficacy to reduce the disease incidence and induced systemic resistance in coleus and ashwagandha under glasshouse condition. Among the various bioformulations, strains RB50 and RB31 were found to be effective in increasing plant growth parameters, *viz.* plant height, number of branches, fresh and dry weight of shoot and root, length of root, yield of coleus and ashwagandha plants and also in decreasing number of galls (RKI) and disease incidence in glasshouse and field conditions.