APPROACHES TOWARDS THE INTEGRATED DISEASE MANAGEMENT OF *PHYTOPHTHORA* INFECTION OF BLACK PEPPER (*PIPER NIGRUM* L.)

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BY

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CERTIFICATE

I hereby certify that, the thesis entitled "APPROACHES TOWARDS THE INTEGRATED DISEASE MANAGEMENT OF *PHYTOPHTHORA* INFECTION OF BLACK PEPPER (P. *nigrum* L.) submitted by Mr.Rajan.P.P for the award of the degree **Doctor of Philosophy** in Botany at the University of Calicut, contains the results of bonafide research work carried out by him at Indian Institute of Spices Research, Calicut- 12, under my supervision and guidance. No part of this thesis has been submitted to any other University for the award of any other degree or diploma. All sources of help received by him during the course of this investigation has been duly acknowledged

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DECLARATION

I, Rajan.P.P, do hereby declare that the thesis entitled "APPROACHES TOWARDS THE INTEGRATED DISEASE MANAGEMENT OF PHYTOPHTHORA INFECTION OF BLACK PEPPER (P.*nigrum* L.)" submitted by me for the award of the degree **Doctor of Philosophy** in Botany to the University of Calicut, is a bonafide research work carried out by me at Indian Institute of Spices Research, Calicut-12, under the supervision and guidance of Dr.YR.Sarma, Principal Scientist and Head, Division of Crop Protection, Indian Institute of Spices Research, Calicut-12. This thesis or part of it has not been submitted to any other University for the award of any other degree or diploma. All sources of help received by me during the course of study have been duly acknowledged.

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Rajan PP Rees

CONTENTS

INTRODUCTION

Rajan P.P "Approaches towards the integrated disease management of phytophthora infection of black pepper (Piper nigrum L.)" Thesis. Department of Botany, University of Calicut,1999

INTRODUCTION

PLATE 1a: HEALTHY BLACK PEPPER PLANTATION

PLATE 1b: FOOT ROT INFECTED BLACK PEPPER PLANTATION

7A

1A

Black pepper (*Piper nigrum* L.), the "King of spices" is a native of the Western Ghats of India (Malabar coasts of South Western India) and belongs to the family *Piperaceae*. Black pepper occurs in the hills of Assam and north Burma. It is also grown in Indonesia, Malaysia, Brazil, Sri Lanka, Thailand, Madagascar, Jamaica and China. India is the leading producer and exporter of black pepper. Pepper is cultivated both as monocrop and as mixed crop. *Erythrina indica* is a popular live standard on which pepper is trailed in pure crop system. In mixed cropping, it is trailed on arecanut, coconut and on silver oak, the shade tree in tea and coffee plantations in Kerala, Karnataka and Tamil Nadu states of South India. Kerala ranked first both in area and production (179590 ha and 55,370 tones 1996-97) and Karnataka is the second major producer in India. During 1996-1997, India exported black pepper worth of Rs. 4165.22 lakhs.

Piper nigrum L. is a perennial woody climber with adventitious roots arising from each node which would help to cling on to supporting standards. Plant reaches a height of 3 meter in general and occasionally it may reach upto ten meter or more in height, especially on silver oak standards. The mature vine has a bushy columnar appearance and is about one and half meter in diameter. It prefers the lataritic to sandy loamy soil with a pH ranging from 5 - 6.5. The crop is rainfed in Kerala, but in Karnataka irrigation is given in areca-pepper mixed cropping system.

India had monopoly in pepper production in the world, but now this position has dwindled because of low productivity compared to Indonesia and Malaysia. Diseases and pests are the major production constraints. Thus the efficient and economically viable disease and pest management strategies are the thrust areas to boost up the black pepper production in India.

The production of black pepper is hampered by the diseases both in Indian and other pepper growing countries. Among the serious diseases affecting in pepper, foot rot caused by *Phytophthora capsici* and slow decline are seriously affecting this crop not only in India but

also in other pepper growing countries caused by fungal - nematode complex (*Radophalus similis, Meleidogyne incognita* and *P.capsici*). On global scale an annual crop loss of $4.5-7.5 million has been reported due to foot rot alone (de Waard 1979).

This disease was known as early as in 1902 from India, when severe vine deaths were noticed in Wynad region of erst while Madras State (Menon - 1949). This was investigated by Barber (1902, 1903, 1905) and Butler (1906, 1918) but the investigations were inconclusive and the etiology remained unresolved. Lack of selective media to isolate *Phytophthora* might be the reason why the diagnosis of causal agent was remained unresolved for a long time. Isolation from infected roots and collar of black pepper remained difficult till 1977 (Tsao and Guy 1977). Although the isolation of *Phytophthora* was reported years back from Mysore area (Venkata Rao- 1929), and it was Samraj and Jose (1966) who reported the "*Phytophthora* wilt" of black pepper in Kerala adopting Muller's (1936) identification as *P . palmivora var. piperina.* In India detailed investigations on this disease were carried out only since then (Sarma and Nambiar 1982). The disease has became a night mare to pepper farmers.

All parts of black pepper vine are susceptible to *P. capsici* and this made the disease management most elusive. According to Schwinn (1983), the infections caused by *Phytophthora* belong to three categories namely (1) infection on aerial parts like leaves and fruits (2) infection on shoot, which are locally systemic and (3) infection on crown and roots. *Phytophthora* infections in black pepper can be broadly classified into three categories viz; (1) aerial infection :- infection on runner shoots, leaf , spike and stem (2) collar infection :- infection on the main stem at or just above the soil level (3) root infection :- infection occurs on fine feeder roots ultimately culminating in the collar infection.

Large scale use of agrochemicals to check *Phytophthora* infections in black pepper has been the subject of concern to consumers and especially to the importers. Pathogen being soil borne in nature, any single approach of disease management would be unsuccessful. An integrated approach involving cultural practices, chemical, biological control coupled with

host resistance would be more practical to contain this disease. However the development of field technology for efficient and reliable integrated management of soil borne plant pathogens in general and *Phytophthora* spp. in particular at present is lacking. Therefore an attempt has been made in the present investigation to integrate cultural, chemical (Potassium phosphonate), biological (*Trichoderma virens*, *Verticllium tenerum* and *Trichoderma harzianum*), organic amendment (coffee pulp) , plant extract (Garlic and Mustard), optimum doses of NPK, and host resistance / tolerance (P24) to control the disease.

Since the disease is soil borne in nature , antagonistic micro organisms would play a major role in keeping the population of pathogen below the threshold level . Though there are many reports in using biocontrol organisms in checking soil borne plant pathogens, studies on biocontrol of *Phytophthora* foot rot in black pepper are recent. For better success of biological control, survival and proliferation of antagonists in soil is most important.

Several workers recommended one percent Bordeaux mixture, some systemic fungicides and contact fungicides to control the pathogen. But so far no chemical has been found give complete control of this disease. When fungicides are applied in to the soil to check the pathogen or to reduce the population of different groups of plant pathogens , this might also negatively affect the other beneficial soil micro organisms, which are otherwise support the crop system. Hence there is need of a chemical to kill or reduce the pathogen population to low level as well as cause no harm to other beneficial soil micro organisms. Chemicals like Potassium phosphonate, which are compatible with biocontrol agents would be ideal candidate.

Prophylactic spraying the foliage with Bordeaux mixture and drenching the basing with either Bordeaux mixture or copper oxychloride in May-June (pre-monsoon) and again during August - September (post monsoon) is the recommended package.

Application of soil amendments at the base of pepper vine is a common practice among farmers, which support the growth and proliferation of micro organisms, water holding capacity of soil, suppress the pathogen population and enhance the root regeneration .

PLATE 1c: FOOT ROT – DEVASTATED BLACK PEPPER PLANTATION

PLATE 1d: PHYTOPHTHORA CAUSED FOLIAR INFECTION ON BLACK PEPPER

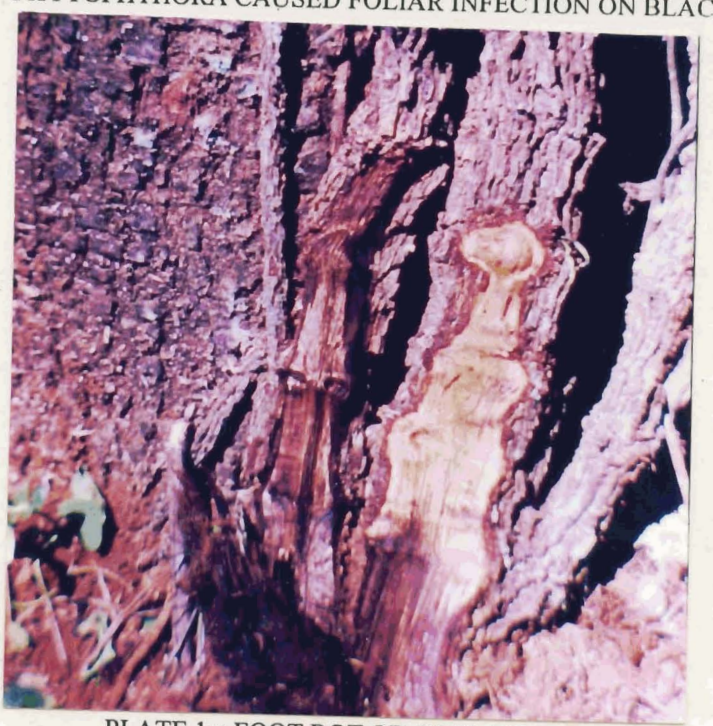


PLATE 1e: FOOT ROT OR COLLAR ROT

Coffee pulp/husk , a bi-product in coffee industry is being used as one of the components in integrated disease management. Nutrition on host and its effect on the disease development has not been studied in black pepper - *Phytophthora* pathosystem. A pot culture experiment was conducted to study the effect of NPK fertilizer on disease incidence.

Even though Western Ghats of India is the center of origin of black pepper, high degree of host resistance has not been identified in any cultivar. An open pollinated seedling progeny black pepper, P24 (Sarma et al, 1996) has been found moderately tolerant foot rot pathogen and this also being included as a component of integrated management, in the present study.

Investigations have been undertaken with the following objectives to develop IDM for foot rot of black pepper

1. Rhizosphere studies of black pepper with respect to the seasonal microbial fluctuation with special emphasis on *Trichoderma* population.
2. Isolation of micro organisms from rhizosphere of healthy black pepper vines from different pepper growing areas of South India, their *in vitro* effects on *P. capsici* to identify their antagonistic potential and their *in vivo* efficacy in pot culture.
3. Use of different soil amendments against root rot pathogen of black pepper.
4. *In vitro* efficacy of botanicals (Garlic and Mustard extract) on different phases of *P.capsici* and their *in vivo* effects (pot culture) on *P.capsici*.
5. *In vitro* efficacy of agro-chemicals on different phases of *P.capsici* and their *in vivo* effects (pot culture) on *P.capsici*
6. Compatibility of *Trichoderma* and *Verticillium tenerum* with Potassium phosphonate, a systemic fungicide
7. Effect of host nutrition on foot rot pathogen of black pepper
8. Integrated approach of the disease by integrating chemical (Potassium phosphonate), organic amendment (decomposed coffee pulp), plant extract (Garlic and Mustard), nutrition and host resistance /tolerance (P 24)
9. Field evaluation of efficient *Trichoderma* spp for its disease suppressiive potential.

REVIEW OF LITERATURE

Rajan P.P "Approaches towards the integrated disease management of phytophthora infection of black pepper (Piper nigrum L.)" Thesis. Department of Botany, University of Calicut,1999

REVIEW OF LITERATURE

Phytophthora foot rot of black pepper, earlier known as "Quick wilt" (Nair and Sarma, 1988) caused by a soil borne fungus *Phytophthora capsici* (= *P.palmivora*- MF_4) (Stamps *et al*, 1990; Sarma *et al*, 1982; Tsao *et al.*, 1985) is a serious pathogen and a major concern for the farming community in all the pepper growing countries including India. Status of this important malady on black pepper is reviewed.

HISTORY AND DISTRIBUTION:

In India, the disease was known as early as in 1902 when severe vine deaths were noticed in Wynad region of erstwhile Madras state (Menon, 1949). Later it was investigated by Barber (1902, 1903 & 1905) and Butler (1906 & 1918) but the investigations were inconclusive and the etiology remained unresolved. The difficulties in isolation of *Phytophthora* in earlier days in the absence of selective madia (Tsao and Guy, 1977) might have been the major factor in correct diagnosis of the causal agent. Although *Phytophthora* in black pepper was recorded during 1929 (Venkata Rao, 1929) the first authentic record of wilt of black pepper due to *Phytophthora* in India was in 1966 (Samraj and Jose, 1966) from Kerala. In Karnataka the disease was noticed in 1929 (Venkata Rao, 1929).

This disease was very serious and destructive in nature in Indonesia a century ago (Muller, 1936; Soepartono, 1953). In Malaysia, the disease was reported as "sudden death" in 1929 (Holl, 1929). Due to the serious outbreak, the research was initiated on the problem in Malaysia during 1952 (Robertson, 1953). Later *Phytophthora* was isolated from diseased pepper vines and was named as *P.palmivora* (Holliday and Mowat, 1957; Holliday, 1960; Holliday and Mowat, 1963). This disease has also been reported from Brazil (Holliday, 1965), Jamaica (Leather, 1967), Thailand (Tsao and Tummakate, 1977) and Malagasy Republic (de Waard, 1979). Recently, the disease has been observed in severe form in Uttar Kannada and Shimoga districts of Karnataka state, India (Sastry, 1982; Hegde, 1983; Dutta, 1984; Hegde and Hegde, 1987)

ECONOMIC IMPORTANCE

In India, Samraj and Jose (1966) recorded the death of pepper vines upto 20 percent and Nambiar and Sarma (1977) reported that, 20 - 30% crop loss in Cannanore and Calicut districts of Kerala. Crop loss survey conducted for three years (1982 - 1984) in Calicut, including Wynad and two years (1985 - 1986) in Cannanore districts of Kerala, has shown that foot rot incidence is causing vine deaths of about 1,88,947 (3.7%) and 10,16,425 (9.4%) amounting to an annual loss of 119 and 905 metric tones of black pepper in Calicut and Cannanore districts respectively (Balakrishnan *et al.*, 1986; Anandaraj *et al.*, 1988b). In Indonesia, up to 20% crop loss has been reported due to this disease (Sitepu and Kasim, 1988).

In Sarawak (1953 - 56), the loss was about 7000 tones amounting to £17 million (Holliday and Mowat, 1963). Ten percent death of vine was reported from West Borneo (Leafman, 1934) due to this disease. In Lampung, an outbreak of foot rot occurred during 1967 - 68, which destroyed 40 - 50% of pepper crop (de Waard, 1979). The overall loss due to this disease in all pepper growing countries was estimated to be $ 4.5 -.7.5 million per annum (de Waard, 1979). This disease appeared in severe form during 1978 in Karnataka state of south India (Dutta, 1984; Sastry and Hegde, 1991).

BIOLOGY AND EPIDEMIOLOGY OF *PHYTOPHTHORA*

BIOLOGY OF *PHYTOPHTHORA*

Kasim, 1978 reported that, the *P.capsici* zoospores get encysted within 2 hours at 5 - 10^{0}C and 35 - 50^{0}C. Maximum germination of zoospores and germ tube growth was noticed at 30^{0}C and least was at 10 and 45^{0}C. In *P.capsici*, thermal death point was high, ranging from 45 - 47^{0}C and this temperature was very critical temperature for the host. According to Alizadeh and Tsao (1985), light plays and important role in the production of sporangia in *P.capsici*. In dark sporangial production was less and they were not easily dislodged from pedicel.

Optimum growth of *P.dreschleri* var *cajani* was obtained at 30^0C, pH 6.5 in Mehrotra's medium (Pal and Grewal, 1976b). Singh and Chauhan (1988) reported that, temperature has got a vital role in the germination of zoospores of *P.dreschleri* f. sp. *cajani*. Chauhan and Singh (1991a), reported that, maximum zoospore germination of *P.dreschleri* f.sp *cajani* noted at pH 7.5 in dark. In *P.dreschleri*, oospore formation was high within 36 hours at 25^0C (Singh and Chauhan, 1988).

EPIDEMIOLOGY

Although the speciation of *Phytophthora* infecting black pepper remained controversial, its taxonomic status as *P.capsici*, Leonian (amend Alizadeh and Tsao, 1983) has been resolved (Tsao *et al.*, 1985). The fungus is soil borne and all the parts of black pepper are prone to infection. Infected plant debris in the soil and infected dried up vines in the gardens appear to be the primary source of inoculum. Since *Phytophtora* being a wet weather pathogen, the activity of the pathogen is association with moisture regimes both in the soil and aerial portions of the vine. Disease starts in the field during the South - West monsoon and continues up to August and later during North - West monsoon during September - October. During the early showers, new tender foliage and tender roots, are highly prone to infection. The early showers and consequent soil moisture would trigger extensive root proliferation, coinciding with the build up of *Phytophthora* propogules in the soil, thus creating highly conducive conditions for disease development. The disease has two important phases, viz; aerial and soil phase (Sarma *et al.*, 1991).

AERIAL PHASE

Due to soil splashes, the tender runner shoots spreading on the ground and the tender leaves at the base of the vine are the first to contact infection, resulting in rotting of shoots or dark brown lesions on the leaves with fast advancing margins. In the presence of free moisture on the leaves, these lesions sporulate abundantly. Due to the intermittent showers, the infection gradually spreads from the lower to the upper regions of the bush, 'hopping' in a 'ladder' like fashion through rain splashes (Ramachandran *et al.*, 1990). In early investigations, Muller (1936) described the appearance of symptoms as concentric lesion

development on leaves. The lesions are gray centers surrounded by alternating dark and light brown zones with peripheral water soaked margins. The zonation appeared due to the prevalence of intermittent wet and dry weather. If wet condition prolonged, no concentric rings appeared. Presence of fimbriate margin at outer, peripheral side of the lesion had been found to be characteristic (Holliday and Mowat, 1963).

In inoculated leaves, symptoms appeared within 24 hours as pale colored water soaked lesions. Lesions coalesced, expanded rapidly, covering the large areas of the lamina. Time taken for lesion development varied from 24 - 48 hours, depending upon the maturity of the leaves. Faster defoliation occurred under low temperature (20 - 24^{0}C) and high ralative humidity (90 - 97%). If unfavorable weather prevails there may be concentric zonations (Nambiar and Sarma, 1977). The infected leaves shed prematurely before the entire lamina is covered by the spots. Spike infection is very common in rainy season - July - September (Oliveira and Pereira, 1983). Tip of the spikes get discolored due to infection (Holliday and Mowat, 1963) and later the entire spike get darkened and fall. Tender berries also get infected. Occasionally, a few berries only showed infection in a spike (Nambiar and Sarma, 1977). Pathogen spread from foliar region to root system through stem resulted in heavy defoliation and death (Holliday and Mowat, 1963; Nambiar and Sarma, 1977). Foliage infections though occur both in pure plantation and also in mixed plantation, they are often noticed in areca-pepper or coconut-pepper mixed cropping system (Sarma *et al.*, 1991&1992). This might be because of the conducive microclimatic condition that prevail under the canopy.

SOIL PHASE

The soil inoculum level decreased from the base of vine with increase in distance and depth (Ramachandran *et al.*, 1986). The distribution of *Phytophthora* inoculum in soil in relation to disease incidence in black pepper has been reported (Sastry and Hegde, 1982). Root infection being under ground, it remains unnoticed and foliar yellowing symptom would appear only after sufficient degeneration of root system. During monsoon, the pathogen build up enhances the rotting of feeder root system leading to vine death. Pathogen enters

the main roots through the feeder root system. When feeder roots decayed, vine starts showing the initial symptoms as yellowing of leaves. The effect of age on root infection, studied under field stimulated microplot conditions clearly brought out that root infection at advanced stages would lead to foot rot leading to vine death (Anandaraj *et al.*, 1994). When the pathogen attack on collar region through the roots, light yellow interveinal chlorosis observed, especially on the upper leaves. This is due to the poor absorption of nutrients and water from the soil. Gradually the whole foliage turned yellow. Even if a portion of root system is healthy, the plant survives with reduced canopy. Rate of root regeneration and root infection determines the speed of decline and death of the vines. After the monsoon, if root system is not enough to support the vine, the vine collapses with wilting and drying of leaves observed (Anandaraj *et al.*, 1988a). Runner shoots or stolons which spreads on the ground from mother plant also play a major role in disease spreading. The creeping stolons get infected and infection advances to the root system later to collar region. Once collar region get infected, the entire vine wilts and defoliation occurs without yellowing (Anandaraj *et al.*, 1988a).

Severe infection on *Piper betle* due to *P.parasitica* var *piperina* obtained at the range of 20 - 24^{0}C (Selvaraj *et al* 1973). According to Maiti and Sen, 1982 the foot rot of betle vine observed at less than 22^{0}C. But Venkata Rao *et al.* (1969) reported that, betle vine wilt appeared continuous day temperature attained minimum at 23^{0}C, and rain fall has not influenced on disease incidence. Selvaraj *et al.* (1973) reported that, wilt disease of betle vine was severe at the soil temperature ranged from 20 to 24^{0}C. Pal and Grewal (1976b) reported that, the maximum number of sporangia were noticed at 25 - 30^{0}C and pH range of 6 - 6.5. Lucas (1965) reported that *Phytophthora nicotianae* var *nicotianae* in tobacco prefer high soil moisture for its growth, but according to Apple (1952) ample moisture was good enough to cause the disease on host. Upto 35% crop loss on betel vine was occurred when there was of 6mm. or above daily rainfall in three districts of West Bengal, India (Maiti and Sen, 1982).

Zentmyer and Mircetich (1966) reported that, *P.cinnamomi* can survive a long time in the

soil under high moisture level. The percentage of rainy days had significant positive correlation with infection index and increase in lesion size of *Phytophthora dreschleri* f. sp *cajani* and it was positively correlated with the mortality of plants (Agrawal and Khare, 1987). Singh and Chauhan, 1985 emphasized the importance of drainage in the field and reported that disease incidence due to *P.dreschleri* f. sp. *cajani* was sever in the field where the water accumulation was more during the rainy season.

Phukan and Baruah (1989a), published that at 20^0 C and at 100% RH was suitable for the growth of *P.infestans* (Mont) de Bary. Bambawale *et al.* (1991) reported that, in Punjab (India) the late blight of potato due to *P.infestans* occurred at less than 20^0C temperature and 80% RH and there was no correlation with rain where dew appeared to be the alternate source of moisture. According to Russell (1969), the ample rain in winter season had devastating effect on the potato crop which were exposed to high humidity and un protected by fungicides.

Reuse of rain water could encourage the spread of *P.cinnamomi* (Brawne, 1987). Liyanage *et al.*, (1983) reported that, a small quantity of water on the surface of rubber pods were enough for proliferation and dissemination of sporangia.

Both high soil salinity and increased water content favored stem rot of citrus root stocks by *P.citrophthora* (Sulistyowati and Keane, 1992). Chlamydospores and oospore production of *P.cactorum* was noticed at 4^0C within 20 days (Darmono and Parke, 1990).

ISOLATION OF *PHYTOPHTHORA*

Phytophthora capsici, the causal organism of foot rot disease of black pepper can be isolated from infected regions. Due to the high phenol content present in root and collar region, the isolation is difficult compared to leaves (Sarma and Nambiar, 1982). Before the discovery of selective medium, the successful isolation was made by using plane agar medium (Holliday and Mowat, 1963). Infected roots and hardy stems, infected materials

kept for leaching in running water for 24 hours (Sarma and Nambiar, 1982) and isolation made successfully by using CMA-PVPH medium (Tsao and Guy, 1977). Addition of 100ppm. of nystatin and 200ppm. ampicillin to rye B agar medium suppressed the fungal and bacterial contaminants and better isolation was made (Sato and Kato, 1993).

Isolation of *Phytophthora capsici* was made from soil by using different baits. Black pepper leaves (Muller, 1936, Kueh and Khew, 1982), black pepper leaf discs (Ramachandran *et al*, 1986), apples (Holliday and Mowat, 1963), castor seeds (Narasimhan and Ramakrishnan, 1969 and Sastry, 1982), root pieces of *Colocasia esculanta* (Satyaprasad and Rama Rao, 1980), leaf lets of *Albizia falcataria* (Anandaraj and Sarma, 1990) were used. A number of baiting techniques and selective media to isolate and quantify *Phtophthora* from soil have been developed (Tsao, 1983, Dhingra and Sinclair, 1985). A number of selective media containing antibacterial antibiotic and selective antifungal agents have been tried for isolation of *Phytophthora* from soil and plant tissues (Eckert and Tsao, 1960; Kuhlman and Hendrix, 1965; Flowers and Hendrix, 1969; Tsao and Ocana, 1969; Sneh, 1972; Fujisawa and Masago, 1975; Masago *et al.*, 1977; Tsao and Guy, 1977)

PATHOGENICITY

Zoospore suspension has been used by several workers to establish the pathogenicity of *Phytophthora* spp. on their respective hosts (Turner, 1967; Mehrotra, 1972; Kroll and Elide, 1981; Mc Donald and Duniway, 1978; Sastry, 1982; Dutta, 1984; Cho *et al.*, 1987). *Phytophthora cinnamomi* zoospores suspension has used as main source of inoculum (Zentmyer, 1980). It was reported that 5×10^2 zoospores were good enough to cause infection on black pepper (Freire and Bridge, 1985).

QUANTIFICATION OF *PHYTOPHTHORA* IN SOIL

Various species of *Phytophthora* which cause foot rot and root rot of black pepper, black pod of cocoa and black shank of tobacco are generally isolated from the upper soil horizons and it was believed that soil borne inoculum is most important in causing disease

epiphytotics (Thorold, 1955; Holliday and Mowat, 1963; Okaisabor, 1971; Onesirosan, 1971; Flowers and Hendrix, 1972; Nambiar and Sarma, 1977). In addition, the inoculum levels of several soil borne species of *Phytophthora* and *Pythium* and infection of several hosts were correlated (Mitchell, 1978). Presence of pathogen at above horizon is more likely to cause early disease out break (Cho *et al.*, 1987) and quantity of inoculum in soil becomes important (Luz and Mitchell, 1994).

A number of selective media and baiting techniques were employed by several researchers for the quantitative estimation of different *Phytophthora* species from soil (Tsao, 1983; Dhingra and Sinclair, 1985). Tsao (1960) used a serial dilution end point method for estimating the disease potentials of *Phytophthora citrophthora*. According to Duncun (1976), the most probable number analysis (MPN) with the baiting technique allowed comparisons between inoculum levels of different plots or treatments. Sastry (1982), found a direct relation between the percentage of baits colonized by *P.palmivora* and inoculum level in the soil.

CULTURAL PRACTICES

In view of soil borne nature of the disease, greater precaution need be exerted to maintain nursery hygiene to ensure disease free rooted cuttings for the better establishment in the field and longivity in black pepper against *P.capsici* (Sarma *et al.*, 1987 & 1992). Incorporation of biocontrol agents in solarised nursery mixture is being popularised (Sarma and Anandaraj, 1998). To reduce the inoculum levels of pathogen in the field, removal of affected vines along with root system and burning off and also maintenance of green cover in the field and pruning off the runner shoots of branches adjacent to the ground level has been emphasized to reduce the chances of foliar infection due to soil and rain splash (Sarma and Anandaraj, 1998). Sastry and Hegde (1988) reported the importance of burning off of infected leaves and twigs to avoid the spread of foot rot disease of black pepper in the fields. Thareja *et al.*, (1989) emphasized the importance of drainage system in tomato fields and also reported that, maximum infection was noticed in the area where direct contact of plants

with soil or near the ground, due to the splash dispersal of *P.nicotianae* var *parasitica* inoculum.

CHEMICAL CONTROL

In general copper fungicides have been reported to be highly inhibitory to pythiaceous fungi. Bordeaux mixture - a contact fungicide has been recommended to use against the foot rot of betle vine and black pepper by many workers (Dastur, 1927 & 1935; Uppal, 1931; Asthana, 1947; Subramaniyan and Venkata Rao, 1970; Narasimhan *et al.*, 1976; Nair and Sasikumaran, 1991).

In black pepper, spraying the foliage with Bordeaux mixture, drenching the soil - around the base of the vines with Bordeaux mixture or copper oxychloride and application of Bordeaux paste to the collar region during May - June (pre-monsoon) and repeating the spraying and drenching again during August - September (post - monsoon) is the recommended package (Sasikumaran *et al.*, 1981; Mammooty *et al.*, 1991; Ramachandran *et al.*, 1990). Foot rot incidence was significantly reduced with the Bordeaux mixture pasting on foot region and spraying and drenching (Nair *et al.*, 1993). Harper (1974) reported that, soil drenching with cuprous oxide reduced the number of dying plants in the field over a five months period. Application of Bordeaux mixture alone and their combinations with copper oxychloride and metalaxyl found significant effect on disease control (Malebennur *et al.*, 1991). In an *in vitro* assay Sastry (1982) reported that, 1% Bordeaux mixture inhibited the growth and sporulation of *P.meadii*, the capsule rot pathogen of cardamom. Bordeaux mixture (1%) spraying twice in June and August gave good control on capsule rot of cardamom (Nambiar and Sarma, 1977).

Out of 5 fungicide formulations tested (Bordeaux mixture, DM-45, Blitox 50, Brestanol and Dithane Z78), Bordeaux mixture was found very effective on control of late blight pathogen (Navase and Dhande, 1982). Fungicides tested against *P.parasitica*, the foot rot pathogen of

betle vine, 1% Bordeaux mixture was found most effective on controlling of the disease (Raj *et al.*, 1973). Ayyavoo and Samiyappan (1984) reported that, application of Bordeaux mixture 0.1% or 1% was good enough to control the foot rot incidence of betle vine in the field. Drenching the soil with Bordeaux mixture at monthly intervals gave effective control and increased yield, followed by COC (0.25%) and dexan (0.5%) - (Narasimhan *et al.*, 1976). Reddy and Mohan (1984) reported that, out of 24 fungicides tested for their bio-efficacy on controlling black pod of cocoa and copper fungicides performed best. Application of COC @ 3 applications per year could control the black pod pathogen (Figueiredo and Lellis, 1980).

Sonoda *et al.* (1990), found that the production of phytotoxin was induced in citrus due to the application of copper fungicides. To over come the problem of phytoalexin production it was suggested that, apply fosetyl Al 14 days earlier than the application of copper fungicides and it could reduced the phytotoxin production in hosts.

According to Rao (1985), single spray of 1% BM in combination with 0.5% Zinc sulphate was effective throughout the rainy season and decreased the incidence of rotting by *P.nicotianae* var *parasitica* to 5.6 - 6.8%, compared with 97.5 - 100% in unsprayed fruits of coorg mandarin. It was also reported that application of 1% Bordeaux mixture alone could not able to control the disease effectively. Sarkar *et al.* (1985) reported that, *P.palmivora* infection was reduced up to 82.15% by the application of 1% Bordeaux mixture in bell pepper.

Systemic fungicides for the control of Oomycetes fungi were developed in sixties. Chloroneb was introduced in 1967 was the first fungicide to show selective toxicity to Oomycetes (Bruin and Edgington, 1983). In 1969, etridiazole (Anon, 1966) was introduced mainly for the control of soil borne species of *Phytophthora, Pythium* and other fungi affecting turf grass, vegetables, fruits, cotton, ground nut and ornamentals. The chemical control of disease caused by Oomycetous fungi had taken a new turn with the introduction of highly

effective chemicals against them in mid seventies. Since then, the information on these chemicals were reviewed by many workers (Schwinn, 1979; Straub and Hubele, 1980; Schwinn, 1983; Bruin and Edgington, 1983; Schwinn and Urech, 1986; Cohen and Coffey, 1986; Schwinn and Staub, 1987). Phenylamides (acylalanines) introduced during seventies (Urech et al, 1977), having four sub classes (Cohen and Coffey, 1986) namely acylalanines, bytyrolactones, thiobutyrolactones and oxazolidinones constitute one of the important groups of fungicides. Detailed investigations have been carried out on the effect of metalaxyl, Methyl D, L - N - (2,6 - dimethyl phenyl) - N - (2' methoxy acetyl) alaninate, a systemic fungicide to control the *Phytophthora* infections on various crops. Metalaxyl is reported to inhibit both protein and nucleic acid synthesis (Fisher and Hayes, 1982) besides reducing nuclear division. According to Fisher and Hayes (1982) respiration, wall synthesis and membrane permeability remained unaltered in treated mycelia of *Phytophthora nicotianae, P.palmivora* and *Pythium ultimum*. As metabolites of the fungicides were not seen in both the fungus and the in medium, metalaxyl is believed to be the primary toxic agent and it is reported to reduce the uptake of labelled uridine and thiamine into RNA and DNA. Hence Fisher and Hayes (1984) concluded that matalaxyl might inhibit RNA polymerase. Ramachandran *et al.* (1988), conducted an extensive study on the effect of metalaxyl on different species of *Phytophthora* affecting on plantation crops. In green house and field trials, Kasim (1986) could get good control on foot rot incidence of black pepper by using Ridomil, followed by Alliette, Dithane M45, and Delsene MX 200. Sastry and Hegde (1987) reported that, foliar spray with metalaxyl gave good control on foliar infection of black pepper due to *P.capsici*. Ramachandran and Sarma (1985) studied the efficacy of 3 systemic fungicides against the foot rot pathogen of black pepper and reported that ridomil (metalaxyl) treated plants showed least root necrosis and no death was noticed followed by Terrazole and Alliette.

Ramachandran and Sarma (1985) evaluated five systemic fungicides viz; metalaxyl, fosetyl-Al, ethazole, propamocarb and oxyadixyl for their bio-efficacies on different phases of '*P.palmivora*' (MF_4) and the field evaluation of first three fungicides. From *in vitro* assays, it was reported that ethazole and metalaxyl were the most toxic to the growth of fungal

mycelium. On sporulation, ethazole followed by metalaxyl, fosetyl Al and oxadixyl were effective. Among three fungicides tested in the field, metalaxyl gave good control of the disease and suppressed *P.palmivora* population.

Spraying and drenching of black pepper with ridomil at 0.08%, 2 weeks before and after inoculation gave good control on *Phytophthora* infection (Kueh, 1984). Effective control on black shank of tobacco due to *P.parasitica* Dastur var. *nicotianae* Tucker (Vasilakakis *et al.*, 1979), and *P.infestans* on tomato plants by metalaxyl was reported (Cohen *et al.*, 1979). According to Edgington *et al.*, (1980), matalaxyl was effective against *Pythium* and *Phytophthora*.

Mycelial growth of *P.cotrophthora* and *P.capsici* was completely inhibited by metalaxyl at 25ppm. and *P.palmivora* at 50ppm. Lethal concentrations of metalaxyl for *P.citrophthora* and *P.palmivora* was reported as 75ppm. (Campelo *et al.*, 1984). Extensive work has been done on the action of metalaxyl on *P.infestans* De Bary, the late blight pathogen of potato (Mantecon and Escande, 1985; Berggren, 1985; Kozlovski and Suprun, 1989; Cohen and Samoucha, 1989; Easton and Nagle, 1985; Tedle, 1985).

In a green house trial Garibaldi and Timietti (1980), reported that metalaxyl @ 50g/plant used as soil drench, 2 days before inoculation protected the plants throughout the trial. Ridomil applied as a single soil drench containing 0.25µg/litre was sufficient to protect the tomato plants from *P.infestans*. Penetration and initial establishment of *P.infestans* in leaves and fruits of tomato was observed, to achieve most efficient control on blight incidence in green house plants and must be treated with chemical either before or within the first 2 days after the inoculation (Cohen *et al.*, 1979). Growth and sporangial germination of *P.dreschleri* f sp. *cajani* was inhibited by the low concentration of metalaxyl. Growth was completely inhibited by at 0.5 µg/ml. and sporangial germination at 1 µg/ml. (Chaube *et al.*, 1987). Extensive studies have been carried out on control of *P.dreschleri* by metalaxyl (Chauhan and Singh, 1987; Bisht *et al.*, 1988; Agrawal, 1987; Singh and Chauhan, 1992; Kannaiyan and Nene, 1984)

Ramraj and Vidhyasekaran (1983) reported that metalaxyl inhibited the production of pectic enzymes by *P.parasitica* var *piperina* in *Piper betle* . Root tissue of wilted betle vine were reported to contain C_1 and Cx enzymes, and these enzymes were also produced in cultures of *P.parasitica* var *piperina*. The Cx production was completely inhibited and inactivated when cultures incubated with etridiazole formulations, which is highly inhibitory to production of C_1 enzyme (Ramraj and Vidhyasekaran, 1982).

Soil treatment with 100ppm metalaxyl could give effective control on *P.parasitica* var *nicotianae* infection in tobacco (Bhatt and Patel, 1989). *P.palmivora* infection in cocoa fields could be controlled effectively by metalaxyl (Mc Gregor, 1982). Metalaxyl or cuprous oxide spray could effectively control the pod rot and canker of cocoa (Holderness, 1992). Low concentrations of metalaxyl was highly inhibitory to mycelial growth, sporangial formation, chlamydospore and oospore formation both in *P.parasitica* and *P.citrophthora* (Farih *et al.*, 1981). *Phytophthora citrophthora*, the causal agent of gummosis of citrus was controlled by soil drenching with metalaxyl and fosetyl aluminium. Stem lesions were reduced by the application of 50µg/litre of metalaxyl (Farih *et al.*, 1981). Root rot of rough lemon due to *P.nicotianae* var *parasitica* could control by soil drenching of metalaxyl and up to 1000ppm. it was not phytotoxic to 2yrs old rough lemon seedlings (Lee and Wicks, 1982). Utkhede (1984c) reported that soil drenching with metalaxyl mancozeb application around the base of naturally infected trees, prevented the further spread of *P.cactorum* in apple trees.

Out of 11 fungicides tested for their bio-efficacies, metalaxyl and alliette (fosetyl aluminium) completely inactivated the mycelium of *P.cactorum* in the soil within 2 days and completely inhibited the production of sporangia and oospores (Rana and Gupta, 1984). Ellis *et al.* (1982) reported that, metalaxyl at lower concentration inhibited the growth, sporulation and zoospore germination of apple collar rot pathogen *P.cactorum*. Soil drenched with metalaxyl prevented the infection of apple trees in green house. *P.syringae*, the apple fruit rot fungus was inhibited by metalaxyl in fields (Edney and Chambers, 1981). Significantly effective

control on *P.capsici*, the pepper blight pathogen was noticed by metalaxyl. In an *in vitro* assay, mycelial growth in solid and liquid media and sporangial germination were inhibited even at low concentrations of metalaxyl, where as sporangial germination was inhibited at higher concentrations (Sung and Hwang, 1988). Lee and chung (1989) studied the effect of metalaxyl on growth of *P.capsici,* the fruit rot pathogen red pepper and reported that metalaxyl MZ was more effective on inhibiting the growth of *P.capsici* than alliette F. From pot culture studies Tamietti and Ritucci (1986) reported that, metalaxyl showed much effective and persistent acivity against the foot rot pathogen - *P.capsici* - of capsicum.

Recent studies have demonstrated that some simple phosphorous compounds have powerful and selective antifungal properties, with good selective activity against oomycete plant pathogens in higher plants. This compound triggered a resistant reaction in the host (Bompeix *et al.*, 1981 & Guest, 1984). Antifungal activity of phosphorous acid have been proved very early (Thizy *et al.*, 1978). Coffey and Bower, 1984 and Fenn and Coffey, 1984 reported that phosphorous acid compounds showed antifungal activity against oomycete fungi and has little or no activity against the majority of other fungi. Potassium phosphonate was investigated for its antifungal properties against a range of fungi grown on liquid and solid media (Coffey and Bower, 1984; Fenn and Coffey, 1984; Dolan and Coffey, 1988). Phosphonate has got high selectivity against certain *Phytophthora* species (Fenn and Coffey, 1984). Sporangial development of *P.palmivora* was inhibited (EC50) even at 0.1µg/ml. (Dolan and Coffey, 1988). Oospores and chlamydospore production of *P.cinnamomi* inhibited at higher concentration-50ppm. (Coffey and Joseph, 1985). Coffey and Joseph (1985) and Dolan and Coffey (1988) emphasised the selective interference with key biosynthesis events on zoosporangia production by *Phytophthora.*

It is known that phosphorous compounds in plant tissues degrade easily (Piedallu and Jamet, 1985; Saindrenan *et al.*, 1985). Tomato leaves when treated with 400 µg/ml. of phosphonate compound and analyzed after 48 hours contained 14µg/g of ethyl phsophonate and 358 µg /g of phosphonate on fresh weight basis (Fenn and Coffey, 1988). Very low

level of ethyl phosphonate was detected in seedlings treated with potassium phosphonate (Ouimette and Coffey, 1989).

A single pre-planting dip of pine apple sucker was effective against *P.cinnamomi* and *P.parasitica* for 18 months (Rohrbach and Schenck, 1985). In avocado, Darvas *et al.*, (1984) and Pegg *et al.*, (1985) reported that, two application with potassium phosphonate as trunk injection could control *P.cinnamomi* and resulted in enhanced growth of host in the following season.

Adams and Conrad (1953) demonstrated that, the microorganisms presented in the soil could oxidize the phosphonates into phosphate. Micoorganisms including bacteria, fungi and actinomycetes can apparently utilize the phosphonate as a phosphorous source (Casida, 1960; Malacinski and Konetzka, 1966).

Dimethomorph or commonly known as acrobat, which was firstly described in 1988 (Albert *et al.*, 1988) was highly effective in the control of downey mildews and diseases caused by different species of *Phytophthora*. Kuhn *et al.* (1989a & b) reported that, dimethomorph is active against *Phytophthora* species *in vitro*, where it inhibits radial growth of mycelium with ED50 values typically in the range of 0.25 - 0.75 micromol. Albert *et.al.* (1988) and Kuhn *et al.* (1989a) found that, dimethomorph acts as a fungicide compound and not as a fungistatic compound. Kuhn *et al.* (1991) has conducted an excellent study on its mode of action on different *Phytophthora* species and reported that, the formation of periodic constrictions along treated hyphae with the dimethomorph, producing a beaded morphology, stunting of hyphae and stimulated the formation of short lateral branches. The most important finding was the extensive proliferation and aberrant deposition of cell wall material and which lead to the formation of false septa. Tomat (1992) and Thomas *et al.* (1992) supported the findings of Kuhn *et al.* (1991) reported that, dimethomorph acts on fungus by interfering with the biochemical processes regulating the cell wall formation.

Aureofungin, chemically known as a heptaene compound, produced from streptomycete –

Streptoverticillium cinnamoneus var *terricola*. Aureofungin reported to be very effective *in vitro* against *P.palmivora* and *P.citrophthora* (Agarwala and Thirumalachar, 1967; Agarwala and Sharma, 1975; Bedi and Dhaliual, 1970; Bedi *et al.*, 1969; Capoor and Marathe, 1970). Its *in vitro* efficacy against *Pythium debaryanum* and *P.myriotylum* was established (Capoor and Marathe, 1970). Field efficacy of aureofungin to control of leaf rot of pan due to *P.parasitica* var *piperina* was reported (Chaurasia *et al.*, 1973).

Aureofungin was also reported to be effective against *Phytophthora* diseases viz; fruit rot of guava (Sohi, 1975), citrus gummosis (Desai *et al.*, 1966) and abnormal leaf fall of rubber (KAU, 1976). Seed dip of rhizomes in 100ppm. aureofungin solution before planting them in the field was found effective against rhizome rot of ginger caused by *Pythium debaryanum*(Haware *et al.*, 1973).

BIOLOGICAL CONTROL

The potential of biocontrol of plant pathogens has been reviewed excellently (Garret, 1965; Baker and Cook, 1974; Cook and Baker, 1983; Baker, 1987 & 1992). Biological control is the use of organisms, genes or gene products to regulate a pathogen and can be used with strategies intended to keep (1) inoculum density below an economic threshold level (2) retard or exclude infection (3) maximize the plant's system for self defence (Cook, 1988). The mechanism of biocontrols on plant pathogens have been reviewed extensively and the main mechanisms identified are antibiosis, lysis, competition and mycoparasitism. (Cook and Baker, 1983; Papavizas and Lumsden, 1982).

Several toxic metabolites are produced by antagonists against pathogens *in vitro* and in soil (Wright, 1956). Many researchers dealing with *Trichoderma* noticed that hyphae of the antagonists parasitize on the hyphae of pathogens brought about several morphological changes viz; coiling, haustoria, disorganization of host cell contents and penetration of the host (Cook and Baker ,1983). It is reported that, cell wall degrading enzymes such as mycolytic enzymes being produced by many biocontrol agents (Cook and Baker, 1983).

Studies on biocontrol of plant pathogens have started during the early period of 20th century. First paper on biological control was published in 1926 (Sanford, 1926), on microbial and soil factors affecting the pathogenicity of *Actinomyces scabies* on potato.

In India, Mehrotra *et al.* (1990) and Mehrotra (1992) reviewed the biocontrol strategy for the control of *Phytophthora* disease of various crops with special emphasis on betle vine *Phytophthora*, *P.nicotianae* var *piperina*. Tiwari and Mehrotra (1974) studied the colonization ability of *Trichoderma viride* and *Aspergillus terreus* on infected root and petiole sections of *P.betle* in fumigated soil and reported that, *T.viride* population was increased in fumigated soil and gave better control against *P. nicotianae* var *piperina*. Sharma and Tiwari (1981) studied the phylloplane microflora infected by *P.infestans* on *Solanum khasianum*, and reported that, healthy leaves had more number of microorganisms than diseased leaves. Halsall (1982) studied the microorganisms in suppressive soil of eucalyptus forest. Suppressive soil from wet sclerophyll eucalyptus forest in Tallaganda, NSW, contained more actinomycetes than conducive soil. All *Streptomyces* isolates isolated from suppressive soil showed antagonistic activity against *P.cinnamomi* and *P.cryptogea*.

Duvenhage *et al.* (1991) isolated antagonistic microorganisms from suppressive soil from avocado plantation. Out of 48 soils studied, 12 found suppressive in nature and had more actinomycetes, bacteria and fungal populations. Out of 50 microbes evaluated 5 bacteria, 4 fungi and 6 actinomycetes were found significant in reducing root rot of avocado caused by *P.cinnamomi*. Broadbent and Baker (1974) studied the suppressiveness of avocado soil. Disease suppressive soil showed higher populations of bacteria and actinomycetes. Mycelial growth and sporangial formation was poor in suppressive soil compared to disease conducive soil.

Among the many potential antagonistic soil inhabitants, members of the genus *Trichoderma* have been studied extensively (Dennis and Webster, 1971; Papavizas, 1982; Wood and Tviet, 1955; Boosalis, 1964; Baker, 1968; Baker and Cook, 1974; Cook, 1977).

Gliocladium roseum was reported antagonistic to *P.palmivora*, and it colonized on sporangia and chlamydospores of the pathogen (Lim and Chan, 1986). Their light microscopic studies, SEM and TEM investigations showed that process of parasitism as coiling, penetration and proliferation of the mycoparasite within the host spores followed by destruction of host cytoplasm. Out of 96 fungi, 174 actinomycetes and 576 bacterial isolates isolated from rhizosphere and non-rhizosphere areas of 5 major capsicum growing areas, *T.harzianum*, *Pseudomonas cepacia* and *Bacillus polymyxa* have been sorted out as promising antagonists agents against *P.capsici* (Jee *et al.*, 1988). *Trichoderma* and *Gliocladium* isolates were found to be potential antagonist of *P.cactorum* causing root and crown rots of apple (Smith *et al.*, 1990; Lederer *et al.*, 1992; Orlikowski and Schmidle, 1985; Roiger and Jeffers, 1991). Pasini *et al.* (1991) tested 62 soil samples for their suppressive nature against *P.cryptogea*, foot rot pathogen of *Gerbera* and 7 samples were found suppressive and the suppressiveness of soil was correlated with the antagonistic effect of *Trichoderma* spp.

Efficacy of *T.harzianum* against *P.cryptogea* causing foot rot of *Gerbera* has been reported (Duskova, 1992). Orlikowski (1994) studied the biocidal property of *Trichoderma* and *Gliocladium* spp. against *P.cryptogea* and reported that 10^9 spores of *T.viride* applied to pits 10 days before inoculation with *P.cryptogea*, could control foot rot of *Gerbera* effectively. *T.harzianum* impregnated on clay granules could control damping off of pineapple seedlings due to *P.cinnamomi* (Kelley, 1976).

Culture filtrates of *G.roseum*, *T.harzianum* and *T.roseum* inhibited the mycelial growth of *P.megasperma* f sp. *glycinea* (Al-Heeti and Sinclair, 1988). In an *in vitro* study, culture filtrates of *Chaetomium globosum*, *G.virens* and *T.viride* were found antagonistic and mycoparasitic to *P.cinnamomi*, *P.cactorum*, *P.fragariae* and *P.nicotianae*. Culture filterates of *T.viride*, *Aspergillus niger* and *A.flavus* suppressed the sporangial formation of *P.parasitica* var *piperina* in *Piper betle* (Vyas *et al.*, 1981; Chile, 1982). In an *in vitro* assay, culture filtrates of *Myrothecium noridum*, strongly inhibited the growth of *P.nicotianae* var *parasitica*, *P.syringae* and *P.capsici* (Tuset *et al.*, 1990). Antagonistic

property of *Neocosmospora vasinfecta* (Von.Arx) Cannon and Howkswork on *P.capsici* (Turhan and Grossmann, 1988). *Penicillium aurantiogriseum* and *Fusarium equiseti* were reported as antagonists against *P.infestans* (Jindal *et al.*, 1988). Krishnakumar *et al.* (1987) isolated *Penicillium aurantiogriseum*, *T.koningii* and *Mucor hiemalis* from potato phylloplane.as antagonists of *P.infestans*..

Bacterial isolates antagonistic to the growth and multiplication of serious soil borne pathogens have been reported (Brown, 1974; Hutchins, 1980; Merriman *et al.*, 1975; Sneh *et al.*, 1977; Utkhede and Rahe, 1980). By using Kings (B) and D4 media, Ryu *et al.* (1991) isolated 926 rhizosphere bacteria and 63 isolates were found antagonistic to *P.capsici*. Galindo (1992) studied the efficacy of *Pseudomonas fluorescens* isolates against *P.palmivora*, *in vitro* and *in vivo* and reported that it was more effective than copper oxychloride and chlorothalonil. The fluctuation of bacteria depends on RH and rainfall. *Pseudomonas cepacia, Bacillus polymyxa* and *Bacillus* sp. were found effective against *P.nicotianae* and *P.capsici* (Cho, 1987).

Antagonistic effect of bacterial isolates on *P.cactorum* were studied extensively (Utkhede, 1984a&b; Utkhede and Gounce, 1983; Marchi and Utkhede, 1994; Utkhede and Smith, 1993). *In vitro* efficacy of *Rhizobium* sp. was studied on *Fusarium* spp.; *Pyrenochaeta terrestris*, *Colletotrichum destructum*, *P.cactorum* and *Coniothyrium* sp. (Drapeau *et al.*, 1973). Gupta and Utkhede (1987) studied the nutritional requirements of antagonistic bacteria *Enterobacter aerogenes* and *Bacillus subtilis* and they found that addition of $(NH_4)H_2PO_4$ increased the production of antagonistic substances. Results indicated that use of N and P fertilizer increased the production of antifungal substances by the agonistic bacteria in soil.

For better antagonistic effects against *P.cactorum*, the temperature and pH requirements were 14 - 21^{0}C and 3 - 5 for *E.aureogenes* and 21 - 28^{0}C and 5 - 8 for *B.subtilis* respectively. Optimum growth in sterized soil was observed at 18^{0}C for *E.aerogenes*. and 25^{0}C for *B.subtilis*. Fosetyl did help in bacterial multiplication at lower temperature and

metalaxyl at higher temperature. Light and electron microscopic studies conducted by Malajczuk *et al*, (1977) reported that, *Pseudomonas* spp., *Bacillus* spp. and *Streptomyces* spp. lysed the hyphae and inhibited the production of zoospores and its release of *P.cinnamomi* in soil.

Turnbull *et al.* (1992) reported that *Pseudomonas cepacia* reduced the root rot caused by *P.cinnamomi*. Myatt *et al.* (1993) screened 1000 bacterial isolates *in vitro* and *in vivo* against *Phytophthora* root rot pathogen of chick pea. They reported that 31 isolates out of 1000 delayed or limited the decay of chick pea seedlings disease in pasteurized soil. The most effective isolates included *P.cepacia* (7 strains) and *P.fluorescens* (2 strains).

Several actinomycetes have been isolated and tested against soil borne plant pathogens. Lee *et al.* (1990) studied the activity of *Streptomyces parvullus* against *P.capsici*. The active compound responsible for the antagonistic activity was purified by ion-exchange, adsorption and gel-permeation and partial column chromatography techniques and identified as polyoxin. Ahn and Hwang (1992) isolated actinomycetes antagonists to *P.capsici* from rhizosphere soil of 6 capsicum growing areas. Actinomycetes, antagonistic to *P.meadii*, causing fruit rot of rubber, were isolated from soils of rubber growing areas and reported that soil samples had more antagonistic actinomycetes (Kochuthresiamma *et al.*, 1988).

Treatment of tomato seedlings with culture filtrates of *Streptomyces aurantiacus, S. griseus* and *S. longissimus,* before sowing reduced the infection due to *Fusarium oxysporum* and *P.parasitica* (Tsintasadze and Tsilosani, 1973). Chung and Hong (1991) studied the action of two strains of *Streptomyces* sp. (Strain11 &20) on *Fusarium oxysporum* f sp. *vasinfectum* and *P.nicotianae* var.*parasitica* and they reported that the culture filtrates of actinomycetes lysed the mycelia and inhibited the spore germination.

Verticillium tenerum a saprophytic fungus, proved its antagonistic ability against the foot rot pathogen of black pepper, *P.capsici* (Rajan and Sarma, 1997) and as hyper parsite on *Rhizoctonia solani* (Turhan, 1990).

The role of VAM in nutrient uptake, growth promotion, tolerance to biotic and abiotic stress in crop plants has been reviewed (Sieverding, 1991). The association of VAM with root system of black pepper and the growth promoting activities was reported (Manjunath and Bagyaraj, 1982; Bopaiah and Khader, 1982). Role of VAM and *Trichoderma* on *Phytophthora* root rot suppression in black pepper nurseries has been proved (Anandaraj and Sarma 1994; Sarma *et al.*, 1996). Nambiar and Sarma (1979) isolated *Trichoderma* spp. from the roots of healthy black pepper vines and also noted the lysis of mycelium of black pepper isolate of *Phytophthora* when the *Trichoderma* sp. over grown on the test fungus.

MASS MULTIPLICATION AND DELIVERY SYSTEM OF *TRICHODERMA* AND *GLIOCLADIUM*

Apart from isolation and identification of potential biocontrol agents, their mass multiplication and delivery systems are important for the successful exploitation of biocontrols. Different solid media for the mass production of *Trichoderma* and *Gliocladium* have frequently been used (Davet *et al.*, 1981; Elad *et al.*, 1980a; Elad *et al.*, 1980b). Use of different carrier media were tried by many workers, bark pellets (Sundheim, 1977), wheat bran plus peat (Sivan *et al.*, 1984), barley grains (Abd-El Moity and Shatla, 1981). Composted hard wood bark was used for multiplication of *Trichoderma* and *Gliocladium* (Hoitink, 1980; Nelson and Hoitink, 1983; Nelson *et al.*, 1983). Ricard (1981), mass multiplied and commercialized the *Trichoderma* and *Glicocladium* as mycofungicide for field application. Hunt *et al.* (1971), tried motor oil for *Trichoderma* formulation and successfully inoculated on pine stumps during tree cutting.

Growth media for liquid formulation included such as inexpensive products like glucose, starch, hydrolyzed corn and soy products, whey and molasses (Kenney and Couch, 1981). Use of inexpensive liquid media such as molasses and brewers yeast to produce viable inocula of *Trichoderma* and *Gliocladium* with a deep tank fermentor system for large scale industrial production has been emphasized recently (Papavizas *et al.*, 1985). A dry

formulation of *Trichoderma* and *Gliocladium* was prepared by air drying the fungal mats, grinding them and diluting the powder with the commercially available pyrax as a carrier (Papavizas, 1984). Lewis and Papavizas (1984) refined the techniques of incorporation of antagonistic fungi in nutrient carrier (bran) with alginate to provide a food base in intimate contact with the antagonist.

Mukhopadhay (1987) has given a list of growth media used for mass multiplication of *Trichoderma* species. Backman and Rodriguez-Kabana (1975) formulated a diatomaceous earth granule impregnated with a 10 percent molasses solution and was found suitable for growth and delivery of *Trichoderma harzianum*. Kausalya Gangadharan and Jeyarajan (1988) used tapioca 'thrippe' for mass multiplication of *T.viride* and *T.harzianum*. Mukhopadhyay (1987) tried a mixture of wheat bran, sawdust and tap water to multiply *T.harzianum* and used against damping off of seedlings of tobacco caused by *Pythium aphanidermatum*. Padmanabhan and Alexander (1987) used *T.viride* multiplied in sand-sorghum medium for the control of root rot of sugar cane seedlings caused by *P.graminicolum*. Subram. Upadhyay and Mukhopadhyay (1986) used sorghum grains substrate for mass multiplication of *T.harzianum*.

ESTABLISHMENT AND PROLIFERATION OF FUNGAL ANTAGONISTS IN SOIL

Locke *et al.* (1984) got excellent control of Fusrium wilt of chrysanthemum by the addition of conidial suspension of *T.viride* as soil mix. The biocontrol agent, *T.viride* was applied as conidia @10^4 conidia/cm^2 to the pasturized (at 82^0C for two hours) soil mix, which helped in rapid colonization of antagonist in soil mix and prevented the reinvasion of the pathogen. Lewis and Papavizas (1984) described that, *Trichoderma* and *Gliocladium* and other potential antagonistic fungi proliferated abundantly in various natural soils when added as young mycelia in intimate contact with a food base (sterile moist bran inoculated with conidia and allowed to incubate for one to three days before addition to soil), but not as conidia with or without bran. Proliferation (upto 10^6 fold) and subsequent establishment in

soil depended on inoculum age and how it is added in relation to food base. Lewis and Papavizas (1984) reported that, alginate pellets containing fermentor biomass preperations of *Trichoderma* and *Gliocladium*, in a food base (bran) stimulated the great increase in population. Dry formulation of *Trichoderma* and *Gliocladium* by fermentation technique appeared to proliferate greatly. The conidial number was increased in soil from $5x10^5$ to $6-7x10^6$ per gram of soil (Papavizas *et al.*, 1984). The unique ability of young hyphae but not conidia of *Trichoderma* and *Gliocladium* to proliferate from thorough colonized substrate or from alginate pellets might be due to their insensitivity to fungistasis.

EFFECT OF ORGANIC SOIL AMENDMENTS

Addition of soil amendments into soil may alter the soil microbial population. Zentmyer (1963) noted that, addition of alfalfa and cotton waste (gin trash) increased the soil microorganisms and suppressed the *P.cinnamomi* Rands. infection in avocado root. On contrary Jeyarajan *et al.* (1987) reported that addition of neem and neem products reduced the fungi, bacteria and actinomycetes population in the soil and suppressed the *P.capsici* infection on betle vine. Tsao and Oster (1981) studied the effect of urea and chicken manure on *Phytophthora*, they reported that the formation of ammonia and nitrous acid from amendments, found toxic to the fungus. Nam *et al.* (1988) reported that amendments with 5-10% of arrow roots, leaf tissue, rice polish and wheat bran stimulated the growth of *Capsicum annum* and its inhibitory effect on growth of *P.capsici in vitro*.They also reported that, application of organic amendments together with antagonists greatly enhanced the disease suppressive effect. Crown rot of capsicum due to *P.capsici* reduced significantly by addition of 10% compost of sewage sludge and survival of pathogen was not affected by compost but it enhanced the total microbial population which suppressed the activity of pathogen (Lumsden *et al.*, 1983). Singh and Vyas (1984) studied the effect of 5 oil cakes viz; *Brassica compestris* L., *Linum usitatissimum* L., *Ricinus communis* L., *Azadirachta indica* Juss. and *Madhura indica* Gmel. on *P.parasitica* var *nicotianae* and they found that mustard oil cake was fungi toxic causing upto 51.2% inhibition.

Inhibitory effect of soil amendments on *P.cinnamomi* was studied by several workers (Hoitink *et al.*, 1977; Rosas Romero *et al.*, 1986; Sivasithamparam 1981; Nesbitt *et al.*, 1979). Spencer and Benson (1982) studied the effect of pine bark, hard wood bark compost and peat amendment on lupin root rot by different *Phytophthora* species. Huang (1991) obtained very good control on *Phytophthora* blight of cucumber, *Pythium* damping off, club root rot of crucifers (*Plasmopara brassica*) and *Fusarium* wilt of water melon by using a S-H mixture consisted of 4.4% bagasse, 8.4% rice husk, 4.25% oyster shell powder, 8.25% urea, 1.04% potassium nitrate, 13.16% calcium sulphate and 60.5% mineral ash (slag). Enhancing effect of organic amendments on antagonistic organisms were emphasized by Linderman (1989). Stover (1962) and Hubber & Watson (1970) emphasized the effect of organic amendments and green manures in the control of soil borne plants pathogens.

EFFECT OF PLANT EXTRACTS

Smale *et al.* (1964) have carried out a survey of green plants showing antifungal properties against fungi and bacteria. Chamount and Jolivet (1978), have tested 100 extracts of vegetable origin against seven plant pathogenic fungi and reported that *Fusarium oxysporum* and *P.cinnamomi* were most resistant to the action of these plant extracts. Chamount (1979) tested aqueous extracts of eight flowering plants against 51 fungi and found that *Phytophthora* and *Pythium* were among the most resistant ones. Singh (1972) tested 10 plant extracts on plant pathogens. Whitefield *et al.* (1981) reported, the root extract of *Acacia pulchella* R.Br. showed high inhibitory effects on growth, sporangial production, sporangial germination and zoospore germination of *P.cinnamomi* Rands. They characterized the volatile compounds responsible for inhibitory action as 2 & 3 methyl butanol, hexanol, pentanol, 2, 3 methylbutanol, 4-methyl ascetophenone and carbon disulphide.

Pathak and Dixit (1984) studied the antifungal and antimicrobial activity of essential oils extracted from *Glossocardia bosvallia* DC, and reported that it showed good antifungal action against *P.parasitica*. Gennari *et al.* (1987) emphasized the activity of parthenolide,

extracted from *Tanacetum vulgare* on *P.capsici*. Chauhan and Singh (1991b) conducted a study on avtivity of 5 plant extracts on *P.dreschleri* f.sp. *cajani*, and reported the feasibility of garlic and onion extracts in fields to control the pigeon pea wilt. Johri *et al.* (1994) reported that, the use of *Ammi majus* extracts to control the *P.palmivora* in betle vine. Wagner and Flores (1994) studied the effect of Taxol and related compounds obtained from *Taxus* spp. on several fungi, their study showed that the extracts got good inhibitory effect on growth of *Phytophthora* spp., *Pythium* and *Rhizoctonia solani*. Gerrettson *et al.* (1976) found that growth and pathogenicity of *P.cinnamomi* were inhibited by the bark extract of *Pinus radiata* D.Don.

Vasyukova *et al.* (1977) found that, both deltozid and deltonin (second saponins from the rhizome of deltoid yam) were inhibitory to zoospore of *P.infestans*. Leaf extract of six plant species were tried against *P.palmivora* and found that *Xylia xylocarpe* (Rosb.) Taub. was the most effective one (Hegde, 1983).

Ajoene, a compound derived from garlic (*Allium sativum* L.) was found highly inhibitory to *P.dreschleri* f.sp. *cajani* (Singh *et al.*, 1992). Zoospore germination of *P.dreschleri* f.sp.*cajani* was found inhibited by the extracts of garlic and onion at 5,000 and 10,000 ppm. respectively (Chauhan and Singh, 1991b). Zuberi (1987) reported the antifungal activity of garlic on *Aspergillus flavus* spores. A clear zone of inhibition was noted in garlic extract treated plates. Kohlos *et al.* (1993) reported the antifungal action of garlic on *Inonotus obliquus*. Antimicrobial activity of garlic has been well established by many workers (Rees *et al.*, 1993; Hughes and Lawson, 1991; Dalaha and Garagust, 1985; Jain, 1993; Weber *et al.*, 1993; Dhaliwal and Dhaliwal, 1971). Compound responsible for antimicrobial activity of garlic has been isolated, purified and identified as allicin (di allyl thiosulphinate) (Barone and Tansey, 1977). Later on Pandy *et al.* (1990) Yoshida *et al.* (1987) and Singh *et al.* (1990 & 1992) have isolated a compound called ajoene from garlic extract, which showed high inhibitory action against fungi.

Ernest Guenther (1978), has well documented the major constituents of mustard (*Brassica*

compestris L.*)* and he reported that, allyl isothiocyanide is the principal constituent of mustard oil and this compound showed high antimicrobial properties.

COMPATIBILITY OF BIOCONTROLS WITH AGROCHEMICALS

Integrated disease management (IDM) has became more relevant in the present crop protection strategies and biocontrol has became very important component, especially soil borne plant pathogens. Hence the compatibility of biocontrol agents with fungicides received considerable attention in recent years.

Fungicide application into soil will destort the existing equilibrium of microorganisms in the soil. Due to the fungicides, some get killed and some may over come the situation. Organisms escaped tend to multiply and proliferate in the soil. The biological equilibrium will change better for worse (Martin,1950). Saprophytic soil fungi influence survival/pathogenicity of soil borne pathogens by competition, antagonism or parasitism (Warcup, 1951; Weindling *et al.*, 1950). Soil application of metalaxyl to control avocado root rot enhanced the suppression of *P.cinnamomi* disease without affecting its biological antagonists (Malajczuk *et al.*, 1983). Chandra and Bollen (1961) demonstrated that soil application of nabam (100ppm.) and mylone (150ppm.) significantly reduced the number of fungal propogules in the soil. Corden and Young (1965) studied the effect of Vapam, metasol, mylone and nabam on soil fungi and found that a drastic reduction in the number of fungal propogules in the treated soil compared to untreated soil. Waksman and Starkey (1923) reported that fungal colonies developing on plates from fungicide treated soil represented relatively few species as compared to those in untreated soils. Quantitative studies revealed that certain genera like *Aspergillus, Penicillium, Fusarium* and *Trichoderma* became abundant in most of the treated soils. It was noted that indigenous of introduced *Trichoderma* sp. have greater tolerance to most of the broad spectrum fungicides and greater colonizing capacity than other soil competitors (Munnecke, 1972). Richardson (1954) demonstrated that *Trichoderma* and *Penicillium* in thiram treated soil have constantly better survivability and multiplication. Davet (1981) confirmed from his work

that, *Trichoderma harzianum* has got beneficial effect in thiram treated soil. Spores of *T.hamatum*, *T.harzianum* and *T.viride* isolates can tolerate exposure to methamsodium in dilution up to 350µg active ingredient when incorporated along with the fumigant and applied (Lewis and Papavizas, 1984).

Metalaxyl was reported non-toxic to *T.harzianum, in vitro*, in contrast benimidazole fungicides. Benomyl strongly inhibited the growth of *Trichoderma* spp. in culture even at the concentration of 0.5mg/litre. Captan, chlorothalonil, chloroneb and PCNB were not inhibitory to *Trichoderma* (Abd El moity *et al.*, 1982). Papavizas (1981) demonstrated the compatibility of metalaxyl with *T.harzianum* by the infusion of pea seeds with this fungicide before planting, with conidia of *T.harzianum*, which improved the survival of conidia and even increased the CFU in the rhizosphere compared to rhizosphere of plants where seed covered with conidia only.

Casida (1960), Malacinski and Konetzka (1966) demonstrated the effect of potassium phosphonate on disease suppression and its effect on other soil microorganisms. They reported that, different soil microorganisms including bacteria and actinomycetes can apparently utilize phosphonate as phosphorus source. Wongwathanarat and Sivasithamparam (1991) reported that, potassium phosphonate has got no negative effect on beneficial microorganisms in soil and it is compatible with *T.harzianum*. Rajan and Sarma (1997) proved the compatibility of potassium phosphonate with eight species of *Trichoderma* in an *in vitro* study even at 1200 ppm.

The population of different group of bacteria are generally altered by fungicide application in soil. They are reduced in number for a period, then multiply rapidly, usually the numbers exceeding those in untreated soil. Part of the rise in number might be due to decomposition of the chemical (Mattews,1924). After reaching a maximum, bacterial numbers fall towards those of untreated soil (Waksman and Starkey, 1923). The fall is some times very slow taking over a year. Treatment of cotton seeds with Agrosan GN had increased the bacterial population in the rhizosphere of cotton seedlings (Pugashetty and Rangaswami, 1969) for

the first seven days. An initial increase in bacterial population in Dithane-M45 and captan treated soil has been reported by several workers (Agnihotri, 1971; Balasubramanian, *et al.*, 1973; Cram and Vaarteya, 1957; Domsch, 1959). All concentrations (2.5, 5, 10 and 20ppm.) of aretan and lower concentrations of bavistin (5 to 20ppm.) stimulated bacterial counts (Sinha, *et al* 1979a & 1980b).

Contrary reports exist showing reduced population of bacteria following fungicide application. Nauman (1972) observed that vapam, dozomet and allyl alcohol when added to soil inhibited proliferation of bacterial population in the soil. Detrimental effect of worlex to the proliferation of soil bacteria was noticed during the first two week. Potassium phosponate was found compatible with *Enterobacter aerogenes* (Utkhede and Smith, 1993).

Yatzawa *et al.* (1960) observed that, allyl alcohol at or 112.5litre/ha. inhibited actinomycetes population. Similar inhibition was observed due to metham (Bollen *et al.*, 1954), indar (Sinha and Singh, 1979), carbendazim (Sinha *et al.*, 1980). Contrary in this, Roslyeky (1980) showed little initial effect on the actinomycete population following worlex application at recommended rates. Pugashetty and Rangaswami (1969) while studying the rhizosphere microflora of cotton seedlings as influenced pre-treatment of cotton seed with agrosan-GN, observed a reduction in the actinomycetes counts in initial stage of plant growth, but not in the later part of the growth. Balasubramanian *et al.* (1973) reported the compatibility of Dithane M-45 with different antagonistic actinomycetes. An appreciable increase in the population of actinomycetes in soil, treated with aretan has been reported (Hofer, 1958).

Malatozuk *et al.* (1983) reported that, metalaxyl stimulates the lytic capacities of soil micro organisms, antagonistic to *P.cinnamomi*. Bailey and Coffey (1985) reported that the composition and levels of microbial populations (bacteria, fungi and actinomycetes) of similar soils either active or inactive in the break down of metalaxyl, did not differ.

EFFECT OF HOST NUTRITION IN RELATION TO DISEASE INCIDENCE

Rhizosphere organisms and pathogens depend on nutrients present at rhizosphere areas for their food. Addition of chemical fertilizers one way or other influence the microbial equilibrium of rhizosphere as well as it may support or suppress the soil borne plant pathogens. The effect of plant nutrient solutions on late blight pathogen was studied extensively (Main and Gallegly, 1964; Borys, 1964). Sawicka (1993) and Rudkiewicz *et al.* (1983) reported that higher dose of nitrogen (200kg.nitrogen/ha) increased the haulm infection in potato by *P.infestans*. Increased dose of NPK and excessive nitrogen reduced the rishitin concentration related to host resistance in potato tubers, which caused high infection by *P.infestans* (Stroikov *et al.*, 1980). Graded doses of nitrogen and potassium showed increased susceptibility to infection in potato by *P.infestans* (Phukan, 1993). Phukan and Baruah (1989b) reported that increased concentration of potassium showed more susceptibility to *P.infestans*. Inhibitory action of phosphorus against *P.infestans* has been emphasized by Szczotka *et al.* (1973). Sharma and Sohi (1983) described that higher dose of nitrogen resulted good yield but it enhanced the infection due to *P.nicotianae* in tomato. They also reported that increased phosphorus yielded healthy fruits and less disease incidence. Nema (1990) studied the effect of graded dose of NPK on *Phytophthora parasitica* var *piperina* infection on betle vine and reported that all doses of P and K reduced the disease intensity while N enhanced the disease incidence in the field. Dirks *et al.* (1980) studied the effect of fertilizer on incidence of *P.megasperma* var *sojae* on soya beans, they reported that increased dose of chemical fertilizer enhanced the disease incidence in the field. Hoitink *et al.* (1986) reported that nitrogen concentration in the soil support the *Phytophthora* infection in rhododendron. Utkhede (1984d) studied the effect of ammonium sulphate, ammonium nitrate, calcium nitrate, urea and sewage sledge on *P.cactorum* infection of apple and reported that all the amendments enhanced the disease incidence.

HOST RESISTANCE

Host resistance is one of the major components of IDM and with great practical value. The centre of origin of black pepper is Western Ghats of India and it is expected that host resistance for *P.capsici* would be available in the center of origin. However high degree of resistance has not been located so far. Muller (1936) reported the black pepper variety Belantung from Indonesia as resistant to foot rot. Indian pepper cultivar Uthirankotta and the Indonesian varieties Djambi and Belantung reported to possess appreciable resistance (Holliday and Mowat, 1963). Ruppel and Almeyda (1965) reported that out of five *Piper* species tested, *P.aduncum* L., *P.scabrum* Sw., and *P.treleasanum* Britt. and Wils showed partial resistance. Albuquerque (1968) reported resistance in *Piper colubrinum* Link., *P.obliquum* and Balankotta were found to be resistant (Turner, 1971). In Ghana, *Piper quineese* has been reported to be resistant (Anonimous, 1977). Sarma and Nambiar (1982) screened different *Piper* species against *P.palmivora* (=*P.capsici*) and reported that *P.colubrinum* was apparently resistant. Sarma and Nambiar (1979) tested 40 Indian cultivars including Uthirankotta and 45 wild types adopting root dip inoculation technique and reported that all of them as susceptible. However *Phytophthora* tolerant lines of black pepper have been reported (Sarma *et al.*, 1996). Hegde (1984) conducted screening of seven cultivars in wilt sick plot and could not get a single resistant plant. Dutta (1984) tested the seedlings raised from seeds and cuttings of healthy black pepper vines survived in the badly infected gardens and reported that none of them were resistant.

INTEGRATED DISEASE MANAGEMENT (IDM)

Integrated disease management would be the ideal strategy to tackle the complex and elusive soil borne problems like foot rot of black pepper, since any single approach would be of little consequence to contain the disease. Nursey hygiene, phytosanitation and other cultural practices, chemical, biocontrol measures coupled with host resistance are important components of IDM, that would reduce the pesticide load into the environment. Out of the various components of IDM, biocontrol programmes are of high priority in managing soil

borne plant pathogens. Curl *et al.* (1976) observed that ineffective amounts (1-2 µg/g soil) of PCNB applied together with *T.harzianum* Rifai controlled *Rhizoctonia solani* Kuhn. more effectively than did *T.harzianum* alone in cotton seedling disease in the green house. Henis *et al.* (1978) obtained green house control of *R.solani* damping off of radish by integration of PCNB (4µg/g soil) and *T.harzianum*. Lewis and Papavizas (1981) have reported that field control of root rot of cucumber caused by *R.solani* by integration of chlorothalonil with *T.harzianum* and cultural practices. Lewis and Papavizas (1981) obtained field control of root rot of cucumber and crown rot of pepper caused by *P.capsici* by integration of chlorothalonil with metalaxyl respectively with *T.harzianum*. Chandra (1984) reported that integration of both chemical and biological control measures showed a synergistic effect on the control of damping off in sugar beet. Mukhopadyay *et al* (1986) also obtained successful control of damping off of tobacco and egg plants by application of *Trichoderma* preparation to soil and integrating it with matalaxyl seed treatment. Stankova-Opocenska and Dekker (1970) reported that treatment of cucumber seed with fungicide (6-azauracil) at lower dose resulted in significant increase in the number of bacteria in the rhizosphere and control the damping off of cucumber seedlings caused by *Pythium debaryanum* Hesse.

Sarma *et al.* (1988) emphasized the importance of integrated disease management of *Phytophthora* infection in black pepper by using cultural, chemical, biological coupled with host resistance. Utkhede and Smith (1993) described the long term effect of chemical and biological treatment on crown rot of apple trees caused by *P.cactorum*. They reported that the integration of fungicides (metalaxyl, fosetyl-Al, mancozeb, copper+sulphur and captafol), along with *Enterobacter aerogenes* applied as soil drench and trunk drench reduced the infection. Utkhede and Smith (1991) reported that, metalaxyl along with *E.aerogens* significantly reduced the *P.cactorum* in apple orchards. Raicu and Stan (1976) discussed the feasibility of controlling the *P.(nicotianae) parasitica* infection in tomato by integrating the chemical and cultural methods.

MATERIALS AND METHODS

Rajan P.P "Approaches towards the integrated disease management of phytophthora infection of black pepper (Piper nigrum L.)" Thesis. Department of Botany, University of Calicut,1999

MATERIALS AND METHODS

1.1a). PATHOGEN ISOLATION

The pathogen, *Phytophthora capsici*, was isolated from infected plant parts and cultures were maintained on carrot agar for different experiments. For isolation , selective medium (PVPH - Tsao and Guy, 1987) was used. Antibiotics solution consisted of Pimaricin, Vancomycin, PCNB and Hymexazole was prepared as given below in sterile double distilled water and incorporated into Corn Meal Agar (Hi-Media) medium.

Composition of PVPH medium:

Pimaricin	-	10mg/100ml
Vancomycin	-	200mg/100ml
PCNB	-	100mg/100ml
Hymexazole	-	50mg/100ml

All antibiotics dissolved in 100ml sterile double distilled water, and stored in amber colored bottle in refrigerator as stock solution for further use. Antibiotic solution (PVPH) 10ml. was added to 90ml molten (40 - 50^0c) Corn Meal Agar medium and distributed into petri plates. This was done in laminar flow under sterile condition . Since Pimaricin is light sensitive, antibiotic solution preparation and incorporation to CMA was done without the presence of direct light.

Infected plant parts and soil collected from field were brought into the laboratory for isolation. Infected roots and woody stem pieces fastened in a cheese cloth and tied to a running tap water for 48 hours to leach off phenoles. Infected leaf samples and plant parts were surface sterilized with 0.1% mercuric chloride for one minute and washed thoroughly by passing through sterile distilled water for 3 -4 times and were blotted in sterile blotters and plated on CMA - PVPH medium . The plates were incubated in dark for 48 - 72hrs.

I.1b) ESTIMATION OF DISEASE POTENTIAL INDEX (DPI) OF *P.CAPSICI*

Since the direct estimation of *Phytophthora capsici* in soil was unsuccessful, disease potential index was estimated as per the method of Tsao (1983) which was adopted by Anandaraj and Sarma (1990) as follows by using the leaves of *Albizia falcataria* as bait. Infected soil samples were serially diluted with sterile soil and at each level 25g of soil mixture was taken and suspended in 100ml. distilled water. *Albizia* leaves were put on the surface of soil suspension and incubated for 48 - 72hrs. at low temperature (28 $\pm$ 1^{0}C). Albizia leaves took up infection by *Phytophthora* after 48 - 72 hours and isolation was done. Infection on *Albizia* leaves was confirmed by noticing the formation of sporangia round the lamina of leaves. Infected leaves were washed in running water to remove the adhered soil particles, suraface sterilized with 0.1% mercuric chloride, passed through sterile distilled water for 4 - 5 times and inoculated on CMA-PVPH medium as described earlier.

2. STANDARDIZATION OF PROTOCOLS

For multiplication and growth studies of *P. capsici* , carrot agar medium was used (Griffin, 1977) . Carrot agar medium was prepared as follows:

Carrot	-	200g
Agar	-	17g
Distilled water	-	1000ml

200gm. carrot was weighed and washed thoroughly and cut into small pieces. This was later mixed with about 200ml. of distilled water and blended in a blender. The extract was squeezed through muslin cloth and was made up to 1 litre with distilled water. To this 17g agar was added, boiled for 15 - 20mts, redistributed into 250ml flasks @150ml/flask and sterilized at 120^{0}C for 15 mts. This was used for all growth studies. *Phytophthora* cultures stored in carrot agar slants and kept in BOD at 20^{0}c for further studies .

2.1. STANDARDIZATION OF MEDIA FOR ISOLATION OF RHIZOSPHERE MICRO ORGANISMS

For isolation of soil micro organisms (Fungi, Bacteria and Actinomycete) from rhizosphere soil, different media were tried to standardize the best medium for isolation of different microbes. For fungi, four media which are commonly used in mycological studies were tried.

2.1.1. FOR FUNGI

1. **Rose Bengal Agar medium, modified** (Martin, 1950 and modified by Tsao, 1964)

Dextrose	-	10.00g
Yeast extract	-	0.50g
KH_2PO_4	-	0.50g
K_2HPO^4	-	0.50g
$MgSO_4.7H_2O$	-	0.50g
Peptone	-	0.50g
Rose Bengal	-	0.05g
Agar	-	17.00g.
Distilled water	-	1000ml
Streptomycin	-	0.03g

All the ingredients dissolved in distilled water distributed into 250ml conical flasks @150ml/flask. Required quantity (1.55g) of agar was added to each flask and autoclaved at 120^0C and 15lb.pressure for 15 minutes. Required quantity of streptomycin was added from the stock solution to the molten medium only at the time of pouring into petri plates.

2. **Corn Meal Agar medium** (Riker and Riker -1936) - (Hi-media)

Corn Meal	-	20g
Peptone	-	20g
Dextrose	-	20g
Agar	-	17g
Distilled water	-	1000ml

Corn meal was cooked at about 60^0c for one hour in 500ml.of distilled water and the solution was filtered through a cheese cloth and later added to melted agar (in 500ml. distilled water). The volume was adjusted to 1000ml. and distributed into 250ml. conical flasks. The medium was sterilized as described earlier.

3. **Czapek- Dox Agar medium:** (Raper and Thom- 1949)

$NaNO_3$	-	2.0g
K_2HPO_4	-	1.0g
$MgSO_4.7H_2O$	-	0.5g
KCl	-	0.5g
$FeSO_4.7H_2O$	-	10.0g
Sucrose	-	30.0g
Yeast Extract	-	1.0g
Agar	-	15.0g
Distilled water	-	1000ml.

The sucrose was added just prior to final sterilization

4. **Malt Extract Agar medium:** (Raper and Thom, 1949)

Agar	-	20g
Malt Extract	-	20g
Dextrose	-	20g
Peptone	-	1g
Distilled water	-	1000ml.

2.1.2. FOR BACTERIA

For isolation of bacteria from soil, two commonly used media were tried to find out the best media which can be used for isolation of soil bacteria

1. **Soil Extract Agar medium** (Allen, 1957)

Agar	-	15.0g
Glucose	-	1.0g

K_2HPO_4	-	0.5g
Soil Extract	-	100.0ml.
Tap water	-	900.0ml.
pH	-	6.8-7.0

Soil Extract was prepared by taking 1000g. sieved garden soil with 1000ml. of tap water and autoclaved for 30 minutes. About 0.5g., $CaCO_3$ was added and soil suspension was filtered through a double fold filter paper and clear extract was collected for media preparation. All ingredients including soil extract, dissolved in 900ml. tap water and boiled. Medium was distributed in 250 ml conical flasks and autoclaved. Before autoclaving pH of medium was adjusted to 6.8-7.0.

2. **Nutrient Agar medium -** (Hi-media)

Twenty gram of ready made formulation of nutrient agar medium, obtained from Hi-media was suspended in 1000ml. double distilled water and cooked. The pH was adjusted to 6.8-7.0 before autoclaving, medium was distributed in 250 ml. conical flasks and sterilized as described earlier.

2.1.3. FOR ACTINOMYCETES

For the isolation of actinomycetes from rhizosphere soil, three actinomycete isolation media were tried

1. **Actinomycetes Isolation Agar medium** : (Hi-media)

Ready made formulation of actinomycete isolation agar media obtained from Hi-media, dissolved @20g/litre of double distilled water and stirred with a glass rod. Glycerol was added @5ml/litre and boiled. Prepared medium was distributed in 250ml. conical flasks and sterilized as described earlier .

2. **Starch Casein Agar medium** : (Kuster and Williams, 1964)

Starch	-	10.00g
Casein (vitamin free)	-	0.30g
KNO_3	-	2.00g
NaCl	-	2.00g
K_2HPO_4	-	2.00g
$MgSO_4.7H_2O$	-	0.50g
Ca CO_3	-	0.02g
FeSO4.7H2O	-	0.01g
Agar	-	18.00g
Distilled water	-	1000ml.
pH	-	7.0 - 7.2

All ingredients dissolved in 1000ml. double distilled water and boiled. The boiled medium distributed in 250ml conical flasks and sterilized. Nystatin and Actidione (50mg/ml each) were added to the cooled (40^0C)liquid medium before pauring into plates.

3. **Jensen's Agar medium** (Jensen, 1930)

Dextrose	-	2.0g
Casein	-	0.2g
K_2HPO_4	-	0.5g
$MgSO_4.7H_2O$	-	0.2g
$FeCl_3.6H_2O$	-	Trace
Agar	-	15.0g
Distilled water	-	1000ml
pH	-	6.5 - 6.6

All ingredients dissolved in distilled water and pH was adjusted to 6.5 - 6.6 and boiled. The boiled medium distributed in conical flasks and sterilized.

2.2. STANDARDIZATION AND MASS MULTIPLICATION OF *P.CAPSICI* INOCULUM

2.2.1. MASS MULTIPLICATION OF *P.CAPSICI* INOCULUM

An experiment was set up to study the feasibility of carrot broth-sand media, which supported the growth and reproduction of pathogen. Incubation period to attain maximum DPI of pathogen was also monitored. Sieved sand (3mm) 500g was filled in poly propylene bags (12x 8)and moisturized with 150ml carrot broth (carrot broth was prepared as described earlier). The sand - carrot broth mixture was autoclaved for one hour at 121^0C and 15 lb pressure. After cooling , each bag was inoculated with five culture discs (5.0mm) from 48hrs old culture and incubated at 28 ± 1^0c . *Phytophthora* population as DPI was monitored (Anandaraj and Sarma. 1990) every five days intervals, and continued for one month.

2.2.2. STANDARDIZATION OF INOCULUM DOSE TO INDUCE ROOT ROT IN BLACK PEPPER

A pot culture study was conducted to standardize optimum dose of inoculum to induce high root rot in black pepper. For the study , a susceptible cultivar >Subhakara= was used. Rooted runner shoots were collected from bamboo splits and single node cuttings were planted in 12" pots filled with potting mixture and potting mixture consisted of sieved forest soil, sand and farm yard manure (FYM) at a ratio of 3:1:1. The plants which attained a six months growth (of 5-6 leaves stage) were used for the study. Ten plants of uniform growth were maintained for each treatment. Inoculum dose of 0.5%, 1.0%, 2.0%, 3.0%, 4.0%and 5.0% were imposed on the test plants during during June-July, coinciding with South-West monsoon and plants without inoculum were maintained as control. Four months after inoculation plants were uprooted. Observations were recorded for percentage of root rot,

fresh and dry weight of root and shoot were monitored. The inoculum dose which induced highest root rot and death was used for all pot culture experiments.

3. RHIZOSPHERE STUDIES

FLUCTUATION IN RHIZOSPHERE MICROBIAL POPULATION IN BLACK PEPPER GARDEN

A detailed rhizosphere study was under taken to monitor the fluctuations of *Trichoderma*, total fungi, bacteria and actinomycete population over a period of time at different depths (0-10cm, 10-20cm and 20 -30cm). Ten years old pure pepper garden - hybrid, Panniyur-1 trailed on *Erythrina indica* was selected for the study. The garden was partially infected with foot rot disease and few vines were dead previously. Experiment was started during January 1992 and continued for one year . Fifty healthy and ten diseased vines were selected randomly for sampling . During the study no chemicals or fertilizers were applied . For the comparison of rhizosphere microbes, 50 non rhizosphere areas were also taken in between the vines. Non - rhizosphere areas were selected at 1m. away from the base of the vines. One month prior to the commencement of the experiment, all the weeds along with the root system were removed from the non-rhizosphere to avoid the influence of the root system of weeds on microbial population and weed free condition in the garden was maintained through out the year. Soil samples were collected with the help of an auger (5cm diameter). From each sampling spot , three samples were collected. Five composite samples of each depth was taken from both rhizosphere and non rhizosphere areas. Same procedure of sampling was followed in diseased vines also. Sampling was done in every alternate months . In addition to the microbial population, pH of the soil , soil moisture level and *Phytophthora* population as DPI in diseased rhizosphere soils were also monitored. Soil sample processing and serial dilution was done as described earlier. *Trichoderma* population was monitored by using *Trichoderma* specific medium (Elad and Chet-1983).

***Trichoderma* Specific medium** (TSM)- (Elad and Chet - 1983).

$MgSO_4.7H_2O$	:	0.2g.
K_2HPO_4	:	0.9g
Ammonium nitrate	:	1.0g
Potassium chloride	:	0.15g
Glucose	:	3.0g
Agar	:	15.0g
Dexan- 60 wp or Metalaxyl	:	0.3g
Rose Bengal	:	0.05g
Chloramphenicol	:	0.25g
Distilled water	:	1000ml.

4. ISOLATION AND IDENTIFICATION OF BIOCONTROL AGENTS

4.1 PROCESSING OF SOIL SAMPLES FOR MICROBIOLOGICAL STUDIES

Rhizosphere soil samples with feeder roots were collected from bases of healthy black pepper vines. Soil particles adhering to feeder roots was tapped into wax paper and air dried for 24hrs. and sieved through 1mm. sieve. One gram soil was weighed from each sample and added to 9ml sterile distilled water. For thorough dispersion of soil in water, suspension stirred for 10 minutes. While revolving soil particles in water, 1ml. suspension was taken and added to another 9ml. sterile distilled water kept in vial aseptically by using sterile pipette and serially diluted (Warcup, 1955). For estimation of soil microbes, third (10^3), fifth (10^5) and sixth (10^6) dilution was used for fungi, actinomycetes and bacteria respectively. One ml. of soil suspension was added to sterile petri dish and about 18ml molten agar medium (40-45^{0}c) was added and the plates were rotated gently for even distribution of soil particles in medium. Three replications were maintained for each sample. Plates were incubated at 26- 28^{0}C for bacteria and fungi and 35- 40^{0}C for actinomycetes. Enumeration of

bacteria and fungi was done after 48hrs respectively. Actinomycetes colonies were counted only after 5 days .

Rhizophere soil samples along with feeder roots of pepper were collected from different pepper growing areas of south India . Soil particles adhered to feeder roots were collected and air dried over night. Feeder roots were cut into small pieces and washed thoroughly with sterile distilled water and plated on PDA medium for isolation of fungi.

Potato Dextrose Agar medium (Riker and Riker, 1936)

Peeled potato	:	200g
Dextrose	:	20g
Agar	:	17g
Distilled water	:	1000ml

Peeled potato of 200g was cooked in 500ml of distilled water for half an hour. The decanted extract was made up 1000ml. with distilled water. To this agar and dextrose was added and boiled, distributed into 250ml. conical flasks @150ml/flask and autoclaved for 15 minutes at 120^0C and 15 lb. pressure.

For bacterial isolation Nutrient agar medium (Hi-media) and for fluorescent pseudomonads modified Kings (B) medium was used.

Modified Kings (B) Medium (Geels and Schippers,1983)

Proteose peptone no.3	:	20.0g
K_2HPO_4	:	1.5g
$MgSO_4.7H_2O$	:	1.5g
Glycerol	:	10.0g
Agar	:	15.0g

Distilled water	:	1000ml
Cyclohexamide	:	100ppm
Ampicillin	:	50ppm.
Chloramphenicol	:	12.5ppm.

The prepared medium was boiled and distributed the medium into 250 ml . conical flasks and sterilized as described earlier. Cyclohexamide, Ampicillin and Chloramphenicol were added just before pouring the petri plates.

Feeder roots were plated both in PDA and Kings (B) media and incubated at 28 ± 1^{0}C for 48 hr. Fungal colonies appeared were subcultured into PDA slants. Fluorescent bacterial colonies viewed under UV light in Kings (B) medium were subcultured onto nutrient agar slants.

Air dried soil samples were sieved and serial dilution and plating was done for different microbes as described earlier. Predominant fungal and Actinomycetes colonies were isolated and maintained on PDA. Bacterial colonies appeared on soil extract medium were isolated and maintained on nutrient agar slants.

4.2. SCREENING OF TEST ORGANISMS FOR THEIR ANTAGONISM TO *P.CAPSICI*

a *IN VITRO*

Dual culture plate technique was adopted for initial screening of antagonists against *P.capsici.* Culture discs (0.5cm) from 48hrs old *P.capsici* placed at 3.5cm apart from center on carrot agar plates and culture discs of 0.5cm. of 48hrs old test fungus, grown on PDA were placed at 3.5 cm apart from *P.capsici* culture disc. Actinomycetes and bacterial cultures were streak at 3.5 cm. away from *P.capsici* culture and incubated at 28 ±1^{0}C.

Short listed isolates based on their antagonism were maintained at 20^0C in BOD incubator for further study. Bacterial isolates were maintained on YGCA. fungal cultures on PDA and actinomycete cultures on NA.

Yeast Glucose Calcium carbonate Agar medium - (YGCA)

Yeast Extract	:	10g
Glucose	:	10g
Calcium carbonate	:	20g
Agar	:	20g
Distilled water	:	1000ml

Yeast Extract , Glucose and agar added to the distilled water , while boiling calcium carbonate added little by little and stirred continuously to avoid charring .Slants were prepared and autoclaved. After autoclaving , the tubes were rotated between hands to ensure uniform distribution of $CaCO_3$ in medium and slants were made.

Rhizosphere organisms showed antagonistic property against *P.capsici* was stored in tubes and all subcultured to plates for pure cultures . Fungal and actinomycetes cultures were subcultured in PDA and Bacterial cultures in nutrient agar and 48hrs. old cultures were used for studies . Molten carrot agar medium poured into petri dishes and allowed to cool. Culture discs (0.5cm) of 48 hrs. old *P.capsici* were taken with the help of a cork borer. One disc each was placed on carrot agar medium 1cm. apart from the side of the plate and culture disc (0.5cm) of test fungi was placed 1cm. apart from side of the petri plate as just opposite to the pathogen (*P.capsici*). For bacteria and actinomycetes, *P.capsici* culture discs placed at centre of carrot agar plates as described earlier. and test organisms - bacteria and actinomycetes - were streaked 3.5cm. apart from the center of the plates. For each organism, three replications were maintained. Carrot agar with *P.capsici* disc served as control. Plates were incubated at 28 ± 1^0C. for 72hrs.

Radial growth of *P.capsici* in each plates was taken at every 24 hr. intervals. Incubation percentage was calculated in each treatment by using the formula formulated by Arora and Dwivedi (1979) as

$$S : \frac{C - T}{C} \times 100.$$ (S= percentage of inhibition, C= control reading, T= reading in treatment)

All test organisms were stored in tubes and incubated in BOD for further studies. Subculturing was done periodically. Fungal and actinomycetes were subcultured in every alternate months and bacterial cultures subcultured in every month.

4.3. *IN VIVO* EVALUATION OF ANTAGONISTS/HYPERPARASITES FOR THEIR DISEASE SUPPRESSIVENESS

Bioefficacy of antagonistic organisms against root rot pathogen was evaluated in plants raised in poly bags. Rooted runner shoots of susceptible cultivar of black pepper, Subhakara were raised as mentioned earlier, but in poly bags (6x10") filled with 1kg of nursery mixture. Cuttings reached at the growth stage of 5-6 leaves (six months old) were used for the experiment. For each isolate 5 cuttings were maintained and experiment was RBD.

4.3.1. INOCULUM PREPARATION

Fungi: Twenty seven isolates of *Trichoderma* and 104 of other fungal isolates were used for the bioefficacy study. All the fungal antagonists stored in CMA slants were subcultured into PDA plates. Broken sorghum seed were moistened with tap water (100ml. water/250g. seed) and filled in 8x12" polypropylene bags @ 250g./bag. Bags were autoclaved for one hour at 121^{0}C and 15lb. pressure. Culture discs (0.5cm.) were collected from 48hrs. old culture plates and inoculated the bags @5discs/bag. Five bags were maintained for each isolate and bags were incubated at room temperature (32 - 35^{0}C) for 20days. For each cutting, 20g. of inoculum was added around the root system, after removing the upper layer of the soil and later replaced the soil. For each isolate, five cuttings were maintained. Irrigation was done on alternate days with sterile tapwater.

Bacteria: All antagonistic bacterial isolates (16) stored in YGCA slants and cultures were streaked on to nutrient agar (Hi-media) plates for single cell colonies and incubated for 48hours at 28 ± 1^{0}C. Single celled colonies were isolated and streaked on NA medium for mass multiplication. For each isolate, five plates were maintained. To each plate about 10ml of sterile water was added, bacterial growth was scraped and added to a beaker. This was repeated twice. The whole inocula was thoroughly shaken and made upto 500ml. The bacterial suspension was added @100ml/cutting and five cuttings were maintained for each isolate. Two sets of control plants viz; i. Plants with inoculum pathogen inoculum alone, ii. Plants without pathogen inoculum as absolute control. Irrigated on alternate days with sterile tap water.

PATHOGEN: Virulent isolate of *P.capsici* was mass multiplied in sand-carrot broth as described earlier was used as inocula. Fifteen days after application of antagonists, 2% *P.capsici* incocula was applied at the base of the cuttings, around the root system after removing the upper layer of the soil mixture from the base and later replaced. Six months after the application of various antagonists, the experiment was concluded. Plants were uprooted and following observations were recorded as per the treatments.

1. Root rot (%)
2. Fresh weight of the root
3. Dry weight of the root
4. Height of the plants
5. No. of leaves
6. Fresh weight of the shoot
7. Dry weight of the shoot

4.4. FIELD EVALUATION OF BIOCONTROL AGENTS FOR DISEASE SUPPRESSION OF PHYTOPHTHORA FOOT ROT OF BLACK PEPPER

Biocontrol antagonists isolated from rhizosphere of black pepper were tried for their bio efficacy against the foot rot pathogen *P.capsici*. Based on the results obtained from *in vivo* screening, a field experiment was set up at Pulpally in Wynad district of Kerala state, where the foot rot incidence was very severe for the last many years. Partially foot rot affected black pepper garden (monocrop) was selected . Area selected was well drained and no irrigation was practiced and rainfed. The garden consisted of 10 years old Karimunda cultivar which is susceptible to *P.capsici*.

Mass multiplication of biocontrol agents were carried out as mentioned earlier. The CFU of each BCA isolate was noted before application as

1. *Trichoderma virens* (P12) = 5×10^5,
2. *Trichoderma hamatum* (T3) = 5×10^5,
3. *Trichoderma virens* (P72) = 6×10^5,
4. *Trichoderma harzianum* (P26) = 6×10^5,
5. *Trichoderma polysporum* (MT) = 5×10^5,

For each isolate 4 replications @12vines/plot were maintained. Between the treatments a border raw of vines, without the biocontrol application were maintained. Biocontrol inocula were applied before the onset of monsoon (during May), and application of the antagonists were carried out for three consecutive years (1994-1996). Soil application of BCA's were done after mixing 50g. of BCA with 1kg. of neem cake/vine. Inocula were applied at the base of the vines, around the base of the vine and earthed up to avoid the disturbances to root systems. Apart from this one basket full (10kg.) of FYM was applied per vine as farmers' practice. All cultural practices such as shade regulations, runner shoot pruning and minimum tillage were carried out. Soil samples were collected before application of BCA to monitor

the pathogen population (DPI), pH of the soil, soil moisture and other microbial load in the soil. Different species/isoates of *Trichoderma* were included in the experiment. Individually as well as their combinations were used for their effects on foot rot infection.

Treatments:

1. *Trichoderma virens* (P12)
2. *Trichoderma hamatum* (T3)
3. *Trichoderma virens* (P72)
4. *Trichoderma harzianum* (P26)
5. *Trichoderma polysporum* (MT)
6. Mixture of *T.virens* (P72), *T.harzianum* (P26) and *T.polysporum* (MT)
7. Control

Application of BCA was done as once in a year during June after receiving a pre-monsoon shower. Seasonal fluctuation of microbial population (0 - 20cm.depth) in the treated soils were monitored and compared with control at bimonthly intervals. Disease incidence and health of the vines were monitored every alternate months and data was analyzed.

5. CHEMICAL CONTROL

Three systemic fungicides were tested against *P.capsici* both *in vitro* and *in vivo*. Fungicides viz; Dimethomorph (acrobat), Potassium phosphonate (akomin) and Aureofungin(aureofungin sol)were studied for their both *invitro* and *in vivo* efficacy for their disease suppression. Technical grades of all these chemicals were used for all the experimental purposes.

Fungicides were tested for their effect on growth, sporulation, sporangial germination and zoospore germination of *P.capsici*.

5.1. *IN VITRO* TESTS

DIMETHOMORPH

Technical grade demethomorph was obtained from Nocil, Bombay. Five thousand ppm. of the solution was prepared by adding 1g. of dimethomorph powder to 100ml. of the sterile distilled water. For dissolution of the powder, the suspension was prepared in 50% alcohol. This was later mixed with required amount of water and kept at room temperature to evaporate the alcohol content. For sterilization, the solution was passed through a sterile G5 filter (0.22 μ) and the filterate was stored in an sterile ambor colored bottle kept in a refrigerator.

POTASSIUM PHOSPHONATE

Potsssium phosphonate, technical grade of the fungicide was obtained from Rallis India Limited, Bangalore, for the experimental purposes. Forty percent (400000ppm.) solution of the chemical was prepared and sterilized by passing through a sterile G5 filter (0.22 μ). The filtrate was collected in a sterile amber colored bottle and stored in refrigerator for further studies.

AUREOFUNGIN

Aureofungin, an antifungal antibiotic, technical grade was obtained from Hindustan Antibiotics Limited,Pimpri, Pune was used for the all experiments. Aureofungin (96.4%) was suspended in distilled water. It was not readily dissolved in water, but soluble in alcohol. Aureofungin powder weighed and dissolved in 50% alcohol and kept in room temperature for one hour to evaporate the alcohol content of the solution. Since aureofungin is a thermoliable in nature, the sterilisation was done by passing the solution through a sterile G5 filter (0.22 μ). Filtrate was collected in a sterile amber coloured bottle and stored at low temperature (refrigerator). To study the effects of dimethomorph, potassium phosphonate and aureofungin both for *in vitro* and *in vivo* studies on *P.capsici*, the methodology mentioned earlier was followed.

5.2. EFFECT OF SYSTEMIC FUNGICIDES ON ROOT ROT OF BLACK PEPPER CAUSED BY *P.CAPSICI*

A POT CULTURE STUDY

Based on the results obtained from the *in vitro* evaluation of different chemicals, a pot culture experiment was set up to study their *in vivo* efficacy. Experiment consisted of 11 treatments. The methodology adopted in similar as mentioned earlier. The experiment was conducted with the following treatments

1.	400ppm. of potassium phosphonate	(Three round foliar spray and soil drench)
2.	800ppm. of potassium phosphonate	,,
3.	1200ppm. of potassium phosphonate	,,
4.	300ppm. of dimethomorph	,,
5.	400ppm. of dimethomorph	,,
6.	100ppm. of aureofungin	,,
7.	200ppm. of aureofungin	,,
8.	300ppm. of aureofungin	,,
9.	1200ppm. of potassium phosphonate	(Two round foliar spray and soil drench)
10.	1200ppm. of potassium phosphonate	(One round foliar spray and soil drench)
11.	Control	

6. COMATIBILITY OF AGROCHEMICAL WITH BIOCONTROL AGENTS

From the results obtained from *in vitro* and *in vivo* chemical assays, potassium phosphonate found effective in suppression of root rot of black pepper. In the studies conducted earlier, different species of *Trichoderma* and *Verticillium tenerum* were found promising in suppression of *P.capsici.* Hence experiments were set up to study the compatibility of potassium phosphonate with different biocontrol agents, in order to integrate chemical and biocontrol methods.

6.1.*TRICHODERMA* SPP.

Nine *Trichoderma* species viz; *T.aureoviride*, *T.hamatum*, *T.harzianum*, *T.koningii*, *T.longibracheatum*, *T.polysporum*, *T.pseudokoningii*, *T.viride* and *T.virens* from culture collection of Indian Institute of Spices Research were used in the present study. Stock cultures of these antagonistic fungi were maintained on PDA slants, stored in BOD incubator at 20^0C for further studies. Potasssium phosphonate formulation was passed through a sterile G5 filter for sterilization and stored in refrigerator for further studies. For all the studies, three concentrations of the chemical viz; 400, 800 and 1200ppm. were used. For each treatment four replications were maintained and experiments were conducted at room temperature.

7. EFFECT OF SYSTEMIC FUNGICIDE ON GROWTH AND SPORULATION OF BIOCONTROL AGENTS

To study the compatibility of potassium phosphonate with different biocontrol agents, three concentrations of the chemical (400,800 and 1200 ppm.) were incorporated in sterile PDA medium at the time of pouring into the petri plates. Culture discs (0.5cm.) of the test isolates were taken from actively growing cultures (48 hr. old) and plated. Culture raised on PDA without potassium phosphonate served as control for each isolate. Growth of each isolate was recorded at 24 hrs. interval upto 72 hours.

To study the effect of potassium phosphonate on sporulation, cultures of different biocontrol agents were raised on PDA incorporating the different concentrations of potassium phosphonate. After 96 hours, 10 discs (0.5cm.) per plate at randomly were taken in 80ml. of sterile double distilled water in 250ml. beakers and agitated thoroughly with the help of a magnetic stirrer and the contents were made upto 90ml.with sterile double distilled water. Spore count was recorded in each treatment with the help of a hoemocytometer.

VERTICILLIUM TENERUM

In the case of *Verticillium tenerum* only growth studies were conducted. The methodologies were adopted as in the case of *Trichoderma spp.*

8. EFFECT OF PLANT EXTRACTS ON *P.CAPSICI*

Botanical pesticides are receiving importance attention due to their eco-friendly nature. Extracts of garlic and mustard were tried against *P.capsici* both *in vitro* and *in vivo.*

8.1. *IN VITRO* STUDIES

Garlic (*Allium sativum* L.) pearls and mustard (*Brassica compestris.* L.) seeds (100g. each) were soaked in 500ml double distilled water for overnight and ground with the help of a grinder. Thee xtracts were passed through a muslin cloth separately and squeezed to get contents completely without the residues. For complete extraction the procedure was repeated six times and the volume of each extract was made upto 1000ml (10%). Extracts were kept undisturbed for three hours to settle down the sediments. The clear upper layer decanted was passed through a sterile G5 filter and stored in refrigerator for further studies. Extracts were tested individually and their combinations against the four phases of *P.capsici* viz; growth, sporulation, sporangial and zoospore germination.

8.1.1. EFFECT OF GARLIC AND MUSTARD EXTRACTS ON GROWTH OF *P.CAPSICI*

To evaluate the bio-efficacy on growth of *P.capsici*, the extracts were incorporated in carrot agar medium, alone and in combinations at the time of pouring into petri plates. Later the plates were inoculated at the centre with *P.capsici* culture disc (0.5 cm) taken from the growing edges of 48 hours old culture. For each treatment three replications were maintained. *Phytophthora capsici* raised on carrot agar medium alone served as control. Plates were incubated at room temperature (28^0C) for 72 hr. Growth was recorded at 24 hours intervals. Inhibition percentage in each treatment was calculated by a formula of Arora and Dwivedi (1979). Experiment was conducted twice.

8.1.2. EFFECT OF GARLIC AND MUSTARD EXTRACTS ON SPORULATION OF *P.CAPSICI*

Different test concentrations of extracts of garlic and mustard, alone and their combinations were prepared in sterile double distilled water and the test solution was added @ 15ml. per plate. Forty eight hours old cultures of *P.capsici* raised in carrot agar medium in dark, was used as source of culture discs. *Phytophthora capsici* discs (0.5cm) from actively growing culture were placed in the test solution @3discs/plate and were incubated under luminiscent light (40w) for sporulation at 20°C for 48 hours. Culture discs in sterile double distilled water served as control. Sporangial count was taken at three microscopic fields at random from each sporulating disc (in petri plates) under 10x magnification. Inhibition percentage of sporulation was calculated in comparison with control. Experiment was repeated twice.

8.1.3. EFFECT OF GARLIC AND MUSTARD EXTRACTS ON INDIRECT GERMINATION OF ZOOSPORANGIA OF *P.CAPSICI*

The experiment was conducted as above and the treated discs were given a cold shock (10^0C) for 10 minutes and later incubated at room temperature for 30 minutes at 28^0C. Observations were recorded on the number of liberated and unliberated sporangia in three microscopic fields at random per disc. Percentage of inhibition of sporangial liberation was calculated in comparison with control. The experiment was conducted twice.

8.1.4. EFFECT OF GARLIC AND MUSTARD EXTRACTS ON ZOOSPORE GERMINATION OF *P.CAPSICI*

The sporulating culture discs of *P.capsici* were taken in a test tube and were given a cold shock as mentioned above to get the release of zoospores. The zoospore suspension was decanted into a separate test tube and agitated. The water was decanted retaining the zoospores settled down at the bottom. Zoospore suspension was incorporated with different concentrations of extracts and made upto 45 ml and distributed @ 15ml. per plate. Suspensions was incubated at room temperature for 24hours and the zoospore germination per microscopic field was recorded . Three observations were made per plate and the inhibition percentage was calculated in comparison with control. The experiment was repeated twice.

8.2. EFFECT OF GARLIC AND MUSTARD EXTRACTS ON DISEASE SUPPRESSION-A POT CULTURE STUDY

Since garlic and mustard extracts showed inhibitory effect on all the phases of *P.capsici in vitro*, a pot culture study was conducted to estimate their *in vivo* efficacy as a bio-fungicide against *Phytophthora* root rot in black pepper. The experiment was conducted during July - August of 1996, as per the details mentioned earlier.

Treatments:

1. 1% garlic extract
2. 2% garlic extract
3. 3% garlic extract
4. 1% mustard extract
5. 2% mustard extract
6. 3% mustard extract
7. 1% garlic extract + *T.harzianum*
8. 2% garlic extract + *T.harzianum*
9. 3% garlic extract + *T.harzianum*
10. 1% mustard extract + *T.harzianum*
11. 2% mustard extract + *T.harzianum*
12. 3% mustard extract + *T.harzianum*
13. 1% garlic extract + 1% mustard extract
14. 2% garlic extract + 2% mustard extract
15. 1% garlic extract + 1% mustard extract + *T.harzianum*
16. 2% garlic extract + 2% mustard extract + *T.harzianum*
17. Control

Root rot(%), fresh weight of root, dry weight of root, fresh weight of shoot and dry weight of shoot

9. EFFECT OF DIFFERENT ORGANIC SOIL AMENDMENTS ON ROOT ROT

Application of different organic soil amendments for black pepper is a common practice among the farmers, to enhance the soil nutrients as well as for betterment of soil texture. A pot culture experiment was conducted to study the influence of organic amendments on disease incidence. Locally available amendments viz; Coffee pulp, Poultry manure, Neem cake and Farm yard manure were used for the study. All amendments were analysed for their NPK and their quantities were adjusted to the recommanded doses of NPK (140 :55:270) by adding required chemical fertilizers. For comparison recommanded doses of NPK also was included as another treatment. The experiments were conducted two consecutive years during June - December period.

HOST: Rooted cuttings of cultivar Subhakara were raised in polythene bags (6x10"), filled with 1kg nursery mixture. Two months old nursery cuttings raised in polythene tubes, grown in soil mixture consisted of forest soil and sand in the ratio of 3:1 was used for the experiments. Cuttings were transplanted in earthern pots (12"), (without disturbing root system). filled with forest soil and sand at the ratio of 3:1@10kg/pot. Plants were irrigated on alternate days with tap water. Plants with 5 - 6 leaves were selected for uniformity and different treatments were imposed. Two controls, one with *P.capsici* inoculum and another without were maintained. RBD design was adopted with ten replicate pots/treatment.

Treatmentwise microbial population viz; fungal, bacterial and actinomycetes colonies were monitored. Fifteen days after imposing different amendments to the pepper plants, CFU of microbes were monitored at the dilution of 10^{-3} for fungi, 10^{-6} for bacteria and 10^{-5} for actinomycetes. Population of microbes were also monitored at the time of uprooting of plants.

Fifteen days after imposing different treatments all plants, except absolute control were uniformly inoculated with 20 days old 2% (200g) inoculum of *P.capsici*, raised in sand carrot broth. Inoculum was applied at the base of plants after removing the upper layer of soil,

without disturbing the root system and later covered. Plants were irrigated to ensure high soil moisture. DPI of *Phytophthora* and pH of treated soil were also monitored every month.

Six months after inoculation, plants were uprooted. Disease incidence, root rot (%), fresh and dry weights of root and shoot were recorded.

10. EFFECT OF HOST NUTRITION ON *PHYTOPHTHORA* ROOT ROT OF BLACK PEPPER

A pot culture study was conducted to study the effect of nutrients on root rot due to *P.capsici* in black pepper. A factorial experiment consisted of 27 treatments includes, three levels of NPK and their combinations (3x3x3) was conducted. Black pepper cuttings of Subhakara cultivar were raised in earthen pots as mentioned earlier.

Six months old plants with uniform growth (6 leaves stage) were selected and 12 plants were maintained for each treatment. Three levels of fertilizer viz. recommended dose (140:55:270), double the recommended dose (280:110:540) and without addition of NPK, in their 27 combinations were used. Fertilizers were applied in three splits. Urea, super phosphate and murite of potash was used as the source of NPK. One month after the application of last split of the fertilizers, plants were inoculated as mentioned earlier. Twenty days old inoculum of *P.capsici* raised in sand-carrot broth was applied to all pots @200g/pot (2%). Six plants were maintained un-inoculated in each treatment for comparison of root rot (%) and other growth parameters. Six months after the application of pathogen, all the treated plants were uprooted to score the root rot incidence (%) and other growth parameters as mentioned earlier.

11. INTEGRATED MANAGEMENT OF *PHYTOPHTHORA* INFECTION IN BLACK PEPPER

Based on the results obtained from various pot culture experiments, a pot culture study was conducted by integrating all components of disease management viz; cultural, chemical and

biological methods coupled with host resistance/tolerance. Total twenty one treatments with one absolute control were included in the experiment with two varieties of black pepper (P24 (tolerant) and KS27(susceptible). Garlic extract (3%), potassium phosphonate (1200ppm.) and coffee husk were common for all the treatments, except control. For each treatment 12 potted plants of same age were maintained.

Design : Split plot

Main plots : Varieties (P24 and KS 27)

Sub plots : Three fertilizer doses ($N_0P_0K_0$, $N_0P_1K_0$ and $N_0P_2K_0$)

Sub sub plots: Biocontrol agents (five)

1. *T.harzianum* (T1)
2. *Trichoderma virens* (T10) + *Verticillium tenerum*(63)
3. *Trichoderma virens* (T10)
4. *Trichoderma virens* (T19)
5. *Trichoderma virens* (T10) +*V.tenerum* (71)
6. *Verticillium tenerum* (63)
7. *Verticillium tenerum* (71)

The plants for the pot culture study were raised as mentioned earlier. First dose of fertilizer was given during the month of June, when the plants were six months old, second during October and third dose was given during the month of February. Application of coffee husk, garlic extract and first round application of potassium posphonate were done during the month of May.

Biocontrol organisms were mass multiplied on sorghum meal as mentioned earlier and was applied in each pot @30g/pot, 15 days after first round application of potassium phosphonate. Second round application of potassium phosphonate done during the last week of June. Utmost care was taken to avoid any disturbance to the root system.

Six plants out of 12 in each treatment were inoculated with *P.capsici* raises on sand - carrot broth, as described earlier. Before the application of pathogen, soil samples were collected from each treatment to monitor the biocontrol and other microbial population. Plants were irrigated daily with tapwater. Second and third round application of potassium phosphonate (1200ppm) was done during August and September. During the course of the study, disease symptoms as foliar yellowing and defoliation were monitored every week.

All the potted plants were uprooted during March, and soil samples were collected at the time of uprooting to monitor the *P.capsici* population as DPI, biocontrol and the microbial status of the soil. Following observations were made at the time of conclusion of the experiment.

1. Root rot (%)
2. Fresh weight of root
3. Dry weight of root
4. Fresh weight of shoot
5. Dry weight of shoot

Readings of treated plants were compared with the untreated plants of the same treatment and data were statistically analyzed.

RESULTS

Rajan P.P "Approaches towards the integrated disease management of phytophthora infection of black pepper (Piper nigrum L.)" Thesis. Department of Botany, University of Calicut,1999

RESULTS

1. STANDARDIZATION OF PROTOCOLS

1.1. STANDARDIZATION OF MEDIA FOR ISOLATION OF RHIZOSPHERE MICRO ORGANISMS

Different culture media were tried for the isolation of soil microbes from rhizosphere soil samples. For isolation of fungi, three semisynthetic media were tried based on the colony forming units (CFU). Rose Bengal Agar medium was found to be the best (CFU = $35.3x10^3$) for the isolation of fungal population from rhizosphere soils followed by Malt Extract Agar medium (CFU = $17.2x10^3$) and Corn Meal Agar medium (CFU = 5x103). For the isolation of rhizosphere bacteria, Soil Extract Agar medium was found to be the best of CFU of 8.7x106. For the isolation of rhizzosphere actinomycetes, Actinomycetes Isolation Agar medium (Hi-media) was found to be the best of CFU of $5.6x10^5$. **Table -I.**

Table I : Relative efficacy of different media for isolation of soil microbes

Fungi	**Media**	**RBA**	**ME**	**CMA**
(CFU/gm. = No. of colonies x 10^3)	No. of colonies	35.3	17.2	5
Actinomycetes	**Media**	**SC**	**JA**	**AIA**
(CFU/gm. = No. of colonies x 10^5)	No. of colonies	0.5	3.4	5.6
Bacteria	**Media**	**SEA**	**NA**	**K(B)**
(CFU/gm. = No. of colonies x 10^6)	No. of colonies	8.7	3.5	0

ME : Malt Extract
CMA : Corn Meal Agar
SC : Starch Casein
JA : Jensen Agar

AIA : Actinomycetes Isolation Agar

SEA : Soil Extract Agar

NA : Nutrient Agar

K(B) : King's(B) medium

RBA : Rose Bengal Agar Medium

1.2. Standardization of mass multiplication of *P.capsici* inoculum to induce root rot in black pepper

For large scale multiplication of *P.capsici* inoculum meant for pot culture experiments, sand-carrot broth medium was found best and cultures incubated for 20 days in temperature of 28±1^{0}C showed highest Disease Potential Index (DPI) **Table -2**. It was noticed that the DPI was increasing upto 20 days after incubation and remained the same even after 30 days. Reduction in DPI was noticed after 30days. Inoculum with highest DPI was used for pot culture study to find out the optimum dose of sand-carrot broth inoculum for the induction of maximum root rot in black pepper. It was found that 20 days old 2% sand-carrot broth inoculum was sufficient to induce maximum root rot (98%) in black pepper, followed by 5% (74%). Least root rot incidence was observed in plants treated with 0.5% of sand-carrot broth inoculum (40.5%). (**Table -4**). High reduction of root mass and over all growth of plants were observed in plants treated with 2% inoculum. DPI was same in all soil samples immediately after application (131072). But 10 days after inoculation the reduction in DPI showed in pots treated with 0.5% inoculum where as in other treatments the DPI increased. Significant reduction of DPI of *Phytophthora* was noticed in all treatments at the time of uprooting of plants (**Table -3**).

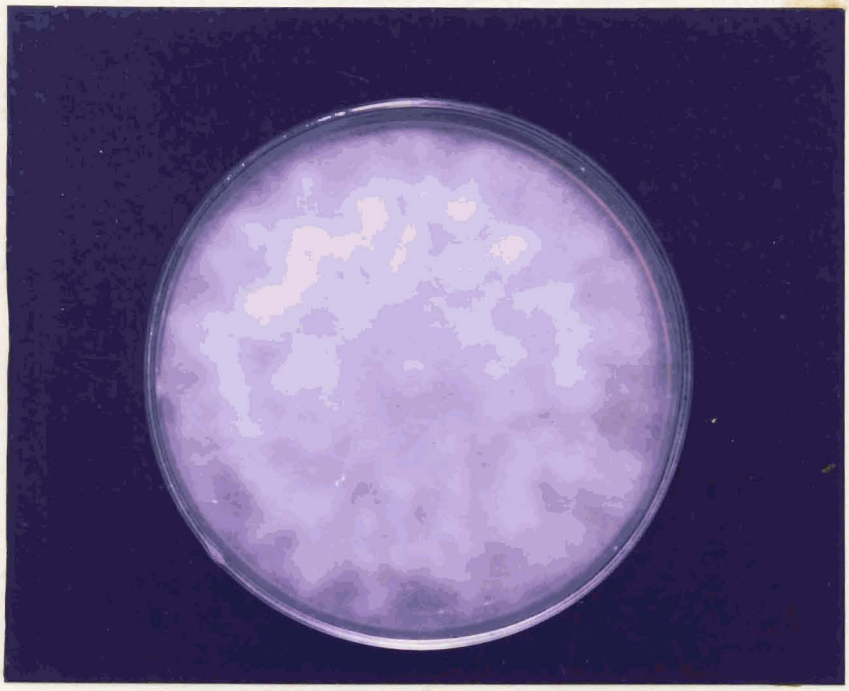


PLATE 2a: ***PHYTOPHTHORA CAPSICI***

PLATE 2b: INDUCTION OF ROOT ROT IN BLACK PEPPER USING CARROT BROTH-SAND INOCULUM OF *P.CAPSICI*
1: 0.5%, 2: 1.0%, 3: 2.0%, 4: 3.0%, 5: 4.0%, 6: 5.0%

11

63A

63B

Table -2: Disease Potential Index (DPI) of sand-carrot broth inoculum of *P.capsici*

Days after incubation	DPI
10 days after incubation	512
15 days after incubation	131296
20 days after incubation	13615104
30 days after incubation	13615104
40 days after incubation	1701888

Table 3: Disease Potential Index of soils, treated with different doses of sand-carrot broth inoculum of *P.capsici*

Treatment	Intervals	DPI
0.5%	Immediately after inoculation (0 hrs.) 10 days after inoculation At the time of uprooting (four months after inoculation)	131072 8201 2
1.0%	Immediately after inoculation (0 hrs.) 10 days after inoculation At the time of uprooting (four months after inoculation)	131072 16384 1
2.0%	Immediately after inoculation (0 hrs.) 10 days after inoculation At the time of uprooting (four months after inoculation)	131072 16384 1
3.0%	Immediately after inoculation (0 hrs.) 10 days after inoculation At the time of inoculation (four months after inoculation)	131072 16384 2
4.0%	Immediately after inoculation (0 hrs.) 10 days after inoculation At the time of uprooting (four months after inoculation)	131072 16384 2
5.0%	Immediately after inoculation (0 hrs.) 10 days after inoculation At the time of uprooting (four months after inoculation)	131072 16384 1
Control	Immediately after inoculation (0 hrs.) 10 days after inoculation At the time of uprooting (four months after inoculation)	0 0 0

Table -4: Effect of different inoculum doses on root rot and growth of black pepper

Treatments (inoculum dose)	Root rot(%)	Fresh weight of root (g)	Dry weight of root (g)	Fresh weight of shoot (g)	Dry weight of shoot (g)
0.5%	40.5	25.5	04.6	170.5	19.7
1.0%	41.0	27.0	04.1	146.5	46.9
2.0%	98.0	06.0	01.4	035.9	23.6
3.0%	71.0	15.3	03.4	081.0	37.5
4.0%	49.5	13.0	03.5	098.0	30.8
5.0%	74.0	16.2	03.9	129.0	49.2
Control	00.0	19.5	05.6	152.5	47.9
CD at 5%	24.8	12.4	3.34	27.37	23.5

2. RHIZOSPHERE STUDIES

2.1. Fluctuation of rhizosphere microflora in a black pepper garden

Rhizosphere study conducted in a pepper garden clearly indicated the fluctuation of soil microbes during different seasons. Maximum fungal population ($72.59x10^3$) in rhizosphere was observed during month of November and least was in May ($26.33x10^3$), where as in non-rhizosphere, maximum fungal population ($46.17x10^3$) was observed during the month of September and least was in the month of January ($15.57x10^3$). Highest bacterial population ($18.88x10^6$) in rhizosphere soil was observed during the month of July and least was in the month of March ($2.08x10^6$), where as in non-rhizosphere, highest bacterial count ($5.05x10^6$) was observed during the month of March and it was significantly low compared to rhizosphere soils and the least bacterial population in non-rhizosphere was noticed in the month of May ($2.43x10^6$). In the case of actinomycetes, highest count in rhizosphere ($2.09x10^5$) was noticed during the month of November and least was observed during the month of July ($0.18x10^5$), where as in non-rhizosphere highest count was noticed ($0.93x10^5$) during the month of September and it was significantly low compared to rhizosphere soils and least count was noticed during the month of March ($0.19x10^5$).

Table -5.

Table 5: Fluctuation of microbial populations in healthy rhizosphere of black pepper

Months	Fungi (x 10^3)		Bacteria (x 10^6)		Actinomycetes(x 10^5)	
	Rhizo-sphere	Non-rhizo-sphere	Rhizo-sphere	Non-Rhizo-sphere	Rhizo-sphere	Non-rhizo-sphere
January	32.81	15.57	07.06	03.05	0.83	0.46
March	47.37	36.44	02.08	05.05	0.66	0.19
May	26.33	27.49	04.68	02.43	0.57	0.44
July	47.07	45.42	18.88	02.89	0.18	0.46
September	39.89	46.17	02.88	05.01	0.50	0.93
November	72.59	19.56	03.05	04.42	2.19	0.57
CD at 5%	11.48		03.56		00.48	

Maximum fungal population in rhizosphere areas of black pepper was observed at the depth of 11-20cm.($47.16x10^3$) during the month of November but during September, when rain fall decreased, maximum fungal population ($46.08x10^3$) was observed slightly deeper layers of soil (21-30cm.), where as in non-rhizosphere, maximum fungal population ($72.59x10^3$) was observed during the month of September at the top layer of soil (0-10cm). Least count of fungal population was observed during November at all the depths (**Table -6**).

Table -6: Fluctuation in soil microflora of healthy rhizosphere and non-rhizosphere areas during different seasons and depths

Months	Fungi (x 10^3)						Bacteria (x 10^6)						Actinomycetes (x 10^5)					
	Rhizosphere			Non-rhizosphere			Rhizosphere			Non-rhizosphere			Rhizosphere			Non-rhizosphere		
	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm
January	02.12	02.90	02.90	15.57	04.10	22.98	06.44	05.83	03.56	02.08	05.04	05.55	0.82	0.73	0.43	0.66	0.19	0.69
March	31.78	41.38	26.91	26.33	27.49	32.36	03.81	06.59	03.55	04.68	02.43	04.42	0.51	0.76	0.50	0.57	0.44	0.62
May	01.61	35.67	46.25	47.07	45.42	52.34	01.41	04.82	10.89	18.88	02.89	12.88	0.08	0.75	0.32	0.17	0.46	0.39
July	39.55	31.94	43.03	39.89	46.17	40.03	04.81	03.78	03.95	02.88	05.01	03.20	0.62	0.51	0.72	0.50	0.93	0.94
Septem.	34.55	27.72	46.08	72.59	19.56	44.33	04.71	02.82	03.73	03.04	04.42	03.45	0.51	0.26	1.38	2.18	0.57	1.69
Novem	44.34	47.16	41.90	47.37	36.44	43.42	00.64	00.90	00.90	03.05	01.27	04.71	0.08	0.12	0.12	0.46	0.17	0.66
CD- 5%	19.88						6.17						0.83					

Table -7: Rhizosphere microflora of diseased and healthy vines

Months	Fungi (x 10^3)						Bacteria (x 10^6)						Actinomycetes (x 10^5)					
	Diseased			Healthy			Diseased			Healthy			Diseased			Healthy		
	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm
January	01.73	02.45	02.45	32.52	03.46	28.93	04.46	04.11	02.53	03.11	01.96	02.20	0.80	0.83	0.22	0.82	0.73	0.43
March	44.21	35.50	25.09	19.67	30.52	34.53	08.76	04.11	08.11	10.44	05.78	08.65	0.76	0.72	0.71	0.51	0.76	0.50
May	01.41	45.94	61.93	107.7	46.09	53.37	00.64	09.52	16.54	05.66	27.42	18.65	0.10	0.99	0.40	0.08	0.75	0.32
July	47.31	46.46	34.11	32.33	125.0	45.77	05.90	07.70	02.95	02.55	03.34	03.10	0.69	0.54	0.96	0.62	0.51	0.72
Septem	37.86	40.23	69.12	61.78	76.46	68.65	06.53	09.06	03.35	03.11	03.66	05.10	0.74	0.76	1.85	0.51	0.26	1.38
Novem	48.89	48.17	55.11	66.44	43.78	73.08	00.79	01.12	01.12	10.46	01.58	05.38	0.13	0.18	0.18	0.08	0.12	0.1
CD at5%	16.92						7.74						1.30					

Table -8: Fluctuation of soil microflora in diseased rhizosphere and nonrhizosphere areas of black pepper

Month	Fungi (x 10^3)						Bacteria (x 10^6)						Actinomycetes (x 10^5)					
	Rhizosphere			Non-rhizosphere			Rhizosphere			Non-rhizosphere			Rhizosphere			Non-rhizosphere		
	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm	10cm	20cm	30cm
Janu.	068.0	055.3	002.3	15.57	04.10	22.98	02.6	02.0	01.0	02.08	05.04	05.55	1.0	0.0	0.0	0.66	0.19	0.69
March	105.0	041.0	053.0	26.33	27.49	32.36	03.0	03.0	03.0	04.68	02.43	04.42	0.0	0.0	0.0	0.57	0.44	0.62
May	027.3	022.3	039.3	47.07	45.42	52.34	10.6	08.3	11.6	18.88	02.89	12.88	0.0	1.0	1.0	0.17	0.46	0.39
July	059.0	127.0	047.3	39.89	46.17	40.03	05.3	11.5	02.0	02.88	05.01	03.20	1.6	0.0	0.0	0.50	0.93	0.94
Sept.	053.6	015.6	027.6	72.59	19.56	44.33	03.6	01.0	03.0	03.04	04.42	03.45	2.0	0.0	0.0	2.18	0.57	1.69
Nov.	067.0	027.6	010.3	47.37	36.44	43.42	06.3	01.0	02.3	03.05	01.27	04.71	0.0	1.6	2.6	0.46	0.17	0.66
CD at 5%	19.88						6.17						0.83					

Highest count in bacterial population was observed in rhizosphere during the month of May ($10.89x10^6$) at the depth of 21-30cm. where as in non-rhizosphere highest count was observed at the top layer of soil ($18.88x10^6$) during the month of May. For actinomycetes population no much fluctuation was observed during the seasons and depths (**Table -6**).

During different seasons of the year, healthy rhizosphere areas yielded maximum fungal and bacterial population (**Table -7**), maximum fungal population was observed during the months of May and July at the top layers (0-10 and 11-20cm.) of soils ($107.7x10^3$ and $125.0x10^3$ respectively), where as in diseased rhizosphere areas, maximum fungal population observed during the month of May ($61.93x10^3$) at deeper layer (21-30cm.) of soil. In both the cases, the least number of fungal propogules observed during the month of March. Highest number of bacterial population was observed at the healthy vine bases during the month of May at the depth of 11-20cm. ($27.42x10^6$), where as in diseased vine bases it was significantly low during May ($9.52x10^6$). No much fluctuation in actinomycetes population was observed in either diseased or in healthy vine bases at different depths. Highest population of actinomycetes observed in diseased vine bases during the month of September ($1.85x10^5$) at the deeper layer (21-30cm).

Table -8 clearly indicated the fluctuation of microbial population in diseased rhizosphere areas with non-rhizosphere. Maximum fungal population in diseased rhizosphere areas was observed during July ($127x10^3$) at 11-20cm. depth and least count was noticed during the month of November ($2.3x10^3$) at the depth of 21-30cm. In non-rhizosphere areas maximum fungal population was observed during the month of September ($72.59x10^3$) at 0-10cm.depth and least was observed during the month of November at the depth of 11-20cm. ($4.1x10^3$). Maximum bacterial population was observed in diseased rhizosphere during the month of May and July in all the depths, where as in non-rhizosphere maximum bacterial count was noticed during the month of May at the top layer of soil. No much fluctuation was noticed in actinomycetes population both in diseased rhizosphere and non-rhizosphere areas.

Fluctuation in *Trichoderma* population was noticed in rhizosphere areas during different seasons. Maximum *Trichoderma* population was noticed during the month of July at the depth of 0-10cm and 11-20cm depths (7.22 and $7.27x10^3$ respectively). At the depth of 21-30cm., the *Trichoderma* population was significantly low during the month of March ($0.22x10^3$) Table -9. Irrespective of depths, the maximum population of *Trichoderma* was observed during the month of July ($5.27x10^3$) **Table -10.**

Trichoderma population in healthy and diseased vine showed considerable fluctuation. During the rainy season (May-November), the *Trichoderma* population showed an increase in in rhizosphere of healthy vines (**Table 11**).

Phytophthora population as DPI was monitored from diseased rhizosphere areas. Maximum DPI of pathogen was observed during the month of September (32) but during the months of January and March no *Phytophthora* isolation could made (**Table -12 & Fig – 1)**

Table -9 Seasonal fluctuation of *Trichoderma* population at different depths in rhizosphere of black pepper (CFU=10^3x1g.soil)

Months	Depths		
	10cm	20cm	30cm
January	08.66	01.11	00.44
March	01.49	01.49	00.22
May	00.94	00.11	00.50
July	07.22	07.27	01.33
September	00.94	00.72	01.11
November	00.83	00.11	01.49
CD at 5%	04.198		

Table 10: Fluctuation of *Trichoderma* population in rhizosphere of black pepper during different seasons

Months	*Trichoderma* population (x 10^3/g.soil)
January	3.40
March	1.07
May	0.51
July	5.27
September	0.92
November	0.81
CD at 5%	2.42

Table -11: Fluctuation of *Trichoderma* population in rhizosphere of diseased and healthy vines of black pepper (CFU x 10^3/g.soil)

Months	**Diseased**			**Healthy**		
	10cm	20cm	30cm	10cm	20cm	30cm
January	01.96	02.38	01.07	01.66	00.47	01.49
March	02.04	01.00	00.51	00.11	00.92	00.94
May	00.49	04.20	05.27	05.33	05.21	07.22
July	01.80	01.22	00.92	01.11	00.73	00.94
September	00.85	00.70	00.81	01.00	00.62	00.83
November	00.60	00.85	00.85	04.25	01.21	01.11
CD at 5%	05.93					

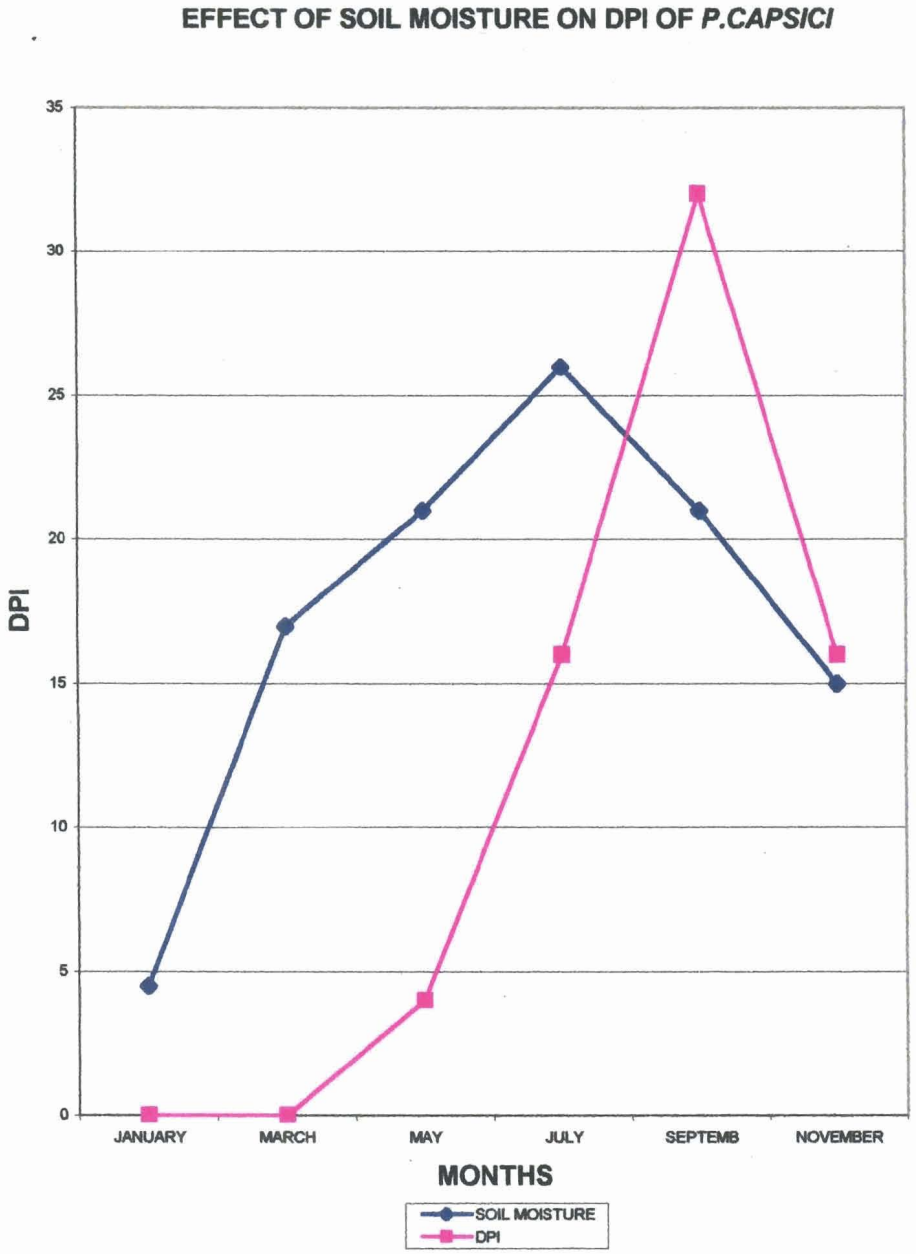

EFFECT OF SOIL MOISTURE ON DPI OF P.CAPSICI
35
30
25
20
15
10
5
0
DPI
JANUARY
MARCH
MAY
JULY
SEPTEMB
NOVEMBER
MONTHS
SOIL MOISTURE
DPI

FIG: 1

15

12A

Table -12: Soil moisture, Rainfall, Soil pH and *Phytophthora* population during rhizosphere study (0-30cm)

Month	Soil moisture		Soil pH		Rainfall (in mm)	*Phytophthora* population as DPI (diseased soil)
	Rhizo-sphere	Non-rhizo-sphere	Rhizo-sphere	Non-rhizo sphere		
January	04.5%	04.0%	6.1	5.9	---	00
March	17.0%	18.0%	5.4	5.0	028.0	00
May	21.0%	22.7%	5.2	5.0	411.0	04
July	26.0%	25.0%	6.0	4.5	1207.0	16
September	21.0%	20.0%	5.2	4.6	128.0	32
November	15.0%	14.5%	6.4	6.6	157.8	16

3. STUDIES ON BIOCONTROL

3.1. ISOLATION,MAINTENANCE AND *IN VITRO* EVALUATION OF ANTAGONISTS

Soil samples collected from different pepper growing areas of South India were analysed for microbial population. Fungal, bacterial and actinomycetes were isolated both from roots and rhizosphere soils (**Table -13**). Total 50 soil samples were tested, out of 50 samples 10 samples were collected from diseased rhizosphere areas from Wynad district of Kerala.

All fungal, bacterial and actinomycetes were tested against *P.capsici* for their antagonistic property against growth of the pathogen (**Table -14, 15, 16 & 17**). With the fungal isolates (other than *Trichoderma*) percentage of inhibition of *P.capsici* ranged from 5-97 and maximum inhibition was observed with isolates of 50,53,63 and 71. Isolates 50 and 53 were identified as *Penicillium* spp. and isolates 63 and 71 were identified as *Verticillium tenerum* (**Table -14**).

All the *Trichoderma* isolates were screened against *P.capsici* for their growth inhibition (**Table -15**) and percentage of inhibition ranged from 33-73.

All rhizosphere bacterial isolates, isolated from feeder roots were screened against *P.capsici* to evaluate their inhibitory effect on growth of *P.capsici*. Out of 16 isolates tested, three isolates (Pf3, Pf4 and B4) showed inhibition zone against *P.capsici*. Percentage of inhibition due to bacterial isolates ranged from 18-70 (**Table -16**).

Out of 12 actinomycetes isolates tested for their in vitro effects on *P.capsici*, six isolates (4,5,6,7,9 & 11) produced inhibition zone against *P.capsici*. Percentage of inhibition ranged from 0-50 (**Table -17**).

3.2. *IN VIVO* EVALUATION OF BIOCONTROL AGENTS

Antagonistic fungal and bacterial isolates were tested *in vivo* against *P.capsici*. Out of 104 fungal isolates tested, three isolates showed more than 95% root protection (isolates No.63, 71 & 74) and these were identified as *Verticillium tenerum* (Iso.63 & 71) and *Penicillium* sp.(74) nine isolates showed more than 90% root protection. More over all these isolates enhanced the overall growth of plants compared to other isolates (**Table -18**) . Out of 27 *Trichoderma* isolates tested *in vivo* against *P.capsici*, two isolates of *T.virens* (*T.virens*10 &19) gave 100% root protection (**Table -19**).

Table -13: Isolation of soil microorganisms from the rhizosphere of Black Pepper vines

Sl. No:	Fungi (Cfu/gm. = No. of colonies x 10^3)	Bacteria (Cfu/gm. = No. of colonies x 10^6)	Actinomycetes (Cfu/gm. = No. of Colonies x 10^5	pH of the Soil	Predominantfungal groups
KARNATAKA SOILS					
1.	15	2	1	7.30	*Penicillium*, Mucorales, *Aspergillus&Trichoderma*
2.	23	3	1	7.00	,,
3.	66	1	1	6.60	Mucorales & *Penicillium*
4.	19	2	1	7.10	,,
5.	09	4	0	7.00	,,
6.	08	5	0	6.80	,,
7.	20	3	1	7.20	*Penicillium* & Mucorales
8.	41	2	0	6.40	,,
9.	48	8	0	7.00	+ *Trichoderma*
10.	28	6	0	7.20	,,
11.	23	4	0	7.10	,,
12.	32	5	0	7.00	,,

13.	31	9	2	6.12	Mucorales & *Aspergillus*
14.	41	2	1	6.50	*Penicillium &Trichoderma*
15.	29	2	1	6.50	Mucorales, *Penicillium & Trichoderma*
16.	127	3	2	6.70	Mucorales
17.	80	6	2	6.80	Mucorales, *Penicillium, spergillus & Trichoderma*
18.	94	9	2	6.90	*Trichoderma*
19.	65	4	1	6.60	,,
20.	84	3	4	6.80	*Penicillium, Mucorales& Trichoderma*

KERALA SOIL

21.	43	18	0	5.73	Mucorales, *Penicillium, Aspergillus&Trichoderma*
22.	87	14	4	5.73	Mucorales, *Penicillium, & Aspergillus*
23.	65	3	2	5.44	Mucorales, *Penicillium, Trichoderma& Aspergillus*
24.	45	6	1	5.73	Two highly potential *Trichodermas* isolated & *Penicillium*

25.	23	2	10	5.5	*Penicillium, Aspergillus & Trichoderma*
26.	32	3	1	5.70	,,
27.	31	4	1	6.50	+ Mucorales
28.	07	4	2	6.80	,,
29.	04	7	0	6.50	,,
30.	08	4	1	6.30	,,
31.	58	1	2	5.60	Mucorales. *Penicillium & Trichoderma*
32.	45	3	1	6.40	,,
33.	45	2	0	6.80	,,
34.	18	3	1	6.70	,,
35.	28	2	2	6.70	,,
36.	32	11	0	6.89	+ *Aspergillus*
37.	37	1	2	6.60	,,
38.	22	6	1	6.90	,,
39.	38	4	3	6.70	,,

40.	17	2	2	6.70	*Penicillium*,Mucorales & *Trichoderma*
41*	36	9	4	6.90	Mucorales & *Trichoderma*
42.	32	3	2	5.80	*Penicillium*,Mucorales & *Trichoderma*
43.	42	5	3	5.60	,,
44.	15	2	2	5.90	*Penicillium*,Mucorales, *Trichoderma&Aspergillus*
45.	35	1	1	5.40	,,
46.	25	0	1	5.60	,,
47.	56	1	3	5.40	,,
48.	21	1	0	5.50	,,
49.	17	1	1	5.40	,,
50.	25	2	2	5.40	,,

*** 41 - 50 From rhizosphere of diseased vines**

Table -14 *In vitro* efficacy of rhizosphere fungal isolates against *P.capsici*

Isolate No.	Inhibition (%)	Isolate No.	Inhibition (%)	Isolate No.	Inhibition (%)
1	25.84	25	27.75	49	71.18
2	21.40	26	97.33	50	98.33
3	39.40	27	18.85	51	21.17
4	23.08	28	23.08	52	14.11
5	11.00	29	18.00	53	97.33
6	39.19	30	29.44	54	26.90
7	34.52	31	70.12	55	19.06
8	19.90	32	19.48	56	98.33
9	90.60	33	18.00	57	93.00
10	44.48	34	18.12	58	21.00
11	19.48	35	18.00	59	20.00
12	30.50	36	13.07	60	26.00
13	56.56	37	07.61	61	42.00
14	37.07	38	11.00	62	46.00
15	54.01	39	11.40	63	92.33
16	34.31	40	05.07	64	13.87
17	40.88	41	33.89	65	13.72
18	18.85	42	25.81	66	20.33
19	49.78	43	36.43	67	13.53
20	33.67	44	45.11	68	12.41
21	38.34	45	34.22	69	13.82
22	38.76	46	64.00	70	25.41
23	48.72	47	72.24	71	97.67

24	27.75	48	22.23	72	17.50
73	15.67	84	11.56	95	14.11
74	16.94	85	11.00	96	14.39
75	22.02	86	15.23	97	13.53
76	22.02	87	10.43	98	21.18
77	13.82	88	10.33	99	14.39
78	13.82	89	12.70	100	17.78
79	11.00	90	33.89	101	10.43
80	12.00	91	21.18	102	17.78
81	02.52	92	10.71	103	15.80
82	05.00	93	16.65	104	18.63
83	13.82	94	13.82		
CD at 5%	4.262				

Out of sixteen bacterial isolates tested for their bio-efficacy against root rot of black pepper, three isolates (Pf2, Pf5 & B-N) gave more than 95% root protection. Out of three isolates two isolates (Pf2 & Pf5) showed more than 98% root protection. Maximum growth in pepper plant was observed in plants treated with an isolate of Pf2 (Chett-1), which enhanced the height (145.8cm), number of leaves (11.3), maximum weight of shoot (34.0g) with least root rot (1.25%), compared to 17.33cm, 01.5, 04.0 and 83.63 respectively in control **Table -20**.

Table -15: *In vitro* inhibition of *P.capsici* by different species of *Trichoderma* isolated from rhizosphere of black pepper

Isolate No	Percentage of inhibition
T.virens -isol-1	49.20
T.virens -isol-2	33.09
T.virens -isol-3	69.48
T.virens -isol-4	52.58
T.virens -isol-5	41.78
T.virens -isol-6	40.04
T.virens -isol-7	73.49
T.virens -isol-8	36.85
T.virens -isol-9	48.69
T.virens -isol-10	53.75
T.virens -isol-11	42.48
T.virens -isol-12	40.37
T.virens -isol-13	44.83
T.virens -isol-14	53.52
T.virens -isol-15	54.69
T.virens -isol-16	38.49
T.virens -isol-17	53.76
T.virens -isol-18	41.08
T.virens -isol-19	53.99
T.virens -isol-20	42.25
T.virens -isol-21	44.59
T.virens -isol-22	56.80
T.aureoviride-1	42.25
T.aureoviride-2	43.65
T.harzianum-1	34.03
T.harzianum-2	59.15
T.polysporum-1	42.25
CD at 5%	3.717

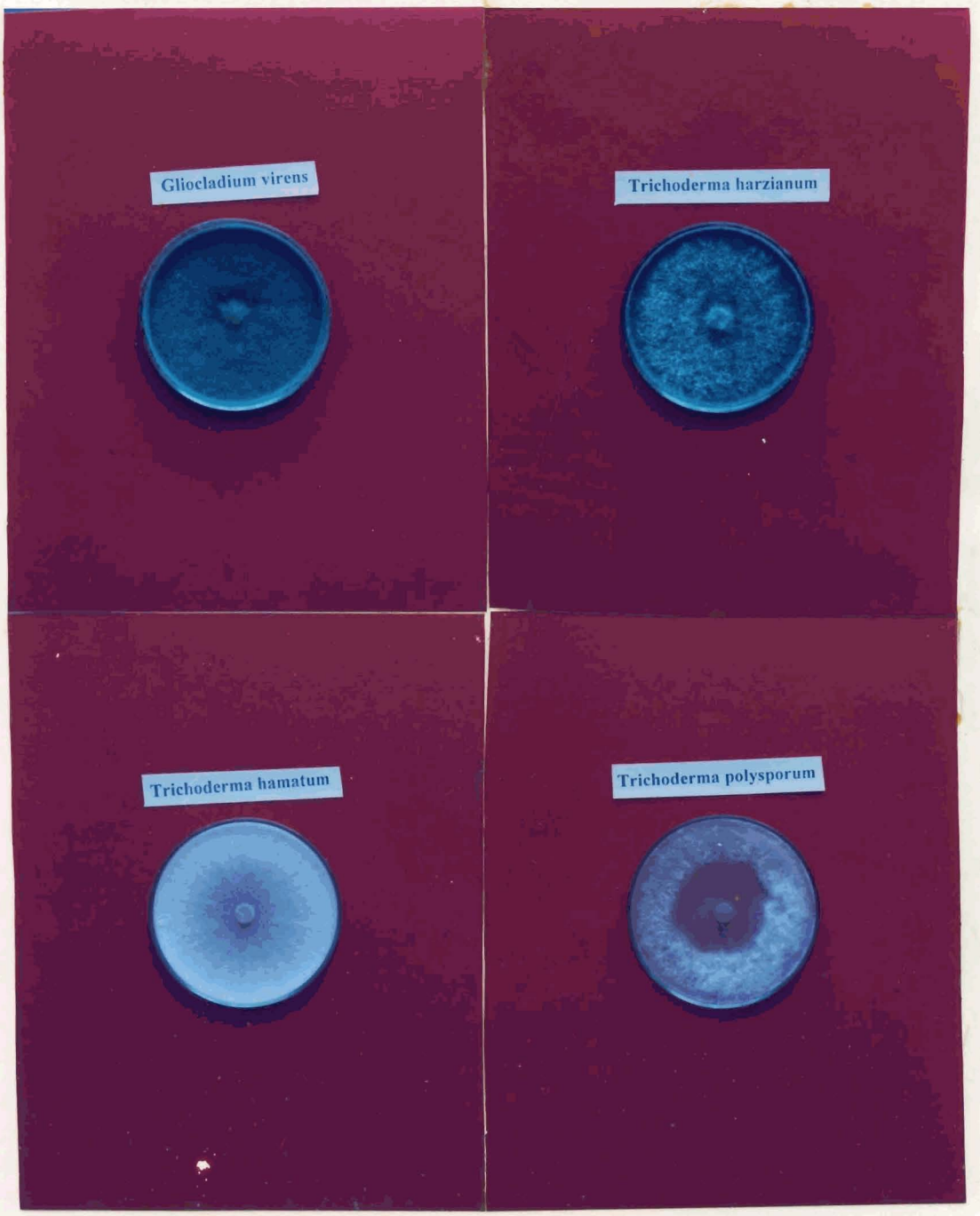


PLATE 3: DIFFERENT SPECIES OF *TRICHODERMA* ASSOCIATED WITH RHIZOSPHERE OF BLACK PEPPER

16 81A

81A

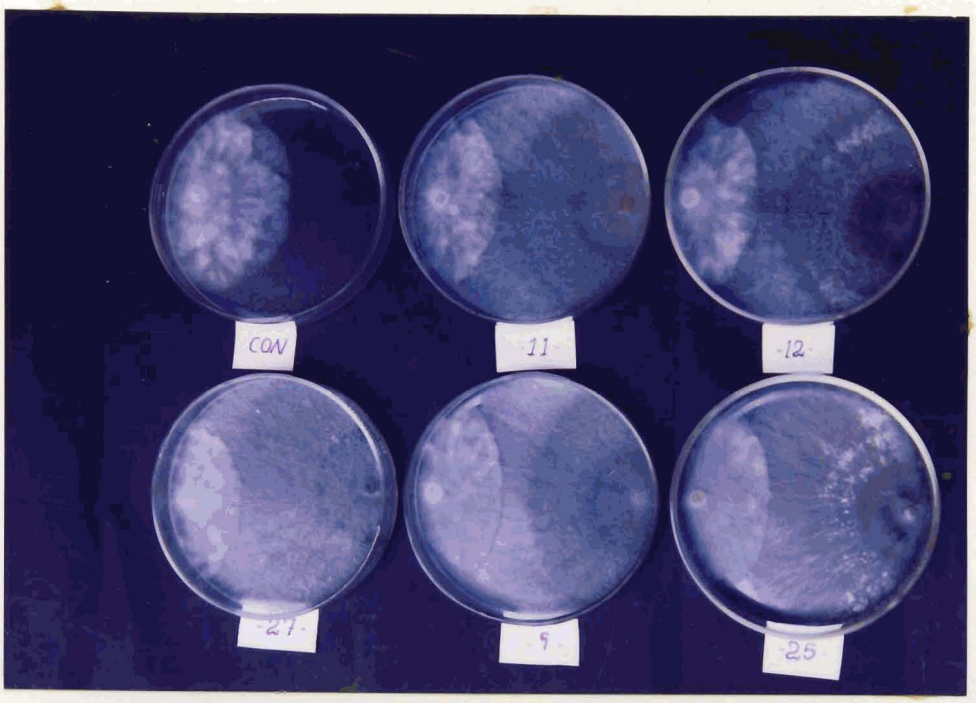


PLATE4a: DUAL CULTURE – *TRICHODERMA X P.CAPSICI*

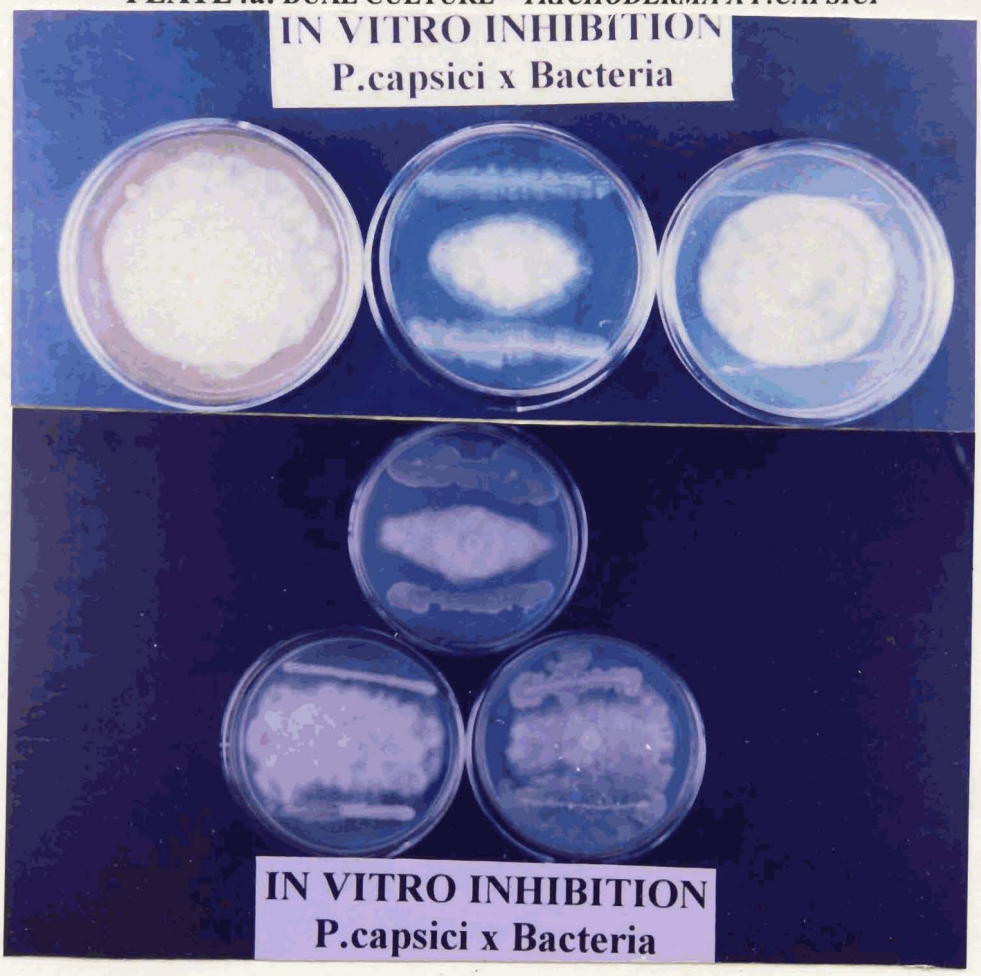


PLATE 4b:DUAL CULTURE – BACTERIA X *P.CAPSICI*

18
81B

Table -16: *In vitro* inhibition of *P.capsici* by bacterial isolates, isolated from rhizosphere of black pepper

Sl.No	Isolates	Inhibition zone (mm)	Inhibition percentage
1	*Pseudomonas fluorescens* (Pf-1)	00.00	00.00
2	Pf-2 (Chett-1)	00.00	39.87
3	Pf-3	02.33	39.85
4	Pf-4 (Chett-2)	05.75	47.85
5	Pf-5 (B-6)	00.00	58.24
6	Pf-6 (B-13/2)	00.00	45.19
7	B-10	00.00	43.96
8	B-X	00.00	42.19
9	B-2/2	00.00	40.49
10	B-4	16.92	70.36
11	B-3	00.00	37.21
12	B-9	00.00	24.94
13	B-11	00.00	29.44
14	B-12	00.00	18.81
15	B-17	00.00	42.33
16	B-N	00.00	32.71
CD at 5%		01.59	07.56

Table -17: *In vitro* inhibition of *P.capsici* by rhizosphere actinomycetes isolated from black pepper

Isolate No.	Inhibition zone	Inhibition percentage
1	00.00	47.03
2	00.00	47.03
3	00.00	43.02
4	04.42	48.04
5	03.67	44.44
6	07.75	46.78
7	06.08	50.55
8	00.00	00.00
9	07.42	36.99
10	00.00	38.50
11	02.00	45.78
12	00.00	39.77
CD at 5%	00.95	07.05

Table -18: *In vivo* evaluation of fungal isolates from rhizosphere on root rot of black pepper

Isolate No.	Root rot (%)	Fresh wt. of root (g)	Dry wt. of root (g)	Height of plant (cm)	Number of leaves	Fresh wt. of shoot (g)	Dry wt. of shoot (g)
1	25.0	02.4	2.00	41.2	07.8	07.3	2.71
2	95.0	00.1	0.08	04.6	00.6	00.8	0.14
3	98.0	00.3	0.13	02.2	00.6	00.4	0.08
4	42.0	01.4	0.21	10.2	03.6	04.8	0.98
5	82.0	00.5	0.07	06.0	01.4	01.8	0.36
6	20.0	02.2	0.54	37.0	03.0	07.6	1.61
7	04.0	07.1	1.13	71.2	05.2	12.0	3.50
8	75.0	01.4	0.21	22.0	02.2	02.8	0.82
9	40.0	02.4	0.57	40.6	06.2	10.0	2.21
10	40.0	02.4	0.56	40.6	06.2	10.0	2.21
11	62.0	01.4	0.31	11.4	01.6	02.2	0.58
12	80.0	02.4	0.60	16.4	00.4	01.6	0.51
13	52.0	02.0	0.40	24.8	05.2	05.2	1.41
14	94.0	00.9	0.23	12.6	03.4	03.4	0.90
15	90.0	00.3	0.09	05.2	01.0	02.0	0.48
16	98.0	00.2	0.06	03.2	00.2	00.8	0.26
17	82.0	00.2	0.06	03.2	00.2	00.8	0.26
18	27.0	02.4	0.50	81.2	11.0	16.0	2.53
19	98.0	01.4	0.42	11.0	01.2	00.9	0.31
20	80.0	00.8	0.14	09.0	00.2	01.2	0.31
21	90.0	00.4	0.13	15.6	01.6	01.8	0.26
22	02.0	02.1	0.54	40.8	07.0	09.2	1.96
23	100.0	00.0	0.00	0.00	00.0	00.0	0.00
24	94.0	00.6	0.21	18.4	01.6	02.2	0.72
25	96.0	00.8	0.26	17.6	04.0	04.0	1.36

26	88.0	01.3	0.46	20.8	02.0	03.3	0.98
27	100.0	00.0	0.00	0.00	00.0	00.0	0.00
28	60.0	00.8	0.25	24.0	04.8	06.4	1.94
29	20.0	00.8	0.34	24.8	07.0	09.0	2.00
30	96.0	00.4	0.11	06.0	00.0	00.6	0.18
31	52.0	02.6	0.45	18.0	03.0	05.0	1.41
32	66.0	01.9	0.37	24.6	05.8	07.0	1.65
33	72.0	01.0	0.34	20.0	03.6	04.2	1.24
34	88.0	00.8	0.33	23.0	02.8	03.8	1.40
35	02.0	02.5	0.50	22.2	02.8	06.2	1.63
36	02.0	02.4	0.50	22.2	02.8	06.2	1.64
37	02.0	02.6	0.48	22.2	02.8	06.2	1.49
38	72.0	01.6	0.31	18.4	02.2	04.4	1.11
39	99.0	00.8	0.15	09.0	01.0	01.8	0.77
40	44.0	01.4	0.71	57.0	06.8	09.8	2.04
41	72.0	00.7	0.22	28.4	02.2	03.0	1.38
42	34.0	03.55	0.53	57.6	05.2	12.5	3.38
43	62.0	01.1	0.45	28.4	02.6	03.4	0.83
44	100.0	00.0	0.00	00.0	00.0	00.0	0.00
45	100.0	00.0	0.00	0.00	00.0	00.0	0.00
46	100.0	00.0	0.00	0.00	00.0	00.0	0.00
47	82.0	00.2	0.05	06.0	02.0	00.6	0.13
48	65.0	02.0	0.44	33.2	04.8	06.6	1.53
49	80.0	00.4	0.08	10.0	01.6	01.8	0.41
50	100.0	00.0	0.00	0.00	00.0	00.0	0.00
51	10.0	04.0	0.78	55.0	04.0	14.0	3.36
52	48.0	01.0	0.30	23.4	02.0	04.2	1.12
53	55.0	00.9	0.17	07.0	00.4	01.6	0.36
54	100.0	00.0	0.00	0.00	00.0	00.0	0.00
55	100.0	00.0	0.00	0.00	00.0	00.0	0.00
56	85.0	00.6	0.30	04.6	00.8	01.0	0.29
57	100.0	00.0	0.00	00.0	00.0	00.0	0.00

58	100.0	00.0	0.00	0.00	00.0	00.0	0.0
59	10.0	01.4	0.38	32.4	01.8	05.0	1.34
60	30.0	03.4	0.75	46.2	05.4	08.2	2.11
61	100.0	00.0	0.00	00.0	00.0	00.0	0.00
62	100.0	00.0	0.00	00.0	00.0	00.0	0.00
63	05.0	06.0	1.90	81.6	11.8	14.8	2.80
64	85.0	00.6	0.22	18.0	01.8	02.8	0.84
65	98.0	00.6	0.19	12.0	00.8	01.6	0.50
66	98.0	00.6	0.24	05.2	01.0	01.4	0.30
67	100.0	00.0	0.00	00.0	00.0	03.4	0.00
68	29.0	00.4	0.27	23.6	02.4	00.0	1.30
69	35.0	04.0	0.63	17.6	03.6	08.0	2.04
70	98.0	00.2	0.03	06.2	00.6	01.2	0.34
71	00.0	08.7	1.85	70.2	11.8	17.6	5.07
72	100.0	00.0	0.00	00.0	00.0	00.0	0.00
73	55.0	01.3	0.46	14.8	05.0	03.8	0.86
74	00.0	04.0	0.60	27.0	04.0	08.0	1.60
75	88.0	01.4	0.21	13.6	01.8	03.0	0.72
76	100.0	00.0	0.00	00.0	00.0	00.0	0.00
77	28.0	03.2	0.75	24.0	04.0	06.4	1.44
78	90.0	00.5	0.25	12.0	01.2	01.2	0.26
79	68.0	01.6	0.63	19.0	01.8	03.9	0.77
80	100.0	00.0	0.00	00.0	00.0	00.0	0.00
81	100.0	00.0	0.00	00.0	00.0	00.0	0.00
82	100.0	00.0	0.00	00.0	00.0	00.0	0.00

83	80.0	00.8	0.09	08.8	01.6	01.6	0.47
84	88.0	00.3	0.08	04.0	00.4	00.8	0.26
85	100.0	00.0	0.00	00.0	00.0	00.0	0.00
86	100.0	00.0	0.00	00.0	00.0	00.0	0.00
87	86.0	00.8	0.33	18.0	03.6	03.6	1.40
88	82.0	00.2	0.03	08.0	01.8	01.8	0.62
89	100.0	00.0	0.00	00.0	00.0	00.0	0.00
90	50.0	02.4	0.51	23.0	07.4	16.0	3.40
91	100.0	00.0	0.00	00.0	00.0	00.0	0.00
92	42.0	05.2	0.99	16.6	01.4	04.7	1.93
93	100.0	00.0	0.00	00.0	00.0	00.0	0.00
94	100.0	00.0	0.00	00.0	00.0	00.0	0.00
95	99.0	00.6	0.24	03.2	00.2	00.4	0.08
96	100.0	00.0	0.00	00.0	00.0	00.0	0.00
97	60.0	00.6	0.12	13.8	01.8	02.8	0.69
98	75.0	01.6	0.51	12.0	00.8	02.0	0.44
99	05.0	04.2	0.84	42.0	09.6	11.8	2.75
100	80.0	00.7	0.24	05.8	00.2	01.5	0.43
101	100.0	00.0	0.00	00.0	00.0	00.0	0.00
102	100.0	00.0	0.00	00.0	00.0	00.0	0.00
103	96.0	01.0	0.45	16.0	02.0	02.0	0.50
104	100.0	00.0	0.00	00.0	00.0	00.0	0.00
Control	100.0	00.0	0.00	00.0	00.0	00.0	0.00
CD at 5%	06.09	0.305	9.154	3.75	0.536	0.795	0.202

Table -19: *In vivo* evaluation of different *Trichoderma* isolates on root rot of black pepper

Isolates	Root rot (%)	Fresh wt of root (g).	Dry wt. of root (g)	Height of plants (cm)	No. of leaves	Fresh wt. of shoot (g)	Dry wt. of shoot (g)
T.virens -1	46.00	01.00	00.52	33.00	03.80	05.20	01.02
T.virens -2	80.00	00.35	00.03	08.40	01.00	02.80	00.68
T.virens -3	06.00	02.10	00.54	45.60	06.20	10.70	02.54
T.virens -4	20.00	02.20	00.62	35.40	02.80	07.80	01.56
T.virens -5	18.00	01.60	00.63	30.40	08.40	05.20	01.51
T.virens -6	30.00	02.40	00.26	40.20	07.20	09.00	02.02
T.virens -7	23.00	01.30	00.52	17.00	03.80	02.70	00.49
T.virens -8	100.0	00.00	00.00	00.00	00.00	00.00	00.00
T.virens -9	20.00	00.80	00.58	24.00	08.00	01.60	00.53
T.virens -10	00.00	02.00	01.19	40.00	08.00	08.00	05.80
T.virens -11	100.0	00.26	00.19	04.00	01.00	01.00	00.29
T.virens -12	00.00	06.89	01.54	22.20	03.00	08.00	02.81
T.virens -13	50.00	02.10	00.17	51.00	11.20	12.00	02.79
T.virens -14	40.20	02.44	00.28	42.60	02.40	09.60	02.22
T.virens -15	26.00	00.80	00.37	78.40	08.80	13.60	02.26
T.virens -16	100.0	00.00	00.00	00.00	00.00	00.00	00.00
T.virens -17	100.0	00.32	00.02	15.60	00.40	01.20	00.60
T.virens -18	46.00	00.60	00.07	13.20	01.20	02.40	01.26
T.virens -19	00.00	04.67	01.05	48.00	07.00	09.60	01.85
T.virens -20	100.0	00.00	00.00	00.00	00.00	00.00	00.00
T.virens -21	100.0	00.00	00.00	00.00	00.00	00.00	00.00
T.virens -22	97.00	00.20	00.07	14.40	02.40	00.00	00.00
T. aureoviride-1	62.00	01.12	00.42	52.40	02.80	03.80	00.81
T. aureoviride-2	30.00	03.68	00.54	46.20	06.00	08.20	00.92
T.harzianum-1	75.00	01.40	00.50	22.00	02.20	02.80	00.98
T.harzianum-2	97.00	00.20	00.07	14.40	02.40	03.20	02.11
Trichoderma polysporum-1	06.00	02.04	00.26	30.60	04.00	04.60	00.94
Control	100.0	100.0	00.00	00.00	00.00	00.00	00.00
CD at 5%	36.85	1.504	0.4002	27.11	2.939	4.858	1.144

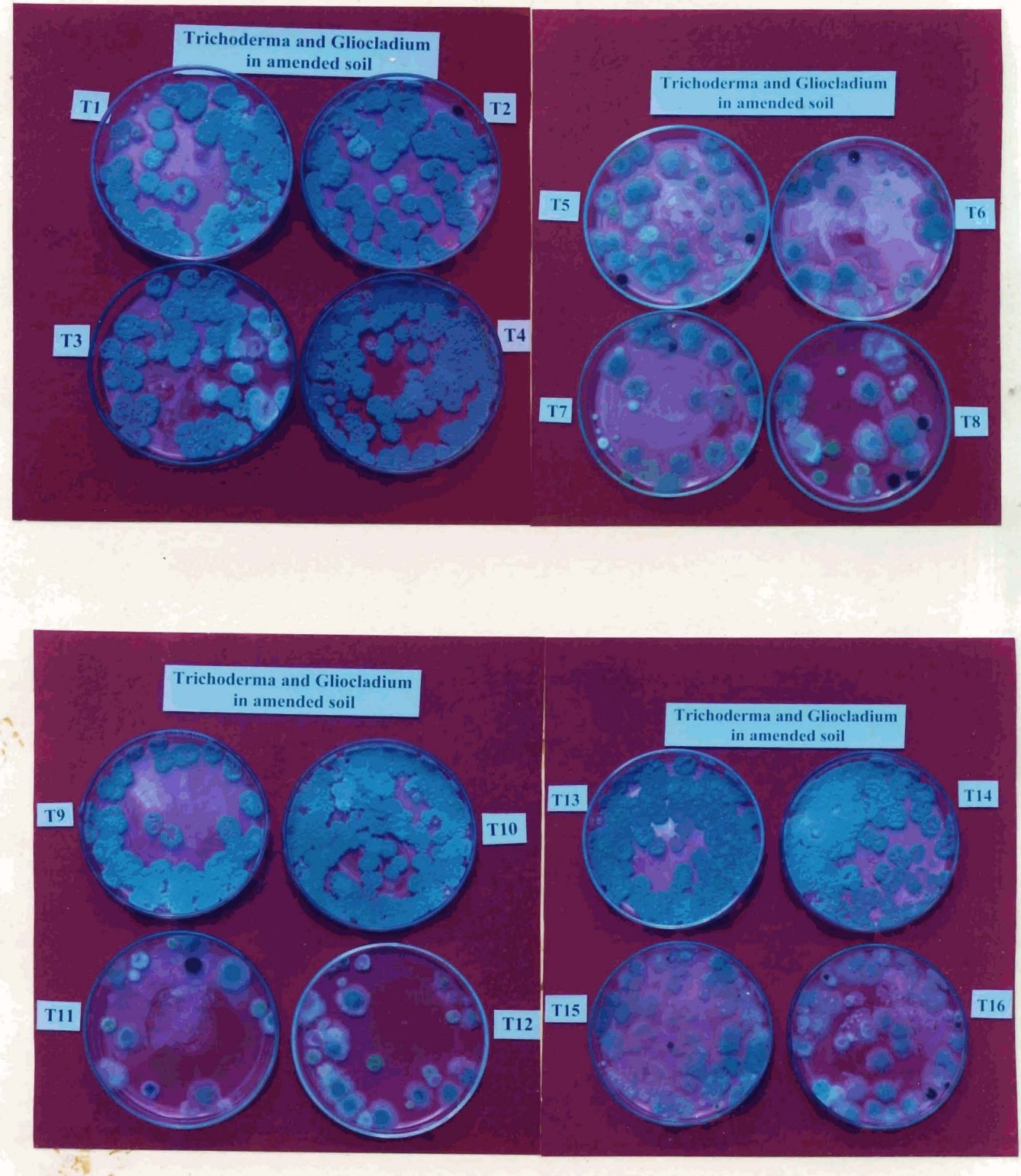
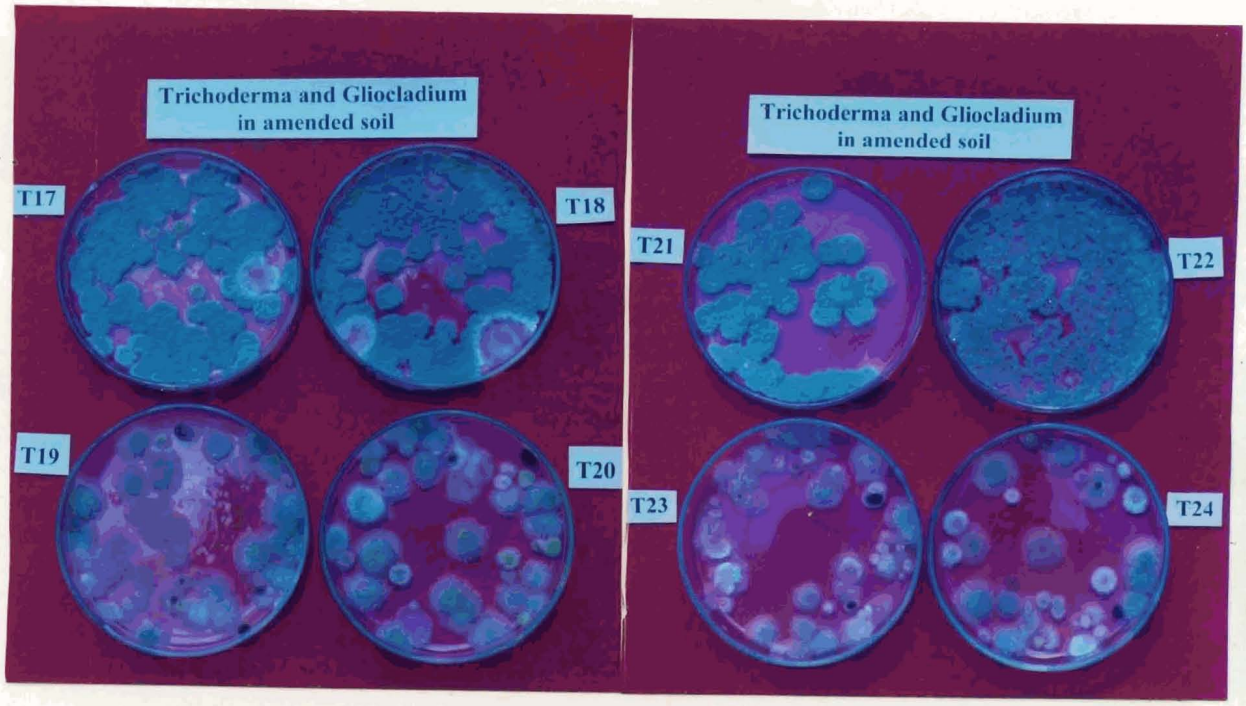


PLATE 5:*TRICHODERMA* ISOLATES RETRIEVED FROM TREATED SOIL USING TRICHODERMA SPECIFIC MEDIUM (TSM)

88A



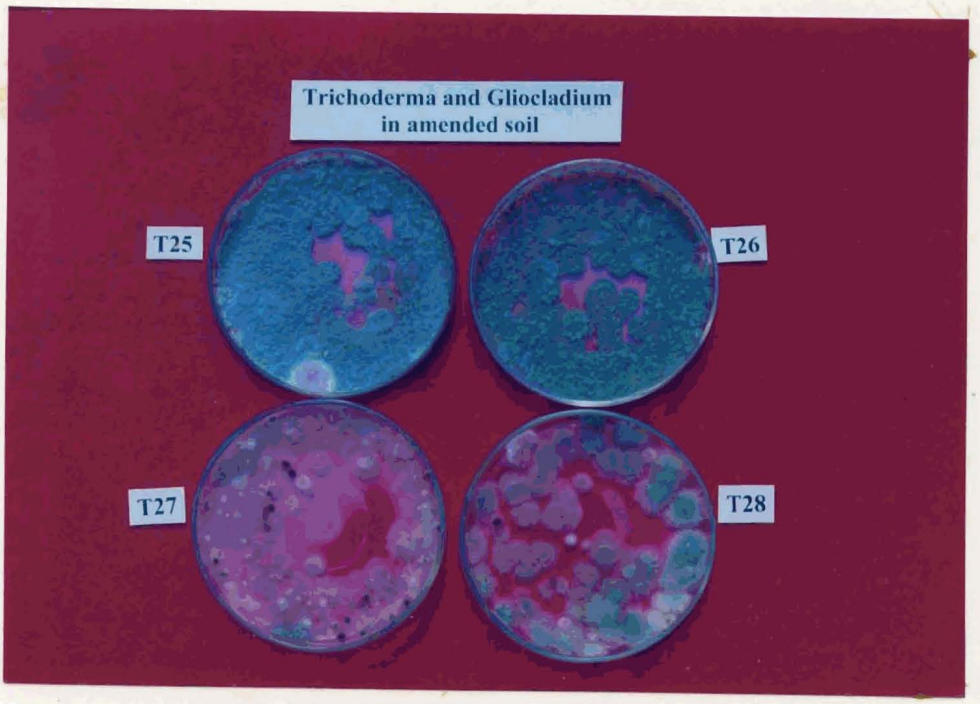


PLATE 5 continue...:*TRICHODERMA* ISOLATES RETRIEVED FROM TREATED SOIL USING TRICHODERMA SPECIFIC MEDIUM (TSM)

Table -20: *In vivo* evaluation of antagonistic rhizosphere bacteria on root rot of black pepper

Isolate	Root rot (%)	Fresh wt. of root (g)	Dry wt. of root (g)	Height of plants (cm)	No.of leaves	Fresh wt. of shoot (g)	Dry wt. of shoot (g)
(Pf-1)	78.13	01.25	00.44	24.00	01.00	05.00	03.75
Pf-2 (Chett-1)	01.25	06.4	04.16	145.8	11.30	34.00	18.13
Pf-3	81.25	01.31	00.61	14.00	02.85	09.63	04.75
Pf-4 (Chett-2)	81.25	01.39	00.59	23.50	01.13	07.13	04.50
Pf-5 (B-6)	01.25	04.94	01.71	41.38	06.50	21.25	12.75
Pf-6 (B-13/2)	23.75	02.67	01.43	45.63	04.50	19.88	12.63
B-10	38.75	02.49	01.53	58.50	03.00	17.13	10.75
B-X	18.75	03.83	01.86	86.75	06.50	20.00	13.50
B-2/2	41.88	02.49	01.43	41.50	04.13	18.00	11.00
B-4	23.13	04.45	01.98	67.13	05.38	18.25	12.13
B-3	37.50	04.24	01.47	95.88	07.30	19.88	12.13
B-9	47.50	01.88	00.94	53.63	02.88	10.00	09.00
B-11	65.63	01.94	00.65	24.25	01.40	08.63	06.25
B-12	16.25	04.47	02.07	114.0	07.50	22.25	14.38
B-17	25.63	02.93	01.21	111.3	04.00	21.75	12.88
B-N	02.50	03.06	01.65	82.63	05.63	21.13	13.63
Control	83.63	00.75	00.36	17.13	01.50	04.00	03.13
CD at 5%	50.61	03.08	01.44	81.15	05.89	15.59	09.32

3.3. FIELD TRIAL

The experiment was started during 1994, during the three years of field study, there was significant reduction in disease incidence in pepper field where biocontrol inoculum was applied. Reduction of disease incidence was gradual. During the second (1995)and third year (1996), least disease index, 0.9 and 1.6 compared to 42.9 and 10.2 in control, was noticed in plots where *T.virens* iso-12 was applied. During 1995, disease index was 0.915 where isolate

P-12 was applied, where as in control the disease incidence was 42.98. During 1996, the disease index was 1.6 and it was significantly lower than in control plants (10.2), followed by *T.hamatum* (iso-3) and *T.virens* (iso-72). **Table -21.**

Table -21: Disease Incidence in a biocontrol agents (BCA) applied black pepper field

Treatments	Disease Index*		
	1994	1995	1996
1.Trichoderma virens-P12	10.458	00.915	01.6
2.Trichoderma hamatum-T3	06.147	03.294	03.2
3.Trichoderma virens-P72	12.278	04.179	04.2
4.Trichoderma harzianum-P26	16.731	10.333	08.1
5.Trichoderma polysporum-MT	34.063	31.734	07.2
6.Mixture of 1,2,3 &4	26.063	45.894	11.2
7.Control	35.551	42.982	10.2
CD at 5%	15.60	20.55	06.18

* Scores from 0 - 4 were allocated for those vines having healthy, 0- 25% yellowing/defoliation 26 - 50%, 51 - 75% and 76 - 100% respectively.

The disease index was not significantly different among various treatments with biocontrol agents. However all the treatments were significantly superior over control (**Fig - 2**). Application of mixture of biocontrol inoculum did not indicate any superiority over the individual organisms on the root rot suppression.

During different seasons of the year maximum BCA population was noticed during July-November. The population of *Trichodrma harzianum* (iso-P26) was significantly higher and persistant in all the seasons compared to all other isolates used. Maximum *T.harzianum* iso-26 population(43.63 x 10^3) was noticed in the month of September and minimum (13.83x10^3) during January **Table -22.**

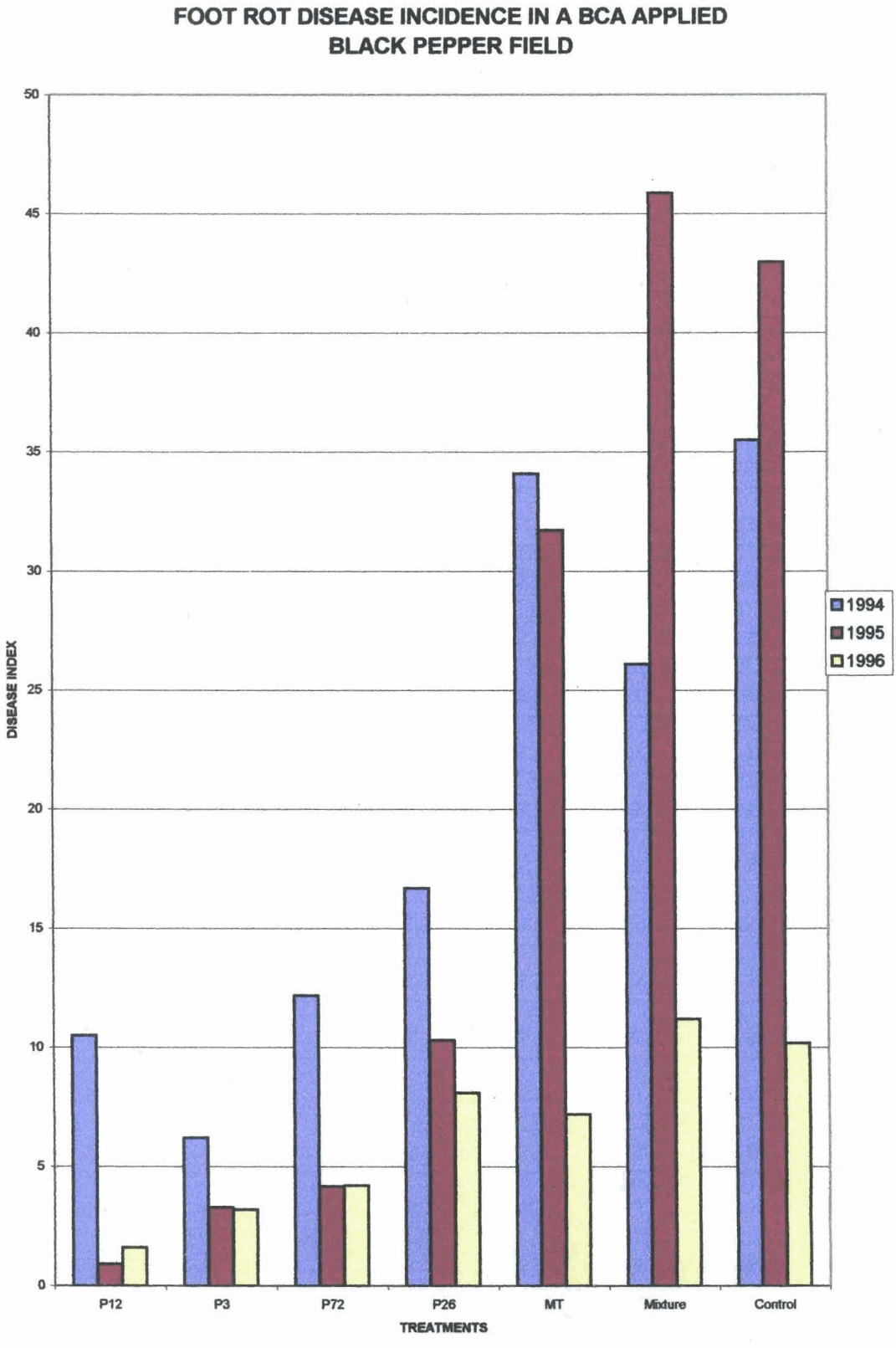

FOOT ROT DISEASE INCIDENCE IN A BCA APPLIED BLACK PEPPER FIELD
DISEASE INDEX
50
45
40
35
30
25
20
15
10
5
0
P12
P3
P72
P26
MT
Mixture
Control
TREATMENTS
1994
1995
1996

FIG : 2

During the years of study, maximum fungal population was noticed during the month of July in 1994 & 1995 and November in the year 1996. **Table -23.**

In the case of bacteria, maximum propogules were noticed during the month of July in 1994, May in 1995 and November in 1996 **Table -24.** No much fluctuation was noticed during different seasons of the year. Mximum actinomycete population was noticed during the month of September in 1994 & 1996 and January in 1995. In general increased microbial load was noticed during wet period and this would have substantially contributed to the difference in levels of root rot.

Table -22: Fluctuation of different isolates of BCA in a pepper field during different months of the year

(CFU at 10^3 dilution)

Isolates	Months					
	Jan.	Mar.	May	July	Sept.	Nov.
T.virens (P-12)	3.07	2.60	1.63	2.40	0.96	1.74
T.hamatum (T-3)	2.78	2.89	2.11	3.00	1.89	2.97
T.virens (P-72)	2.33	10.22	9.82	2.19	2.74	3.74
T.harzianum(P26)	13.85	24.19	22.70	37.52	43.63	40.19
T.polysporum(MT)	3.04	5.37	2.56	5.37	1.85	3.07
Mixture	9.52	6.22	4.52	7.70	4.07	8.70
Control	0.44	0.66	0.81	0.52	0.44	0.55
CD at 5% = 1.708						

Table -23: Fluctuation in fungal population in a BCA applied black pepper field during different months of the years studied

(CFU at 10^{-3} dilution)

Months	Year		
	1994	1995	1996
January	46.11	43.52	45.14
March	33.83	63.52	37.71
May	41.06	37.90	63.52
July	107.0	107.0	46.14
September	40.41	66.29	48.68
November	67.33	65.92	76.59
CD at 5% = 1.118			

During three years of study, *T.harzianum* (iso-26) population was significantly higher in all the years compared to all other isolates used followed by *T.virens* (iso-72). Though disease suppression was low in plots where P12, T3, P72 and MT isolates applied, the survival and persistant effect of *T.harzianum* (iso-P26) was proved as superior (**Fig - 3**).

Table -24: Fluctuation in bacterial population in a BCA applied black pepper field during different months of the years studied

(CFU at 10^6 dilution)

Months	Year		
	1994	1995	1996
January	06.96	04.50	42.60
March	05.36	08.57	20.59
May	02.55	20.48	08.57
July	09.27	09.25	07.77
September	05.87	03.14	05.98
November	03.73	04.20	50.92
CD at 5% = 1.118			

Table -25: Fluctuation in actinomycete population in a BCA applied black pepper field during different months of the years studied

(CFU at 10^5 dilution)

Months	Year		
	1994	1995	1996
January	01.00	02.25	00.85
March	00.55	00.68	00.80
May	00.26	00.79	00.73
July	00.42	00.41	00.58
September	01.61	00.98	01.57
November	00.92	01.19	00.79
CD at 5% = 1.118			

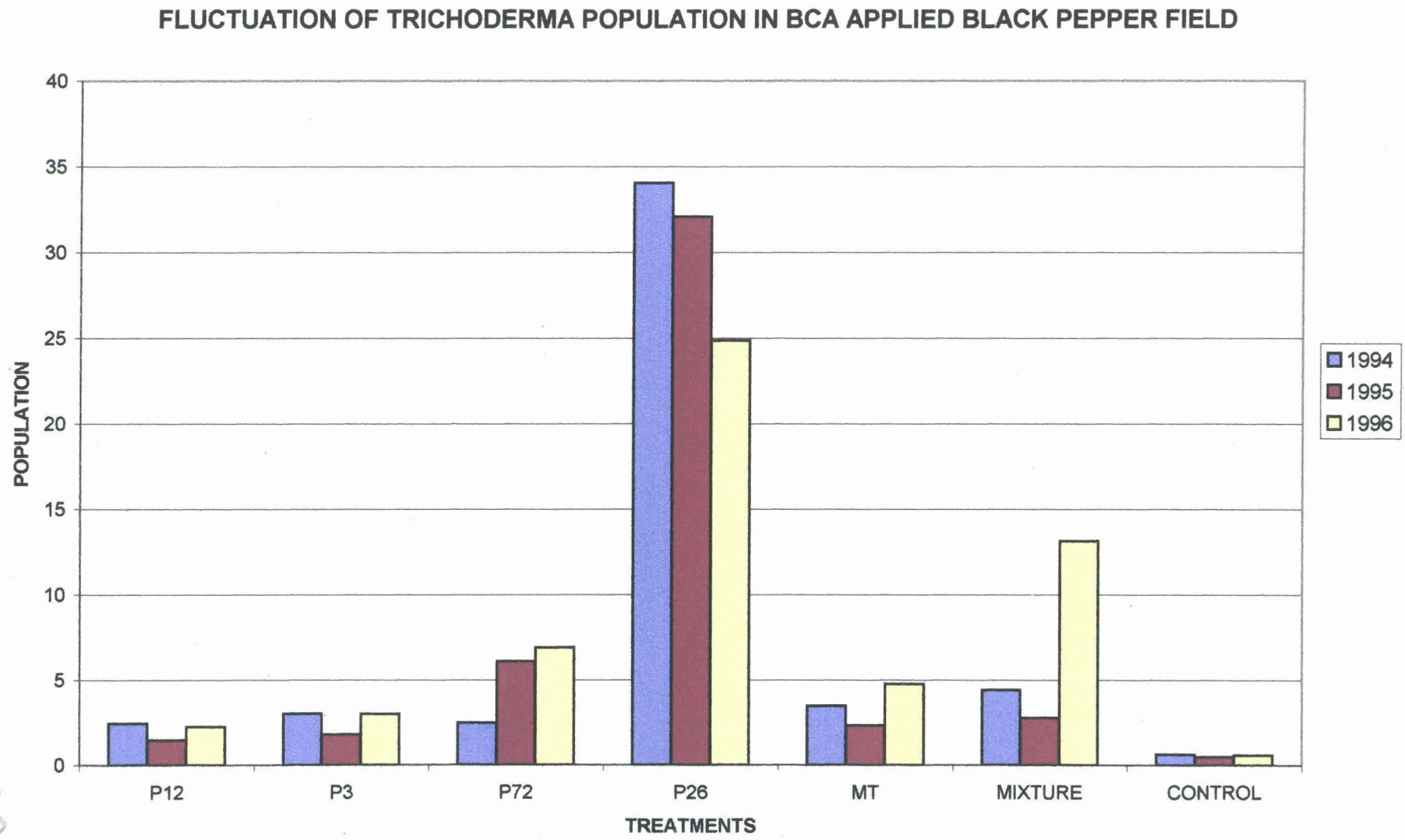

FLUCTUATION OF TRICHODERMA POPULATION IN BCA APPLIED BLACK PEPPER FIELD
POPULATION
40
35
30
25
20
15
10
5
0
P12
P3
P72
P26
MT
MIXTURE
CONTROL
TREATMENTS
1994
1995
1996

FIG : 3

Table -26: Fluctuation of *Trichoderma* in applied black pepper field during the year of study (CFU at 10^{-3} dilution)

Isolates	Years		
	1994	1995	1996
T.virens (P-12)	2.47	1.48	2.26
T.hamatum (T-3)	3.02	1.79	3.00
T.virens (P-72)	2.48	6.11	6.93
T.harzianum (P-26)	34.07	32.09	24.87
T.polysporum (MT)	3.50	2.35	4.78
Mixture	4.43	2.79	13.15
Control	0.65	0.50	0.59
CD at 5% = 1.208			

Population of BCA was higher in upper layer (0-10cm.) of soil compared to deeper layers of soil. Propogules of *T.harzianum* (P26) was significantly higher ($45.26x10^3$) in upper layer of soil compared to all other isolates used **Table -27**.

Table -27: Presence of *Trichoderma* spp. at different depths
(CFU at 10^3 dilution)

Isolates	Depths		
	0 - 10cm.	11 - 20cm.	21 - 30cm
T.virens (P-12)	2.67	1.96	1.57
T.hamatum (T-3)	2.72	3.83	1.26
T.virens (P-72)	8.17	3.87	3.48
T.harzianum (P-26)	45.26	22.87	22.91
T.polysporum (MT)	3.80	4.11	2.76
Mixture	9.83	5.72	4.82
Control	0.65	0.40	0.35
CD at 5% = 1.208			

4. CHEMICAL CONTROL

4.1. *In vitro* evaluation

Three agrochemicals were tested against *P.capsici*, both *in vitro* and *in vivo*. For *in vitro* studies, chemicals were tried against the four phases of *P.capsici* viz; growth, sporulation, sporangial germination and zoospore germination. Dimethomorph has got very good inhibitory property on all phases of *P.capsici* even at very low concentration. Complete inhibition on growth and sporulation was observed at 0.8ppm. level. Sporangial germination was completely inhibited at 700ppm. and zoospore germination at 100ppm. (**Table -28**).

Aureofungin, an antifungal antibiotic has shown complete inhibition on growth at 1000ppm and sporulation at 150ppm. Indirect sporangial germination was inhibited completely at 900ppm and zoospore germination at 20ppm. (**Table - 29**). Potassium phosphonate, a phosphorus acid compound was tested against four phases of *P.capsici*. Complete inhibition on growth was observed at 1000pppm. and sporulation at 7ppm. Complete inhibition on indirect sporangial germination was observed at 1100ppm. and zoospore germination at 110ppm. (**Table –30**).

Three systemic fungicides viz; dimethomorph, heptaene antibiotic (aureofungin) and potassium phosphonate were tested against *P.capsici* both *in vitro* and *in vivo*. Dimethomorph was found very effective on suppression of growth, sporulation, indirect sporangial germination and zoospore germination of *P.capsici*, but aureofungin and potassium phosphonate were found effective on these phases only at higher concentrations.

Table -28: *In vitro* efficacy of dimethomorph on different phases of *P.capsici*

Growth		Sporulation		Sporangial germination (indirect)		Zoospore germination	
Concentration	Inhibition (%)	Concentration	Inhibition (%)	Concentration	Inhibition (%)	Concentration	Inhibition (%)
0.1ppm.	12.57	0.1ppm.	18.01	100ppm.	76.29	10ppm.	57.97
0.2ppm.	27.97	0.2ppm.	78.95	200ppm.	78.60	20ppm.	45.48
0.3ppm.	43.39	0.3ppm.	99.16	300ppm.	96.40	30ppm.	62.10
0.4ppm.	55.20	0.4ppm.	97.73	400ppm.	98.87	40ppm.	74.50
0.5ppm.	79.33	0.5ppm.	97.47	500ppm.	98.87	50ppm.	90.01
0.6ppm.	88.01	0.6ppm.	99.78	600ppm.	99.55	60ppm.	96.81
0.7ppm.	93.60	0.7ppm.	99.83	700ppm.	100.0	70ppm.	97.90
0.8ppm.	100.0	0.8ppm.	100.0	800ppm.	100.0	80ppm.	87.24
0.9ppm.	100.0	0.9ppm.	100.0			90ppm.	98.45
1.0ppm.	100.0	1.0ppm.	100.0			100pp	100.0
CD at 5%	3.368	6.854		6.901		9.127	

Table -29: *In vitro* effects of aureofungin on different phases of *P.capsici*

Growth		Sporulation		Sporangial germination (indirect)		Zoospore germination	
Concentration	Inhibition (%)	Concentration	Inhibition (%)	Concentration	Inhibition (%)	Concentration	Inhibition (%)
100ppm.	76.31	10ppm.	46.91	100ppm.	26.92	5.0ppm.	97.67
200ppm.	83.67	20ppm.	70.39	200ppm.	65.93	10ppm.	99.55
300ppm.	86.25	30ppm.	93.67	300ppm.	91.76	15ppm.	99.87
400ppm.	86.59	40ppm.	96.23	400ppm.	96.15	20ppm.	100.0
500ppm.	88.02	50ppm.	97.44	500ppm.	98.08		
600ppm.	89.37	60ppm.	96.33	600ppm.	98.63		
700ppm.	90.36	70ppm.	99.41	700ppm.	99.18		
800ppm.	90.59	80ppm.	99.42	800ppm.	99.45		
900ppm.	95.76	90ppm.	99.10	900ppm.	100.0		
1000ppm	100.0.	100ppm.	98.59				
		110ppm.	98.61				
		120ppm.	98.82				
		130ppm.	99.41				
		140ppm.	99.94				
		150ppm.	100.0				
CD at 5%	3.617	4.861		11.59		0.5845	

Table -30: *In vitro* effect of potassium phosphonate on different phases of *P.capsici*

Growth		Sporulation		Sporangial germination (indirect)		Zoospore germination	
Concentration	Inhibition(%)	Concentration	Inhibition(%)	Concentration	Inhibition(%)	Concentration	Inhibition(%)
100ppm.	54.08	0.1ppm.	39.57	100ppm.	75.08	10ppm.	17..6
200ppm.	67.54	0.2ppm.	61.33	200ppm.	91.63	20ppm.	15.12
300ppm.	70.59	0.3ppm.	66.62	300ppm.	92.87	30ppm.	15.77
400ppm.	71.87	0.4ppm.	74.14	400ppm.	91.11	40ppm.	17.80
500ppm.	94.23	0.5ppm.	86.40	500ppm.	91.87	50ppm.	32.56
600ppm.	96.15	0.6ppm.	97.84	600ppm.	94.63	60ppm.	57.93
700ppm.	96.39	0.7ppm.	98.34	700ppm.	96.35	70ppm.	98.28
800ppm.	96.63	0.8ppm.	98.86	800ppm.	99.56	80ppm.	98.96
900ppm.	97.11	0.9ppm.	99.37	900ppm.	99.40	90ppm.	99.54
1000ppm.	100.0	1.0ppm.	99.38	1000ppm.	99.92	100ppm.	99.88
		2.0ppm.	99.33	1100ppm.	100.0	110ppm.	100.0
		3.0ppm.	99.64	1200ppm.	100.0		
		4.0ppm.	99.40				
		5.0ppm.	99.52				
		6.0ppm.	99.53				
		7.0ppm.	100.0				
CD at 5%	5.357	13.93		3.680		5.709	

4.2. *IN VIVO* EVALUATION

To evaluate the bio-efficacy of agrochemicals on foot rot of black pepper, a pot culture experiment was conducted. Three concentrations of potassium phosphonate (400, 800 & 1200ppm.) two concentrations of dimethomorph (33 & 400ppm.) and three concentrations of aureofungin (100, 200 & 300ppm.) were tried as three rounds soil drenching and foliar spray. In addition to that, 1200ppm. of potassium phosphonate was also tested as one round and two rounds application. Least root rot (17.78%) and high fresh weigh of root (43.22g) was noticed in plants treated with three round application of 1200ppm. of potassium phosphonate. Among the treatments, plants treated with 200ppm. of aureofungin yielded maximum fresh weight (172g) and dry weight (64.89g) of shoot (**Table -31**).

Table -31: *In vivo* evaluation of agrochemicals on root rot of black pepper

Treatments	Root rot (%)	Fresh wt. of Root (g)	Dry.wt. of root (g)	Fresh wt.of shoot (g)	Dry wt. of shoot (g)
Potassium phosphonate - 400ppm	31.88	26.88	10.75	106.50	37.75
,, -800ppm	23.33	22.00	09.72	093.40	35.67
,, -1200ppm	17.78	43.22	15.00	123.10	46.22
Dimethomorph -300ppm	21.11	17.56	10.00	089.22	42.44
,, -400ppm	23.33	38.61	16.22	117.80	41.11
Aureofungin -100ppm	24.44	12.39	08.00	111.30	39.00
,, -200ppm	20.00	29.61	12.22	172.00	64.89
,, -300ppm	47.78	25.44	08.33	077.44	29.67
*Potassium phosphonate-1200ppm	44.44	18.39	08.77	062.11	33.33
+ ,, -1200ppm	45.56	09.00	04.00	099.67	36.89
Control	74.00	09.90	04.40	040.40	15.20
CD at 5%	36.81	23.72	8.358	83.95	28.03

* Two round application – One round application

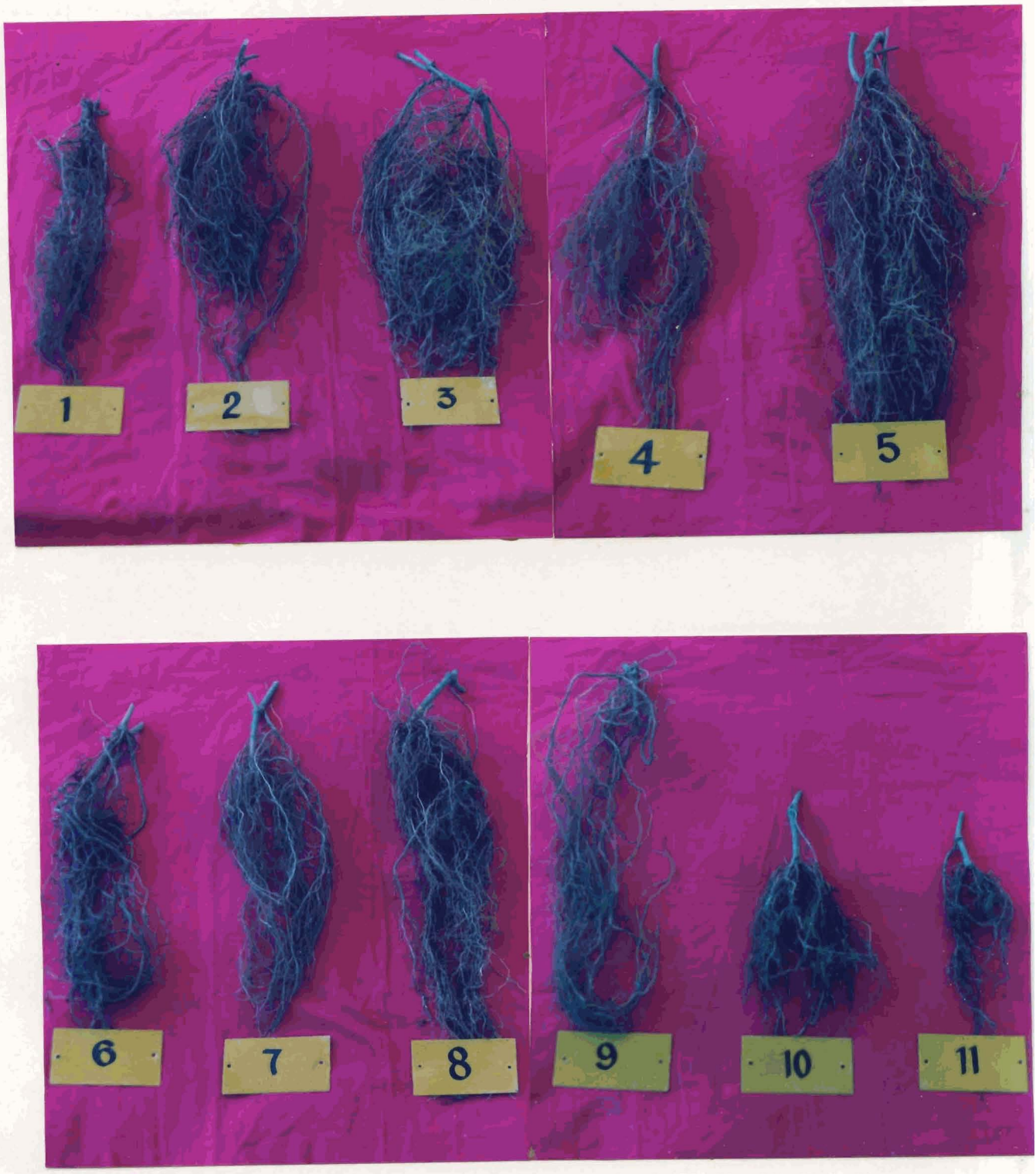


PLATE 6 : EFFECT OF AGROCHEMICALS ON ROOT ROT OF BLACK PEPPER

1=400PPM POTASSIUM PHOSPHONATE, 2=800PPM POTASSIUM PHOSPHONATE
3=1200PPM POTASSIUM PHOSPHONATE, 4=300PPM DIMETHOMORPH,
5=400PPM DIMETHOMORPH, 6=100PPM AUREOFUNGIN, 7=200PPM AUREOFUNGIN
8= 300PPM AUREOFUNGIN, 9=1200PPM POTASSIUM PHOSPHONATE (TWO APPLICATION)
10=1200PPM POTASSIUM PHOSPHONATE (ONE APPLICATION)
11=CONTROL

99A 20

99A

5.1. COMPATIBILITY OF *TRICHODERMA* WITH POTASSIUM PHOSPHONATE

The study clearly indicated that all the *Trichoderma* spp. are compatible with potassium phosphonate based on the growth recorded at 72hrs. Eventhough mild reduction of the growth was noticed at 24hrs and 48hrs with various spcies. It is only *T.hamatum* which showed 24.00mm at 1200ppm potssium phosphonate compared to 45mm in untreated control. The inhibitory effect of potassium phosphonate in *T.harzianum* at 24 and 48hrs is mild (**Table – 32**).

Sporulation was drastically affected at 400, 800 and 1200ppm in *T.polysporum* and *T.hamatum*. In *Trichoderma harzianum*, reduction of spore count (13750, 7503 and 10040 spores/ml) was noticed at 400, 800 and 1200ppm respectively compared to (250616 spores/ml) control (**Table – 33**).

5.2. COMPATIBILITY OF *VERTICILLIUM TENERUM* WITH POTASSIUM PHOSPHONATE

Growth phase of antagonistic *Verticillium tenerum* isolate was tested for their compatibility with potassium phosphonate at three different concentrations (400, 800 & 1200ppm.).There was no significant effect on growth due to the fungicide was noticed even at 1200ppm. level **Table -33**.

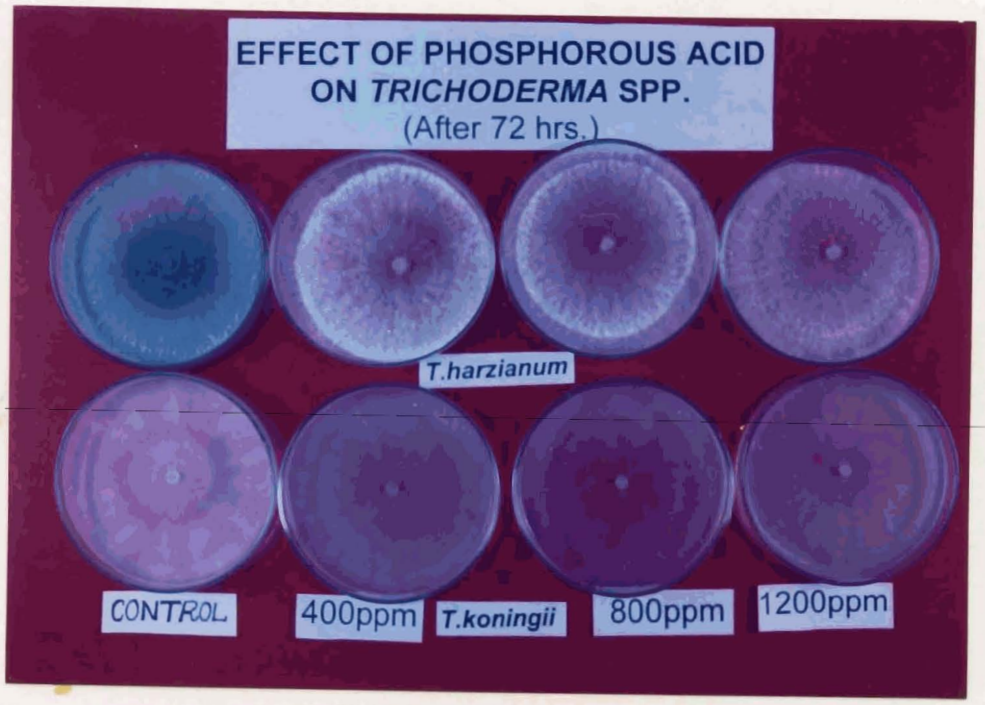
Table-32: Effect of potassium phosphonate on growth of different species of *Trichoderma*

(Radial growth in mm.)

atm.	*T.pseudokoningii*			*T.koningii*			*T.polysporum*			*T.longibracheatum*			*T.hamatum*			*T.harzianum*			*T.aureoviride*			*T.virens*		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
)ppm	19.7	45.0	45.0	17.7	35.5	45.0	12.7	31.5	45.0	19.0	33.9	45.0	16.0	30.4	45.0	30.2	40.6	45.0	16.0	40.6	45.0	20.2	42.1	45
ppm	21.5	43.7	45.0	17.0	32.1	45.0	13.7	31.4	45.0	16.5	30.9	45.0	14.0	27.6	42.0	18.2	35.9	45.0	18.2	40.7	45.0	17.2	30.3	45
)0pp	21.0	45.0	45.0	18.0	33.1	45.0	13.5	28.8	45.0	14.5	26.7	45.0	11.0	22.5	24.0	17.5	32.2	45.0	20.7	34.9	45.0	17.0	30.8	45
ntrol	22.7	45.0	45.0	16.2	34.2	45.0	17.5	37.6	45.0	21.7	39.9	45.0	15.7	32.7	45.0	22.0	45.0	45.0	24.7	45.0	45.0	24.0	45.0	45

CD ($p<0.05$) = 1.354

1=AFTER 24 HOURS. 2= AFTER 48 HOURS. 3=AFTER 72 HOURS



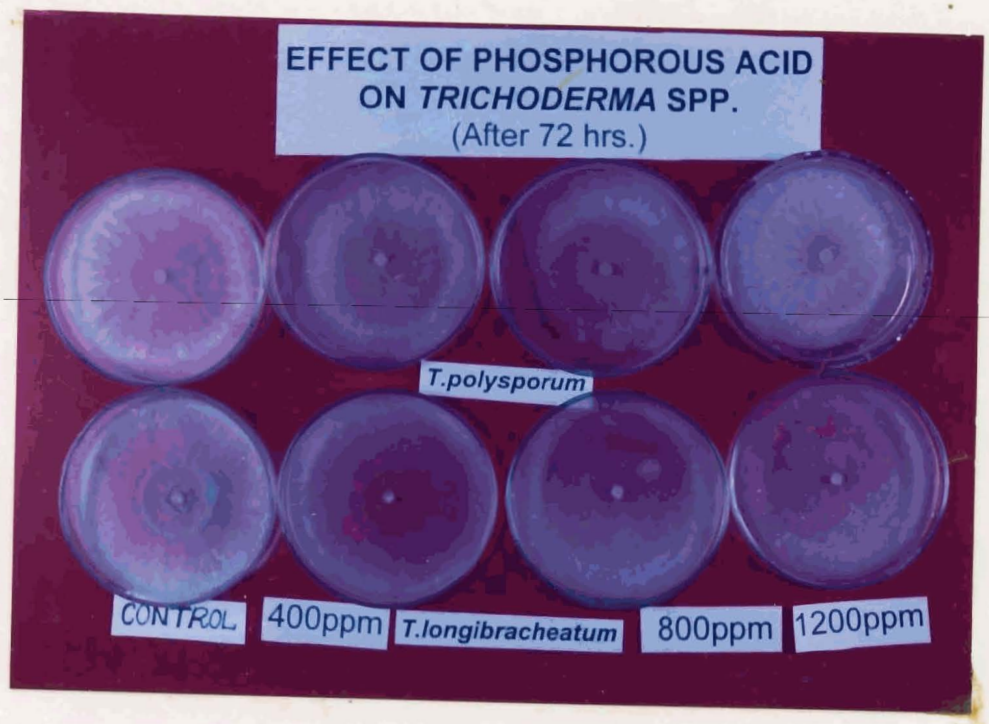


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PLATE 7 : COMPATIBILITY OF *TRICHODERMA* ISOLATES WITH POTASSIUM PHOSPHONATE

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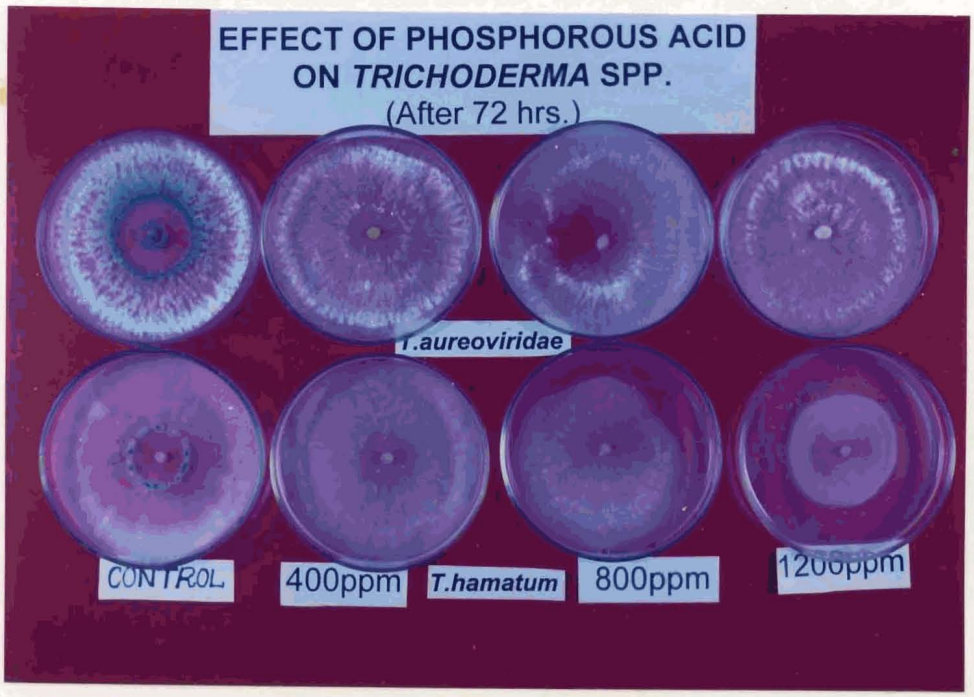

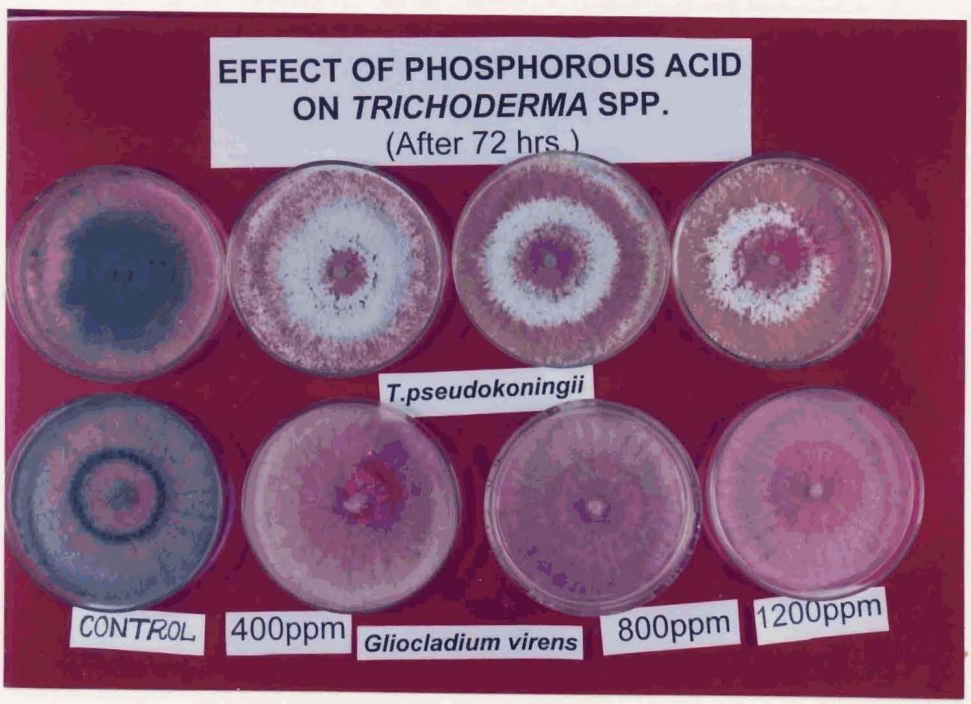

PLATE 7 continue... : COMPATIBILITY OF *TRICHODERMA* ISOLATES WITH POTASSIUM PHOSPHONATE

Table-33: Effect of Potassium phosphonate on sporulation of different species of *Trichoderma*

(Spore count per ml. after 96 hours)

Tr.	*T.pseud*	*T.koni*	*T.poly*	*T.long*	*T.ham*	*T.harzi*	*T.aur*	*T.viren*
Con	10130	12500	62480	2522	3123	250616	17880	87480
400 ppm	342503	1872	00.00	3540	00.00	13750	8750	10620
800 ppm	38120	1252	00.00	3748	00.00	7503	15070	6251
1200 ppm	265033	1248	00.00	2510	00.00	10040	13750	4374
CD (P< 0.05)=30.32								

(Spore count/ml.after 168 hours)

Treatment	*T.polysporum*	*T.hamatum*
Control	63030	31250
400ppm.	18750	8248
800ppm.	13750	1248
1200ppm.	1248	1248
CD (P< 0.05)=12.30		

Table -34:Compatibility of potassium phosphonate on growth of *Verticillium tenerum* (after 120hrs)

Treatments	Growth (mm)
400ppm	21.50
800ppm	21.50
1200ppm	20.25
Control	26.50
CD at 5%	NS

6 - EFFECT OF PLANT EXTRACTS

6.1. *IN VITRO* EFFECTS

Two botanicals viz; garlic and mustard were tried against *P.capsici,* both *in vitro* and *in vivo*. In *in vitro* tests, these aqueous extracts were tried individually and their combinations on four phases of *P.capsici*. Aqueous extract of mustard showed more inhibitory effect on growth of *P.capsici* compared to garlic extract.

Complete inhibition on growth was observed at 4.0% garlic and 3.0% mustard extracts. While in combination, complete inhibition was observed with 2:2% extracts (**Table -35**). This showed synergestic inhitory effect of extracts in combination. Aqueous garlic extract showed maximum inhibitory effect on sporulation. Complete inhibition (100%) of sporulation at 0.5% of garlic extract, but in mustard this level of inhibition noticed only at 1.1%. The combined effect was more than their individual effects and complete inhibition on sporulation noticed even at 0.5:0.5% level. More than 50% inhibiton of sporulation was observed even at 0.1% of garlic extract and 0.3% in mustard extract, but mixture of both the extracts (0.1:0.1%) was enough to attain 50% inhibition (**Table -36**).

The aqueous garlic extract showed complete inhibition of indirect sporangial germination at 0.6% and in mustard extract, this level of inhibition was attained even at 0.4%.Combined extract showed complete inhibition at 0.3:0.3% level. Even at 0.1% both the aqueous extracts showed high inhibition.(**Table -37**).

Aqueous garlic extract at 0.4% and mustard extract at 0.9% showed complete inhibition of zoospore germination.But combined extracts at 0.4:0.4% gave complete inhibition. At 0.1% level, both the extracts did not exhibit any effect on zoospore germination, but in combination, more than 30% inhibition was observed (**Table -38**).

Table -35: Effect of aqueous extract of Garlic and Mustard on growth of *P.capsici* (Percentage of inhibition)

Treatments	Individual		In combination
	Garlic	Mustard	
0.5%	031	008	035
1.0%	041	025	036
1.5%	051	034	043
2.0%	059	088	100
2.5%	066	097	100
3.0%	068	100	100
3.5%	081	100	100
4.0%	100	100	100
CD at 5%	04.1	02.0	02.5

PLATE 8: ***IN VITRO*** **EVALUATION OF GARLIC AND MUSTARD EXTRACTS ON GROWTH OF** ***P.CAPSICI***

GARLIC EXTRACT- 1=0.5%, 2=1.0%, 3=1.5%, 4=2.0%, 5=2.5%, 6=3.0%, 7=3.5%, 8=4.0%

MUSTARD EXTRACT- 1=0.5%, 2=1.0%, 3=1.5%, 4=2.0%, 5=2.5%

COMBINATION- 1=0.5:0.5%, 2=1.0:1.0%, 3=1.5:1.5%, 4=2:2%

Table -36: Effect of aqueous extracts of Garlic and Mustard on sporulation of *P.capsici*

(Percentage of inhibition)

Treatments	Individual		In combination
	Garlic	Mustard	
0.1%	065	000	070
0.2%	081	005	082
0.3%	093	065	090
0.4%	097	081	095
0.5%	100	093	100
0.6%	100	093	100
0.7%	100	094	100
0.8%	100	096	100
0.9%	100	097	100
1.0%	100	097	100
1.1%	100	100	100
1.2%	100	100	100
CD at 5%	05.93	05.75	05.25

Table-37: Effect of aqueous extracts of Garlic and Mustard on indirect sporangial germination of *P.capsici*

(Percentage of inhibition)

Treatments	Individual		In combination
	Garlic	Mustard	
0.1%	075	046	095
0.2%	090	090	095
0.3%	089	099	100
0.4%	094	100	100
0.5%	099	100	100
0.6%	100	100	100
0.7%	100	100	100
CD at 5%	009.7	014.9	011.5

Table -38: Effect of aqueous extracts of Garlic and Mustardon zoospore germination of *P.capsici*

(Percentage of inhibition)

Treatments	Individual		In combination
	Garlic	Mustard	
0.1%	000	000	013
0.2%	071	000	061
0.3%	098	000	091
0.4%	100	000	100
0.5%	100	054	100
0.6%	100	087	100
0.7%	100	095	100
0.8%	100	098	100
0.9%	100	100	100
CD at 5%	04.82	013.2	04.26

6.2. *IN VIVO* EFFECTS

A pot culture experiment was conducted for the evaluation of bio-efficacy of these botanicals on root rot suppression. Extracts of these botanicals were tried individually and also in combination. Combination of these with *T.harzianum* was also tested to study their compatibility with these botanicals. Least root rot incidence (5.22%) was noticed in plants treated with 3% garlic extracts along with *T.harzianum*, followed by 3% mustard extracts along with *T.harzianum* (8.88%). Maximum fresh weight (75.56g) and dry weight (14.67g) of root was noticed in plants treated with 2% mustard extract along with *T.harzianum*. Maximum fresh weight (351g) and dry weight (81.11g) of shoot was recorded in plants treated with 3% mustard extract along with *T.harzianum* (**Table -39**).

Table -39: *In vivo* evaluation of garlic and mustard extracts combination with *T.harzianum*

Treatments	Root rot (%)	Fresh wt. root (g)	Dry wt. Root (g)	Fresh wt. shoot (g)	Dry wt. shoot (g)
1% Garlic extract	53.33	29.89	06.07	221.1	62.33
2% Garlic extract	29.33	53.33	12.80	278.9	76.04
3% Garlic extract	38.78	28.11	06.12	243.3	54.22
1% Mustard extract	68.44	15.78	03.71	210.0	50.56
2% Mustard extract	45.33	30.33	05.46	193.1	33.44
3% Mustard extract	44.44	13.11	02.75	117.8	31.44
1% Garlic extract + *T.harzianum (T.h)*	20.22	45.67	08.86	283.4	70.33
2% Garlic extract + *T.harzianum (T.h)*	50.22	33.33	05.72	129.2	39.22
3% Garlic extract + *T.harzianum (T.h)*	05.22	69.00	13.37	261.9	63.67
1% Mustard extract + *T.harzianum (T.h)*	13.77	66.89	13.60	184.2	50.00
2% Mustard extract + *T.harzianum (T.h)*	11.94	75.56	14.67	299.6	66.89
3% Mustard extract + *T.harzianum (T.h)*	08.88	63.44	12.29	351.0	81.11
1% Garlic extract + 1% Mustard extract	32.33	46.78	08.10	222.2	55.56
2% Garlic extract + 2% Mustard extract	37.89	45.56	08.44	156.3	42.33
1% Garlic extract + 1% Mustard extract +*T.h*	61.67	28.89	06.36	167.4	40.22
2% Garlic extract + 2% Mustard extract +*T.h*	23.11	36.11	08.02	167.0	44.22
Control	69.44	10.11	01.86	048.6	15.22
CD at 5%	33.50	26.49	5.840	124.0	27.01

Table-40: Effect of garlic and mustard extracts on microbial population in treated soil

Treatments	Total fungi ($x10^3$)		*Trichoderma* ($x10^3$)		Bacteria ($x10^6$)		Actinomycetes($x10^5$)	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**
1% Garlic extract	114.7	245.3	00.00	00.00	04.00	02.00	01.33	00.00
2% Garlic extract	046.0	019.67	00.33	00.33	05.33	01.00	01.33	00.66
3% Garlic extract	075.3	019.00	00.33	00.33	02.33	03.33	02.66	01.33
1% Mustard extract	093.3	030.33	00.00	01.00	01.00	15.33	01.00	00.66
2% Mustard extract	071.3	036.33	00.33	06.66	01.00	27.00	00.66	04.33
3% Mustard extract	100.0	034.00	00.66	03.66	03.66	04.33	01.00	01.00
1% Garlic extract + *T.harzianum(T,h)*	092.0	074.00	83.00	72.00	19.33	09.00	00.00	00.33
2% Garlic extract + *T.harzianum (T,h)*	078.6	058.00	03.66	57.33	07.33	07.00	01.33	00.66
3% Garlic extract + *T.harzianum (T,h)*	321.0	018.00	24.67	03.33	07.33	11.67	00.66	00.33
1% Mustard extract + *T.harzianum (T,h)*	066.3	023.33	11.67	05.66	03.00	08.33	00.33	00.66
2% Mustard extract + *T.harzianum (T,h)*	088.3	039.00	23.00	19.67	08.66	05.00	00.66	00.66
3% Mustard extract + *T.harzianum (T,h)*	223.7	065.00	01.33	42.33	12.67	14.33	00.00	00.66
1% Garlic extract + 1% Mustard extract	102.7	027.00	00.33	03.66	05.00	14.33	02.00	02.33
2% Garlic extract + 2% Mustard extract	214.3	023.33	01.33	02.00	15.33	14.00	01.00	01.33
1% Garlic extract + 1% Mustard extract +*T.h*	227.0	015.33	11.00	10.66	16.00	12.33	00.00	03.66
2% Garlic extract + 2% Mustard extract +*T.h*	119.0	024.00	30.67	14.33	28.00	13.00	00.00	04.00
Control	068.3	024.33	00.33	03.33	05.00	02.33	03.66	01.66
CD at 5%	22.21	14.87	11.03	4.011	3.391	3.860	1.272	2.229

1 = 15 days after the application of garlic and mustard extracts **2** = At the time of uprooting of treated plants

There was an increased fungal population was noticed in soil treated with different plant extracts. Maximum fungal population ($321x10^3$) –fifteen days after the application of extracts was noticed in soils treated with 3% garlic extracts along with *T.harzianum*, followed by 1% garlic extract with *T.harzianum* ($227x10^3$). Maximum fungal population noticed at the time of harvest in the soil treated with 1% garlic extract alone ($245x10^3$). Maximum *Trichoderma* population was noticed both at 15 days after inoculation and at the time of up rooting in soils treated with 1% garlic extract with *Trichoderma* isolate ($83x10^3$ and $72x10^3$ respectively).

Maximum bacterial population was noticed in the soil treated with the combination of 2% garlic and 2% mustard extracts along with *Trichoderma* ($28x10^6$) but reduction on bacterial population was noticed at the time of uprooting. There was no much variation on actinomycete population noticed any of the treatment (**Table –40**)

7. EFFECT OF ORGANIC SOIL AMENDMENTS

A pot culture experiment was conducted using four soil amendments to evaluate their effect on *P.capsici*. All the four amendments and forest soil was analysed for their nutritional status (**Table-41**). Neem cake has got maximum nitrogen followed by poultry manure and coffee pulp. Least quantity of N detected in farm yard manure. Maximum P was obtained in poultry manure followed by neem cake and least was noted in coffee pulp. Maximum K was obtained (4%) in coffee pulp followed by poultry manure and neem cake and least in farm yard manure. Maximum growth of shoot and root was recorded in plants treated with coffee pulp. It was noted that coffee pulp enhanced the overall growth and root mass of plants. Poultry manure has got negative effect on growth of plants (**Table -42 & 43**). It was found that *Phytophthora* infection on root (root rot) was almost negligible in plants treated with coffee pulp (10%) and maximum root rot was recorded in plants treated with chemical fertilizers (94.5%) (**Table -44**). Root rot incidence in plants treated with

poultry manure (89.5%) was on par with chemical fertilizers. DPI of *Phytophthora* in amended soils showed that chemical fertilizers were not preventing the proliferation of pathogen and it was on par with control (1024) and least number of propagules were noted in coffee pulp treated soil (4), (**Table -48**).

It was noticed that neem cake enhanced fungal population in soil, followed by poultry manure and farm yard manure but NPK did not support the fungal multiplication (**Table-45**). Fifteen days after the amendment application, poultry manure showed highest number of bacterial population but finally it decreased. Though the soil with farm yard manure gave less number of bacterial count initially, at the time of concluding the experiment, the population was maximum. In chemical fertilizer treated soil the bacterial population was on par with control (**Table -46**). There was an increment in actinomycetes population in soil treated with farm yard manure and least number of actinomycetes propagules were noted in control (**Table -47**).

Table -41: NPK levels of different organic soil amendments and forest soil

Percentage of NPK in amendments			
Treatments	N_2	P_2O_5	K_2O
Coffee pulp	1.620	0.180	4.000
Poultry manure	1.910	0.830	1.800
Neem Cake	2.200	0.700	1.600
Farm Yard Manure	0.400	0.300	0.200
Forest Soil	0.410	0.160	0.190

Table -42: Effect of organic soil amendments on shoot mass

Sl.No.	Treatments	Fresh Wt. (g)	Dry Wt. (g)
1.	Coffee pulp.	07.976	02.212
2.	Poultry manure	03.120	00.771
3.	Neem Cake	03.112	00.806
4.	Farm Yard Manure	03.622	01.105
5.	NPK	03.442	01.169
6.	Control	04.962	01.324
7.	Abosolute control	12.370	02.622
	CD at 5%	02.950	00.760

Table -43: Effect of organic soil amendments on root mass

Sl.No.	Treatments	Fresh Wt. (g)	Dry Wt. (g)
1.	Coffee pulp	05.362	01.462
2.	Poultry manure	00.957	00.285
3.	Neem cake	01.917	00.869
4.	Farm yard manure	02.171	00.685
5.	NPK	02.011	00.651
6.	Control	01.946	00.796
7.	Absolute control	10.184	01.888
	CD at 5%	01.430	00.62

Table -44: Effect of organic soil amendments on root rot

Sl.No.	Treatments	root rot (%)
1.	Coffee pulp	10.00
2.	Poultry manure	89.50
3.	Neem cake	18.20
4.	Farm yard manure	57.00
5.	NPK	94.50
6.	Control	24.500
7.	Absolute control	00.000
	CD at 5%	23.190

Table - 45: Effect of organic soil amendments on fungal population (CFU= Coloniesx10^3)

Sl.No.	Treatments	15 days after amendment application	Time of uprooting
1.	Coffee pulp	071.000	137.433
2.	Poultry manure	026.867	161.400
3.	Neem cake	130.867	212.067
4.	Farm yard manure	018.533	152.067
5.	NPK	015.533	044.500
6.	Control	018.733	050.400
7.	Absolute control	019.967	058.667
	CD at 5%	003.1435	009.405

Table –46: Effect of organic soil amendments on bacterial population (CFU= Coloniesx10^6)

Sl.No.	Treatments	15 days after amendments application	At the time of uprooting
1.	Coffee pulp	04.200	012.000
2.	Poultry manure	26.300	006.967
3.	Neem cake	08.767	027.533
4.	NPK	11.867	201.967
5.	Farm yard manure	02.000	011.167
6.	Control	01.200	001.400
7.	Absolute control	01.300	002.000
CD at 5%		01.595	007.866

Table -47: Effect of amendments on actinomycetes population (CFU= Coloniesx10^3)

Sl.No.	Treatments	15 days after amendments application	At the time of uprooting
1.	Coffee pulp	02.200	10.100
2.	Poultry manure	02.200	03.067
3.	Neem cake	00.000	00.867
4.	Farm yard manure	01.000	19.633
5.	NPK	00.000	00.200
6.	Control	00.333	00.100
7.	Absolute control	00.000	00.000
	CD at 5%	00.226	00.887

Table-48: Disease potential index (DPI) of *P.capsici* in different organics amended soils

Sl.No.	Treatment	DPI		pH
		Initial	Final	
1.	Coffee pulp	2048	4	5.3
2.	Poultry manure	2048	512	7.5
3.	Neem cake	2048	1024	6.3
4.	Farm yared manure	2048	8	6.9
5.	NPK	2048	1024	5.5
6.	Control	2048	1024	6.0
7.	Absolute control	0000	0000	6.0

8. EFFECT OF HOST NUTRITION

A pot culture experiment was conducted to evaluate the chemical fertilizers on root rot of black pepper. Before the commencement of experiment, forest soil which was used for the study was evaluated for its nutritional status (**Table -49**). There was very good root protection noticed in plants treated only with recommended dose of phosphorus and without the addition of nitrogen and potash ($N_0P_1K_0$) with reduced root rot percentage (24.2%). Maximum root rot (96.7%) was noticed in plants treated with the recommended dose of nitrogen and potash without addition of phosphorus ($N_1P_0K_1$). Wherever double the dose of phosphorus added without addition of K along with recommended dose of nitrogen ($N_1P_2K_0$), the root rot was low (38.3%). Treatment with the recommended dose of nitrogen without addition of P and K, the root rot was high (83.3%). In the plants which received double the dose of nitrogen alone without P and K, higher root rot was equally high (90.0%) - (**Table -53**).

Recommended dose of NPK ($N_1P_1K_1$) of did support root rot (50%) and the root rot was almost equal to that in plants without receiving any additional nutrients (50.8%). Where double the recommended dose of NPK used ($N_2P_2K_2$), root rot was high (77.5%).

Addition of potash did not give good protection to the roots against root rot. Wherever potash alone was used ($N_0P_0K_1$), root rot was high (95%) and it was higher than that in treatment with double the dose of K without addition of P and N - $N_0P_0K_2$ (67.5%).

Fresh and dry weight of root was also high in plants treated with double the dose of P with recommended dose of K and N ($N_1P_2K_1$). In plants which received the same dose ($N_1P_2K_1$) fertilizer, fresh and dry weights of root was high in uninoculated plants (58.3g. and 16.2g. respectively) compared to inoculated plants (15.8g. and 4.6g. respectively) **Table - 50**.

Plants received only recommended dose of N and K, without P, the fresh and dry weight of root was low in uninoculated plants (55.2g. and 11.7g. respectively). In the plants of the same treatments the plants inoculated with *P.capsici,* the fresh and dry weight of root was still low (7.2g. and 2.6g. respectively).

Among the inoculated plants, fresh weight of shoot was maximum (260.0g.) in the treated plants received double the dose of N&K and recommended dose of P ($N_2P_1K_2$) . Among the uninoculated plants, fresh and dry weight of shoot was high (462.5g. and 158.3g. respectively) in plants treated with the combinations of double the dose of nitrogen, recommended dose of P and without addition of K- ($N_2P_1K_0$) **Table -51**.

Among the *Phytophthora* infected plants, maximum height (305.7cm.) was observed in plants treated with $N_2P_0K_2$ and among the uninoculated plants, maximum number of leaves (170.80), nodes (218) and height (346.5cm.) was observed in plants

received double the dose of N, recommended dose of P and double the dose of K ($N_2P_1K_2$). But among the in;oculated plants, maximum number of leaves and nodes observed in plants received recommended dose of NPK ($N_1P_1K_1$).Out of 27 treatments no much variation was observed in number of branches - **Table -52**.

Table -49: Nutritional status of forest soil used for pot culture studies

1.	Organic Matter	2.8%
2.	Available Nitrogen	185 ppm.
3.	Phosphorus	1.9 ppm.
4.	Potassium	78 ppm.
5.	Calcium	113 ppm.
6.	Magnesium	51 ppm.
7.	Iron	29ppm.
8.	Manganese	6.3ppm.
9.	Zinc	2.7ppm.
10.	Copper	1.1ppm

Table –50: Effect of Nutrition on *Phytophthora* induced root rot in black pepper

Trmts.	Fresh Wt.Root		Dry Wt.Root	
	+P	--P	+p	--P
$N_0P_0K_0$	18.2	31.5	06.6	04.2
$N_0P_0K_1$	09.7	37.8	02.2	10.8
$N_0P_0K_2$	22.5	34.5	05.8	08.7
$N_0P_1K_0$	40.3	17.8	09.9	05.1
$N_0P_1K_1$	30.3	25.5	06.2	05.2
$N_0P_1K_2$	14.7	31.5	03.7	09.7
$N_0P_2K_0$	21.3	13.5	06.3	03.8
$N_0P_2K_1$	08.7	22.3	02.6	05.9
$N_0P_2K_2$	14.3	21.5	03.3	06.5
$N_1P_0K_0$	09.5	31.8	03.0	10.2
$N_1P_0K_1$	07.2	15.8	02.6	04.6
$N_1P_0K_2$	21.0	29.5	05.4	09.7
$N_1P_1K_0$	25.0	39.3	06.7	07.9
$N_1P_1K_1$	36.5	55.3	08.3	13.1
$N_1P_1K_2$	47.3	73.3	10.2	14.7
$N_1P_2K_0$	42.5	45.0	10.9	12.2
$N_1P_2K_1$	55.2	58.3	11.7	16.2
$N_1P_2K_2$	24.8	47.3	07.7	11.7
$N_2P_0K_0$	21.0	29.0	05.5	07.6
$N_2P_0K_1$	17.2	27.3	04.9	07.3
$N_2P_0K_2$	25.7	31.3	05.7	10.4
$N_2P_1K_0$	18.5	35.8	06.9	10.9
$N_2P_1K_1$	15.0	29.5	03.9	08.8
$N_2P_1K_2$	16.0	38.8	05.6	12.0
$N_2P_2K_0$	19.5	45.5	18.5	11.5
$N_2P_2K_1$	14.3	57.8	03.7	15.5
$N_2P_2K_2$	25.2	39.8	06.7	11.7
CD at 5%	22.0	24.8	N.S	06.6

+P - Inoculated with *P.capsici* --P - Un-inoculated control

Table -51: Effect of nutrition on *Phytophthora* induced root rot on shoot system of black pepper

Trmts.	Fre. wt.Shoot		Dry.wt.Shoot		Height	
	+P	-P	+P	-P	+P	-P
$N_0P_0K_0$	123.0	200.5	027.0	047.3	170.0	246.0
$N_0P_0K_1$	091.7	322.0	028.5	071.5	133.5	280.3
$N_0P_0K_2$	116.5	195.8	035.0	045.0	219.7	220.0
$N_0P_1K_0$	168.7	276.8	049.0	081.0	239.5	215.8
$N_0P_1K_1$	088.7	210.0	025.8	051.0	208.5	189.8
$N_0P_1K_2$	071.3	302.8	025.2	075.0	189.7	240.5
$N_0P_2K_0$	139.2	164.8	038.5	044.8	167.2	180.8
$N_0P_2K_1$	061.3	077.5	019.0	027.5	125.8	157.0
$N_0P_2K_2$	114.5	193.5	029.7	054.8	100.7	149.5
$N_1P_0K_0$	119.5	428.0	056.5	139.5	242.8	270.0
$N_1P_0K_1$	036.2	329.8	011.3	082.8	080.5	251.8
$N_1P_0K_2$	226.7	163.8	066.3	042.8	227.5	276.3
$N_1P_1K_0$	338.0	306.3	088.7	091.8	206.3	234.0
$N_1P_1K_1$	293.8	511.3	076.2	117.5	262.2	263.0
$N_1P_1K_2$	209.2	487.5	081.7	125.8	233.2	237.8
$N_1P_2K_0$	242.7	332.0	099.7	102.5	237.0	228.5
$N_1P_2K_1$	217.7	426.3	089.2	129.5	244.7	247.8
$N_1P_2K_2$	231.3	308.0	079.7	096.0	176.8	174.3
$N_2P_0K_0$	141.2	326.3	052.7	151.0	279.3	289.3
$N_2P_0K_1$	210.0	235.0	072.5	074.5	278.7	272.5
$N_2P_0K_2$	297.5	376.0	125.2	110.3	305.7	309.0
$N_2P_1K_0$	331.2	462.5	138.7	158.3	265.8	325.3
$N_2P_1K_1$	098.2	401.3	043.0	130.3	239.2	290.8
$N_2P_1K_2$	260.0	422.8	092.8	132.5	288.3	346.5
$N_2P_2K_0$	213.2	541.3	072.0	154.3	163.5	247.8
$N_2P_2K_1$	179.0	390.8	051.3	124.5	134.3	174.3
$N_2P_2K_2$	223.7	415.0	072.2	120.3	174.8	187.5
CD at 5%	168.5	234.2	47.6	66.4	93.0	00.47

+P - Inoculated with *P.capsici* -P – Uninoculated control

Table -52: Effect of nutrition on *Phytophthora* induced root rot on shoot system (leaves, nodes and branches) of black pepper

Trmts.	Leaves		Nodes		Branches	
	+P	-P	+P	-P	+P	-P
$N_0P_0K_0$	024.0	053.0	036.3	064.5	3.83	2.25
$N_0P_0K_1$	021.0	096.5	041.2	107.3	2.33	5.25
$N_0P_0K_2$	045.3	063.8	060.2	075.5	4.67	4.50
$N_0P_1K_0$	055.7	048.0	066.2	066.8	4.33	4.25
$N_0P_1K_1$	038.8	067.8	057.3	083.3	4.00	4.00
$N_0P_1K_2$	045.0	075.3	058.7	084.8	2.33	2.75
$N_0P_2K_0$	047.3	037.3	059.3	062.0	3.33	2.75
$N_0P_2K_1$	025.3	025.8	036.5	037.3	2.83	2.75
$N_0P_2K_2$	016.0	036.5	023.0	042.8	2.00	3.25
$N_1P_0K_0$	074.8	114.3	082.8	119.5	3.17	3.75
$N_1P_0K_1$	018.2	053.8	019.5	058.5	0.67	3.25
$N_1P_0K_2$	119.3	148.5	122.0	152.0	3.67	2.25
$N_1P_1K_0$	087.5	107.0	088.8	106.0	3.67	3.28
$N_1P_1K_1$	180.7	180.5	182.0	184.0	4.67	4.25
$N_1P_1K_2$	179.7	185.3	180.2	186.8	5.50	5.50
$N_1P_2K_0$	134.0	129.3	137.0	131.3	4.67	4.00
$N_1P_2K_1$	104.3	102.3	106.2	104.3	4.67	5.75
$N_1P_2K_2$	132.2	138.8	134.5	140.5	4.50	4.00
$N_2P_0K_0$	077.2	071.8	065.5	060.0	4.33	4.75
$N_2P_0K_1$	136.2	134.5	153.0	149.0	4.33	3.75
$N_2P_0K_2$	131.5	136.3	140.8	146.0	5.33	4.75
$N_2P_1K_0$	115.8	137.5	124.0	146.8	4.17	4.25
$N_2P_1K_1$	129.0	178.5	135.7	185.8	4.66	5.50
$N_2P_1K_2$	170.8	210.0	177.5	218.0	4.00	5.75
$N_2P_2K_0$	052.0	086.3	058.2	093.0	3.33	6.50
$N_2P_2K_1$	030.8	035.3	043.0	055.8	3.50	4.75
$N_2P_2K_2$	044.5	040.5	070.0	066.8	5.00	4.00
CDat5%	37.13	20.50	40.19	21.10	2.30	2.10

+P - Inoculated with *P.capsici* ---P – Uninoculated control

Table -53: Effect of nutrition on root rot of black pepper, caused by *P.capsici*

TREATMENTS	ROOT ROT (%)
$N_0P_0K_0$	50.80
$N_0P_0K_1$	95.00
$N_0P_0K_2$	67.50
$N_0P_1K_0$	24.40
$N_0P_1K_1$	54.20
$N_0P_1K_2$	62.50
$N_0P_2K_0$	46.00
$N_0P_2K_1$	90.80
$N_0P_2K_2$	80.00
$N_1P_0K_0$	83.30
$N_1P_0K_1$	96.70
$N_1P_0K_2$	76.70
$N_1P_1K_0$	80.00
$N_1P_1K_1$	50.00
$N_1P_1K_2$	39.20
$N_1P_2K_0$	38.30
$N_1P_2K_1$	43.20
$N_1P_2K_2$	66.70
$N_2P_0K_0$	90.00
$N_2P_0K_1$	88.20
$N_2P_0K_2$	83.30
$N_2P_1K_0$	90.80
$N_2P_1K_1$	90.00
$N_2P_1K_2$	74.20
$N_2P_2K_0$	79.20
$N_2P_2K_1$	78.30
$N_2P_2K_2$	77.50
CD at 5%	36.20

9. INTEGRATED DISEASE MANAGEMENT (IDM)

In general, root rot in tolerant P24 was significantly different on that of susceptible KS27. Thus confirming the tolerance of the variety. Least root rot 1.6%(13), 2.5% (T3 & T9), 3.3%(T6, T12, T16, T19 & T20) in P24, compared similar treatments 13.3%, 45.0%, 29.1%, 33.3%, 24.1%, 12.5%, 11.6 and 11.6% in KS27 respectively (**Table -54**). The effects of higher dose of phosphorus, the garlic extract and potassium phosphonate in various combinations showed comparatively reduced root rot (**Fig - 4**). Though reduction in disease was noticed, the trend varies slightly in different treatments both in P24 and KS27, but the tolerance of P24 was clearly noticed.

Shoot dry weight differed considerably between the two varieties. Maximum dry weight of shoot (105.-g) in inoculated and un inoculated control plants of P24 was noticed in plants treated with *V.tenerum* (71)+ $N_1P_2K_0$ + garlic (3%) + potassium phosphonate (1200ppm) + coffee pulp. In inoculated KS27, maximum dry weight of shoot (52g) noticed in plants treated with *V.tenerum* (71)+ $N_0P_1K_0$ + garlic extract (3%) + potassium phosphonate 1200ppm + coffee pulp, where as in uninoculated control, maximum dry weight (72.1g) of shoot noticed in plants treated with *V.tenerum* (71)+ $N_1P_2K_0$ + garlic (3%) + potassium phosphonate (1200ppm) + coffee pulp (**Table - 58**).

It was noticed that, field tolerant line P24 supported higher growth and proliferation of soil microbes compared to susceptible variety KS27. The overall rhizosphere population level of *Trichoderma*, total fungi, bacteria and actinomycetes were 4.476 x 10^3, 125.032 x 10^3, 51.44 x 10^6 and 0.651 x 10^5 respectively in P24 compared to 1.159 x 10^3,50.921 x 10^3, 9.73 x 10^6 and 0.524 x 10^5 respectively in KS27 (**Table- 59**).

The reduction in the disease combined with increase in growth adds up to the overall improvement of the health of black pepper. The deleterious effect of *Phytophthora capsici*, *Radopholus similis* and *Meloidogyne incognita* on growth of black pepper has been realised (Sarma *et al.*, 1991).

The importance and relevance of identification biocontrol agents with multiple mode of action to check these pathogens and boost up the health and productivity of vines have been highlighted (Sarma *et al.*, 1996). Over and above the isolates which possess the growth promoting activity of the host and deleterious effect on pathogen are to be looked into. Further studies are warranted to identify such organisms with a multiple mode of action, time of application and amendments that support their stability in the soils.

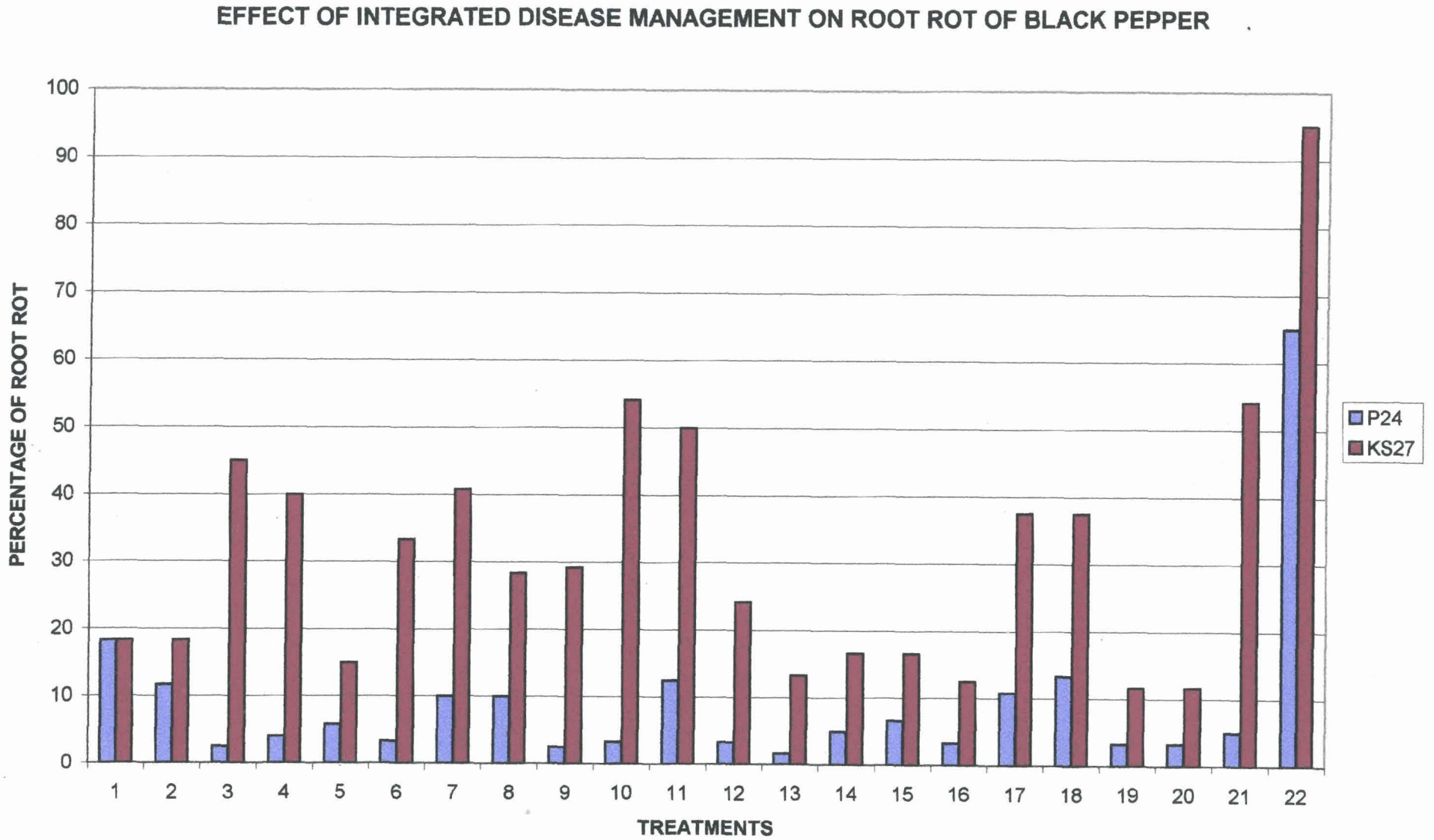
EFFECT OF INTEGRATED DISEASE MANAGEMENT ON ROOT ROT OF BLACK PEPPER
PERCENTAGE OF ROOT ROT
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
TREATMENTS
P24
KS27

FIG : 4

Table -54: Effect of integrated disease management (IDM) on root rot in *Phytophthora* tolerant and susceptible black pepper

Treatments	Root rot (%)		
	P24 (toler)	KS27 (Susc)	+/- over P24 (%)
1. *T.h* (1) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	18.33	18.33	00.00
2. *T.h* (1) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	11.67	18.33	36.33
3. *T.h* (1) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	02.50	45.00	94.44
4. *T.v* (10) +*V.t* (63)+$N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	04.06	40.00	88.50
5. *T.v* (10) +*V.t* (63)+$N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	05.83	15.00	61.13
6 .*T.v* (10) +*V.t* (63)+$N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	03.33	33.33	90.00
7. *T.v* (10) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	10.00	40.83	75.50
8. *T.v* (10) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	10.00	28.33	64.70
9. *T.v* (10) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	02.50	29.17	91.42
10.*T.v* (19) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	03.33	54.17	93.85
11.*T.v* (19) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	12.50	50.00	75.00
12.*T.v* (19) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	03.33	24.17	86.22
13.*T.v* (10) + *V.t* (71) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	01.66	13.33	87.54
14.*T.*v (10) + *V.t* (71) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	05.00	16.67	70.00
15.*T.v* (10) + *V.t* (71) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	06.66	16.67	60.04
16.*V.t* (63) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	03.33	12.50	73.36
17.*V.t* (63) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	10.83	37.50	71.12
18.*V.t* (63) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	13.33	37.50	64.45
19.*V.t* (71) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	03.33	11.67	71.46
20.*V.t* (71) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	03.33	11.67	71.46
21.*V.t* (71) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	05.00	54.17	90.76
22.Control	65.00	95.00	31.57
CD at 5%	9.975		

Table -55: Effect of Integrated disease management (IDM) on fresh weight of root of black pepper (in g.)

Treatments	P24 (tolerant)		KS27 (susceptible)			
	Inoculated	Un-inoculated	Inoculated	Un-inoculated	+/- over P24 (%)	
					Ino	un-inoc
1. *T.h* (1) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	26.00	30.67	06.00	16.16	76.92	47.31
2. *T.h* (1) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	17.67	27.33	07.50	13.33	57.55	51.22
3. *T.h* (1) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	20.00	23.17	19.00	22.67	05.00	02.15
4. *T.v* (10) +*V.t* (63)+$N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	22.00	24.83	13.00	25.00	40.90	-00.68
5. *T.v* (10) +*V.t* (63)+$N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	20.67	22.17	23.00	35.00	11.27	-36.65
6 .*T.v* (10) +*V.t* (63)+$N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	19.33	28.33	11.00	18.00	43.09	36.46
7. *T.v* (10) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	13.83	14.00	09.66	14.86	30.15	-05.78
8. *T.v* (10) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	32.17	33.67	06.33	08.00	80.32	76.23
9. *T.v* (10) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	22.50	27.33	08.16	10.00	63.73	63.41
10.*T.v* (19) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	14.83	17.67	06.66	09.90	55.09	43.97
11.*T.v* (19) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	22.33	25.00	13.67	25.00	38.78	00.00
12.*T.v* (19) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	28.33	33.50	21.33	26.33	24.70	21.40
13.*T.v* (10) + *V.t* (71) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	20.17	20.33	13.00	27.00	35.54	-24.70
14.*T.*v (10) + *V.t* (71) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	21.17	23.33	16.83	29.00	20.50	-19.55
15.*T.v* (10) + *V.t* (71) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	26.50	28.00	06.00	15.83	77.35	43.46
16.*V.t* (63) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	39.17	42.67	12.33	28.83	68.52	32.43
17.*V.t* (63) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	21.33	26.17	09.66	25.17	54.71	03.82
18.*V.t* (63) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	18.50	21.67	16.00	51.00	13.51	-57.50
19.*V.t* (71) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	10.50	10.67	17.83	25.50	03.62	-40.87
20.*V.t* (71) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	21.83	21.88	19.50	51.00	10.67	-57.09
21.*V.t* (71) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	26.33	29.50	10.50	27.50	60.12	06.77
22.Control	10.00	45.00	05.00	16.00	50.00	64.44
CD at 5%	11.52					

Table -56: Effect integrated disease management (IDM) on root dry weight in black pepper (in g.)

Treatments	P24 (tolerant)		KS27 (susceptible)			
	Inoculated	Un-inoculated	Inoculated	Un-inoculated	+/- over P24 (%)	
					Inoculated	Un-inocu
1. *T.h* (1) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	11.50	18.33	10.66	13.66	-07.30	25.47
2. *T.h* (1) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	13.83	14.50	10.06	15.66	27.25	-07.40
3. *T.h* (1) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	09.16	12.83	06.66	10.33	27.29	19.48
4. *T.v* (10) +*V.t* (63)+$N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	12.83	15.67	05.16	08.16	59.78	47.92
5. *T.v* (10) +*V.t* (63)+$N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	09.66	13.67	09.33	13.17	03.41	03.65
6 .*T.v* (10) +*V.t* (63)+$N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	13.33	15.00	07.33	11.33	45.01	24.46
7. *T.v* (10) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	08.00	08.50	03.33	05.50	58.37	35.29
8. *T.v* (10) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	22.17	28.50	03.83	05.83	82.72	79.54
9. *T.v* (10) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	16.33	18.33	06.33	08.53	61.23	53.46
10.*T.v* (19) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	12.83	13.17	04.00	06.33	68.82	51.93
11.*T.v* (19) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	11.17	17.33	08.50	15.00	23.90	13.44
12.*T.v* (19) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	19.50	22.50	09.83	13.33	49.58	40.75
13.*T.v* (10) + *V.t* (71) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	15.33	16.83	09.00	11.33	41.29	32.67
14.*T.*v (10) + *V.t* (71) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	18.50	19.33	12.67	15.66	31.51	18.98
15.*T.v* (10) + *V.t* (71) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	16.50	19.00	03.33	07.55	79.81	60.26
16.*V.t* (63) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	15.67	17.50	06.55	08.89	58.20	49.20
17.*V.t* (63) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	12.33	14.50	05.66	08.00	54.09	44.82
18.*V.t* (63) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	10.17	12.00	08.83	13.67	13.17	-12..21
19.*V.t* (71) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	07.50	09.66	21.17	22.33	-64.57	-56.73
20.*V.t* (71) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	11.83	12.00	13.00	13.67	09.89	-12.21
21.*V.t* (71) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	13.17	14.83	15.16	23.00	15.11	-35.52
22.Control	05.50	09.00	02.00	07.00	63.63	22.22
CD at 5%				5.36		

Table -57: Effect of integrated disease management (IDM) on fresh weight of shoot in black pepper (in g.)

Treatments	P24		KS27			
	Inoculated	Un-inoculated	Inoculated	Un-inoculated	+/- over P24 (%)	
					Inoculated	Un-inocul
1. *T.h* (1) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	131.8	136.7	021.6	017.8	83.61	86.97
2. *T.h* (1) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	110.0	137.0	022.0	028.0	80.00	79.56
3. *T.h* (1) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	091.3	165.3	075.5	147.7	17.85	10.64
4. *T.v* (10) +*V.t* (63)+$N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	047.5	057.3	044.6	068.0	06.10	-15.73
5. *T.v* (10) +*V.t* (63)+$N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	123.7	131.8	041.8	110.2	66.20	16.38
6 .*T.v* (10) +*V.t* (63)+$N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	116.3	119.2	064.0	094.0	52.30	21.14
7. *T.v* (10) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	122.9	128.3	021.3	036.0	82.66	71.94
8. *T.v* (10) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	115.3	135.1	009.0	014.8	92.19	89.04
9. *T.v* (10) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	189.7	190.0	025.5	035.0	86.55	81.57
10.*T.v* (19) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	091.1	125.6	018.8	029.0	79.36	76.91
11.*T.v* (19) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	150.7	218.7	051.6	142.3	65.75	34.93
12.*T.v* (19) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	222.3	295.0	079.8	126.0	64.18	57.28
13.*T.v* (10) + *V.t* (71) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	059.3	165.0	023.0	065.0	61.21	60.60
14.*T.*v (10) + *V.t* (71) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	100.7	206.7	047.5	052.0	52.83	75.09
15.*T.v* (10) + *V.t* (71) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	066.8	076.3	014.1	033.6	78.89	55.96
16.*V.t* (63) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	145.3	171.3	028.3	041.3	80.52	75.89
17.*V.t* (63) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	072.0	156.0	059.0	123.0	18.05	21.15
18.*V.t* (63) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	173.7	225.5	041.8	065.0	76.39	71.17
19.*V.t* (71) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	077.8	142.0	083.3	123.7	-06.60	13.38
20.*V.t* (71) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	255.8	266.5	110.0	136.0	56.99	48.96
21.*V.t* (71) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	169.2	177.0	037.0	058.0	78.13	67.23
22.Control	092.0	110.0	015.0	042.0	83.69	61.81
CD at 5%	79.66					

Table -58: Effect of integrated disease management (IDM) on dry shoot weight in black pepper (in g.)

Treatments	P24 (tolerant)		KS27 (susceptible)			
	Inoculated	Un-inoculated	Inoculated	Un-inoculated	+/- over P24 (%) Inoculated	Un-inocul
1. *T.h* (1) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	036.5	041.6	008.8	010.0	75.89	75.96
2. *T.h* (1) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	029.1	044.8	010.3	011.0	64.60	75.44
3. *T.h* (1) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	029.3	045.0	034.5	056.3	-15.07	-20.07
4. *T.v* (10) +*V.t* (63)+$N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	017.3	018.8	023.1	041.3	25.10	-54.47
5. *T.v* (10) +*V.t* (63)+$N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	044.0	044.5	020.0	064.0	54.54	-30.46
6 .*T.v* (10) +*V.t* (63)+$N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	034.1	039.3	028.5	031.8	16.42	19.08
7. *T.v* (10) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	043.5	045.0	011.5	015.1	73.56	66.44
8. *T.v* (10) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	035.3	037.6	006.8	007.0	80.73	81.38
9. *T.v* (10) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	078.8	079.0	014.6	018.0	81.47	77.21
10.*T.v* (19) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	026.1	033.1	007.1	012.8	70.49	61.32
11.*T.v* (19) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	037.1	095.0	026.8	059.6	27.76	37.26
12.*T.v* (19) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	099.0	105.0	048.6	053.6	50.90	48.95
13.*T.v* (10) + *V.t* (71) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	026.6	048.8	016.0	027.1	39.84	44.46
14.*T.*v (10) + *V.t* (71) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	024.8	096.0	020.1	022.8	18.95	76.25
15.*T.v* (10) + *V.t* (71) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	027.2	032.0	009.1	019.8	66.54	38.12
16.*V.t* (63) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	052.5	063.2	014.5	020.3	72.38	67.87
17.*V.t* (63) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	023.1	057.1	032.5	059.3	-28.92	-03.70
18.*V.t* (63) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	039.0	098.8	022.0	040.0	43.58	59.51
19.*V.t* (71) + $N_0P_1K_0$ + G3% + PP(1200ppm.) + CP	026.6	040.8	052.0	060.1	48.84	-32.11
20.*V.t* (71) + $N_1P_2K_0$ + G3% + PP(1200ppm.) + CP	105.0	112.0	046.0	072.1	56.19	35.62
21.*V.t* (71) + $N_0P_0K_0$ + G3% + PP(1200ppm.) + CP	058.1	060.3	018.6	029.5	67.98	51.07
22.Control	028.0	038.0	008.0	026.0	71.42	31.57
CD at 5%	29.30					

Table – 59: Effect of Integrated Disease Management of black pepper on overall microflora

ORGANISM	P24	KS27	P24	KS27	CD at 5%
	*INITIAL	INITIAL	FINAL	FINAL	
TRICHODERMA	5.716	7.683	4.476	1.159	0.6317
FUNGI	63.825	70.460	125.032	50.921	4.183
BACTERIA	21.190	32.683	51.444	09.730	1.708
ACTINOMYCETES	0.635	0.683	0.651	0.524	0.2183
	**INOCULATED	INOCULATED	UNINOCULATED	UNINOCULATED	
TRICHODERMA	2.968	0.778	4.349	1.524	0.4594
FUNGI	102.3	48.81	64.65	50.92	15.21
BACTERIA	11.70	13.22	32.68	9.683	1.464
ACTINOMYCETES	0.7619	0.2222	0.6825	0.5238	0.2932

* OVERALL EFFECTS ON MICROBES IRRESPECTIVE OF TREATMENTS

** FINAL COMPARISON BETWEEN INOCULATED AND UNINOCULATED CONTROLS IN P24 & KS27.

DISCUSSION

Rajan P.P "Approaches towards the integrated disease management of phytophthora infection of black pepper (Piper nigrum L.)" Thesis. Department of Botany, University of Calicut,1999

DISCUSSION

Phytophthora foot rot and slow decline of black pepper have been identified as major production constraints, not only in India but also in other countries like Indonesia, Malaysia and Brazil where black pepper is grown (Muller, 1936; Soepartono, 1953; Holl, 1929; Robertson, 1953; Holliday, 1965). All parts of black pepper are prone to *P.capsici* infection and the foliar phase and soil phase of disease have been identified (Sarma *et al.*, 1991). Heavy monsoon, soil-borne nature of the disease and poor adoption of phytosanitation have become major bottlenecks and as such disease management has become more elusive. The epidemiological investigations carried out in India (Ramachandran *et al.*, 1991; Anandaraj *et al.*, 1991) have became the basis for sound disease management programme.

Chemical control remained as the main focus of disease management strategy apart from the cultural operations (Ramachandran and Sarma, 1985). Pre-monsoon (May-June) and post-monsoon (August-September) spraying with Bordeaux mixture and drenching with copper oxychloride was the package adopted which provided varying degrees of protection. The contact fungicides like copper oxychloride and Bordeaux mixture are prone to leaching losses during heavy monsoon period (May – June to August-September). In view of leaching off of contact fungicide, alternate systemic fungicides like phenylamides and potassium phosphonates were tested for their efficacy to control *Phytophthora* infection in black pepper (Ramachandran, 1990) ([illegible]). The results clearly indicated that, the metalaxyl was the best among the fungicides tested (Ramachandran, 1990). Metalaxyl also was compatible with quinalphos and endosulfan which were also used against 'pollu' beetle, a pest of black pepper. Metalaxyl was found to be an ideal candidate in IDM. Besides, in recent years, potassium phosphonate has also been found to check *Phytophthora* infection in black pepper, and is systemic in nature.

Both potassium phosphonate and metalaxyl incidentally were found compatible with biocontrol agents and hence were considered ideal in Integrated Disease Management (IDM)

in *Phytophthora* infection in black pepper. The environmental awareness, the possible pesticide residues in produce and stringent pesticide residue monitoring by importing countries made it imparative to look for alternative strategy for disease management. The earlier investigations (Sarma *et al.*, 1991; Nambiar and Sarma, 1977) clearly indicated that a single approach of disease management met with little success. Hence, the importance of integrated disease management approach consisting of cultural, chemical and biological methods coupled with host resistance remained as a practical strategy. In the present investigation these components were studied in detail with greater emphasis on biocontrol in view of its ecofriendly nature and sustainability in agroecosystem. Besides, a *Phytophthora* field tolerant black pepper line- P24, (Sarma *et al.*, 1991) was involved in experimentation in comparison with highly susceptible Karimunda cultivar KS27 (Subhakara).

STANDARDIZATION AND MASS MULTIPLICATION OF *P.CAPSICI* INOCULUM

Mass production of ideal inoculum to induce root rot is the primary prerequisite for conducting experiments to test the efficacy of various treatments superimposed. In the present study for large scale production of *Phytophthora* inoculum carrot broth-sand inoculum was standardized. The high nutrient content of the carrot broth and porosity of sand appeared to have provided ideal environment for *P.capsici* multiplication and this resulted in high Disease Potential Index (DPI) for *P.capsici* by 20 days. This contained sporangia, chlamydospores and mycelia. Most of the workers used zoospore suspension as inoculum source for artificial inoculation (Turner, 1967; Mehrotra, 1972; Kroll and Elide, 1981; Mc Donald and Duniway, 1978; Sastry, 1982; Dutta, 1984; Cho *et al.*, 1987). Out of 6 doses tested, 2% carrot broth inoculum with maximum DPI induced maximum root rot in black pepper. This dose of inoculum was used throughout the pot culture studies. Earlier, Freire and Bridge (1985) used 5 x 10^2 zoospores/ml to induce root rot in black pepper, but in the present study 2% carrot broth - sand inoculum was found superior and simple for disease induction (Table-3).

RHIZOSPHERE STUDIES

For all rhizosphere studies, Martin's Rose Bengal Agar (Martin, 1950), Soil Extract Agar (Allen, 1957) and Actinomycetes Isolation Agar (Hi-media) for fungi, bacteria and actinomycetes, respectively were used throughout (**Table 1**), based on the evaluation of various media tried.

Root health and root regeneration would determine the productivity and longevity of black pepper. Black pepper root system is generally shallow in nature. Rhizosphere studies were undertaken in this programme since root rot is one of the main problems in black pepper. The influence of root system on soil microbes, called rhizosphere effect, results in higher microbial activity unlike that of root free soil. Various nutrients such as sugar, amino acids and vitamins present in root exudates, support the growth and proliferation of microorganisms in the root zone. The production of feeder roots in black pepper showed that, feeder roots are confined to the top 60cm and upto 60cm radius from the base of vine. Activity of feeder root system was more at 0-20cm and 20-40cm depth and distance followed by 40-60cm depth and distance (Anandaraj, 1997). Rhizosphere microflora would exert profound influence on plant growth by decomposing organic matter and thus facilitating the uptake of nutrients by the plants (Johnson and Curl, 1972). This microbial activity also affects the pathogen population in the root zone.

Rhizosphere microflora in a pure black pepper garden showed considerable fluctuation compared to non-rhizosphere areas. In general, microbial activity was higher especially fungi and bacteria, in rhizosphere of black pepper compared to non-rhizosphere during different months studied. This supports the findings of many workers who worked on rhizosphere microorganisms of different plants (Azad *et al.*, 1985; Ridge, 1976). The different exudates excreted by roots and the nutrients liberated during decomposition of sloughed off root cells are utilized by root zone microorganisms resulting in their stimulation. During rainy season decomposition of organic matter takes place affecting uptake of nutrients by the plants. The actinomycete population did show similar trend except during July-September period. This is

in contrast to the study carried out by Ahn and Hawang (1992) who reported higher activity of antibiotic-producing actinomycetes in rhizosphere of bell pepper. Maximum amount of root regeneration in black pepper occured during June-August, which coincided with good soil moisture (Anandaraj, 1997).

Rhizosphere of healthy vines supported higher microbial activity than diseased vines. This is in conformity with the findings of Weste and Vithanage (1976) who reported higher microbial populations (fungi, bacteria and actinomycetes) in the soil where *P. cinnamomi* damage was the least, compared to severely infected Jarrah fields. Maximum fungal and bacterial populations were noticed in rhizosphere of black pepper during the wet season and least population observed during the dry seasons of the year. The density of *P. capsici* population was more around the root zone of infected vines and get reduced by increasing the depth and distance from infected vines (Ramachandran *et al*, 1986). The root regeneration and new flush emergence in black pepper during June-August might be the reason for higher microbial activity and this might be due to exudates released from roots (Anandaraj, 1997).

Black pepper root system is shallow and most roots are concentrated upto 60cm laterally and extend upto 90cm in depth. In healthy rhizosphere of black pepper the microbial activity was high compared to the rhizosphere of diseased vines (**Table - 7**). This is related to the high root density and consequent root exudates compared to poor root activity in diseased vines.

In the present study, maximum fungal and bacterial populations were seen in the top layers (0-10cm) of soil during the rainy season (May – September) compared to dry season (Ocober-April), and is related temperature and soil moisture status.

During the rainy season (May – September), slight increase of *Trichoderma* population was noticed at top layers of soil and reduction was noticed during the dry season at 10cm depth (**Table – 11**). However, it was steady at 20cm and this might be due stablised condition of the soils with regards to moisture and temperature. Trichodermas produce chlamydospores as propagules during adverse conditions especially during dry seasons and these spores germinate during the wet season and more CFU of *Trichoderma* were observed during wet season than dry season (Davet, 1979 & 1981). More *Trichoderma* propagules (CFU) were observed in the rhizosphere of healthy vines than the rhizosphere of diseased vines during wet period. This might be due to high root regeneration and more root exudates at basins of healthy vines which support the proliferation of organisms. High root regeneration and excretion of root exudates might have supported more *Trichoderma* population in the rhizosphere soils of healthy vines.

A positive correlation was noticed between the rainfall and DPI of pathogen in a diseased garden where as it was not in detectable levels in healthy garden (**Table-12** & **Fig-1**). Multiplication of *Phytophthora* mainly depends matric potential in the soil (Duniway, 1983). Pathogen sporulates and multiplies during the rainy season (June - July) and leads to both foliar and root infection. After infection, pathogen multiplies in host tissues and symptoms such as foliar yellowing due to root infection are seen during later months or season. The difference in microbial activity in healthy and diseased vines and also that of *Trichoderma* needs a detailed investigation to understand microbial suppression of *P. capsici*

ISOLATION AND IDENTIFICATION OF BIOCONTROL AGENTS

Every life-supporting soil samples contain beneficial microorganisms that are growth promoting and also inhibitory to specific plant pathogens. Fifty rhizosphere soil samples collected from different pepper gardens of South India were tested for isolation of antagonists. Rhizosphere soils collected from Karnataka state of South India showed neutral or around neutral pH (6.1-7.3) where as Kerala soil were slightly acidic in nature (5.4-6.9). *Penicillium* spp., *Aspergillus* spp., members of Mucorales and *Trichoderma* were

predominant in the collections made which supported the earlier findings of many workers (Maas and Kotze, 1989, Weste and Vithanage, 1976). More *Trichoderma* spp. and *Penicillium* spp. antagonistic to *P.capsici* were isolated from Kerala soils, which indicate that acidic soils support the growth and proliferation of antagonistic organisms than neutral soils.

SCREENING BCA ISOLATES FOR THEIR ANTAGONISM TO *P.CAPSICI*

A large cross section of rhizosphere samples (50) of black pepper both from Kerala and Karnataka, the major pepper growing areas of India were screened for the antagonists and hyperparasites of *P. capsici*. One hundred and four fungal isolates and 27 *Trichoderma* isolates were tested for their growth inhibition of *P.capsici*. Among the fungal isolates tested, four isolates (2 *Penicillium* sp and 2 *Verticillium tenerum*) were found promising. Growth inhibition of *P.capsici* with *Trichoderma* isolates tested ranged from 33-69%. Out of 16 antagonistic bacterial and 12 actinomycetes isolates tested for their growth inhibition, 3 bacterial isolates and 6 actinomycetes isolates produced clear inhibition zone between the antagonist and pathogen. Colour change (slight yellow) observed in the medium especially at the inhibition zone, might be due to the production of antibiotics in the medium by these antagonistic bacteria and actinomycetes (Johnson and Curl, 1972).

IN VIVO EVALUATION

In *In vivo* evaluation of antagonistic fungal isolates, three fungal isolates (63,71 & 74) were found giving 95% root protection against root rot pathogen and increased biomass in the plants treated with these antagonists. Isolate 63 and 71 were identified as *Verticillium tenerum* and their antagonistic ability was proved on *Phytophthora capsici*. *In vivo* efficacy of *Verticillium chlamydosporium* both on *P. capsici* and root knot nematode in black pepper s reported (Veena and Peethambaran, 1997; Sreeja *et al.*, 1996). Hyperparasitism of *V.tenerum* on *R. soloni* was proved by Turhan (1990). Isolate No.74 was identified as *Penicillium* sp., which gave high root rot protection and enhanced the overall growth of host plants. *Penicillium funiculosum* was found antagonistic to *P. parasitica, P.citrophthora* and *P.cinnamomi* and increased growth parameters (Fang and Tsao, 1995). *Penicillium aurantiogriseum* was found antagonistic to *P.infestans* (Jindal *et al*, 1988).

Four species viz; *T.harzianum, T.virens, T.aureoviride* and *T.polysporum* representing among the 27 *Trichoderma* isolates tested in pot culture for their bio-efficacy to contain the root rot. Out of these, three isolates (10, 12 & 19) belonging to *T.virens* gave high root protection and increased root biomass (**Table-19**). Out of 16 antagonistic bacterial isolates tested against root rot, two fluorescent (Pf2, Pf5) and one non-fluorescent bacteria (BN) gave root protection ranging from 18-98% and enhanced the growth of black pepper. Antagonistic effect of bacterial isolates on *P. cactorum* were studied extensively. *E.aerogenes* has been reported highly effective in checking root rot of apple caused by *P. cactorum* (Utkhede, 1984a&b; Utkhede and Gounce, 1983; Marchi and Utkhede, 1994; Utkhede and Smith, 1993).

Rhizobacteria are reported to have more antagonistic effect on soil borne pathogens and enhance the overall growth of host plants (Utkhede, 1984a&b). No correlation was noticed between the production of inhibition zone in the medium and *in vivo* antagonistic efficacy, as reported by Kloepper and Schroth (1981). Introduction of rhizobacteria into rhizosphere soil to support the root system also supported the enhanced growth of host plants (Elad *et al*, 1987, Turner, 1987, Broadbent *et al*, 1977; Geels and Schippers, 1983). However detailed studies are warranted on the mode of action and the presence of their motabolites in the soil if any. Field evaluation of these biocontrol agents is needed to confirm their bioefficacy in reducing root rot.

FIELD TRIAL

Five *Trichoderma* isolates were evaluated in the field for their disease suppression activity for 3 years. Out of five isolates used, *Trichoderma virens* (isolate No.12) showed high protection against the *Phytophthora* infection compared to *T.harzianum, T.hamatum* and *T.polysprum*. However its stability decreased with time (**Table - 22**). There was a gradual reduction in disease incidence in the field where the biocontrols were applied as compared to control, whereas in the area where mixture of the antagonists were applied, there was not much reduction in disease incidence.

The protective effect of all the biocontrol agents tested, though emperically different among themselves, the effects were not significant. However, they differed signficantly over untreated control (**Fig - 2**). The field monitoring of populations of BCA's showed greater population during wet period, thereby indicating the soil moisture in relation to their multiplication. Among the biocontrol agents studied, *T.harzianum* showed greater adaptability in soils ($40x10^3$) compared to *T.virens* and *T.hamatum* (3.74 and $2.97x10^3$)(**Fig - 3**). This has been validated by large scale demonstration trials undertaken in Kerala and Karnataka (Sarma and Anandaraj, 1999). The present study indicated that, application of sorghum based *T.harzianum* along with neem cake supported high population of BCA's and its stability which reflected in better protection of the black pepper vines in the field.

The fungal antagonists when applied to conducive soils reduced the disease potential index (DPI) which resulted in control of azhukal disease of cardamom caused by *P.meadii* (Suseela Bhai *et al.*, 1993). Suseela Bhai *et al.*, (1994) evaluated different carrier media for the ealuation for mass multiplication of biocontrol agents in cardamom fields and further reported that, coffee husk and FYM at the ratio of 1:1 was best for mass multiplication of *T.harzianum* against *P.meadii* of small cardamom (Suseela Bhai, 1998). As reported by Davet (1979 & 81), the BCA population was more during the wet season than the dry season and this might have resulted in pathogen multiplication around the root and consequent protection of the plants (**Table - 22**). The findings of Papavizas (1985) on survival of *T.harzianum* and persistance during the dry season are in confirmity with the present study. Survival ability of the biocontrol agents depend on their competitive saprophytic nature and rhizosphere competence. It also depends their ability to degrade various organic substrates in the soil than metabolic versatility and their resistance to microbial inhibitors depending on prevailing conditions and species or strains involved (Papavizas, 1985). Spraying of spore suspension of *T.viride* could control the early blight of tomato in the field (Kshirsagar, 1995). Efficacy of *T.harzianum* against *P.cryptogea* causing foot rot of *Gerbera* has been reported (Duskova, 1992). Orlikowski (1994) studied the biocidal property of *Trichoderma* and *Gliocladium* spp. against *P.cryptogea* and reported that 10^9 spores of *T.viride* applied to pits 10 days before inoculation with *P.cryptogea,* could

control foot rot of *Gerbera* effectively. *T.harzianum* impregnated on clay granules could control damping off of pineapple seedlings due to *P.cinnamomi* (Kelley, 1976). In black pepper, further studies are needed on the frequency of application of biocontrol inoculum along with organic amendments to ensure its stability under field condition.

CHEMICAL CONTROL

Three agrochemicals viz; **Acrobat (dimethomorph), Aureofungin (heptaene antibiotic)** and **Akomin (potassium phosphonate)** were tested against *P.capsici* both *in vitro* and *in vivo*. In earlier studies, the efficacy of acrobat (dimethomorph), aureofungin (heptaene antibiotic) and potassium phosphonate on *Phytophthora* spp. has been reported (Kuhn *et al.*, 1989 a&b; Agarwala and Thirumalachar, 1967; Coffey and Joseph, 1985). However these were not studied in *Phytophthora* – black pepper system.

In the present study, inhibitory activity of dimethomorph on all the four phases of *P.capsici* was noticed at low concentrations. LD_{50} value for growth inhibition was 0.4ppm., for sporulation 0.2ppm. for indirect sporangial germination 100ppm. and zoospore germination 10ppm. *In vitro* study indicated dimethomorph effects on different phases of *P.infestans* at very low concentrations as reported by Kuhn *et al*, (1989 a&b). Kuhn *et al.* (1989a & b) reported that, dimethomorph is active against *Phytophthora* species *in vitro*, where it inhibits radial growth of mycelium with ED_{50} values typically in the range of 0.25 - 0.75 micromol. Albert *et al.* (1988) and Kuhn *et al.* (1989a) found that, dimethomorph acts as a fungicide compound and not as a fungistatic compound. Dimethomorph (commonly known as acrobat), which was first described in 1988 (Albert *et al.*, 1988) was highly effective in the control of downey mildews and diseases caused by different species of *Phytophthora* (Albert *et al.*,1988; Kuhn *et al.*, 1989a)

Heptaene antibiotic (aureofungin) was tested on four phases of *P.capsici*. Inhibition on growth and sporangial germination was noticed at higher doses, where as inhibition on sporulation and zoospore germination were noticed at very low concentrations. LD_{50} value for growth was 100ppm., and for indirect sporangial germination 200ppm., for sporulation s 20ppm., and for zoospore germination, it was less than 5ppm. Aureofungin is reported to be very effective *in vitro* against *P.palmivora* and *P.citrophthora* (Agarwala and Thirumalachar, 1967; Agarwala and Sharma, 1975; Bedi and Dhaliual, 1970; Bedi *et al.*, 1969; Capoor and Marathe, 1970). Its *in vitro* efficacy against *Pythium debaryanum* and *P.myriotylum* was also established (Capoor and Marathe, 1970). Field efficacy of aureofungin to control leaf rot of pan due to *P.parasitica* var *piperina* was reported (Chaurasia *et al.*.1973). Aureofungin was also reported to be effective against *Phytophthora* diseases viz; fruit rot of guava (Sohi, 1975), citrus gummosis (Desai *et al.*, 1966) and abnormal leaf fall of rubber (KAU, 1976).

Potassium phosphonate was investigated for its antifungal activity against a range of fungi grown on liquid and solid media (Coffey and Bower, 1984, Fenn and Coffey, 1984, Dolan and Coffey, 1988). This agrochemical was also tested against different species of *Phytophthora* (Coffey and Bower, 1984, Bompeix and Saindrenan, 1984). Potassium phosphonate was found very effective against many phytopathogenic fungi. The mode of action on pathogens was studied (Smillie *et al.*, 1989; Guest and Bompeix, 1990). Alteration in nucleotide and pyrophosphate levels in *P.palmivora* has been reported due to exposing to this chemical (Griffith *et al*, 1990). LD_{50} for growth of *P.capsici* was 100ppm. and complete inhibition of growth was noticed at 1000ppm., *Phytophthora cinnamomi*, *P.citricola*, *P.citrophthora* and *P.palmivora* were most sensitive to potassium phosphonate and LD_{50} value for growth was recorded at 5 - 10ppm (Coffey and Ouimette, 1989). For *P.capsici* complete sporangial inhibition was noticed at 7ppm. LD_{50} for indirect sporangial germination was noticed at less than 100ppm and complete inhibition was noticed at 1100ppm. level (**Table – 30**). LD_{50} for zoospore release of *P.cinnamomi* and *P.citricola* was reported to be 6ppm (Coffey and Joseph, 1985). Zoospore germination was the second most sensitive phase to potassium phosphonate, LD_{50} value for zoospore germination was

noticed at 60ppm. and complete inhibition of zoospore germination was noticed at 110ppm level. The results indicated that sporulation and zoospore germination of *P.capsici* were most sensitive to potassium phosphonate. This is in confirmity with previous studies carried out (Ramachandran, 1990). In pine apple, control of *P.cinnamomi* and *P.parasitica* were achieved with a single preplanting dip which was effective for 18 months (Rohrbach and Schenck, 1985). Two applications as trunk injection to avacado resulted in the reversal of the tree decline symptoms due to *P.cinnamomi* infection and restoration of good root and shoot growth in the following season (Darvas *et al.*, 1984; Pegg *et al.*, 1985).

IN VIVO EVALUATION OF AGROCHEMICALS

All the three agrochemicals viz; Dimethomorph, Aureofungin and Potassium phosphonate were evaluated in pot culture for their bio-efficacy on root rot suppression. Least root rot (17.78%) incidence was noticed in plants treated with three application of potassium phosphonate at 1200ppm as soil drench and foliar spray, compared to untreated control (74.0%) (**Table – 31**), all the chemicals showed good root protection against the pathogen. Results indicated that three applications of potassium phosphonate (1200ppm) was superior (17.78%) than one (45.56%) or two (44.44%) applications.

Three rounds application of dimethomorph (300 and 400ppm) gave good root protection,and noticed less root rot (21.11% and 23.33%) which was significantly superior to control plants (74.0%). Three rounds application of aureofungin (100, 200 and 300ppm) gave comparatively good root rot control (24.44, 20.0 and 47.78% respectively). Higher dose of aureofungin (300ppm) did not show superior effect on root protection compared at lower doses (**Table - 31**). In all the cases there was no phytotoxicity on black pepper any of the concentrations tested. *In vivo* efficacy of dimethomorph and aureofungin has been reported earlier also (Albert *et al.*, 1988; Sohi,1975; Desai *et al.*, 1966; KAU,1976). *In vivo* efficacy of potassium phosphonate has been proved by many workers (Rohrbach and Schenck, 1985; Darvas *et al.*, 1984; Pegg *et al.*, 1985)

Since potassium phosphonate is rapidly degraded to phosphate in soils, the use of this antifungal chemical in integrated management system is found more feasible and eco-friendly (Ouimette and Coffey, 1989). Many soil microorganisms such as bacteria and fungi can apparently utilize phosphonate as phosphate source (Casida, 1960, Malacinski and Konetzka, 1966).

Compatibility of Potassium phosphonate with different species of *Trichoderma*

Since potassium phosphonate was found superior over the other two agrochemicals used, its compatibility with *Trichoderma* spp. was studied in detail. Since the emerging strategy is integrated disease management, the compatibility of biocontrol agents with agrochemicals becomes relevant and important in foot rot management in black pepper.

The *in vitro* sensitivity of different species of *Trichoderma* to potassium phosphonate was studied, since *Trichoderma* forms major component of biocontrol. There was a little variation in *in vitro* growth of different isolates during the course of study. After 72 hours, all the species of *Trichoderma* attained maximum radial growth (45mm) in all the treated and untreated plates except in *T.hamtum*. No significant differences were observed in growth of biocontrol agents at different concentrations of potassium phosphonate (**Table- 32**). Out of nine species of *Trichoderma* tested, in *T.pseudokoningii* recorded highest spore load (342503) in 400ppm treated plates (**Table - 33**). In *T.polysporum*, *T.hamatum* the sporulation was not observed in treated plates. In *T.pseudokoningii* it was observed that the fungicide has given some stimulatory effect on sporulation.

Potassium phosphonate did not have any negative effect on the host and plant products (Johri and Chourasia, 1995) and also it is reported that it enhances the overall growth of the plants (Merwe and Dev Vander, 1993) and yield in avocado (Pegg *et al.*, 1987). Potassium

phosphonate treated peach and apricot plants have shown greater defense against *Phytophthora* spp. (Lim *et al*, 1990). This chemical is reported as a very effective against *P.cinnamomi* (Darvas, 1984; Pegg *et al.*, 1990; Lim *et al.*, 1990), *P.cambivora* (Wicks and Hall, 1990), *P.citrophthora* (Afek and Sztejnberg,1989), *P.palmivora* (Flett *et al.*, 1990; Holderness, 1990; Holderness, 1992; Griffith *et al.*, 1993) and *P.infestans* (Yamada et al 1988). This fungicide has been reported to be effective in suppression of *P.capsici* infection in tomato (Fenn and Coffey, 1985). Basipetal mobility of phosphorus acid compound has been proved (Schwinn, 1983; Zentmyer, 1979). Potassium phosphonate was found compatible with *Enterobacter aerogens* (Utkhede and Smith, 1993) and it is also reported that it has no negative effect on other microbial populations of pepper corn and avocado (Wongwathanast and Sivasithamparam, 1991). Compatibility of *Trichoderma harzianum* with potassium phosphonate has been reported (Sharma and Ashok mishra, 1995). The present study has further confirmed the compatibility of *Trichoderma* spp with potassium phosphonate, thereby suggesting their potentiality in IDM of foot rot of black pepper.

EFFECT OF PLANT EXTRACTS

***In vitro* evaluation**:

Use of botanicals in plant disease suppression received greater attention recently because of their ecofriendly nature and feasibility (Fawcett and Spencer, 1970). The root exudates of *Allium* spp. have been reported to inhibit *P.capsici* (Manohara *et al*, 1992). The water and ethanol extracts of *P.colubrinum* and *Chromolaena odorata* on *P.capsici* have been reported inhibitory (Anandaraj and Leela, 1996). The efficacy of garlic extract on suppression of 'slow wilt' was reported earlier (Shivaram, 1991).

Aqueous extracts of garlic and mustard were tested individually and their combinations against the four phases of *P.capsici* viz; growth, sporulation, sporangial and zoospore germination.

Aqueous extract of mustard was more inhibitory to *P.capsici* compared to garlic extract. Complete inhibition of growth was observed at 4.0% garlic and 3.0% mustard extracts. While in combination, complete inhibition was observed in 2:2% extracts indicating their synergestic inhibitory effect (**Table - 35**).

Aqueous garlic extract showed maximum inhibitory effect on sporulation. Complete inhibition on sporulation was seen at 0.5% of garlic extract, whereas in mustard, complete inhibition noticed only at 1.1%. The synergistic inhibitory effect on sporulation was noticed in combined extract (0.5:0.5%) compared to individual effects (**Table - 36**).

Complete inhibition on sporangial germination of *P.capsici* was observed with aqueous garlic extract 0.6% and in mustard extract, same level of inhibition was noticed with 0.4%. Combined extract showed complete inhibition at 0.3:0.3%, thus confirming the previous trend.

Similarly, extracts at 0.4:0.4% gave complete inhibition on zoospore germination. The principal component of garlic is di-allyl thiosulfinate (2-propenyl-2-propenethol sulfinate) and is also called allicin (Cavallito *et al*, 1944) is found inhibiting the enzyme synthesis of the fungus *A.candida* (Barone and Tansey, 1977). Black mustard (*B. compestris* L) has the compound allyl isothicyanate (CH_2=CH-CH_2.N=C=S) which was found to be antimicrobial (Ernest Guenther, 1978).

Under laboratory conditions, the aqueous extracts of both garlic and mustard were found inhibitory to all phases of *P.capsici.* Combinations of both the extracts showed synergestic effects compared to their individual effects on all the phases of *P.capsici.* Combinations of both the extracts at 0.3% level was completely inhibitory to zoospore release. Since both the aqueous extracts showed good inhibition on all phases of *P.capsici* these indicated their potential as a botanical fungicide. Ajoene, a compound derived from garlic (*Allium sativum* L.) was found highly inhibitory to *P.dreschleri* f.sp. *cajani* (Singh *et al.*, 1992). Zoospore

germination of *P.dreschleri* f.sp.*cajani* was found inhibited by the extracts of garlic and onion at 5,000 and 10,000 ppm respectively (Chauhan and Singh, 1991b).

IN VIVO EVALUATION

In vivo evaluation of bio-efficacy of garlic and mustard extracts, individual, their combinations and along with a biocontrol agent (*T.harzianum*) was conducted in pot culture study. Results indicated the reduction in root rot in plants treated with aqueous extracts and more root protection was noticed in plants treated with combinations of extracts and along with biocontrol agent. Root rot of 29.33 and 38.78% was noticed in plants treated with 2 and 3% garlic extracts, whereas in 2 and 3% mustard treated plants root rot was recoded as 45.3 and 44.44% respectively, which was significantly less than control, where root rot recorded was 69.44% but was inferior to garlic extract. When 3% garlic extracts used along with *T.harzianum*, the root rot incidence was reduced to 5.22% offering good root protection. Mustard extract (3%) with *T.harzianum*, recorded only 8.88% root rot, where as in control it was 69.44% (**Table - 39**).

A spurt in the fungal population was noticed in soils treated with 1% garlic extract (245 x 10^3) which was not discernable with increase in concentration. However, similar trend was not noticed in the case of mustard extract with respect to fungal population. The over all effect of extracts on bacterial and actinomycetes is not conspicous. The synergestic effect shown by garlic and mustard extracts *in vitro* evaluation also reflected in *in vivo* effect on root rot. Mustard extracts in combination with *T.harzianum* showed disease suppression ranging from 8.2-18.7% compared to 20.21% in combination with *T.harzianum*. From the results obtained, can be infered disease suppression might be result of cumulative effect, not only due to chemical components of extracts but also combined effect with *T.harzianum*. (**Table**). In general, there is an indication of increase in *Trichoderma* population in soils treated with these extracts

EFFECT OF ORGANIC SOIL AMENDMENTS

The efficacy of organic soil amendments, consequent microbiological changes and their suppressive effect on root rot pathogens have been well established (Tsao and Oster, 1981; Zentmyer, 1963; Jeyarajan *et al*, 1987; Lumsden *et al*, 1983; Singh and Vyas, 1984; Hoitink *et al.*, 1977; Rosas Romero *et al.*, 1986; Sivasithamparam 1981; Nesbitt *et al.*, 1979). Spencer and Benson (1982) studied the effect of pine bark, hard wood bark compost and peat amendment on lupin root rot by different *Phytophthora* species. In the present study the effect of organic soil amendments in comparison with chemical fertilizer, adjusted to recommended dose of NPK, was studied in detail. Amendment of coffee pulp and neem cake showed 10 and 18% root rot compared to 24.5% in untreated control. However the root rot was maximum where chemical fertilizer alone was applied (94.5%) (**Table - 44**). The DPI of *P.capsici* monitored in the present study indicated of its enhancement of root rot. However it is intriguing that FYM which showed DPI 8 of *P.capsici* showed 57% root rot, where as neem cake 512 as DPI but showed 18% root rot. This might be due to enhanced bacterial population in the former that would have increased *Phytophthora*. Additive effect of certain *Pseudomonads* on *Phytophthora* has been reported earlier (Ayers and Zentmyer, 1971) and by chromobacterium (Zentmyer, 1965). In neem cake amended soil, the fungal microflora was enhanced. The better protection might be due to increased antagonists and also possible indirect effect on host defence machanism. This needs further investigation. Since DPI of *P.capsici* was comparatively higher, even though the root rot was less compared to control, the mechanism needs further investigations. Poultry manure has shown root rot suppression in *P.nicotianae* (Tsao and Oster, 1981), in contrast to severe root rot (89.5%) recorded in black pepper (Table-). Root rot incidence in plants treated with poultry manure and neem cake are on par with chemical fertilizers. DPI of *Phytophthora* in amended soils showed that neem cake and chemical fertilizers were not preventing the growth of this pathogen and it was on par with untreated control plants. Least number of propagules were noted in coffee pulp treated soil.

The present study clearly showed that neem cake enhanced the fungal population in soil (212.067 $x10^3$), followed by poultry manure (161.400$x10^3$) and farm yard manure (152.067 $x10^3$) but NPK was not conducive for fungal multiplication (044.500 $x10^3$) and it enhanced the root rot of black pepper. There is a general feeling among black pepper farmers' that chemical fertilizers are enhancing the disease incidences which needs scientific explanation based on the data.

Fifteen days after the amendment application, poultry manure showed highest number of bacterial population (26.3 x 10^6) but later decreased (6.96 x 10^6). Though the soil with farm yard manure gave less number of bacterial count initially (2.00 x 10^6), at the time of uprooting the population was maximum (11.16 x 10^6). The actinomycete population was more in soil treated wth farm yard manure (19.63 x 10^5) and was least in control (0.10 x 10^5). Addition of different soil amendments enhances the microbial population (Nesbitt *et al.*, 1979; Lumsden *et al.*, 1983; Rattink, 1983; Weltzien 1990) growth of the plants and multiplication of antagonists (Nam *et al.*, 1988; Linderman, 1989). *Phytophthora* population was reduced in mustard, castor, neem and mahna cake amended soil (Singh and Vyas, 1984). The soil with compost of pine bark and hard wood bark had suppressed *Phytophthora* population was suppressed (Spring *et al.*, 1980; Spencer and Benson, 1982). Different soil borne diseases caused by *Pythium* sp., *Fusarium* sp., *Phytophthora* sp. and *Plasmopara* sp. in crucifers were reduced by soil amendments (Huang, 1991). It was also reported that soil amendments would help in reduction of *P.cinnamomi* population (Hoitink *et al.*, 1977) and *P.cactorum* (Rana and Gupta, 1985) in infected fields. In *Vigna mungo* fields, increment of yield and antagonistic population againt root rot pathogen was noticed after application of amendments (Chandrasekaran *et al*, 1995). In the present study it was found that coffee pulp amendment greatly reduced the root rot and enhanced the overall growth of the plants. Incidentally coffee pulp supported good growth of *Trichoderma* (Sarma and Anandaraj, 1999).

EFFECT OF HOST NUTRITION

Host nutrition affects considerably its susceptibility/ or other wise to pathogen in several host pathogen combinations. In *Phytopththora* infection involving several crops, variable effects of different nutrients on disease suppression/severity have been reported. In the present study, root rot was not significantly different in plants treated with recommended dose of NPK (140:55:270) and also in plants without receiving any additional nutrients (50.8%)(**Table – 53**). The initial soil nutrient status (**Table - 49**) show high N & K availability and low phosphorus status. Where double the recommended dose of NPK was applied ($N_2P_2K_2$), increased root rot incidence was noticed (77.50%). It was also noticed that, addition of potash did not give good protection against root rot. This might be due to the very high availability of K (78ppm) in the soil used, which has not shown any further response to the addition of fertilizers as recommended and double the recommended doses. The fertilizer application made over the threshold value will have no response on K availability (Datta, 1996) as well as on plant growth as seen in amount of dry matter (shoot) produced. Since the K availability was not a restricting factor in the initial soil used, addition higher dose has not reduced the disease incidence also.

The lowest incidence of root rot was noticed (24.4%) in $N_0P_1K_0$ treatment followed by treatments with double dose of P applied (38.3 – 46%). The application of P to the soil which was initially P deficient (1.9ppm) might have increased the new root growth and thereby decreasing the percentage of root rot incidence.

Fresh and dry weight of root was high in plants treated with double the dose of P and recommended dose of K and N (55.2g). It was also seen in plants received same dose fertilizer that, fresh and dry weights of root was high in uninoculated plants(58.3g) compared to inoculated plants (55.2g). In plants receiving only recommended dose of N and K, without P, the fresh and dry weight of root was low in uninoculated plants (15.8 and 4.6g) and in

plants with same treatments inoculated with *P.capsici* the fresh and dry weight of root was still low (7.2 and 2.6g) (**Table -50**). Among the inoculated plants, fresh weight of shoot was maximum (338g) in the treated plants receiving the recommended dose of N&P without addition of K.

Among the *Phytophthora* infected plants, maximum height (305.7cm) was observed in plants treated with $N_2P_0K_2$ compared to plants treated with $N_2P_1K_2$ (346.5cm) in uninoculated control. Among the uninoculated plants, maximum number of leaves, nodes and height was observed in plants which received double the dose of N, recommended dose of P and double the dose of K. But among the inoculated plants, maximum number of leaves and nodes observed in plants received recommended dose of NPK (**Table - 52**). Variation was observed in number of branches among the treatments. Though the N is an essential nutrient for obtaining high production, the increased usage of nitrogen also supported the disease incidence in most of the crops (Ojha and Mehata, 1970). It was also reported that high nitrogen concentration support the *Phytophthora* incidence in agricultural crops (Pal and Grewal, 1976a; Sharma and Sohi ,1983; Rudkiewicz *et al.*, 1983; Hoitink *et al.*, 1986; Nema, 1990). Increase of potassium concentration in potato leaves lead to increase in susceptibility to *Phytophthora* infection (Phukan and Baruah, 1989b). The present study indicated that phosphorus concentrations in black pepper showed good protection against root rot. Increment of nitrogen concentration without addition of P showed high susceptibility to *P.capsici*. Though the increment of potash gave good growth of the host, it also enhanced the root rot incidence.

Effect of chemical fertilizers on disease incidence was worked out by many workers and reported that, chemical fertilizers enhance the severity of infection by *Phytophthora* spp. as in the case of rosella, *P.parasitica* var *nicotianae* (Olanloyo and Adeniji, 1976). Nitrogen enhanced the buckeye rot in tomato (Sharma and Sohi, 1983), *P.infestans* infection in potato

(Rudkiewicz *et al.*, 1983) and apple crown rot by *P.cactorum* (Utkhede, 1984d). Increasing the soil fertility by addition of chemical fertilizers enhanced disease severity in soya beans (Dirks *et al.*, 1980). Increased nitrogen concentration in the soil enhanced the susceptibility of potato plants to *P.infestans* (Nema, 1990).

INTEGRATED DISEASE MANAGEMENT (IDM)

Integrated disease management involvng cultural, chemical and biocontrol methods combined wth host resistance is a viable strategy. The leads obtained individually in various experiments on disease suppression have been tried in an integrated approach for integrated disease management and was superimposed on *Phytophthora* tolerant (P24) and *Phytophthora* susceptible (KS27) cultivars. The root rot and overall effects on growth of treated plants were recorded which reflected on overall health of the treated black pepper cuttings. Importance of IDM in foot rot management of black pepper has been stressed (Sarma and Anandaraj, 1996) **(Fig – 5).**

It was noticed that, field tolerant line P24 supported higher growth and proliferation of soil microbes compared to susceptible variety KS27. The overall rhizosphere population level of *Trichoderma*, total fungi, bacteria and actinomycetes were 4.476 x 10^3, 125.032 x 10^3, 51.44 x 10^6 and 0.651 x 10^5 respectively in P24 compared to 1.159 x 10^3,50.921 x 10^3, 9.73 x 10^6 and 0.524 x 10^5 respectively in KS27 (**Table -59**).

Reduced root rot incidence was noticed in tolerant line (P24) compared to susceptible cultivar (KS27). Least root rot (1.66%) incidence was recorded in tolerant line with the combination of two biocontrol agents (*T.virens* (10) and *V.tenerum* (71)) along with recommended dose of P without the addition of K and N. Where as in susceptible KS27 with the same treatment 13.33% root rot was noticed, which was significantly superior than control (95%) **Table -54 & Fig – 4.**

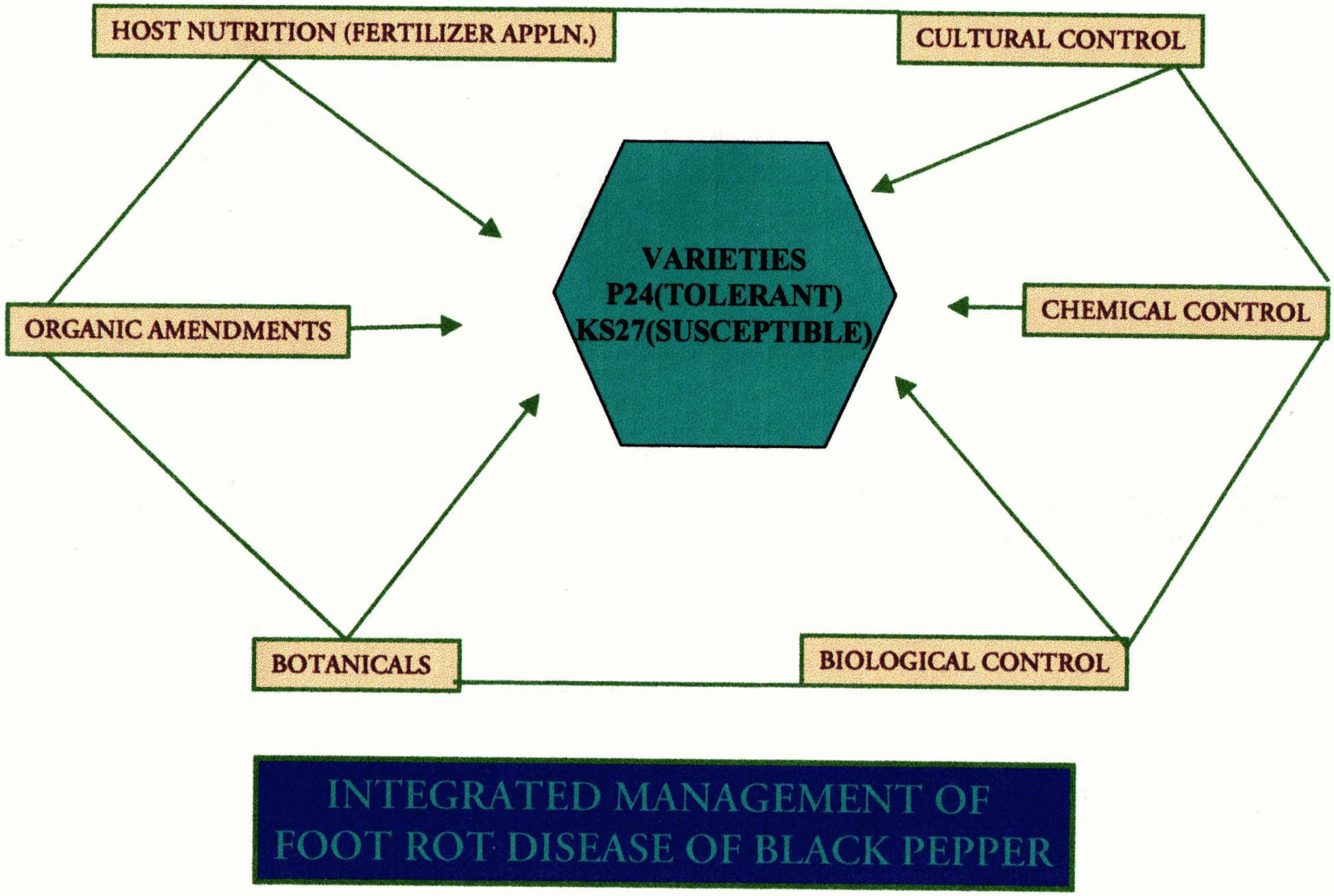
HOST NUTRITION (FERTILIZER APPLN.)
CULTURAL CONTROL
VARIETIES
P24(TOLERANT)
KS27(SUSCEPTIBLE)
ORGANIC AMENDMENTS
CHEMICAL CONTROL
BOTANICALS
BIOLOGICAL CONTROL
INTEGRATED MANAGEMENT OF
FOOT ROT DISEASE OF BLACK PEPPER

FIG : 5

In tobacco, *P.parasitica* infection was reduced with the integrated approach by using organic amendments, fungicides and pressmud (Nanedrappa *et al*, 1992). Chemical and cultural practices could control the *P.parasitica* infection in tomato (Raicu and Stan, 1976). Biological (*Bacillus subtilis*) and chemical control methods (metalaxyl) were integrated together for the control of *P.cactorum* infection in apple (Utkhede and Smith, 1991). Utkhede and Smith (1993) reported that metalaxyl, fosetyl-Al and metalaxyl alternate with *Enterobacter aerogenes* significantly reduced the disease incidence and increased the fruit yield in apple. Integration of biological control and chemical control on *Phytophthora* foot rot in azaleas and rhododendron gave significantly good control (Wills, 1989). Since all the components used in the integrated management programme are compatible with each other, the intergated management is the only long run strategy for the management of this disease.

Since black pepper is the income of medium and poor farmers, the integrated management is the best way for them to manage this menace. Because of the economical feasibility and ecofriendly nature, this method of disease management seems to be more applicable. In the present study the leads obtained on various experiments with biocontrol agents, botanicals and chemicals are good indicators of disease suppression in a system approach. However this needs validation based on the large scale field testing under multilocation. The overall disease suppression and consequent improvement and production of the vines need be assessed in relation to the pathogen – microbial (biocontrol) population in a given locality. It is also important that the efficacy of biocontrol could be realised only where all the cultural practices adopted strictly. Black pepper being a perennial crop the discernable effects of various treatments on overall health and productivity of the vine would be steady and gradual. The increased growth of black pepper plants with some of the bacterial isolates noticed in the present study is an added advantage . The reduction in the disease combined with increase in growth adds up to the overall improvement of the health of black pepper. The deleterious effect of *Phytophthora capsici*, *Radopholus similis* and *Meloidogyne incognita* on growth of black pepper has been realised (Sarma *et al.*, 1991). The importance and relevance of

identification biocontrol agents multiple mode of action to check these pathogens and boost up the health and productivity of vines have been highlighted (Sarma *et al.*, 1996). Over and above the isolates which possess the growth promoting activity of the host and deleterious effect on pathogen is to be looked into. Further studies are warranted to identify such organisms with a multiple mode of action, time of application and amendments that support the stability in the soils

SUMMARY & CONCLUSION

Rajan P.P "Approaches towards the integrated disease management of phytophthora infection of black pepper (Piper nigrum L.)" Thesis. Department of Botany, University of Calicut,1999

SUMMARY & CONCLUSION

1. Rose Bengal Agar medium (Martin, 1950) for fungi, Soil Extract Agar medium (Allen, 1957) for bacteria and Actinomycetes Isolation Agar medium (Hi-media) for actinomycetes were standardised for studying the rhizosphere microflora of black pepper.

2 Twenty days old 2% carrot broth-sand inoculum was found best for the induction of root rot in black pepper.

3. Rhizosphere of black pepper showed higher population of fungi and bacteria compared to non-rhizosphere areas. Healthy rhizosphere areas had more microbial population especially species of *Trichoderma* than the diseased rhizosphere areas.

4. *Trichoderma, Penicillium* and *Aspergillus* spp. were predominant in rhizosphere of black pepper. *Trichoderma* and other fungal populations were noticed more at upper layers of soil than deeper layers. Multiplication of *Trichoderma* and other soil microbes was more during rainy season than dry season especially at top layers of soil.

5. Twenty seven isolates of *Trichoderma*, 104 isolates of other fungi, 16 isolates of bacteria and 12 isolates of actinomycetes were short listed as antagonists by dual culture technique. Short listed antagonistic fungal and bacterial isolates were tested in pot culture for their bio-efficacy on root rot suppression. Three *Trichoderma* spp. (10,12 & 19) and three other fungal isolates (63, 71 & 74) were found very effective against *Phytophthora* infection in black pepper. Out of 16 bacterial isolates tested two flourescent psudomonads (Pf2, Pf5) and one non-flourescent pseudomonads (BN) were found effective on root rot suppression.

6. The suppressive effect of *Verticillium teneum* on *Phytophthora* induced root rot is reported for the first time in India.

7. Four locally available organic soil amendments (coffee pulp, poultry manure, neem cake and farm yard manure) were tested for their disease suppressive effects on *Phytophthora,* in comparison with recommended dose of NPK (140:55:270). Least root rot was noticed in plants treated with coffee pulp followed by neem cake compared to NPK treated plants.

8. Aqueous garlic and mustard extracts were tested against *P.capsici*, both *in vitro* and *in vivo*. Four important phases of *P.capsici* viz; growth, sporulation, sporangial germination and zoospore germination were inhibited by aqueous garlic and mustard extracts at low concentrations and their effects were synergestically inhibitory when they were used in combination. Pot culture studies indicated the suppressive effect of these extracts on root rot incidence, increased multiplication and proliferation of *Trichoderma.*

9. Three systemic fungicides viz; dimethomorph, heptaene antibiotic (aureofungin) and potassium phosphonate were tested against *P.capsici* both *in vitro* and *in vivo.* Dimethomorph was found very effective on suppression of growth, sporulation, indirect sporangial germination and zoospore germination of *P.capsici*, but aureofungin and potassium phosphonate were found effective on these phases only at higher concentrations. Out of these chemicals evaluated in pot culture studies, potassium phosphonate at 1200ppm. (three applications) was found most effective on disease suppression with 17.78% root rot compared to 23.33% in dimethomorph (400ppm) and 47.78% in aureofungin (300ppm).

10. Nine species of *Trichoderma* and *Verticillium tenerum* were found compatible with potassium phosphonate at 1200ppm.

11. Three levels ($N_0P_0K_0$, $N_1P_1K_1$, $N_2P_2K_2$) of fertilizers and their combination were evaluated for their effect on disease incidence. Least amount of root rot incidence

(24.44%) was noticed in plants treated with recommended dose of phosphorus without the application of nitrogen and potash. Phosphorus application showed disease suppressive effect.

12. Of the five biocontrol agents field evaluated for their disease suppressive potential, isolate P12 (*T.virens*) showed root rot suppression where disease index recorded 1.6% compared to 10.2% in control. Isolate P26 (*T.harzianum*) was found to be the best, among the isolates used for survival and proliferation.

13. In IDM study conducted by incorporating all the leads coupled with a disease tolerant (P24) and susceptible (KS27) black pepper, P24 showed minimum root rot (1.66%) compared to KS27 (13.3%).

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