FIELD EVALUATION OF ABIOTIC STRESS TOLERANT STRAINS OF *Trichoderma harzianum* AND *Pseudomonas fluorescens* FOR *Phytophthora* DISEASE MANAGEMENT IN BLACK PEPPER (*Piper nigrum* L.)

By

RIMA K. R.

(2016-11-126)

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR-680 656

KERALA, INDIA

2020

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THESIS

Submitted in partial fulfilment of the

requirement for the degree of

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DEPARTMENT OF AGRICULTURAL MICROBIOLOGY

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KERALA, INDIA

2020

DECLARATION

I hereby declare that this thesis entitled **"Field evaluation of abiotic stress tolerant strains of *Trichoderma harzianum* and *Pseudomonas fluorescens* for *Phytophthora* disease management in black pepper (*Piper nigrum* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Place : Vellanikkara

Date : 25-08-2020

Rima K. R.

(2016-11-126)

CERTIFICATE

Certified that this thesis entitled "**Field evaluation of abiotic stress tolerant strains of *Trichoderma harzianum* and *Pseudomonas fluorescens* for *Phytophthora* disease management in black pepper (*Piper nigrum* L.)**" is a record of research work done independently by Rima K R, (2016-11-126) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Place : Vellanikkara
Date : 25-08-2020

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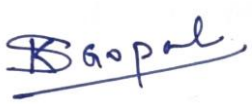

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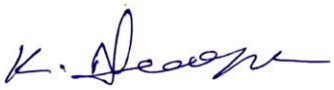

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Introduction

1. INTRODUCTION

India is known as the spice bowl of the world with the amazing richness and diversity of spices which attracted foreign traders in ancient times. Among the spices, black pepper *(Piper nigrum* L.) is renowned as the 'King of Spices'. Black pepper, obtained from dried berries is an important condiment of international commerce for India and other producing countries (Ravindran, 2000). It is a perennial climbing vine originated in the tropical evergreen forests of the Western Ghats.

India is one of the major producers, consumers and exporters of black pepper in the world (IISR, 2015). Vietnam, Brazil, Indonesia, Malaysia, Thailand and Sri Lanka are the other black pepper growing countries in the world. Wayanad (Ambalawayal), Calicut, Kannur (Panniyur), Idukki (Pampadumpara) and Thrissur in Kerala; Coimbatore (Valparai) and Nilgiris in Tamil Nadu and Kodagu in Karnataka are the areas where it is cultivated in India.

Black pepper is a small holder's crop in most of the countries and more than 1 million farmers depends on it for their livelihood (UN, 1995). Kerala accounts over 50 per cent of India's total output followed by Karnataka and Tamil Nadu (Yogesh and Mokshapathy, 2013). The productivity of the black pepper in India started to decline due to *g*lobal climatic changes, low input of mineral nutrients, poor yielding local cultivars, non-availability of superior varieties, non-adoption of agronomic practices, losses due to incidence of diseases, pests, heat and drought (Radhakrishnan *et al.,* 2002). Black pepper is an important spice crop of Kerala which is highly sensitive to abiotic stresses like climate changes. Black pepper has been reported to be the most vulnerable spice crop to climate change (Rao, 2011).

An efficient method should be developed to mitigate the biotic and abiotic stresses for increasing the productivity of black pepper. Venkateshwarlu and

Shanker (2009) carried out an extensive research to develop heat and drought tolerant varieties, shifting the crop calendars, resource management practices etc. Many of these technologies could not reach the farmers due to high cost of establishment and maintenance. Gradually, research focussed on effective, low-cost and eco-friendly technology with microorganisms, gained importance to help the plants withstand abiotic and biotic stresses. Therefore, plant growth promoting rhizosphere microorganisms could be a potential tool for growth promotion, disease management and abiotic stress tolerance for mitigating the abiotic and biotic stresses on the crop.

There are reports where the soil beneficial microorganisms have been found to help the plants to overcome abiotic stresses. Certain microbial species and strains enhance plant tolerance to abiotic stresses such as drought, salinity, nutrient deficiency or excess (Yang *et al*., 2009). Plant growth promoting rhizosphere microorganisms could improve plant performance under stressed environment and enhance the yield both directly and indirectly (Dimkpa *et al*., 2009).

Different species of *Trichoderma* have the potential to control soil-borne plant pathogens more effectively than chemicals (Papavizas, 1985) and they also exhibit plant growth promoting activity (Duffy *et al*., 1996). *Trichoderma harzianum* and *Trichoderma viride* have been used as biological control agents against *Phytophthora capsici* in black pepper (Anandaraj and Sarma, 1995). *Pseudomonas fluorescens* were found efficient in root-rot suppression in black pepper (*Piper nigrum* L.) caused by *Phytophthora capsici* infected cuttings. The results also showed the effectiveness of *P. fluorescens* strains in rejuvenation of infected black pepper cuttings and rhizobacteria-mediated root proliferation in black pepper (Paul *et al*., 2005). Wahyuno *et al*. (2016) reported that an application of *Trichoderma* and *Pseudomonas fluorescens* individually or in combination was advantageous for plant protection and plant growth. However, the microorganisms themselves are vulnerable to abiotic and biotic stresses. Therefore, the abiotic stress tolerant and efficient strains of beneficial microorganisms have to be developed for

black pepper to overcome changes due to microclimatic variables and soil parameters.

In an earlier study in the department of Agricultural Microbiology, abiotic stress tolerant isolates of *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) were identified for growth promotion and disease management in black pepper (*Piper nigrum* L.) under pot culture studies (Vithya, 2017). Their performance under field conditions may not be the same as obtained under pot culture studies. Hence, a study was undertaken on "Field evaluation of abiotic stress tolerant strains of *Trichoderma harzianum* and *Pseudomonas fluorescens* for *Phytophthora* disease management in black pepper (*Piper nigrum* L.)" with an objective to study the effect of micro-climatic and soil parameters on abiotic stress tolerant strains of *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) under field conditions so as to develop an abiotic stress tolerant inoculant for the benefit of black pepper growing farmers.

Review of literature

2. REVIEW OF LITERATURE

Spices have played a pivotal role in the history of human civilization. India is renowned as 'the land of spices' in the world. Black pepper (*Piper nigrum* L.) known as “the king of spices” is an important spice commodity of commerce and trade in India since pre-historic period. Tropical evergreen forests of the Western Ghats of India is considered to be its origin (Thangaselvabal *et al*., 2008). It is also called as “black gold” due to its durability and value. India is one of the major producer, consumer and exporter of black pepper in the world (IISR, 2015). Black pepper is a perennial vine grown for its berries extensively used as spice and in medicine. It is commonly known as “Kali Mirch” in Urdu and Hindi, “Pippali” in Sanskrit, “Milagu” in Tamil.

Black pepper cultivation is mainly restricted to India, Vietnam, Brazil, Indonesia, Malaysia, Thailand and Sri Lanka in the world. As per the International Pepper Community, the global output of pepper in 2015 is estimated as 374,500 tonnes. The production in India during 2015 is forecasted to be around 70,000 tonnes which is almost double the production in 2014. Kerala stands first in the production of black pepper (30.5 MT) followed by Karnataka (19.2 MT) and Assam (2.6 MT) in India during 2015-16 (India Agristat). Kerala accounts over 50 per cent of India’s total output followed by Karnataka and Tamil Nadu (Yogesh and Mokshapathy 2013). In productivity, Karnataka (0.6 MT/ha) is followed by Kerala (0.4 MT/ha). India exported 21,250 t of black pepper products worth of Rs. 94,002 lakhs to various countries during 2013-14.

2.1 Uses of black pepper

Black pepper is a flowering vine that is cultivated for its fruit. Black pepper is widely used in food items as a spice. The chemical piperine, present in black pepper, causes the spiciness. It is having medicinal properties to fight

against cold, flu and infection, to increase blood circulation, to improve digestive health, to lower blood pressure, to improve oral health, to improve brain health, to energize, to retrieve muscle aches and stiffness. It is a carminative and prevents dyspepsia and flatulence. It was used as an ideal additive to diets given to malarial patients. It may promote even weight loss. It is also bacteriostatic and anti-blood coagulant. In the ***Syriae book of medicine*** of fifth century, black pepper is prescribed for such illness as constipation, earache, gangrene, heart disease, hoarseness, indigestion, insect bites, insomnia, hernia, joint pain, liver problems, lung disease, sun burn, oral abscesses, tooth decay and tooth aches. Black pepper corns were used in Ayurveda, Siddha and Unani medicines in India.

2.2 Climate and soil

Black pepper is a plant of humid tropics which requires high rainfall and humidity. For its cultivation, hot and humid climate of sub-mountainous tracts of Western Ghats is ideal. It grows well between 20° North and South latitudes, and up to 1500 m above sea level. The favourable temperature range is found to be 23°C- 32°C and the ideal temperature is about 28°C. However, optimum soil temperature for root growth is 26°C – 28°C (Wahid and Sitepu, 1987).

Relative humidity of the range 75 - 80 per cent is preferred by the crop. For black pepper, a well distributed annual rainfall in the range of 1250 - 2000 mm is ideal. Though it thrives well in red laterite soil in its natural habitat, it can be grown in a wide range of soils with a pH ranging from 5.5 – 6.5. In the hills, at an elevation of 800-1500 m above sea level, the crop is mostly grown on shade trees in coffee, cardamom and tea plantations.

2.3 Constraints in black pepper cultivation

In terms of area and production, India occupies the first place among the major black pepper producing countries in the world. But our productivity is low compared to other producing countries. Continuous cultivation of poor yielding varieties, existence of sterile and unproductive vines, losses due to

pests, diseases and drought, inadequate supply of quality planting material, non-adoption of appropriate agronomic practice, poor transfer of technology and price fluctuations are the major reasons of low productivity. Radhakrishnan *et al.* (2002) reported that global climatic changes, low input of mineral nutrients, poor yielding local cultivars, non-availability of superior varieties, non-adoption of agronomic practices, losses due to incidence of diseases, pests, heat and drought are the prominent factors contributing for the low productivity of black pepper in India. Regeena (2016) reported that ensuring the planting of improved high yielding varieties like Panniyur 1 to Panniyur 8 depending on availability of sunlight, proper and scientific crop management, ensuring prophylactic measures for pest and disease management, promoting good agricultural practices and a more regulated system of planting with standards of uniform height will be helpful in improving overall production and productivity. She also suggested that proper awareness given to pepper producers on post-harvest handling and value addition of pepper will also help them to realize better income from pepper crop.

At a time when black pepper production has seen a drastic fall due to climate change, the unexpected rise in the price of pepper has brought joy to farmers in Kerala (Senthilkumar and Swarupa, 2018). Binoy Kurian, a spice merchant in Kattappana, told that the influx of black pepper from Vietnam and Sri Lanka was the main reason for the fall in pepper price in the Indian market. The compound annual growth rate of the climatic variables showed that, rainfall and maximum temperature had negative growth trend, while minimum temperature had positive growth trend in Idukki and Wayanad districts (Kumar *et al.*, 2017). Incidence of a biotic and abiotic stresses has increased due to climate changes and its impact on the productivity of black pepper will be perceived all over the world.

An eco-friendly, cost-effective and an efficient method should be developed to overcome these constraints. Here, the beneficial rhizosphere microorganisms play a vital role to mitigate the abiotic stresses, to improve growth promotion and for disease management in crop plants.

2.4 *Trichoderma* sp.

Trichoderma are free- living fungi usually found in all climatic zones. This genus was described by Christiaan Hendrik Persoon in 1794. The *Trichoderma* are imperfect filamentous fungi, with teleomorphs belonging to the order of Hypocreales under the division of Ascomycota. The typical habitats of these fungi include soil and rotting wood (Samuels 1996; Druzhinina *et al.*, 2006). They are known as successful colonizers of their habitats, efficiently fighting their competitors. They are the most widespread and culturable fungi in the soil. Rapid growth rate in culture and the production of numerous spores (conidia) in varying shades of green characterize fungi in this genus. The *Trichoderma* isolates were differentiated by growth rate of mycelia and colony appearance, as well as microscopic morphological features, including phialides and phialospores. Conidia are produced in phialides. Conidial walls are slimy, and masses of the spores stick together at the tip of the phialide. Samuels *et al.* (2002) documented mycelial growth of various *Trichoderma* green mould isolates and demonstrated that only *T. harzianum* grew well and sporulated at the temperature of 35°C, whereas *T. atroviride* displayed slower growth under these conditions.

Discoveries reveal that they are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi, protect plants from diseases (Naseby *et al.*, 2000; Harman *et al*., 2004). Some strains establish robust and long-lasting colonizations of root surfaces and penetrate into the epidermis and a few cells below this level. The species flourishes best when large quantities of healthy roots are available. So they have developed many mechanisms for attacking other fungi and for improving plant and root growth. These are highly interactive in root, soil and foliar environments. *Trichoderma* is a good model to study biocontrol because it is ubiquitous, easy to isolate and culture, grow rapidly on many substrates, affect a wide range of plant pathogens, is rarely pathogenic on higher plants, acts as a mycoparasite, competes well for food and site, produces antibiotics and has an enzyme system capable of attacking a wide range of plant pathogens (Islam *et al.*, 2008).

2.4.1 Effect of *Trichoderma* on plant growth promotion

Worldwide traditional agricultural practices are consequently being affected by various problems such as diseases, pests, droughts, decreased soil fertility due to use of hazardous chemical pesticides, pollution and global warming. Chemical methods pollute the atmosphere, damage the environment, leave harmful residues, and can lead to the development of resistant strains among the target organisms with repeated use. Therefore, chemical methods are not economical in the long run (Naseby *et al.*, 2000). There arise a need for some eco-friendly biocontrol agents that may help to resolve some of these problems. Harman *et al.* (2004) suggested that biological control which is the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection.

Trichoderma sp. are among the most studied fungal biocontrol agents and commercially marketed as biopesticides, biofertilizers and soil amendments (Harman, 2000; Harman *et al.*, 2004; Lorito *et al.*, 2004). It is well reported that some strains promote plant growth, increase nutrient availability, improve crop production and enhance disease resistance (Harman *et al.*, 2004). The use of *Trichoderma* in agriculture can provide numerous advantages depending upon the strain: (i) colonization of the rhizosphere by the biocontrol agents (‘‘rhizosphere competence’’) allowing rapid establishment within the stable microbial communities in the rhizosphere; (ii) control of pathogenic and competitive/deleterious microflora by using a variety of mechanisms; (iii) improvement of the plant health and (iv) stimulation of root growth (Harman *et al.*, 2004).

In maize plants, *Trichoderma* inoculation affected root system architecture, which was influenced in increased yield of plants. Root biomass production and root hair development are enhanced (Bjorkman *et al.*, 1998; Harman *et al.*, 2004b). Papavizas (1985) reported that different species of *Trichoderma* have the potential to control soil-borne plant pathogens more

effectively than chemicals and they also exhibit plant growth promoting activity (Duffy *et al.,* 1996). Jisha *et al.* (2000) reported that *T. harzianum* and *Pseudomonas fluorescens* (IISR-6) promoted growth and vigour of black pepper, ginger and cardamom and suppressed soil-borne fungal pathogens in field conditions. They also suggested that *Trichoderma* has been very effectively used for the control of large number of soil-borne plant pathogens like *Phytophthora, Rhizoctonia, Sclerotium, Pythium, Fusarium, Sclerotinia* and *Gaumannomyces.* Plant growth-promoting rhizobacterium such as *Bacillus subtilis* Cohn and the plant-growth promoting fungus (PGPF) *Trichoderma harzianum* Rifai are used in different crop plants as biocontrol agents for management of different pathogens (Murphy *et al.,* 2003; Harman, 2011; Woo *et al.,* 2006). Bae *et al.* (2009) proved that the *T. hamatum* increased tolerance of cocoa plants to water deficit through increasing root growth that provided greater water resources to treated plants and delayed the onset of water deficit in these plants. Yedidia *et al.,* (1999) pointed out that presumed mechanisms involved in the stimulation of plant growth by *Trichoderma* sp. include interactions with plant roots similar to mycorrhizae, in which *Trichoderma* penetrates and colonizes root tissues without eliciting specific defense responses against the colonizing strain. Liliana *et al.* (2009) found a small proportion of *Trichoderma* strains that could stimulate early stages of growth in bean plants, potentially leading to the use of these strains as novel bioinoculants in agriculture with potential for increased crop yields. Aggarwal *et al.* (2001) showed that the isolates of *Trichoderma viride* improved growth of wheat crop such as shoot length, root length and 1000 g weight.

2.4.2 Mechanism of growth promotion of plants by *Trichoderma* sp.

According to Saba *et al.* (2012) *Trichoderma* spp. are endophytic plant symbionts that are widely used as seed treatments to control diseases and to enhance plant growth and yield. They produce or release a wide variety of compounds that induce localized or systemic resistance responses in plants and this explains their lack of pathogenicity to plants. *Trichoderma* strains have long been accepted as biological agents, for the control of plant disease and for their

ability to increase root growth and development, crop productivity, resistance to abiotic stresses, and uptake and use of nutrients. Antagonistic microorganisms such as *Trichoderma*, reduce the growth survival or infection caused by pathogen by different mechanism like competition, antibiosis, microparasitism, hyphal interactions and enzyme secretion. In addition to the beneficial effects that occur in direct interactions with plant disease agents, some *Trichoderma* species are also able to colonize root surfaces and cause substantial changes in plant metabolism (Harman *et al.*, 2004).

Hoitink *et al.* (2006) reported that the mechanisms of action used by *Trichoderma* (competition, antibiosis, parasitism and systemic-induced resistance) are influenced by concentration and availability of nutrients (carbohydrates in lignocellulosic substances, chitin, lipids, etc.) within the soil organic matter.

Chet and Inbar (1994) stated that some *Trichoderma* biocontrol agents produce highly efficient siderophores that chelate iron and stop the growth of other fungi.

According to Harman *et al.* (2004), strains of *Trichoderma* added to the rhizosphere protect plants against numerous classes of pathogens due to the induction of resistance mechanisms similar to the hypersensitive response (HR), systemic acquired resistance (SAR), and induced systemic resistance (ISR) in plants.

Limon *et al.* (1999) documented that *T. harzianum* transformants overexpressing Chit33 chitinase constitutively inhibited the growth of *R. solani* under both repressing and derepressing conditions; the antagonist tests demonstrated that this chitinase also has an important role in mycoparasitism. Biocontrol efficacy of *Trichoderma harzianum* isolates against *Gaeumannomyces graminis* var. *tritici* is related to the production of pyrone-like antibiotics (Ghisalberti *et al.* 1990).

Howell (2003) reported that biocontrol of *Botrytis cinerea* by *T. harzianum* has been attributed in part to the action of proteases produced by the biocontrol agent that inactivate hydrolytic enzymes produced by this pathogen on bean leaves. Synergism among lytic enzymes and between enzymes and antibiotics suggests formulations to test mixtures of *Trichoderma* transformants that produce different enzymes, in order to improve the antagonistic effects of biocontrol agents on phytopathogenic fungi.

Another mechanism proposed to explain biocontrol activity by *Trichoderma* species is resistance induction in the host plant by treatment with the biocontrol agent. To support this concept Yedidia *et al.* (1999), in their study, demonstrated that inoculating roots of 7-day-old cucumber seedlings in an aseptic hydroponic system with *Trichoderma harzianum* (T-203) spores to a final concentration of 10^5 per ml initiated plant defense responses in both the roots and leaves of treated plants. They also discovered that hyphae of the biocontrol fungus penetrated the epidermis and upper cortex of the cucumber root.

2.5 *Pseudomonas fluorescens*

Pseudomonas is a gram-negative, motile aerobic rod shaped bacteria that are widespread throughout nature and characterised by elevated metabolic versatility and presence of a complex enzymatic system (Franzetti and Scarpellini, 2007). Fluorescent Pseudomonas spp. can generally be visually distinguished from other Pseudomonads by their ability to produce water soluble yellow-green pigment (Daniel and Fergan, 1992).The optimum range of temperature for growth of *Pseudomonas fluorescens i*s 25–30°C.

According to Ridgway and Safarik (1990), some species of *Pseudomonas* are medically important because they are considered to be opportunistic pathogens for humans and animals while other groups like Phytopathogens are very important in agriculture. *Pseudomonas* strains which produce fluorescens under UV light are known as fluorescent pseudomonads.

Fluorescent pseudomonads are extracellular PGPR (ePGPR) (Bhattacharyya and Jha, 2012).

Pseudomonas has the ability to grow in simple media at the expense of a great variety of simple organic compounds, without organic growth factors. For the isolation of most species of *Pseudomonas*, King's B media is suitable. King *et al.*. (1954) developed the first selective media (King's A and King's B) for the isolation of Fluorescent Pseudomonads.

Pseudomonas species had an important role in plant growth promotion and plant health and it had been reviewed for the biofertilizer, biostimulator and phytopathogen biocontrol activities. According to Schroth and Hancock (1982), among the various rhizospheric bacteria, *Pseudomonas* sp. were aggressive colonizers of the rhizosphere of various crop plants. It had a wide array of antagonistic activity against plant pathogens (Siddiqui *et al.,* 1999).

2.5.1 Effect of *Pseudomonas fluorescens* on plant growth promotion

Fluorescent pseudomonads are the most exploited bacteria among the PGPRs for biological control of soil borne and foliar plant pathogens. According to Kloepper *et al*. (1980), *Pseudomonas fluorescens* are some of the effective candidates for biological control of soil-borne plant pathogens owing to their rhizosphere competence, plant growth promotion and disease suppression. Fluorescent Pseudomonads is found to be largest and potentially most promising group of plant growth promoting rhizobacteria involved in the biocontrol of plant diseases (Kloepper *et al.,* 1988). *Pseudomonas* induces resistance in plants against fungal, bacterial, and viral diseases (Liu *et al.*, 1995).

Van peer and Schippers (1988) reported an increase in root and shoot fresh weight for tomato, cucumber, lettuce and potato as a result of bacterization with *Pseudomonas* strains. Van peer *et al.* (1991) reported that application of *Pseudomonas* sp. strain WCS 417r in carnation protected plants systemically against *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *dianthi.* Kloepper

et al. (1980) reported growth and yield enhancement of potato by seed inoculation with fluorescent pseudomonads in field trials.

Srivastava and Upadhyay (2010) isolated *P. fluorescens* (strain Psd) from the rhizosphere of *Vigna mungo* and evaluated for its multiple plant growth promoting and biocontrol against *Fusarium oxysporum*. The study conducted by Diby *et al.* (2005) demonstrated the ability of *Pseudomonas fluorescens* strains as rejuvenating agents in black pepper cuttings, which had already been infected naturally.

2.5.2 Mechanism of growth promotion of plants by *Pseudomonas fluorescens*

Pseudomonas species are important plant growth promoting rhizobacteria used to improve crop yield by various mechanisms. The production of Indole Acetic Acid (IAA) is found to be direct method of plant growth promotion (Vasanthakumar and McManus, 2004). Siderophore producing *Pseudomonas* sp. plays an important role in stimulating plant growth and controlling several plant diseases. Siderophore production, (fluorescent yellow-green pigment), namely pseudobactin, limits the availability of iron necessary for the growth of pathogens (Kloepper *et al.*, 1980). Phosphate solubilization (Wu *et al.*, 2005), HCN production (Defago *et al.*, 1990), ACC deaminase, root elongation, degradation of toxic compound (Bano and Musarrat, 2003) are other direct mechanisms for growth promotion of plants. *Pseudomonas* acts as biological control agent for phytophathogens such as *Aspergillus niger* and *A. flavus* (Dey *et al.*, 2004). Biological control mechanisms of pathogens by fluorescent pseudomonads usually involve competition for nutrients, production of bacterial metabolites such as iron chelating siderophores, hydrogen cyanide (HCN), antibiotics, extracellular lytic enzymes and induced systemic resistance (O'Sullivan and O'Gara, 1992; Van Loon *et al.*, 1998).

According to Tryfinopoulou *et al.* (2002), mupirocin is a polyketide antibiotic which has been isolated and characterized from the soil bacterium *P.*

fluorescens NCIMB 10586. Karunanithi *et al*. (2000) reported a native isolate of *P. fluorescens* producing an antibiotic substance, pyrrolnitrin, which inhibited the growth of *Macrophomina phaseolina* by producing an inhibition zone of 12mm. Weller and Thomashow (1993) suggested that production of metabolites such as antibiotics, siderophores and HCN was the primary mechanism of the biocontrol for many Pseudomonads.

Zehnder *et al.*, (2001) reported that many strains of Pseudomonads could indirectly protect the plants by inducing systemic resistance against various pest and diseases. Trivedi *et al.* (2008) examined *Pseudomonas corrugata*, for its antagonistic activities against two phytopathogenic fungi, *Alternaria alternata* and *Fusarium oxysporum*.

According to Lee *et al.* (2001), *Pseudomonas* strains which induced resistance systemically in watermelon to gummy stem rot, are tested on their induced systemic resistance (ISR) related characteristics. Press *et al.* (1997) defined induced resistance as active resistance dependent on the host plant's physical or chemical barriers, activated by biotic or abiotic agents. Mathiyazhagan *et al.* (2004) discovered that *P. fluorescens* (ENPF1) and *P. chlororaphis* isolate (BCA) promoted plant growth and induce systemic resistance against stem blight pathogen *Corynespora cassiicola* in *P. amarus* under *in vitro* conditions. In a study by Verhagen *et al*. (2010), the ability of *Pseudomonas fluorescens* CHA0 and *Pseudomonas aeruginosa* 7NSK2 to induce resistance in grapevine against *Botrytis cinerea* is demonstrated. Barka *et al.* (2000) concluded that the induced systemic resistance was triggered by *P. fluorescens* (EP1) against red rot caused by *Colletotrichum falcatum* on sugar cane.

2.6 Abiotic stress tolerance of *Trichoderma* sp.

In a study conducted in the Department of Agricultural Microbiology, *Trichoderma harzianum* was found to be the most promising abiotic stress tolerant culture among various other cultures promoting growth of black pepper

under pot culture (Vithya, 2017). Daniel *et al.* (2011) identified a strain of *Trichoderma viride* as a potential agent of abiotic stress and chemical fungicide tolerance. Amalraj *et al*. (2011) proved that *Trichoderma* sp. was tolerant to abiotic stresses like temperature, salinity and drought in *Vigna mungo*. Reetha *et al.* (2014) concluded that the *Trichoderma harzianum* grew better at 25-30 °C and very slow growth at 37 ºC. Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system had been reported by Ahmad *et al.* (2015). According to Mastouri *et al.* (2010) treatment of tomato seeds with *Trichoderma harzianum* was found to have several beneficial effects, from accelerating seed germination and enhancing seedling vigour to amelioration of abiotic stresses such as water, salinity and heat. They also suggested that *T. harzianum* probably acts by inducing protection in the host against oxidative stress. Rice seedlings on treatment with *Trichoderma* showed delayed wilt response to drought along with a concomitant delay in water stress, induced physiological changes such as leaf greenness, stomatal conductance and photosynthesis (Shukla *et al*., 2012).

According to Limon *et al.* (2004), growth of *Trichoderma* is more efficient in acidic than alkaline soils and they modify the rhizosphere soil by acidifying the soil. Jackson *et al.* (1991) reported that *T. harzianum* isolate showed optimum mycelial growth between pH 4.8-6.8. Upadhyay and Raj (1979) proved that *Trichoderma* sp. prefer and grow well in the soils with acidic pH. According to Rousk *et al.* (2009), acidic pH favoured fungal growth than alkaline pH.

It is reported that certain *Trichoderma* species also promote plant growth through the activity of ACC deaminase other than ACC deaminase producing bacteria thereby reducing ethylene biosynthesis, which is also linked to degradation of DELLA proteins. It is found that IAA production from *Trichoderma* contributes to exogenous IAA stimulated ethylene synthesis through the activation of ACC synthase, which in turn triggers an increase in ABA synthesis (Stepanova *et al.,* 2007).

2.7 Abiotic stress tolerance of *Pseudomonas fluorescens*

Plants are always exposed to abiotic stresses, such as drought, temperature stress, salinity stress which are the most important problems related with plant growth and development affecting agricultural demands. Inoculation of plants with PGPR has been found effective under drought stress environment (Chanway and Holl, 1994) to increase productivity. An understanding of interactions between plants and microorganisms having influence on plant growth and stress tolerance is needed.

According to Vivekananthan *et al*. (2004), soil borne fluorescent pseudomonads had excellent root colonizing ability, catabolic versatility and produced a wide range of enzymes and metabolites that favour the plant to withstand under varied biotic and abiotic stress conditions. In a study conducted by Sandhya *et al.,* (2010) all the *Pseudomonas* sp. inoculated treatments showed significantly higher plant growth in terms of root and shoot length and dry biomass as compared to uninoculated control under both drought stress as well as no stress conditions. In the same study, as compared to uninoculated seedlings, inoculated seedlings showed significantly lower activities of antioxidant enzymes, ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) under drought stress, indicating that inoculated seedlings felt less stress as compared to uninoculated seedlings.

Pseudomonas fluorescens can produce a wide range of enzymes and metabolites helping the plants to withstand varied biotic and abiotic stresses (Saravanakumar *et al*., 2011). Tsuji *et al*. (1982) reported that *Pseudomonas cepacia* and *Pseudomonas fluorescens* showed high population in the temperature range of 28 to 37 ºC and grew at high pH values. Christen and Marshall (1984) concluded that the growth of the *Pseudomonas* sp. was at optimum temperature and pH might have influenced the bacterial enzyme production as a result of which the *Pseudomonas* sp. could tolerate high temperature and acidic pH. Ali *et al.* (2009) documented that a *Pseudomonas* strain AKM-P6 possessing plant growth promoting properties was found

tolerant to elevated temperatures (47-50ºC) in sorghum seedlings. Soesanto *et al*. (2011) suggested optimum temperature for *Pseudomonas fluorescens* as 35°C and optimum pH as 7-8. Chu *et al.* (2019) reported that *Arabidopsis thaliana* plants inoculated with *Pseudomonas* PS01 survived under salt stress conditions up to 225 mM NaCl, while all non-inoculated plants were dead above 200 mM NaCl. Schnider *et al.* (2001) reported that *P. fluorescens* can survive under dry conditions and hyper osmolarity and for this adaptation, the gene AlgU is a crucial determinant. Inoculation of plants with *Pseudomonas* was found to alleviate salinity effects on plant growth and development by reducing the uptake of toxic ions, inducing systemic resistance, producing phytohormones, improving nutrient uptake and establishing root colonization (Sarma and Saikia, 2013; Bano and Fatima, 2009).

2.8 Effect of micro-climatic and soil parameters on population of *Trichoderma* sp.

Reetha *et al.* (2014) conducted an experiment to study the effect of temperature and pH on the growth of *Trichoderma harzianum*. They prepared cultures of *Trichoderma* at different temperatures such as 25 °C, 30 °C, 35 °C, 45 °C and also at different pH such as 5, 6, 7 and 7.5. Faster growth of *Trichoderma harzianum* was observed at 25- 30 °C while the growth was very slow at above 35 °C and there was no growth at 45 °C. Optimum temperature of *Trichoderma* was observed between 25 and 30 °C, approximately 28 °C by radial growth. The maximum growth of *Trichoderma harzianum* was recorded at pH 7-7.5 and the minimum growth was observed at pH 5.

Chaudhary *et al.* (2016) revealed that the population of *Trichoderma* was negatively correlated with soil temperature and most of the fungal species were positively correlated with the soil moisture. Knudsen and Bin (1990) conducted a study on the effect of soil moisture and temperature on the growth of *Trichoderma harzianum* from the alginate pellets. They showed that temperature had a significant positive effect on radial growth rate. Danielson and Davey (1973) suggested that the optimum temperature for growth differs

among the *Trichoderma* species. They reported that most *Trichoderma* strains are mesophilic and cannot protect germinating seeds from soil borne diseases caused by cold-tolerant strains of plant pathogenic fungi during cold autumn and spring conditions.

Jayaraj and Ramabadran (1999) carried out a study to know the effect of soil moisture on the growth of *Trichoderma harzianum*. They noticed that, out of six moisture levels, the highest population (98.3 $\times 10^3$ cfu/g) and competitive saprophytic ability (0.218) were noticed at 10 per cent moisture levels followed by 5 per cent (82.7) (0.197) and 25 per cent (50.5) (0.180) levels. The lowest (1 %) as well as higher levels of moisture (25, 50 and 80 %) were not suitable for the persistence and multiplication of *Trichoderma* propagules in soil. The least population (8.5×10^3 cfu/g) and CSA (0.063) was recorded at 80 per cent level of moisture.

Ahmad and Baker (1987) conducted an experiment in which cucumber seeds were treated with conidia of *Trichoderma harzianum.* Then the isolate could be detected in rhizosphere soil of pH 5, 6, 7 at temperatures of 19, 26 and 33 °C. They found higher population densities at pH 5 than in 7.

According to Mishra and Khan (2015), *Trichoderma viride* was favoured by acidic pH while poor mycelia development was observed in alkaline medium. They mentioned that alkaline pH had inhibitory effect on the growth and development of mycelia and pigmentation of the fungus. *Trichoderma viride* could grow at a wide range of temperature between 20-30 °C. Best growth and sporulation occurred at 28 °C with colony diameter of 2.6 cm and optimum at pH 6 with 3.7 cm colony diameter and optimum relative humidity for sporulation was obtained at 80 per cent.

2.9 Effect of micro-climatic and soil parameters on population of *Pseudomonas fluorescens*

Bowers and Parke (1993) studied the effect of soil temperature on the colonization of *Pseudomonas fluorescens* in the pea roots. Soil was maintained

as various sections at different temperatures such as 16, 20, 24 and 28 °C and the inoculated seeds were sown in them. It was found that expeditious root growth was at the temperature of 24 °C and the optimum temperature for root colonisation of *Pseudomonas fluorescens* was at 16 °C. Guo *et al*. (2013), concluded that population of soil bacteria is negatively correlated with soil temperature.

In a study conducted by O'Callaghan *et al.* (2001), establishment of *Pseudomonas fluorescens* was significantly affected by both soil temperature and soil moisture content. It was found that the population decline was much greater at 20 °C than at the temperatures of 10 °C and 15 °C, with populations falling to below the level of detection at all soil moisture levels after 54 days. Also, least population was recorded at the driest soil. *Pseudomonas fluorescens* could not be recovered from most of the samples incubated at 20 °C when sampled at 82 days. Nevertheless, the data indicate the sensitivity of the bacterium to soil environmental factors.

Loper *et al*. (1985) concluded that soil temperature influenced rhizosphere populations of *Pseudomonas* sp. without the addition of water and the survival of bacterial cells may be maximized at lower temperatures, which may be due, in part, to reduced competitive microbial activity. According to Davies and Whitbread (1989), soil temperature had a significant, but complex effect on colonization and that the complexity may be a result of differential effects of a variety of biotic components in the system.

Koche (2012) studied on effect of temperature on growth of *P. fluorescens.* The isolates of *P. fluorescens* were inoculated at different temperatures which ranges from 4 to 45°C and growth was measured after 48 h of incubation by turbidometry. 30°C was found to be suitable temperature for growth of *P. fluorescens.*

Gamliel and Khan (1991) reported an inverse relationship between soil pH and population densities of *Pseudomonas fluorescens*. While Chiarini *et al.*

(1998) showed that soil pH was positively correlated with the rhizospheric population density.

Several workers monitored the persistence of *Pseudomonas* populations in soil as part of studies on strains with biological control potential. Ownley *et al.,* (2003) inferred that take-all disease caused by *Gaeumannomyces graminis* var. *tritici* in wheat could be controlled by *Pseudomonas fluorescens* 2-79RN10, but the level of protection in the field varied from site to site. The relative importance of soil properties on disease suppression was evaluated based on the correlation between sixteen soil properties and disease suppression. It can be found that biocontrol activity was positively correlated with pH and negatively correlated with organic matter content, total carbon and total nitrogen.

Several studies have reported increases in soil microbial biomass under elevated carbon dioxide (Berntson and Bazzas, 1997; Hungate *et. al.*, 1996; Rice *et al.*, 1994). Marilley *et al.* (1999) recorded an increased dominance of pseudomonads in the rhizosphere of *Lolium perenne* and a decreased dominance in the rhizosphere of *Trifolium repens* under elevated atmospheric CO_2 content.

Montealegre *et al.* (2002) studied the influence of increased atmospheric CO_2 on soil microbial population, activity and on microbial composition. According to them, changes in population of soil microbial community are likely to occur under elevated atmospheric CO_2, but the extent of those changes depend on plant species. There was no impact of CO_2 concentration on population of bacteria in bulk soil under perennial ryegrass, but population increased about 1.4 fold under white clover.

Wessendorf and Lingens (1989) stated that *Pseudomonas fluorescens* (R1) failed to persist for long periods in natural soil. The decline of *Pseudomonas* population might be greater than expected as Pseudomonads are typically rhizosphere colonizers and population decline might have been slower in the presence of plant roots. However, the data indicated the sensitivity of the bacterium to soil environmental factors.

Duffy and Defago (1997) mentioned that the interaction between a plant pathogen and a biocontrol agent could be influenced by soil properties. According to Bowers and Parke (1993), the rhizosphere presents a complex set of conditions, and with the interactive nature of environmental determinants, the task of determining the direct effect of any one variable on any aspect of microbial activity, such as colonization, which itself is a sum of other microbial processes, is complicated.

2.10 Effect of micro-climatic and soil parameters on functional efficiency of *Trichoderma* sp. and *Pseudomonas fluorescens*

Arshad and Frankenberger (1992) and Bric *et al*. (1991) reported that there are numerous microbiota actively involved in the synthesis of auxins in pure culture and in soil. It can be found, in general, that microorganisms isolated from the rhizosphere and rhizoplane of various crops are more active in producing auxins than those from root-free soil (Brown, 1972; Dvornikov *et al*., 1970; Kampert *et al.*, 1975; Purushothaman *et al.,* 1974). Production of auxin in rhizosphere soil is most likely because of the abundance of substrates and microorganisms. Sarwar *et al.* (1992) documented that auxin production by *Pseudomonas* sp. was increased from 25 to 40°C and maximum auxin producing bioactivity was observed at 40°C.

Maleki *et al.* (2010) stated that *in situ* condition in the natural rhizosphere influences survival, growth and production of secondary metabolites by the biocontrol strains.

Tailor and Joshi (2012) investigated the effect of temperature on production of siderophore and showed that *Pseudomonas fluorescens* strain from sugarcane rhizosphere produced highest siderophore at a temperature of 29 °C and at pH 7.

In a study conducted by Ram *et al.* (2018), effect of pH and temperature on IAA production by *Trichoderma* sp. was found out. Maximum IAA production was found at a pH of 3. A progressive increase in production of IAA

by *Trichoderma* sp. was observed from pH 3 to 7 and a reduction of IAA production was observed from pH 8 onwards. In the case of temperature, maximum IAA was produced at 30 °C followed by 35 °C and 40 °C. Hasan (2009) and Nathan *et al.* (2017) found maximum IAA production at the optimal temperature range of 25-30 °C.

Mishra *et al.* (2013) documented that *Pseudomonas* sp. strain (PGERs17) showed the production of siderophore and HCN at 15 °C temperature and also showed that the isolate was able to retain its functional traits even at 4 °C, which was the lower temperature extreme for its growth, while higher values for all parameters were recorded at a temperature of 28 °C.

Ownley *et al*. (2003) conducted a study on identification of soil factors that influence biocontrol ability of *Pseudomonas fluorescens* (2-79RN10). They concluded that level of protection varied from site to site. They realized that biocontrol activity of *Pseudomonas* strain against disease caused by *Gaeumannomyces graminis* var. *tritici* was positively correlated with soil pH and negatively correlated with exchangeable acidity, percent organic matter (OM), total carbon and total nitrogen. It was reported that biocontrol activity was mainly associated with siderophore production.

2.11 *Phytophthora* foot rot disease in black pepper

Sarma (2003) suggested that quick wilt disease of black pepper caused by *Phytophthora capsici* is a very serious disease and it caused big economic loss to the farmers. It was Muller who first called the disease as foot rot (1937). *Phytophthora* is a soil-borne fungal pathogen. According to Tsao (1988), although 17 diseases have been reported in in India, foot rot caused by *Phytophthora* Leonian emend Alizadeh and Tsao is a major one.

Nair and Sarma (1988) reported that the term *Phytophthora* foot rot was given precedence over ‘quick wilt’ or ‘foot rot’ of black pepper at the International Community Workshop on Joint Research for the Control of Black Pepper Diseases in 1988 at Goa, India. Quick wilt is used because of the

external symptoms of disease when the collar of vine was infected and killed within a few days. The term quick wilt is used because of the external symptoms of disease when the collar of vine was infected and killed within a few days. According to Anandaraj (2000), the disease has become one of the major limiting factors of black pepper wherever the crop is grown since its first report in Indonesia in 1885 (Erwin and Ribeiro, 1996).

In India, foot rot disease is prevalent in Kerala, Karnataka, Tamil Nadu states and recently in Assam (Sarkar *et al.*, 1985). Mammootty *et al.* (1991) documented high foot rot disease incidence in the month of July (39.08 %) when rainfall, number of rainy days and relative humidity were high. Incidence of the dreaded *Phytophthora capsici* foot rot in the nursery, was a problem in most pepper growing regions of Kerala, where conditions were suitable for disease build-up especially during the southwest monsoon season (Sarma *et al.*, 1996).

Thomas and George (1992) recorded partial wilting of black pepper vines up to 62 per cent and complete vine death up to 41.5 per cent in the hilly terrains of Western ghats. Generally loss of vines due to the disease is reported to be from 5 to 20 % (Manohara *et al.*, 2004), but it is found significantly higher in India (Shamarao and Siddaramaiah, 2002) and Indonesia (Sitepu and Mustika, 2000). Erwin and Ribeiro (1996) reported *Phytophthora capsici* as the true cause of foot rot of black pepper in many countries.

2.12 Effect of *Trichoderma* sp. and *Pseudomonas fluorescens* on foot rot disease management in black pepper

Rajan *et al*. (2002) demonstrated *Trichoderma harzianum* for its biocontrol activity against *Phytophthora capsici* causing foot rot disease of black pepper. Shashidhara *et al.* (2009) carried out a field study to control *Phytophthora* disease in black pepper using integrated disease management practices, in that they identified that application of Metalaxyl MZ 72 WP integrated with bioagents *T. harzianum, P. fluorescens* and a plant product neem

cake performed better in reduction of intensity of leaf infection, collar infection and wilt incidence.

Different species of *Trichoderma* have the potential to control soil-borne plant pathogens more effectively than chemicals (Papavizas, 1985) and they also exhibit plant-growth promoting activity (Duffy *et al.*, 1996). *Trichoderma harzianum* and *Trichoderma viride* have been used as biological control agents against *Phytophthora capsici* in black pepper (Anandaraj and Sarma, 1995).

Six strains of *Pseudomonas fluorescens* were found efficient in root-rot suppression in black pepper (*Piper nigrum* L.) caused by *Phytophthora capsici* infected cuttings. Drenching with *P. fluorescens* in soil before the planting of black pepper reduced wilt disease caused by *P. capsici* under greenhouse conditions (Anith, 2002). Wahyuno *et al*. (2016) reported that an application of *Trichoderma* and *Pseudomonas fluorescens* individually or in combination was advantageous for plant protection and plant growth.

Anandaraj and Peter (1996) reported that several antagonistic beneficial microorganisms like *Trichoderma* and *Gliocladium* were present in the black pepper rhizosphere. Paul *et al.* (2005) isolated *Pseudomonas fluorescens* from black pepper roots and reported that they produced volatile and non-volatile metabolites and inhibited the growth of *Phytophthora capsici.*

Materials and methods

3. MATERIALS AND METHODS

The study on "Field evaluation of abiotic stress tolerant strains of *Trichoderma harzianum* and *Pseudomonas fluorescens* for *Phytophthora* disease management in black pepper" was carried out during 2016-2018 at the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara. The materials used and methodologies adopted in this study are presented below.

3.1 Purification and maintenance of the microbial isolates

Trichoderma harzianum (CKT) and *Pseudomonas fluorescens* (PAP) strains (isolated from Chelakkara and Pazhayannur respectively in Thrissur district) which were identified as abiotic stress tolerant isolates in the previous studies conducted in the department of Agricultural Microbiology along with KAU reference cultures of *Trichoderma viride* and *Pseudomonas fluorescens* were purified and maintained using standard procedures. *Pseudomonas* strains were streaked on King's B agar medium till the pure and single isolated colonies were obtained. Pure cultures were further maintained on King's B agar slants in refrigerator at 4° C and as glycerol stock (broth culture containing 40% glycerol) at -80°C in deep freezer. *Trichoderma* colonies developed were transferred to potato dextrose agar (PDA) medium in Petri plates. Pure cultures of the fungi obtained were maintained in PDA slants under refrigerated condition. *Phytophthora capsici* which causes foot rot disease in black pepper was used for artificial inoculation in the pot culture experiment. It was purified and and periodically subcultured in PDA medium in petri plates and further maintained in PDA slants.

3.2 Morphological and cultural characterization of the isolates

Morphological and cultural characteristics such as colony shape, colour of the colony, cell shape and fluorescence production and gram staining of *Pseudomonas fluorescens* were observed (Holt *et al*., 1994).

The morphological characterization including conidia production, colour of the conidia and number of the concentric ring formation of *Trichoderma harzianum* were observed in the PDA medium. Arrangement of the phialids and spores of the fungi was observed under microscope as described by Shah *et al*. (2012).

3.3 Screening of abiotic stress tolerant *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) against *Phytophthora* disease of black pepper under pot culture studies

The experiment was conducted to evaluate the efficiency of the abiotic stress tolerant *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) for plant growth promotion and disease management in black pepper under pot culture. The experiment was carried out during the months of April – October 2017.

Test crop : Black pepper (*Piper nigrum* L.)
Variety : Panniyur-1
Design : CRD
Replications: 3

Treatment details

T_1 : *Trichoderma harzianum* (CKT isolate)
T_2 : *Pseudomonas fluorescens* (PAP isolate)
T_3 : *Trichoderma viride* (KAU reference culture)
T_4 : *Pseudomonas fluorescens* (KAU reference Culture)
T_5 : Package of Practices Recommendations of KAU (2016)
T_6 : Organic Package of Practices of KAU (2009)
T_7 : Chemical treatment [Kocide (Copper hydroxide) @ 2g l^{-1}]
T_8 : Absolute control
T_9 : Pathogen control

3.3.1 Preparation of potting mixture and planting of black pepper cuttings

Top soil, river sand and cattle manure were mixed in the ratio 1:1:1 to prepare the potting mixture. Sterilized potting mixture was used for the current study. Polythene bags with the size of 6×4 inches were filled with the potting mixture. Healthy two noded black pepper cuttings were used for planting.

3.3.2 Preparation and application of *Pseudomonas* sp. inoculum

An individual colony was picked from the agar plates of respective *Pseudomonas* strains and inoculated into 3 ml of King's B broth medium taken in test tubes. These were incubated for 2 – 4 days and then transferred into 300 ml of King's B broth contained in 500 ml conical flasks. After incubation for 48 hours at 28 ± 2 °C, respective 300 ml inocula were mixed with 1 kg of sterilized talc powder separately for preparing the talc based bioinoculant. Prepared formulations were air dried and packed. The inocula were applied at the time of planting of pepper cuttings.

3.3.3 Preparation and application of *Trichoderma* sp. inoculum

Five millimetre disc from each of *Trichoderma* were inoculated in 100 ml potato dextrose broth in 250 ml conical flasks. These were kept for incubation at 28±2 °C for 7 days. Then the inocula were thoroughly mixed with 1 kg of sterilized talc powder separately for preparing the carrier based formulation. Mixed formulations were air dried and packed. The inocula were applied at the time of planting.

3.3.4 Artificial inoculation with *Phytophthora*

Two hundred gram of carrot was cut into small pieces and sterilized in a conical flask. It was then inoculated with *Phytophthora* culture. For that, eight millimeter mycelial disc from the five day old culture of *Phytophthora* was taken. The inoculated carrot pieces were applied on root on which a slight injury was given

using a sterile blade. That portion was covered with soil again. Finally, the whole plant was covered with polythene bag sprinkled inside with water.

3.4 Observations under pot culture experiment

Observations on chemical analysis of potting mixture, micro-climatic parameters, soil parameters, biometric characters, per cent foot rot disease incidence and per cent foot rot disease index were recorded under the pot culture studies.

3.4.1 Chemical analysis of potting mixture

Organic carbon per cent (Walkley and Black, 1934), available nitrogen, available phosphorus (Bray and Kurtz, 1945) and available potassium (Jackson, 1973) of the potting mixture before inoculation and 6 months after inoculation were analyzed.

3.4.1.1 Organic Carbon

Organic carbon was determined by using Walkley and Black wet digestion method (Walkley and Black, 1934). One gram of ground and 0.5 mm sieved potting mixture sample was taken in 500 ml conical flask. After adding exactly 10 ml of 1 N $K_2Cr_2O_7$, the flask was swirled to disperse the soil in the solution. Rapidly, 20 ml of concentrated H_2SO_4 was added. Immediately, the flask was swirled gently until the soil and the reagents were mixed and kept for 30 minutes on the asbestos sheet. In order to stop the reaction, 200 ml of distilled water was added to the flask. 3-4 drops of ferroin indicator was added to the solution and the contents in the flask were titrated with 0.5 N ferrous ammonium sulphate taken in a burette. When the solution approaches end point, a dark green colour was observed. At this point, ferrous ammonium sulphate was added drop by drop until the colour turned to red from blue. A blank was also prepared in the same manner without soil sample.

$$\text{Organic Carbon (\%)} = \frac{(\text{meq } K_2Cr_2O_7 - \text{meq Fe } (NH_4)_2 SO_4) \times 0.003 \times 100 \times 1.3}{\text{Weight of soil (g)}}$$

3.4.1.2 Available Nitrogen

The potting mixture sample was air dried under shade. It was ground and sieved through 2 mm stainless sieve. 5 g was taken from the sample and transferred to Kjeldahl's flask. After placing 1 g paraffin and a few glass beads along with potting mixture in Kjeldahl's flask, the Kjeldahl nitrogen distillation assembly was set. 0.32 % $KMnO_4$ and 2.5 % NaOH (25 ml each) were added automatically by distillation unit programme. 25 ml of 2 % boric acid solution was transferred along with mixed indicator into 250 ml Erlenmeyer flask. Ammonia was released and was collected by dipping the outlet of the condenser tube into the boric acid solution. The distillation process was started by automatic distillation unit for 9-12 minutes. Pinkish colour turned to green in the conical flask due to the absorption of ammonia. Then the green distillate was titrated with 0.02 N H_2SO_4 and the colour turned to pink (initial colour) at the end point. Blank sample was also made without potting mixture simultaneously. The blank and sample titre values were noted and the available nitrogen in the potting mixture sample was estimated using the following equation.

$$\text{Available nitrogen (kg ha}^{-1}) = \frac{R \times N \times Z \times \text{Weight of one hectare of soil}}{\text{Sample weight (g)} \times 1000}$$

3.4.1.3 Available phosphorus

Available phosphorous was extracted using Bray No. 1 (Bray and Kurtz, 1945), with 0.03 N NH_4F and 0.025 N HCl. Five grams of potting mixture was added to a 250 ml conical flask. Then 50 ml of Bray No.1 reagent was added and shaken for five minutes. Whatman No. 42 filter paper was used for filtering. 7.5 ml of 0.8 M (10ml, 4%) boric acid (50 g H_3BO_3 per litre) was added to 5 ml of the extract in order to avoid interference of fluoride. Estimation was done by reduced molybdate blue colour method (Olsen *et al*., 1954).

Five milliliter of the extract was pipetted into a 25 ml volumetric flask. It is diluted to approximately 20 ml. Four millilitre of reagent B was added and the volume was made up with distilled water and the contents were shaken well.

Intensity of colour was read after 10 minutes at 660 nm. The colour was stable for 24 hours and the maximum intensity was developed within 10 minutes. The concentration of phosphorous in the sample was calculated using standard curve.

Different concentrations of P at 1, 2, 3, 4, 5 and 10 ml of 2 μg ml^{-1} P solution in 25 ml volumetric flasks were made for the preparation of standard curve. Five millilitre of the extracting reagent (Bray No.1) was added. Colour was developed as described above by adding reagent B. The concentration *vs*. absorbance curve was plotted on a graph paper.

$$\text{Available Phosphorus (mg kg}^{-1}\text{ soil)} = \frac{\text{Absorbance for sample}}{\text{Slope of standard curve}} \times \frac{50}{5} \times \frac{25}{5}$$

3.4.1.4 Available Potassium

Flame photometric method (Jackson, 1973) was used for the estimation of available potassium. The sample of air-dried potting mixture was passed through a 2 mm sieve. Five gram of sample was mixed with 25 ml of neutral normal potassium acetate for five minutes and allowed to stand for a few seconds. It was then filtered through Whatman No. 42 filter paper. Potassium content of the extract was determined using flame photometer after necessary settings and calibration of the instrument.

Standard curve for potassium was prepared with series of stock solutions of potassium chloride to give concentrations of 5 to 20 μg ml^{-1} of K. The reading was set as zero for the blank and at 100 for 20 μg ml^{-1} of K after attaching the appropriate filter and adjusting the gas and air pressure. The curve was prepared by plotting the readings against different concentrations (5, 10, 15, and 20 μg ml^{-1} of K).

$$\text{Available Potassium (mg kg}^{-1}\text{ soil)} = \mu\text{g K per ml of aliquot} \times \frac{25}{5}$$

3.4.2 Enumeration of beneficial microflora

Monthly isolation and counting of beneficial microflora were done in the Petri dish with respective dilution by serial dilution and plating method and were expressed as colony forming units per gram of soil (cfu/g) (Sutton, 2011).

Under aseptic conditions, ten gram of soil sample was transferred into 250 ml conical flask containing 90 ml sterilized distilled water. The contents were mixed by shaking for five minutes. One ml of aliquot was taken and transferred to a test tube containing 9 ml of sterilized distilled water. Before further dilution, the suspension was shaken for one minute for homogenization. Dilutions upto 10^{-6} were prepared for the isolation of microorganisms. One ml of the respective dilutions was pipetted out and transferred into sterile Petri dishes aseptically. The plates were rotated in clockwise and anti-clock wise manner for the uniform mixing of the aliquot with the agar media. The mixture was allowed to solidify and kept for incubation. The number of colonies in the respective agar media were observed and recorded. It is then calculated as to obtain the result in number of colony forming units per gram of soil (cfu/g).

3.4.3 Micro- climatic parameters

Micro-climatic parameters were soil temperature and soil moisture. Observations were recorded at monthly intervals.

3.4.3.1 Soil temperature

Soil temperature was measured with soil thermometer (Rokade *et al*., 2014). The soil temperature was recorded at 9.00 am in the morning and 2.30 pm in the afternoon. The mean soil temperature was calculated at monthly intervals.

3.4.3.2 Soil moisture

Gravimetric method (Black, 1965) was used to measure soil moisture. For measuring the soil moisture, Al-tin was weighed and that weight was recorded

(tare). Soil sample was placed in the tin (about 10 g) and the weight was recorded (wet soil + tare). The soil sample taken in the tin was placed in the oven at 105 ^{0}C and was dried for 24 h overnight. Then the sample was weighed and that weight was recorded (dry soil + tare). The last step was repeated for five times until no difference come between any consecutive measurements of the weight (dry soil + tare).

$$\text{Moisture per cent in soil on oven dry basis} = \frac{\text{Weight of moisture in sample} \times 100}{\text{Weight of oven dry sample}}$$

3.4.4 Soil parameters

Soil parameters were soil pH and soil respiration and were recorded at monthly intervals.

3.4.4.1 Soil pH

Soil pH is a measure of hydrogen ion (H^+) activity in the soil solution defined as -log 10 of the hydrogen ion [H^+] concentration. Ten gram of 2 mm sieved air dried soil was taken in a 50 ml beaker with 25 ml water. Then the suspension was stirred well and kept for half an hour. Again it was stirred and soil pH was measured in a 1:2.5 fresh soil-water suspension using a pH meter (Jackson, 1973).

3.4.4.2 Soil respiration

Soil respiration was done by Alkali trap method (Chhonkar *et al*., 2007) at monthly intervals during the pot culture studies. Initially, moisture content of the samples was determined gravimetrically. Forty gram moist soil was taken in a 50 ml capacity beaker and water was added up to its field capacity. The beaker was kept inside the glass jar. Five millimetre of 0.5 N NaOH was taken in a scintillation vial and kept into the jar just beside the beaker. Then 2-3 ml of water was added in the bottom of the jar and the lid was closed to make air tight. One blank was kept without soil. The jars were kept in the incubator at 28^{0}C or 37^{0}C. The jars were taken out from the incubator after 24 hours of incubation. 0.5 N NaOH was transferred from the vial to conical flask after washing of the vials several times. Few drops of saturated $BaCl_2$ solution was added into the NaOH solution with

phenolphthalein indicator. The contents in the conical flask were titrated against 0.5 N HCl slowly until the pink colour just disappeared and recorded the titre value. The soil respiration was expressed as mg CO_2 g^{-1} $day.^{-1}$

CO_2 evolved was calculated by using the following equation:

Soil respiration = (Blank value – Titre value) × (Normality of acid × Equivalent weight of C in CO_2)

3.4.5 Biometric observations

The biometric observations on the plant such as number of leaves, plant height, number of lateral branches, number of nodes and internodal length at monthly intervals were recorded at monthly intervals.

3.4.5.1 Number of leaves

Total number of leaves per plant was counted at monthly intervals for six months and average values were calculated.

3.4.5.2 Plant height

The distance from the base of the plant to the tip of the plant was taken as the plant height at monthly intervals and expressed in centimetre (cm).

3.4.5.3 Number of lateral branches

Total number of lateral branches was counted in each plant at monthly intervals and mean value was taken.

3.4.5.4 Number of nodes

The number of nodes per plant was counted at monthly intervals and mean value was taken.

3.4.5.5 Internodal length

The distance between two consecutive nodes was taken as internodal length at monthly intervals and expressed in centimetre (cm).

3.4.6 Per cent disease incidence

Incidence of *Phytophthora* foot rot in the nursery was recorded frequently after one week of challenge inoculation and the per cent disease incidence was calculated as follows (Amalraj *et al*., 2010).

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

3.4.7 Per cent disease index

Each plant was checked for the foot rot disease severity at 60 days after challenge inoculation with *Phytophthora capsici* based on a score chart from 0-4 (Jimenez *et al*., 2012) as shown in Table 3.1.

Per cent disease index was calculated using the formula suggested by James (1971).

$$\text{Per cent disease index} = \frac{\text{Sum of all disease ratings} \times 100}{\text{Total no. of ratings} \times \text{Maximum disease score}}$$

3.8 Score chart for severity of *Phytophthora* foot rot in black pepper

Score	Description
0	Healthy plants (0-24 % infection)
1	Plants with only their leaves wilting ((25-49 % infection)
2	Plants which show severe wilting symptoms (50-74% infection)
3	Plants with less wilting but severely dry leaves (75-90% infection)
4	Dead plants (91-100% infection)

3.5 Screening of abiotic stress tolerant strains of *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) against *Phytophthora* disease of black pepper under field conditions

The experiment was conducted to evaluate the performance of the abiotic stress tolerant strains of *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) for growth promotion and disease management in black pepper under field conditions. The experiment was conducted during the months of November 2017 – May 2018.

Test crop : Black pepper (*Piper nigrum* L.)
Variety : Panniyur-1
Design : CRD
Replications: 4

Treatment details

T_1 : *Trichoderma harzianum* (CKT isolate) + KAU POP 2016
T_2 : *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016
T_3 : *Trichoderma viride (*KAU ref. culture) + KAU POP 2016
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016
T_5 : *Trichoderma harzianum* (CKT isolate)
T_6 : *Pseudomonas fluorescens* (PAP isolate)
T_7 : *Trichoderma viride (*KAU ref. culture)
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)
T_9 : Package of Practices Recommendations (KAU, 2016)
T_{10}: Organic Package of Practices (KAU, 2009)
T_{11}: Chemical treatment [Kocide (Copper hydroxide) @ 2g l^{-1}]
T_{12}: Absolute control

A plot of area 567 m^2 was taken for the field study. Field was levelled and prepared well for the cultivation of black pepper. Standards of karayam (*Garuga pinnata*) was used in the field. Pits of size 50 cm×50 cm ×50 cm was prepared on

northern side of standards 15 cm away from it. Black pepper plants of six months old were planted. Inoculants and manures were applied according to the treatment details given above.

3.6 Observations under field experiment

Observations on chemical analysis of field soil, micro-climatic parameters, soil parameters, enumeration and functional efficiency of beneficial microorganisms, biometric characters and the per cent foot rot disease incidence were recorded under the field study.

3.6.1 Chemical analysis of field soil

Analyses of organic carbon (Walkley and Black, 1934), available nitrogen, available phosphorus (Bray and Kurtz, 1945) and available potassium (Jackson, 1973) in the soil before inoculation and 6 months after inoculation were done. The procedure for the chemical analysis of soil is as described in the section 3.4.1.

3.6.2 Enumeration of beneficial microflora from soil

Monthly isolation and counting of beneficial microflora were done in the Petri dish with respective dilution by serial dilution and plating method and were expressed as colony forming units per gram of soil (cfu/g) (Sutton, 2011).

Under aseptic conditions, ten gram of soil sample was transferred into 250 ml conical flask containing 90 ml sterilized distilled water. The contents were mixed by shaking for five minutes. One ml of aliquot was taken and transferred to a test tube containing 9 ml of sterile distilled water. Before further dilution, the suspension was shaken for one minute for homogenization. Dilutions upto 10^{-6} were prepared for the isolation of microorganisms. One ml of the respective dilutions was pipetted out and transferred into sterile Petri dishes aseptically. The plates were rotated in clock wise and anti-clock wise manner for the uniform mixing of the aliquot with the agar media. The mixture was allowed to solidify and kept for incubation. The number of colonies in the respective agar media were observed and

recorded. It is then calculated as to obtain the result in number of colony forming units per gram of soil (cfu/g).

3.6.3 Micro- climatic parameters

The micro-climatic parameters were soil temperature and soil moisture. Observations were recorded at monthly intervals.

3.6.3.1 Soil temperature

Soil temperature was measured with soil thermometer (Rokade *et al*., 2014). The soil temperature was recorded at 9.00 am in the morning and 14.30 pm in the afternoon. The mean soil temperature was calculated at monthly intervals.

3.6.3.1 Soil moisture

Gravimetric method (Black, 1965) was used to measure soil moisture. For measuring the soil moisture, Al-tin was weighed and that weight was recorded (tare). Soil sample was placed in the tin (about 10 g) and the weight was recorded (wet soil + tare). The soil sample taken in the tin was placed in the oven at 105°C and was dried for 24 h overnight. Then the sample was weighed and that weight was recorded (dry soil + tare). The last step was repeated for five times until no difference come between any consecutive measurements of the weight (dry soil + tare).

$$\text{Moisture per cent in soil on oven dry basis} = \frac{\text{Weight of moisture in sample} \times 100}{\text{Weight of oven dry sample}}$$

3.6.4 Soil parameters

The soil parameters were soil pH and soil respiration and were recorded at monthly intervals.

3.6.4.1 Soil pH

Soil pH is a measure of hydrogen ion (H+) activity in the soil solution defined as -log 10 of the hydrogen ion [H+] concentration. Ten gram of 2 mm sieved air dried soil was taken in a 50 ml beaker with 25 ml water. Then the suspension was stirred well and kept for half an hour. Again, it was stirred and soil pH was measured in a 1:2.5 fresh soil-water suspension using a pH meter (Jackson, 1973).

3.6.4.2 Soil respiration

Soil respiration was done by Alkali trap method (Chhonkar *et al*., 2007) at monthly intervals during the pot culture studies. Initially, moisture content of the samples was determined gravimetrically. Forty gram moist soil was taken in a 50 ml capacity beaker and water was added up to its field capacity. The beaker was kept inside the glass jar. Five millimetre of 0.5 N NaOH was taken in a scintillation vial and kept into the jar just beside the beaker. Then 2-3 ml of water was added in the bottom of the jar and the lid was closed to make air tight. One blank was kept without soil. The jars were kept in the incubator at 28^0C or 37^0C. The jars were taken out from the incubator after 24 hours of incubation. 0.5 N NaOH was transferred from the vial to conical flask after washing of the vials several times. Few drops of saturated $BaCl_2$ solution was added into the NaOH solution with phenolphthalein indicator. The contents in the conical flask were titrated against 0.5 N HCl slowly until the pink colour just disappeared and recorded the titre value. The soil respiration was expressed as mg CO_2 g^{-1} day^{-1}

CO_2 evolved was calculated by using the following equation:

Soil respiration = (Blank value – titre value) × (Normality of acid × Equivalent weight of C in CO_2)

3.6.5 Biometric observations

The biometric observations on the plant such as number of leaves, plant height, number of lateral branches, number of nodes and internodal length at monthly intervals were recorded at monthly intervals.

3.6.5.1 Number of leaves

Total number of leaves per plant was counted at monthly interval for six months and average value was taken.

3.6.5.2 Plant height

The distance from the base of the plant to the tip of the plant was taken as the plant height at monthly interval and expressed in centimetre (cm).

3.6.5.3 Number of lateral branches

Total number of lateral branches was counted in each plant at monthly intervals and mean value was taken.

3.6.5.4 Number of nodes

The number of nodes per plant was counted at monthly intervals and mean value was taken.

3.6.5.5 Internodal length

The distance between two consecutive nodes was taken as internodal length at monthly intervals and expressed in centimetre (cm).

3.6.6 Disease incidence

No *Phytophthora* infection was found in the field. However, *Colletotrichum* sp. infection is found.

3.6.7 Screening of *Pseudomonas* sp. for functional efficiency and antagonistic activity under *in vitro*

The isolates obtained after monthly enumeration were screened for plant growth promoting (PGP) activities like production of indole acetic acid (IAA), ammonia, HCN and siderophore and the isolates *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) were tested for antagonistic activity against *Phytophthora capsici* under *in vitro*.

3.6.7.1 Screening for IAA production

Pseudomonas isolates obtained after enumeration were inoculated in sterile Luria - Bertani broth supplemented with tryptophan at the rate of 1 mg/ml. The tubes were incubated in the dark for seven days. After incubation, the cultures were centrifuged at 3000 rpm for 30 minutes and the supernatant was collected. 4 ml of Salkowski reagent was added to the supernatant. The development of pink colour indicated production of IAA (Ahmad *et al*., 2008).

3.6.7.2 Screening for ammonia production

Screening of the *Pseudomonas* isolates for ammonia production was carried out by inoculating the isolates in 4 per cent peptone water and incubating them at 28^0C for 48 hours. After incubation, 0.5 ml of Nessler's reagent was added to the tubes. Formation of yellow to brown colour indicated the presence of ammonia (Cappuccino and Sherman, 1992). Based on the intensity of colour produced, the reaction was rated as follows:

Yellow: Weak (+)

Orange: Moderate (++)

Brown: High (+++)

3.6.7.3 Screening for HCN production

The screening of *Pseudomonas* isolates for their ability to produce hydrogen cyanide was done by the method introduced by Bakker and Schipper (1987). Bacteria were streaked on King's B agar media supplemented with glycine at the rate of 4.4 g/l. Sterile filter paper dipped in picric acid solution (2.5 g of picric acid; 12.5 g Na_2CO_3, 1000 ml distilled water) was placed on the upper lid of the Petri plate. The plates were incubated at 28°C for 5 days. A colour change in the filter paper from yellow to light brown, brown or reddish brown represented weak (+), moderate (++) or high (+++) reaction respectively.

3.6.7.4 Screening for siderophore production

Bacterial isolates were screened for siderophore production by using Chrome Azurol Sulfonate (CAS) assay (John and Thankavel, 2015). For making the assay medium, 60.5 mg CAS was dissolved in 50 ml distilled water, which was then mixed with Iron (III) solution (1mM $FeCl_3.6H_2O$ in 10mM HCl). The solution was then gradually added to 72.9 mg hexadecyltrimethyl ammonium bromide (HDTMA) dissolved in 40 ml distilled water. Then a blue/green coloured solution was obtained. This solution was mixed with 100 ml nutrient agar, which was then used for screening. The *Pseudomonas* isolates were spot-inoculated on the media and kept at 30°C for 4-5 days of incubation. Formation of yellow to orange coloured halo around the colony indicated the capability of the isolates for siderophore production.

3.6.7.5 Testing antagonistic activity of Pseudomonas fluorescens **(PAP)**

Isolate *Pseudomonas fluorescens* (PAP) was screened for antagonistic activity against *Phytophthora capsici* (pathogen) under *in vitro* by dual culture method (Utkhede and Rahe, 1983). Potato dextrose agar (PDA) was allowed to solidify in sterilized Petri plates. 8 mm sized mycelial disc of *Phytophthora capsici* was inoculated at the centre of the Petri dish 48 hours prior to inoculation of *Pseudomonas fluorescens*. PAP isolate was inoculated as a line of streak on either

side of the pathogen leaving 2.25 cm from the edge of the Petri dish. Plate with *P. capsici* alone was served as the control. Three replications were maintained for this purpose. Observations on growth of the pathogen were taken at regular intervals upto 5 days. The per cent inhibition of the mycelial growth of the pathogen was calculated using the formula suggested by Vincent.

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

Where,

C: Growth of pathogen in control (mm)

T: Growth of pathogen in dual culture (mm)

3.6.8 Screening of *Trichoderma* sp. for functional efficiency under *in vitro*

3.6.8.1 Screening for IAA production

Czapek dox broth supplemented with 0.5 % tryptophan was prepared, sterilized and cooled. *Trichoderma* isolates were then inoculated into the broth and incubated for a period of 15 days at 28°C. Fully grown cultures were then centrifuged at 3000 rpm for 30 minutes. The supernatant obtained was then tested with Salkowski reagent. Development of pink colour indicated the production of IAA.

3.6.8.2 Sreening for Ammonia production

Freshly grown cultures were inoculated in 4 per cent peptone water in each test tube and incubated at 28°C for 4 days. After incubation, 0.5 ml Nessler's reagent was added in each tube. Development of yellow to brown colour indicated positive reaction (Cappuccino and Sherman, 1992).

3.6.8.3 Screening for HCN production

Trichoderma isolates were spot-inoculated on King's B media amended with glycine at the rate of 4.4 gl^{-1} (Bakker and Schipper, 1987). Sterile filter paper saturated (Whatman No.1) saturated with picric acid solution (2.5 g of picric acid; 12.5 g of Na_2CO_3, 1000 ml of distilled water) was placed in the upper lid of the Petri plate. The dishes were sealed with parafilm and incubated at 28 °C for 5 days. A change of colour of the filter paper from yellow to light brown, brown or reddish brown was recorded as weak (+), moderate (++) or high (+++) reaction respectively.

3.6.8.4 Screening for siderophore production

Trichoderma isolates were assayed for siderophore production in Chrome Azurole Sulfonate (CAS) agar medium (John and Thankavel, 2015). The *Pseudomonas* isolates were spot-inoculated on the media and kept at 30^0C for 5-6 days of incubation. Development of yellow to orange halo around the colony was considered as positive for siderophore production.

3.6.8.5 Antagonistic activity of Trichoderma harzianum **(CKT)**

Trichoderma harzianum (CKT) was tested for antagonistic activity against *Phytophthora capsici* by dual culture method (Skidmore and Dickinson, 1976). The organisms were inoculated as dual culture giving due consideration for the growth rate of both the pathogen and the antagonist. Mycelial disc (8mm) of *Phytophthora capsici* from seven days old culture grown on PDA was inoculated aseptically on one side of a Petri dish and incubated at room temperature for 48 hours. After that, 8 mm mycelial disc of *Trichoderma harzianum* was inoculated in the same PDA plate, 3.5 cm away from the pathogen disc and incubated. Three replications were maintained for that. The pathogen grown in monoculture served as control. The per cent inhibition of mycelial growth of the pathogen was calculated as mentioned in 3.6.7.5.

3.7 Statistical Analysis

Agricultural statistical package WASP 2.0 was used to do the analysis of variance. To find out the effect of micro-climatic and soil parameters on the functional efficiency of microflora, the data were cross tabulated for any two characters that were deemed associated and the dependency of one character on the other was measured through significance of chi-square statistics computed. Correlation studies were done using SPSS package.

Results

4. RESULTS

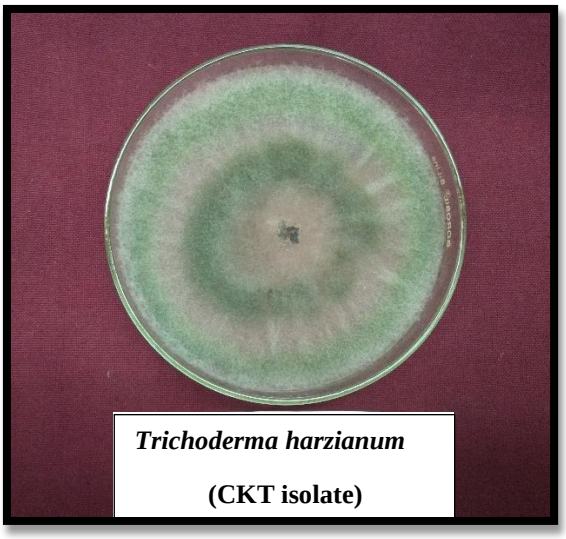
The present study was conducted to evaluate the efficiency of the abiotic stress tolerant strains of *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) for plant growth promotion and *Phytophthora* disease management in black pepper and to assess the effect of micro-climatic and soil parameters on those strains under field condition. The experiment was carried out in the plot allotted by the Department of Plantation crops and Spices in College of Horticulture, Vellanikkara. Evaluation was also conducted under pot culture in the Department of Agricultural Microbiology. The experimental results obtained are presented in this chapter.

4.1 Screening of abiotic stress tolerant strains of *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) for growth promotion and *Phytophthora* disease management under pot culture experiment

Evaluation of the abiotic stress tolerant strains of *Trichoderma harzianum* (CKT isolate) (Plate 1) and *Pseudomonas fluorescens* (PAP isolate) (Plate 2) for plant growth promotion and disease management in black pepper was carried out under pot culture based on chemical analysis of soil (organic carbon, available nitrogen, available phosphorus and available potassium), enumeration of *Trichoderma* sp. and *Pseudomonas fluorescens*, micro-climatic and soil parameters (soil temperature, soil moisture, soil pH and soil respiration), per cent disease incidence and biometric parameters (number of leaves, plant height, number of branches, number of nodes and internodal length).

4.1.1 Population of *Trichoderma* sp. and *Pseudomonas fluorescens* at monthly intervals under pot culture studies (sterile)

The population of *Trichoderma* sp. and *Pseudomonas fluorescens* at monthly intervals under pot culture are presented in Table 1. No population was recorded in



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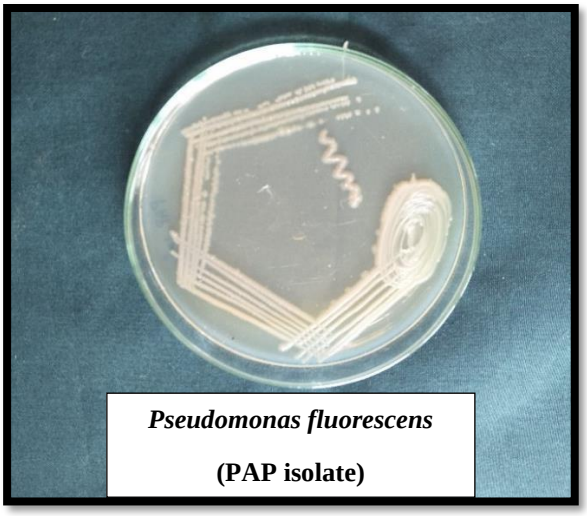
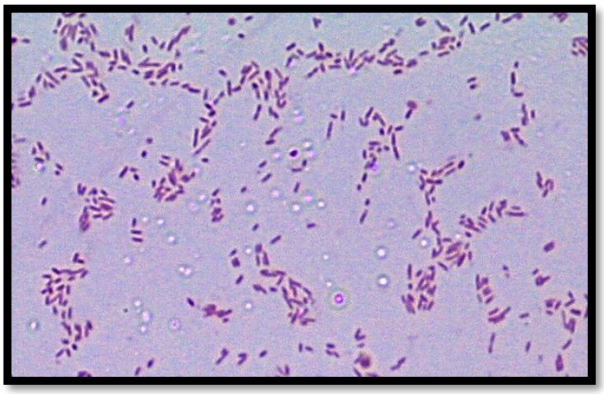
***Trichoderma harzianum* (CKT)**

Microscopic view of *Trichoderma harzianum*

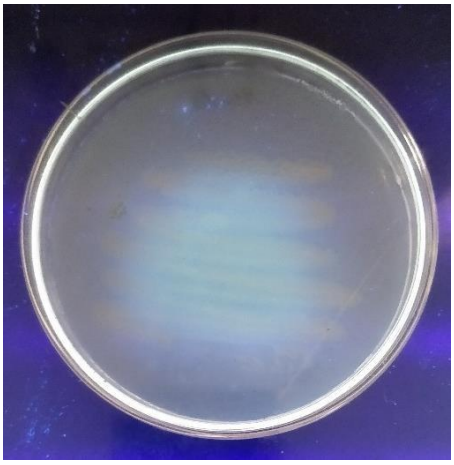
Plate 1 : Morphological and cultural characteristics of *Trichoderma harzianum*



***Pseudomonas fluorescens* (PAP)**



Microscopic view of *Pseudomonas fluorescens*



***Pseudomonas fluorescens* under uv light**

Plate 2 : Morphological and cultural characteristics of *Pseudomonas fluorescens*

Table 1: Population of *Trichoderma* sp. and *Pseudomonas fluorescens* at monthly intervals (cfu g^{-1}) under pot culture (sterile)

Isolates	30 DAP	60 DAP	90 DAP	120 DAP	150 DAP	180 DAP
T_1 :*Trichoderma harzianum* (CKT)	7.0×10^5 (0.846^b)	3.5×10^5 (0.701^b)	7.5×10^4 (0.869^b)	3.5×10^4 (0.541^b)	7.0×10^3 (0.842^b)	3.0×10^3 (0.460^b)
T_2 :*Pseudomonas fluorescens* (PAP)	4.5×10^6 (1.641^a)	2.5×10^6 (1.392^a)	6.5×10^5 (1.746^a)	3.0×10^5 (1.460^a)	5.5×10^4 (1.725^a)	2.5×10^4 (1.372^b)
T_3 :*Trichoderma viride* (KAU reference culture)	6.0×10^5 (0.774^b)	2.0×10^5 (0.541^b)	6.0×10^4 (0.723^b)	2.5×10^4 (0.377^b)	5.0×10^3 (0.693^b)	2.0×10^3 (0.301^b)
T_4 :*Pseudomonas fluorescens* (KAU reference culture)	6.5×10^6 (1.867^a)	3.0×10^6 (1.453^a)	8×10^5 (1.912^a)	4.0×10^5 (1.593^a)	7×10^5 (1.894^a)	4.5×10^4 (1.586^a)
T_5 :Package of Practices Recommendations (KAU, 2016)	a	a	a	a	a	a
T_6 :Organic Package of Practices (KAU,2009)	a	a	a	a	a	a
T_7 :Chemical treatment (Kocide 2g L^{-1})	a	a	a	a	a	a
T_8 :Absolute control (No inoculants, no pathogen)	a	a	a	a	a	a
T_9 :Control (No inoculants, with pathogen)	a	a	a	a	a	a
$CD_{(0.05)}$	0.195	0.297	0.178	0.248	0.185	0.175

Initial *Trichoderma* population: Absent
Initial *Pseudomonas fluorescens* population: Absent
Each value represents an average of three replications
Logarithmic transformed values are given in parentheses
DAP-Days After Planting
a – Absent

the potting mixture initially. After planting, population was present only in treatments T_1 [*Trichoderma harzianum* (CKT)], T_2 [*Pseudomonas fluorescens* (PAP)], T_3 [*Trichoderma viride* (KAU reference culture)] and T_4 [*Pseudomonas fluorescens* (KAU reference culture)].

There were significant differences among different treatments at 5 per cent level of significance. At 30 DAP, maximum population was found in treatment T_4 (6.5×10^6 cfu g^{-1}) and T_2 (4.5×10^6 cfu g^{-1}) and minimum in T_3 (6.0×10^5 cfu g^{-1}) and T_1 (7.0×10^5 cfu g^{-1}). Highest population at the time of harvest was found in treatments T_4 and T_2 (4.5×10^4 and 2.5×10^4 cfu g^{-1} respectively) and lowest in the case of treatments T_3 and T_1 (2.0×10^3 cfu g^{-1} and 3.0×10^3 cfu g^{-1} respectively). However, the population of *Trichoderma* sp. and *Pseudomonas fluorescens* declined gradually from 30 DAP to 180 DAP.

4.1.2 Effect of different treatments on micro-climatic and soil parameters under pot culture studies

4.1.2.1 Effect of different treatments on soil temperature under pot culture studies (sterile)

Soil temperature was recorded in different treatments at monthly intervals under pot culture experiment (Table 2). Soil temperature did not show significant differences among different treatments statistically. Overall, the temperature ranged from 29.1 °C to 32.8 °C.

4.1.2.2 Effect of different treatments on soil moisture under pot culture studies (sterile)

Soil moisture in different treatments under pot culture in sterile condition at monthly intervals are presented in Table 3. Initial soil moisture per cent in the sterilized potting mixture was 31.5. Treatments were significantly different in soil moisture content at 5 per cent level of significance statistically.

Treatment T_6 (Organic Package of Practices) showed highest soil moisture content (35.7 %) at 30 DAP followed by treatments T_1 [*Trichoderma harzianum* (CKT)] and T_2 [*Pseudomonas fluorescens* (PAP)] which were (34.4 % and 34.1 % respectively) on par with T_6. Lowest soil moisture content (26.2 %) was recorded in the case of treatment T_7 (Chemical treatment).

At 180 DAP, highest soil moisture content (36.4 %) was recorded in the treatment T_6 (Organic Package of Practices) followed by T_1 (CKT) which is (35.8 %) on par with T_6. Lowest soil moisture (28.2 %) was showed by T_7 (Chemical treatment).

4.1.2.3 Effect of different treatments on soil pH under pot culture studies (sterile)

Effect of different treatments on soil pH was recorded at monthly intervals under pot culture studies (Table 4). It shows a gradual decrease in pH from 30 DAP to 180 DAP. Statistically, there were no significant differences between the treatments. However, initial pH of the sterilized potting mixture was 7.2.

4.1.2.4 Effect of different treatments on soil respiration (CO_2 evolution) under pot culture studies (sterile)

Soil respiration was recorded in different treatments at monthly intervals under pot culture experiment (Table 5). Soil respiration decreased gradually from 30 DAP to 180 DAP except for treatments T_7 (Chemical treatment), T_8 (Absolute control) and T_9 (Control). There were significant differences among the treatments statistically at 5 per cent level of significance.

Highest soil respiration (11.92 mg CO_2 g^{-1} day^{-1}) at 30 DAP was showed by the treatment T_6 (Organic POP) followed by the treatments T_1 (*Trichoderma harzianum*), T_4 [*Pseudomonas fluorescens* (KAU reference culture)], T_2 [*Pseudomonas fluorescens* (PAP)] and T_3 [*Trichoderma viride* (KAU reference culture)] which were on par with T_6 (11.84, 11.72, 11.68 and 11.56 mg CO_2 g^{-1} day^{-1}

Table 2: Soil temperature in different treatments at monthly intervals under pot culture studies (sterile)

Soil temperature (°C)						
Treatments	**May**	**June**	**July**	**August**	**September**	**October**
T_1 :*Trichoderma harzianum* (CKT isolate)	32.3	29.5	29.4	30.5	31.3	32.2
T_2 :*Pseudomonas fluorescens* (PAP isolate)	32.1	29.3	29.4	30.3	31.2	32.1
T_3 :*Trichoderma viride* (KAU ref. culture)	32.2	29.1	29.2	30.4	31.3	32.0
T_4 :*Pseudomonas fluorescens* (KAU ref. culture)	32.3	29.4	29.3	30.3	31.1	32.2
T_5 :Package of Practices Recommendations (KAU, 2016)	32.8	29.7	29.9	30.8	31.7	32.6
T_6 :Organic Package of Practices (KAU,2009)	32.4	29.5	29.6	30.5	31.4	32.3
T_7 :Chemical treatment (Kocide 2g L^{-1})	32.6	29.6	29.7	30.6	31.5	32.4
T_8 :Absolute control (No inoculants, no pathogen)	32.5	29.5	29.5	30.6	31.4	32.3
T_9 :Control (with pathogen)	32.4	29.5	29.4	30.6	31.3	32.3
$CD_{(0.05)}$	NS	NS	NS	NS	NS	NS

Initial potting mixture temperature: 33°C

Each value represents an average of three replications

NS – Non- significant

DAP-Days After Planting

Table 3: Soil moisture in different treatments at monthly intervals under pot culture studies (sterile)

Soil moisture (%)						
Treatments	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 :*Trichoderma harzianum* (CKT isolate)	34.4^{ab}	36.8^{ab}	35.4^{ab}	34.8^{ab}	35.6^{ab}	35.8^{ab}
T_2 :*Pseudomonas fluorescens* (PAP isolate)	34.1^{ab}	35.6^{bc}	34.2^{bc}	33.4^{bc}	34.8^{bc}	34.9^{bc}
T_3 :*Trichoderma viride* (KAU ref. culture	33.6^{bc}	35.3^{bc}	34.1^{bc}	33.3^{bc}	34.1^{bc}	34.7^{bc}
T_4 :*Pseudomonas fluorescens* (KAU ref. culture)	33.2^{bc}	34.7^{cd}	34.8^{bc}	33.6^{bc}	34.5^{bc}	34.1^{bc}
T_5 :Package of Practices Recommendations (KAU,2016)	30.5^{cd}	29.2^{cd}	32.8^{cd}	32.4^{cd}	32.6^{cd}	32.2^{cd}
T_6 :Organic Package of Practices (KAU,2009)	35.7^{a}	37.4^{a}	38.4^{a}	36.5^{a}	37.3^{a}	36.4^{a}
T_7 :Chemical treatment (Kocide 2g L^{-1})	26.2^{e}	26.8^{f}	27.5^{e}	26.9^{e}	27.6^{e}	28.2^{e}
T_8 :Absolute control (No inoculants, no pathogen)	27.8^{de}	29.7^{de}	30.4^{de}	30.7^{de}	31.8^{de}	30.7^{de}
T_9 :Control (No inoculants, with pathogen)	27.4^{de}	28.5^{e}	29.8^{de}	30.4^{de}	31.7^{de}	30.5^{de}
$CD_{(0.05)}$	0.15	0.18	0.16	0.17	0.17	0.18

Initial soil moisture: 31.5 %

Each value represents an average of three replications

DAP-Days After Planting

Table 4: Soil pH in different treatments at monthly intervals under pot culture studies (sterile)

Treatments	Soil pH					
	30 DAP	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 :*Trichoderma harzianum* (CKT isolate)	7.2	7.2	7.1	7.2	7.0	7.1
T_2 :*Pseudomonas fluorescens* (PAP isolate)	7.3	7.3	7.2	7.1	7.0	7.0
T_3 :*Trichoderma viride* (KAU ref. culture	7.3	7.2	7.1	7.1	7.1	7.0
T_4 :*Pseudomonas fluorescens* (KAU ref. culture)	7.2	7.3	7.2	7.2	7.0	7.1
T_5 :Package of Practices Recommendations (KAU,2016)	7.6	7.6	7.5	7.5	7.5	7.4
T_6 :Organic Package of Practices (KAU,2009)	7.5	7.5	7.4	7.4	7.3	7.3
T_7 :Chemical treatment (Kocide 2g L^{-1})	7.4	7.4	7.3	7.3	7.2	7.2
T_8 :Absolute control (No inoculants, no pathogen)	7.2	7.2	7.1	7.2	7.1	7.1
T_9 :Control (No inoculants, with pathogen)	7.2	7.1	7.2	7.1	7.1	7.1
$CD_{(0.05)}$	NS	NS	NS	NS	NS	NS

Initial soil pH: 7.2

Each value represents an average of three replications

NS – Non- significant

DAP-Days After Planting

Table 5: Soil respiration (CO_2 evolution) in different treatments at monthly intervals under pot culture studies (sterile)

Soil respiration (mg CO_2 g^{-1} day^{-1})						
Treatments	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 :*Trichoderma harzianum* (CKT isolate)	11.84^{ab}	11.50^{a}	10.52^{ab}	10.56^{ab}	9.68^{ab}	8.71^{a}
T_2 :*Pseudomonas fluorescens* (PAP isolate)	11.68^{ab}	11.25^{ab}	10.35^{ab}	10.42^{ab}	9.16^{ab}	8.45^{ab}
T_3 :*Trichoderma viride (*KAU ref. culture)	11.56^{ab}	11.17^{ab}	10.44^{ab}	10.37^{ab}	9.48^{ab}	8.18^{ab}
T_4 :*Pseudomonas fluorescens* (KAU ref. culture)	11.72^{ab}	11.32^{ab}	10.43^{ab}	10.46^{ab}	9.54^{ab}	8.52^{ab}
T_5 :Package of Practices Recommendations (KAU, 2016)	8.26^{bc}	9.73^{bc}	9.12^{bc}	8.94^{bc}	6.83^{bc}	6.24^{bc}
T_6 :Organic Package of Practices (KAU,2009)	11.92^{a}	11.54^{a}	10.63^{a}	10.76^{a}	9.72^{a}	8.74^{a}
T_7 :Chemical treatment (Kocide 2g L^{-1})	0.81^{c}	0.86^{c}	1.03^{c}	1.01^{c}	1.24^{c}	1.22^{d}
T_8 :Absolute control (No inoculants, no pathogen)	0.89^{c}	0.90^{c}	1.06^{c}	1.04^{c}	1.28^{c}	1.25^{d}
T_9 :Control (No inoculants, with pathogen)	0.84^{c}	0.92^{c}	1.09^{c}	1.05^{c}	2.16^{c}	2.34^{c}
$CD_{(0.05)}$	0.92	0.52	0.51	0.80	0.82	0.86

Initial soil respiration: 0.83 mg CO_2 g^{-1} day^{-1}

Each value represents an average of three replications

DAP-Days After Planting

respectively). Lowest soil respiration (0.81 mg CO_2 g^{-1} day^{-1}) was given by treatment T_7 (Chemical treatment).

At 180 DAP, highest soil respiration (8.74 mg CO_2 g^{-1} day^{-1}) was recorded in treatment T_6 followed by treatments T_1, T_4, T_2 and T_3 (8.71, 8.52, 8.45 and 8.18 mg CO_2 g^{-1} day^{-1}) and lowest in T_7 (1.22 mg CO_2 g^{-1} day^{-1}).

4.1.3 Effect of micro-climatic and soil parameters on the population of *Trichoderma* sp. and *Pseudomonas fluorescens* under pot culture studies (sterile)

Micro-climatic and soil parameters such as soil temperature, soil moisture, soil pH and soil respiration were correlated (Spearman's correlation) with the population of *Trichoderma harzianum* (CKT), *Trichoderma viride (*KAU ref. culture), *Pseudomonas fluorescens* (PAP isolate) and *Pseudomonas fluorescens* (KAU ref. culture). It was found that population of all the four microbial isolates were negatively correlated with soil temperature and positively correlated with soil moisture. There was no significant correlation between the population of microbes and soil pH and soil respiration (Table 6).

Table 6: Correlation of microclimatic and soil parameters with population of *Trichoderma* sp. and *Pseudomonas fluorescens* under pot culture studies (sterile)

Parameters	Correlation coefficient			
	Population of *Trichoderma harzianum* (CKT)	**Population of *Trichoderma viride* (KAU ref. culture)**	**Population of *Pseudomonas fluorescens* (PAP)**	**Population of *Pseudomonas fluorescens* (KAU ref. culture)**
Soil temperature	- 0.284*	- 0.239*	- 0.205*	- 0.331*
Soil moisture	0.213*	0.357*	0.382*	0.426*
Soil pH	0.194	0.276	0.405	0.294
Soil respiration	0.018	0.362	0.114	0.318

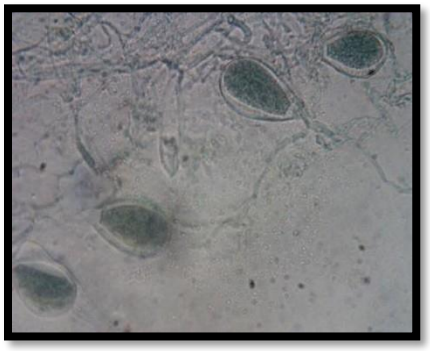
*Correlation significant at 5 % level of significance

4.1.4 Effect of different treatments on per cent disease incidence and per cent disease index of foot rot of black pepper under pot culture studies (sterile)

All plants except T_8 (Absolute control) were challenge inoculated with *Phytophthora capsici* (Plate 3). Per cent disease incidence and per cent disease index were recorded at 7 and 14 DAI (Table 7, Plate 4a, 4b). Minimum disease incidence (0%) was observed in the case of treatments T_8 (Absolute control) and T_7 (Chemical treatment). Also, treatments T_1 [*Trichoderma harzianum* (CKT)] and T_2 [*Pseudomonas fluorescens* (PAP)] showed low per cent disease incidence (16.67% each) and per cent disease index (10% and 15 % respectively) at 7 DAI. Disease incidence at 7 DAI was maximum (100%) in the treatment T_9 (Control) followed by treatments T_5 (KAU POP, 2016) and T_6 (Organic POP) (50% each). At 7 DAI, maximum foot rot disease index (85%) was recorded in the treatment T_9 (Control) followed by treatments T_5 (KAU POP, 2016) and T_6 (Organic POP) (45, 40% respectively). Least per cent disease index (0%) was recorded in the case of treatments absolute control and chemical treatment. Treatments T_1 [*Trichoderma harzianum* (CKT)] and T_2 [*Pseudomonas fluorescens* (PAP)] showed low per cent disease incidence (16.67% each) and per cent disease index (10% and 15 % respectively) at 7 DAI.

At 14 DAI, minimum disease incidence and disease index (0%) were observed in the case of treatment absolute control. Highest per cent disease incidence (100%) was shown by the treatment control followed by treatments KAU POP 2016 and organic POP (66.67% each). Treatments T_1 [*Trichoderma harzianum* (CKT)] and T_2 [*Pseudomonas fluorescens* (PAP)] recorded low per cent disease incidence (16.67% each) and per cent disease index (20% each) at 14 DAI. Maximum per cent disease index was recorded in the treatment control (100%) followed by treatments KAU POP 2016 and organic POP (60 and 50 %).

Phytophthora capsici



Microscopic view of *Phytophthora capsici*

Plate 3 : Cultural and morphological characteristics of *Phytophthora capsici*

Table 7: Effect of different treatments on per cent disease incidence and per cent disease index of foot rot of black pepper 60 DAI under pot culture studies (sterile)

Treatments	Per cent disease incidence		Per cent disease index	
	7 DAI	14 DAI	7 DAI	14 DAI
T_1 :*Trichoderma harzianum* (CKT isolate)	16.67	16.67	10	20
T_2 :*Pseudomonas fluorescens* (PAP isolate)	16.67	16.67	15	20
T_3 :*Trichoderma viride (*KAU ref. culture)	33.33	50	30	40
T_4 :*Pseudomonas fluorescens* (KAU ref. culture)	26.67	50	20	35
T_5 :Package of Practices Recommendations (KAU, 2016)	50	66.67	45	60
T_6 :Organic Package of Practices (KAU,2009)	50	66.67	40	50
T_7 :Chemical treatment (Kocide 2g L^{-1})	0	16.67	0	20
T_8 :Absolute control (no inoculants, no pathogen)	-	-	-	-
T_9 :Control (no inoculants, with pathogen)	100	100	85	100

DAI – Days after inoculation with *Phytophthora capsici*

Table 8: Population of *Phytophthora capsici* in the potting mixture at 14 DAI

Treatments	Population of *Phytophthora capsici* in the potting mixture at 14 DAI (cfu g^{-1} soil)
T_1 :*Trichoderma harzianum* (CKT isolate)	0.1×10^2
T_2 :*Pseudomonas fluorescens* (PAP isolate)	0.2×10^2
T_3 :*Trichoderma viride (*KAU ref. culture)	0.5×10^2
T_4 :*Pseudomonas fluorescens* (KAU ref. culture)	0.4×10^2
T_5 :Package of Practices Recommendations (KAU, 2016)	0.7×10^2
T_6 :Organic Package of Practices (KAU,2009)	0.7×10^2
T_7 :Chemical treatment (Kocide 2g L^{-1})	0.1×10^2
T_8 :Absolute control (no inoculants, no pathogen)	0
T_9 :Control (no inoculants, with pathogen)	1.3×10^2

Each value represents an average of three replications

T_1 :*Trichoderma sp.*

(CKT isolate)

T_2 :*Pseudomonas* sp.

(PAP isolate)

T_3 :*Trichoderma sp.*

(KAU ref. culture)

T_4 :*Pseudomonas sp.*

(KAU ref. culture)

T_5 :POP (KAU, 2016)

Plate 4a. Black pepper plants at 14 days after artificial inoculation with *Phytophthora capsici*

T_6 :Organic POP

(KAU, 2009)

T_7 :Chemical treatment

(Kocide 2g L^{-1})

T_8 :Absolute control

T_9 :Control

Plate 4b. Black pepper plants at 14 days after artificial inoculation with *Phytophthora capsici*

Also, population of *Phytophthora capsici* in the potting mixture in every treatments were recorded at 14 DAI (Table 8). Treatments *Trichoderma harzianum* (CKT) and chemical treatment (Copper hydroxide) recorded least population of *P. capsici* (0.1×10^2 cfu g^{-1} soil) followed by *Pseudomonas fluorescens* (PAP) (0.2×10^2 cfu g^{-1} soil). Treatment T_9 (Control) recorded highest population of *P. capsici* (1.3×10^2 cfu g^{-1} soil). However, in the case of treatment T_8 (Absolute control), no *P. capsici* population was recorded. Altogether, isolate *Trichoderma harzianum* (CKT) performed well in the case of *Phytophthora* disease management followed by *Pseudomonas fluorescens* (PAP).

4.1.5 Effect of different treatments on biometric parameters under pot culture studies

4.1.5.1 Effect of different treatments on number of leaves at monthly intervals under pot culture studies (sterile)

Number of leaves per plant was recorded in every treatment at monthly intervals under pot culture experiment (Table 9). There were no significant differences among the treatments in the case of number of leaves up to 120 DAP.

Highest number of leaves (5.85) at 150 DAP was recorded in plants treated with Package of Practices Recommendations (KAU, 2016) followed by treatments T_1 (CKT) and T_2 (PAP) (5.45 and 5.15 respectively) which were on par with T_5. Lowest number of leaves (3.70) was observed in treatment T_9 (Control).

At 180 DAP, maximum number of leaves (6.55) was recorded in the treatment T_5 (KAU POP, 2016) followed by treatments T_1 (CKT) and T_2 (PAP) (6.35 and 6.05 respectively) which were on par with T_5. Minimum number of leaves (0.00) was observed in the case of treatment T_9 (Control).

4.1.5.2 Effect of different treatments on plant height at monthly intervals under pot culture studies (sterile)

Plant height per plant recorded under pot culture experiment at monthly intervals are presented in Table 10. There were no significant differences among the treatments up to 90 DAP.

Maximum plant height (23.28 cm) at 120 DAP was recorded in the treatment T_5 (KAU POP, 2016) followed by the treatments T_1 (CKT) and T_2 (PAP) (22.85 cm and 22.60 cm respectively) which were on par with T_5. Treatment T_9 (Control with pathogen) recorded minimum plant height (18.40 cm).

At 180 DAP, highest plant height (28.46 cm) was recorded in the treatment T_5 (KAU POP, 2016) followed by treatments T_1 (CKT) and T_2 (PAP) (28.18 cm and 27.85 cm respectively) which were on par with T_5. Lowest plant height (15.42 cm) was recorded in the case of treatment T_9.

4.1.5.3 Effect of different treatments on number of nodes at monthly intervals under pot culture studies (sterile)

Number of nodes per plant was recorded under pot culture experiment at monthly intervals (Table 11). There were no significant differences among the treatments up to 120 DAP.

4.1.5.4 Effect of different treatments on internodal length at monthly intervals under pot culture studies (sterile)

Internodal length of black pepper plants was recorded at monthly intervals under pot culture (Table 12). There were no significant differences between the treatments in the case of internodal length up to 150 DAP.

Table 9: Effect of different treatments on number of leaves at monthly intervals under pot culture studies (sterile)

Treatments	Number of leaves					
	30 DAP	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 :*Trichoderma harzianum* (CKT isolate)	1.00	1.95	3.25	4.20	5.45^{ab}	6.35^{ab}
T_2 :*Pseudomonas fluorescens* (PAP isolate)	1.00	1.85	3.20	4.05	5.15^{ab}	6.05^{ab}
T_3 :*Trichoderma viride* (KAU ref. culture)	1.00	1.60	2.80	3.85	4.25^{cd}	5.30^{cd}
T_4 :*Pseudomonas fluorescens* (KAU ref. culture)	1.00	1.65	2.85	3.95	4.70^{bc}	5.60^{bc}
T_5 :Package of Practices Recommendations (KAU, 2016)	1.00	2.15	3.50	4.35	5.85^{a}	6.55^{a}
T_6 :Organic Package of Practices (KAU,2009)	1.00	1.80	3.15	4.00	4.80^{bc}	5.65^{bc}
T_7 :Chemical treatment (Kocide 2g L^{-1})	1.00	1.45	2.65	3.75	4.15^{cd}	4.90^{cd}
T_8 :Absolute control (No inoculants, no pathogen)	1.00	1.40	2.55	3.65	4.10^{d}	4.85^{d}
T_9 :Control (No inoculants, with pathogen)	1.00	1.30	2.50	3.55	3.70^{e}	0.00^{e}
$CD_{(0.05)}$	NS	NS	NS	NS	0.41	0.58

NS – Non-significant

Each value represents an average of three replications

DAP-Days After Planting

Table 10: Effect of different treatments on plant height at monthly intervals under pot culture studies (sterile)

Treatments	Plant height (cm)					
	30 DAP	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 :*Trichoderma harzianum* (CKT isolate)	13.18	16.52	19.73	22.85^{ab}	25.14^{ab}	28.18^{ab}
T_2 :*Pseudomonas fluorescens* (PAP isolate)	13.02	16.27	19.55	22.60^{ab}	24.97^{ab}	27.85^{ab}
T_3 :*Trichoderma viride* (KAU ref. culture)	12.90	15.63	18.74	20.3^{cd}	22.13^{cd}	25.29^{cd}
T_4 :*Pseudomonas fluorescens* (KAU ref. culture)	12.93	15.84	18.97	20.76^{cd}	22.74^{cd}	25.67^{cd}
T_5 :Package of Practices Recommendations (KAU, 2016)	13.65	17.36	20.16	23.28^{a}	25.54^{a}	28.46^{a}
T_6 :Organic Package of Practices (KAU,2009)	13.14	16.18	19.22	21.67^{bc}	23.26^{bc}	26.38^{bc}
T_7 :Chemical treatment (Kocide 2g L^{-1})	12.37	14.80	17.82	18.96^{de}	20.85^{de}	22.54^{de}
T_8 :Absolute control (No inoculants, no pathogen)	12.03	14.26	17.73	18.83^{de}	20.63^{de}	22.26^{de}
T_9 :Control (No inoculants, with pathogen)	11.94	14.20	17.10	18.40^{e}	20.51^{e}	15.42^{a}
$CD_{(0.05)}$	NS	NS	NS	3.13	3.24	3.48

NS – Non-significant

Each value represents an average of three replications

DAP-Days After Planting

Table 11: Effect of different treatments on number of nodes at monthly intervals under pot culture studies (sterile)

Treatments	Number of nodes					
	30 DAP	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 :*Trichoderma harzianum* (CKT isolate)	2.00	3.15	3.75	4.05	5.25^{ab}	6.25^{ab}
T_2 :*Pseudomonas fluorescens* (PAP isolate)	2.00	2.95	3.50	3.95	4.90^{ab}	6.05^{ab}
T_3 :*Trichoderma viride* (KAU ref. culture)	2.00	2.50	3.05	3.55	4.45^{bc}	5.30^{bc}
T_4 :*Pseudomonas fluorescens* (KAU ref. culture)	2.00	2.60	3.20	3.75	4.60^{bc}	5.40^{bc}
T_5 :Package of Practices Recommendations (KAU, 2016)	2.00	3.35	3.95	4.45	5.65^{a}	6.55^{a}
T_6 :Organic Package of Practices (KAU,2009)	2.00	2.75	3.25	3.80	4.75^{bc}	5.45^{bc}
T_7 :Chemical treatment (Kocide 2g L^{-1})	2.00	2.35	2.95	3.45	3.95^{cd}	4.85^{cd}
T_8 :Absolute control (No inoculants, no pathogen)	2.00	2.30	2.85	3.40	3.85^{cd}	4.80^{d}
T_9 :Control (No inoculants, with pathogen)	2.00	2.25	2.80	3.30	3.75^{d}	2.25^{e}
$CD_{(0.05)}$	NS	NS	NS	NS	0.73	0.95

NS – Non-significant

Each value represents an average of three replications

DAP-Days After Planting

Table 12: Effect of different treatments on internodal length at monthly intervals under pot culture studies (sterile)

Treatments	Internodal length (cm)					
	30 DAP	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 :*Trichoderma harzianum* (CKT isolate)	2.43	2.96	3.57	3.96	4.54	5.21^{a}
T_2 :*Pseudomonas fluorescens* (PAP isolate)	2.38	2.92	3.45	3.84	4.42	5.13^{ab}
T_3 :*Trichoderma viride (*KAU ref. culture)	2.26	2.87	3.20	3.72	4.27	4.84^{bc}
T_4 :*Pseudomonas fluorescens* (KAU ref. culture)	2.34	2.81	3.24	3.78	4.36	4.95^{bc}
T_5 :Package of Practices Recommendations (KAU, 2016)	2.45	3.13	3.75	4.12	4.71	5.46^{a}
T_6 :Organic Package of Practices (KAU,2009)	2.22	2.85	3.32	3.80	4.16	5.10^{ab}
T_7 :Chemical treatment (Kocide 2g L^{-1})	2.18	2.72	3.14	3.67	4.08	4.53^{bc}
T_8 :Absolute control (No inoculants, no pathogen)	2.17	2.68	3.00	3.62	3.97	4.36^{c}
T_9 :Control (No inoculants, with pathogen)	2.16	2.64	2.96	3.54	3.94	3.92^{d}
$CD_{(0.05)}$	NS	NS	NS	NS	NS	0.17

NS – Non-significant

Each value represents an average of three replications

DAP-Days After Planting

At 180 DAP, treatment T_5 (KAU POP, 2016) showed maximum internodal length (5.46 cm) followed by treatments T_1 (CKT), T_2 (PAP) and T_6 (Organic POP) which were (5.21 cm, 5.13 cm and 5.10 cm respectively) on par with T_5. Lowest internodal length (3.92 cm) was recorded in the case of treatment T_9.

Maximum number of nodes (5.65) at 150 DAP was recorded in the treatment T_5 (KAU POP, 2016) followed by treatments T_1 (CKT) and T_2 (PAP) (5.25 and 4.90 respectively) which were on par with T_5. Minimum number of nodes (3.75) was recorded in the case of treatment T_9.

At 180 DAP, maximum number of nodes (6.55) was recorded in the treatment T_5 followed by the treatments T_1 and T_2 (6.25 and 6.05 respectively) which were on par with T_5. Treatment T_9 showed least number of nodes (2.25).

4.1.5.5 Effect of different treatments on number of lateral branches at monthly intervals under pot culture studies (sterile)

Number of lateral branches per plant was recorded at monthly intervals under pot culture experiment (Table 13). There were no lateral branches formed up to 120 DAP. After that, there were lateral branches. However, treatments were not significantly different among each other.

Table 13: Effect of different treatments on number of lateral branches at monthly intervals under pot culture studies (sterile)

Treatments	Number of lateral branches	
	150 DAP	180 DAP
T_1 :*Trichoderma harzianum* (CKT isolate)	0.16	0.33
T_2 :*Pseudomonas fluorescens* (PAP isolate)	0.16	0.16
T_3 :*Trichoderma viride* (KAU ref. culture)	0	0
T_4 :*Pseudomonas fluorescens* (KAU ref. culture)	0	0
T_5 :Package of Practices Recommendations (KAU, 2016)	0.33	0.33
T_6 :Organic Package of Practices (KAU,2009)	0.16	0.16
T_7 :Chemical treatment (Kocide 2g L^{-1})	0	0
T_8 :Absolute control (No inoculants, no pathogen)	0	0
T_9 :Control (No inoculants, with pathogen)	0	0
$CD_{(0.05)}$	NS	NS

NS – Non-significant

Each value represents an average of three replications

DAP-Days After Planting

4.1.6 Effect of different treatments on nutrient status under pot culture studies

Organic carbon, available nutrients (Nitrogen, Phosphorus and Potassium) and pH of potting mixture before planting and six months after planting were recorded in Table 14. Initial sterilized potting mixture contained high level of organic carbon (2.10%), medium level of available nitrogen (470.42 kg ha^{-1}), high level of available phosphorus (52.46 kg ha^{-1}), high level of available potassium (295.18 kg ha^{-1}) and neutral pH (7.2).

Table 14: Initial and final nutrient status under pot culture studies

INITIAL NUTRIENT STATUS										
Item	**Organic Carbon (%)**		**Available Nitrogen (kg ha^{-1})**		**Available Phosphorus (kg ha^{-1})**		**Available Potassium (kg ha^{-1})**		**pH**	
Initial potting mixture sample	2.10	High	470.42	Medium	52.46	High	295.18	High	7.2	Neutral
FINAL NUTRIENT STATUS										
Treatments	**Organic Carbon (%)**		**Available Nitrogen (kg ha^{-1})**		**Available Phosphorus (kg ha^{-1})**		**Available Potassium (kg ha^{-1})**		**pH**	
T_1 : *Trichoderma harzianum* (CKT isolate)	2.45	High	548.86	Medium	65.48	High	316.74	High	7.1	Neutral
T_2 : *Pseudomonas fluorescens* (PAP isolate)	2.34	High	524.15	Medium	63.57	High	312.86	High	7.0	Neutral
T_3 : *Trichoderma viride* (KAU ref. culture)	2.36	High	528.64	Medium	61.23	High	305.43	High	7.0	Neutral
T_4 : *Pseudomonas fluorescens* (KAU ref. culture)	2.39	High	535.36	Medium	60.78	High	310.85	High	7.1	Neutral
T_5 : Package of Practices Recommendations (KAU, 2016)	2.54	High	608.24	High	78.32	High	357.68	High	7.4	Neutral
Package of Practices (KAU,2009)	2.66	High	552.27	Medium	67.84	High	332.26	High	7.3	Neutral
T_7 : Chemical treatment (Kocide 2g L^{-1})	1.97	High	441.38	Medium	49.67	High	295.87	High	7.2	Neutral
T_8 : Absolute control	2.14	High	479.36	Medium	51.58	High	297.52	High	7.1	Neutral
T_9 : Control (with pathogen)	2.19	High	490.56	Medium	52.51	High	299.34	High	7.1	Neutral

After six months of planting, highest organic carbon (2.66% - high) was observed in the treatment T_6 (Organic Package of Practices). Lowest OC (1.97 % - high) was recorded in the treatment T_7 (Chemical treatment). Maximum available nitrogen (608.24 kg ha^{-1} - high) was observed in the treatment T_5 (KAU POP, 2016). Lowest N was found in the treatment T_7 (Chemical treatment) and which is 441.38 kg ha^{-1} (medium). Highest available phosphorus (78.32 kg ha^{-1} - high) and available potassium (357.68 kg ha^{-1} high) were recorded in the case of treatment T_5 (KAU POP, 2016). Lowest P and K were estimated in treatment T_7 (Chemical treatment) and they were 49.67 kg ha^{-1} (high) and 295.87 kg ha^{-1} (high) respectively. Highest pH (7.4) was recorded in the treatment T_5 (KAU POP, 2016) and lowest (7.0) in the case of treatments T_2 [*Pseudomonas fluorescens* (PAP)] and T_3 [*Trichoderma viride* (KAU ref. culture)].

4.1.7 Selection of best treatment based on biometric and biological parameters under pot culture studies

On the basis of biometric (number of leaves, plant height, number of lateral branches, number of nodes and internodal length) and biological parameters (soil respiration), the best treatment was found out by pooled ranking method using statistical methods (Table 15). T_1 (*Trichoderma harzianum*) is ranked first followed by T_2 (*Pseudomonas fluorescens*) and T_5 (KAU POP, 2016) under pot culture studies.

4.2 Screening of abiotic stress tolerant strains of *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) for growth promotion and *Phytophthora* disease management under field condition

Six months old black pepper plants were planted in the plot (Plate 5) allotted by the Department of Plantation crops and Spices in College of Horticulture, Vellanikkara. Evaluation of the abiotic stress tolerant strains of *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) for plant growth promotion and disease management in black pepper was carried out under

Table 15: Selection of best treatment based on biometric and biological parameters under pot culture studies

Treatments	Number of leaves	Plant height (cm)	Number of branches	Number of nodes	Internodal length (cm)	Soil respiration (mg CO_2 g^{-1} day^{-1})	Ranking by pooled ranking method
	180 DAP	180 DAP	180 DAP	180 DAP	180 DAP	180 DAP	
T_1 : *Trichoderma harzianum* (CKT isolate)	6.35^{ab}	28.13^{ab}	0.33	6.25^{ab}	5.21^{ab}	8.71^{a}	1
T_2 : *Pseudomonas fluorescens* (PAP isolate)	6.05^{ab}	27.85^{ab}	0.16	6.10^{ab}	5.13^{ab}	8.45^{ab}	2
T_3 : *Trichoderma viride* (KAU ref. culture)	5.30^{bc}	25.29^{bc}	-	5.30^{bc}	4.48^{bc}	8.18^{ab}	5
T_4 : *Pseudomonas fluorescens* (KAU ref. culture)	5.60^{bc}	25.67^{bc}	-	5.40^{bc}	4.95^{bc}	8.52^{ab}	5
T_5 : Package of Practices Recommendations (KAU, 2016)	6.55^{a}	28.46^{a}	0.33	6.55^{a}	5.46^{a}	6.24^{bc}	3
T_6 : Organic Package of Practices (KAU,2009)	5.65^{bc}	26.38^{bc}	0.16	5.45^{bc}	5.10^{ab}	8.74^{a}	4
T_7 : Chemical treatment (Kocide 2g L^{-1})	5.20^{bc}	22.54^{cd}	-	4.85^{cd}	4.53^{bc}	1.22^{d}	6
T_8 : Absolute control (no inoculants, no pathogen)	5.15^{c}	22.26^{cd}	-	4.80^{cd}	4.36^{bc}	1.25^{d}	7
T_9 : Control (no inoculants, with pathogen)	0.00^{d}	15.42^{d}	-	2.25^{d}	3.92^{c}	2.34^{c}	8

field conditions based on chemical analysis of soil (organic carbon and available nitrogen, available phosphorus and available potassium), population of *Trichoderma* sp. and *Pseudomonas fluorescens*, functional efficiency of *Trichoderma* sp. and *Pseudomonas fluorescens*, micro-climatic and soil parameters (soil temperature, soil moisture, soil pH and soil respiration), per cent disease incidence and biometric parameters (number of leaves, plant height, number of lateral branches, number of nodes and internodal length).

4.2.1 Population of *Trichoderma* sp. and *Pseudomonas fluorescens* in soil at monthly intervals (cfu g^{-1}) under field condition

The population of *Trichoderma* sp. and *Pseudomonas fluorescens* at monthly intervals under field condition is presented in Table 16. The population gradually declined from 30 DAP to 180 DAP. Initial population of *Trichoderma* sp. and *Pseudomonas fluorescens* was absent in the field soil.

Population was present in only treatments T_1 [*Trichoderma harzianum* (CKT) + KAU POP 2016], T_2 [*Pseudomonas fluorescens* (PAP) + KAU POP 2016], T_3 [*Trichoderma viride* (KAU reference culture) + KAU POP 2016], T_4 [*Pseudomonas fluorescens* (KAU reference culture) + KAU POP 2016], T_5 [*Trichoderma harzianum* (CKT)], T_6 [*Pseudomonas fluorescens* (PAP)], T_7 [*Trichoderma viride* (KAU reference culture)] and T_8 [*Pseudomonas fluorescens* (KAU reference culture)] after 30 days of planting. However, the population of *Trichoderma* sp. and *Pseudomonas fluorescens* declined gradually from 30 DAP to 180 DAP.

Statistically, there were significant differences among different treatments at 5 per cent level of significance. At 30 DAP, maximum population was found in the treatment T_6 (7.5×10^5 cfu g^{-1}) followed by T_8 (6.5×10^5 cfu g^{-1}) and minimum in T_3 (4×10^4 cfu g^{-1}). Highest population at the end of the experiment was found in treatment T_6 (2.5×10^3 cfu g^{-1}) followed by T_8 (2×10^3 cfu g^{-1}). Lowest population was recorded in treatment T_2 (2.0×10^2 cfu g^{-1}) and no population was found in the treatment T_3 at

Plate 5 : Overview of the field

Table 16: Population of *Trichoderma* sp. and *Pseudomonas fluorescens* in soil at monthly intervals (cfu g^{-1})

Isolates	30 DAP	60 DAP	90 DAP	120 DAP	150 DAP	180 DAP
T_1: *Trichoderma harzianum* (CKT) + KAU POP 2016	4.5×10^4 (0.65)	2×10^4 (1.26)	5×10^3 (0.69)	2.5×10^3 (1.39)	6.5×10^2 (0.81)	3.5×10^2 (0.65)
T_2: *Pseudomonas fluorescens* (PAP) + KAU POP 2016	5×10^5 (1.69)	3.5×10^5 (2.54)	4.5×10^4 (1.65)	6×10^3 (1.77)	4.5×10^2 (0.64)	2×10^2 (0.46)
T_3: *Trichoderma viride* (KAU reference culture) + KAU POP 2016	4×10^4 (0.56)	2.5×10^4 (0.86)	3×10^3 (0.46)	1.5×10^3 (0.65)	2.5×10^2 (0.39)	0×10^2 (0.00)
T_4: *Pseudomonas fluorescens* (KAU reference culture) + KAU POP 2016	4.5×10^5 (2.65)	3×10^5 (2.46)	4×10^4 (2.15)	2.5×10^4 (2.26)	3×10^3 (1.46)	1.5×10^3 (1.22)
T_5: *Trichoderma harzianum* (CKT)	6×10^4 (0.76)	3.5×10^4 (1.54)	7×10^3 (0.84)	5.5×10^3 (1.74)	8×10^2 (0.90)	4.5×10^2 (0.74)
T_6: *Pseudomonas fluorescens* (PAP)	7.5×10^5 (2.81)	5×10^5 (2.69)	6.5×10^4 (2.39)	4.5×10^4 (2.69)	6.5×10^3 (1.81)	2.5×10^3 (1.55)
T_7: *Trichoderma viride* (KAU reference culture)	5.5×10^4 (0.74)	3×10^4 (1.46)	6×10^3 (0.77)	3.5×10^3 (1.54)	5.5×10^2 (0.74)	3×10^2 (0.59)
T_8: *Pseudomonas fluorescens* (KAU reference culture)	6.5×10^5 (1.87)	4×10^5 (2.59)	5.5×10^4 (1.81)	2.5×10^4 (2.65)	5×10^3 (1.69)	2×10^3 (1.41)
T_9: Package of Practices Recommendations (KAU, 2016)	0	0	0	0	0	0
T_{10}: Organic Package of Practices (KAU,2009)	0	0	0	0	0	0
T_{11}: Chemical treatment (Kocide 2g L^{-1})	0	0	0	0	0	0
T_{12}: Absolute control	0	0	0	0	0	0
CD $_{(0.05)}$	0.19	0.24	0.16	0.17	0.13	0.16

Initial *Trichoderma* population : Absent, Initial *Pseudomonas fluorescens* population : Absent
Each value represents an average of three replications, Logarithmic transformed values are given in parentheses

the end of the experiment. However, population of *Trichoderma* sp. and *Pseudomonas fluorescens* were absent in the case of treatments T_9 (Package of Practices Recommendations), T_{10} (Organic Package of Practices), T_{11} (Chemical treatment) and T_{12} (Absolute control) throughout the experiment.

4.2.2 Screening of *Trichoderma* and *Pseudomonas fluorescens* for functional efficiency and antagonistic activity

All the isolates obtained were screened for ammonia, IAA, HCN and siderophore production at monthly intervals for a period from December 2017 to May 2018 (Table 17 - Table 20) and antagonistic activity of *Trichoderma harzianum* and *Pseudomonas fluorescens* were screened.

4.2.2.1 Ammonia production

Isolates obtained from the treatments T_1 (CKT+ KAU POP 2016), T_4 [*Pseudomonas fluorescens* (KAU ref. culture) + KAU POP], T_5 (CKT) and T_8 [*Pseudomonas fluorescens* (KAU ref. culture)] produced ammonia at a high intensity at 30 DAP (December, 2017) and 60 DAP (January, 2018) (Plate 6). Treatments T_2 (PAP + KAU POP), T_3 (*Pseudomonas fluorescens* (KAU ref. culture) + KAU POP)], T_6 (PAP) and T_7 [*Trichoderma viride* (KAU reference culture)] recorded medium ammonia production (Table 17). Thereafter, production of ammonia declined gradually. At 150 and 180 DAP no ammonia was produced.

4.2.2.2 Indole Acetic Acid production

All the eight isolates obtained recorded high production of IAA (Table 18) at 30 DAP (December, 2017) and 60 DAP (January, 2018) (Plate 7). Later, IAA production decreased gradually. At 180 DAP (May, 2018), only two isolates T_5 (CKT) and T_6 (PAP) showed IAA production and with a low intensity. No IAA was produced by the other isolates at 180 DAP.

4.2.2.3 Hydrogen cyanide production

All the eight isolates (T_1, T_2, T_3, T_4, T_5, T_6, T_7 and T_8) obtained at 30 DAP (December, 2017) recorded HCN production (Plate 8) at a medium intensity (Table 19). At 60 DAP, only two isolates (T_5, $T_{8)}$ produced HCN and at a medium level. No HCN production was recorded from 90 DAP (February, 2018) to 180 DAP (May, 2018).

4.2.2.4 Siderophore production

Among the eight isolates obtained at 30 DAP (December, 2017), only three isolates (T_5, T_6 and T_8) produced siderophore (Table 20). At 60 DAP, siderophore production was recorded by the isolates obtained from treatments T_6 and T_8 (Plate 9). Thereafter, siderophore production was found to be negative from 90 DAP to 180 DAP.

4.2.2.5 Antagonistic activity of Trichoderma harzianum **(CKT)** *and Pseudomonas fluorescens* **(PAP)** *in vitro*

Antagonistic activity of *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) against *Phytophthora capsici* were tested *in vitro* (Plate 10) and found out to be antagonistic with per cent inhibition of 64.3% and 57.1% respectively.

4.2.3 Effect of different treatments on micro-climatic and soil parameters under field condition

4.2.3.1 Effect of different treatments on soil temperature under field condition

Soil temperature was recorded at monthly intervals from December 2017 to May 2018 under field condition (Table 21). Soil temperature ranged from 25.8 – 31.8°C. Initial soil temperature in the field soil was 25.4°C. However, there were no significant differences among the treatments.

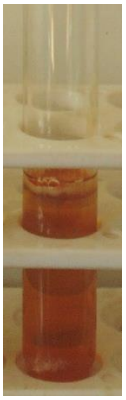
Table 17: Screening of *Trichoderma* sp. and *Pseudomonas fluorescens* for ammonia production

Ammonia production						
Isolates	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 : *Trichoderma harzianum* (CKT) + KAU POP 2016	+++	+++	++	++	-	-
T_2 : *Pseudomonas fluorescens* (PAP) + KAU POP 2016)	++	++	++	++	-	-
T_3 : *Trichoderma viride* (KAU reference culture) + KAU POP 2016	++	++	++	++	-	a
T_4 : *Pseudomonas fluorescens* (KAU reference culture) + KAU POP 2016	+++	+++	++	++	-	-
T_5 : *Trichoderma harzianum* (CKT)	+++	+++	++	++	-	-
T_6 : *Pseudomonas fluorescens* (PAP)	++	++	++	++	-	-
T_7 : *Trichoderma viride* (KAU reference culture)	++	++	++	++	-	-
T_8 : *Pseudomonas fluorescens* (KAU reference culture)	+++	+++	++	++	-	-
T_9 : Package of Practices Recommendations (KAU, 2016)	a	a	a	a	a	a
T_{10}: Organic Package of Practices (KAU,2009)	a	a	a	a	a	a
T_{11}: Chemical treatment (Kocide 2g L^{-1})	a	a	a	a	a	a
T_{12}: Absolute control	a	a	a	a	a	a

a: No isolate obtained

- Negative, + Low, ++ Medium, +++ High

T. harzianum (CKT)+POP



P. fluorescens (PAP)+POP

T. viride (KAU ref.)+POP

P. fluorescens (KAU ref.)+POP

T. harzianum (CKT)

P. fluorescens (PAP)

T. viride (KAU ref.)

P. fluorescens (KAU ref.)

Control

Plate 6 : Screening for ammonia production

Table 18: Screening of *Trichoderma* sp. and *Pseudomonas fluorescens* for indole acetic acid production

IAA production						
Isolates	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 : *Trichoderma harzianum* (CKT) + KAU POP 2016	+++	+++	+++	+	+	-
T_2 : *Pseudomonas fluorescens* (PAP) + KAU POP 2016	+++	+++	+++	+	+	-
T_3 : *Trichoderma viride* (KAU reference culture) + KAU POP 2016	+++	+++	+	+	+	a
T_4 : *Pseudomonas fluorescens* (KAU reference culture) + KAU POP 2016	+++	+++	+	+	+	-
T_5 : *Trichoderma harzianum* (CKT)	+++	+++	+++	+	+	+
T_6 : *Pseudomonas fluorescens* (PAP)	+++	+++	+++	+	+	+
T_7 : *Trichoderma viride* (KAU reference culture)	+++	+++	+++	+	+	-
T_8 : *Pseudomonas fluorescens* (KAU reference culture)	+++	+++	+++	+	+	-
T_9 : Package of Practices Recommendations (KAU, 2016)	a	a	a	a	a	a
T_{10}: Organic Package of Practices (KAU,2009)	a	a	a	a	a	a
T_{11}: Chemical treatment (Kocide 2g L^{-1})	a	a	a	a	a	a
T_{12}: Absolute control	a	a	a	a	a	a

a: No isolate obtained

- Negative, + Low, ++ Medium, +++ High

T. harzianum (CKT) + KAU POP

P. fluorescens (PAP) + KAU POP

T. viride (KAU ref. culture) + KAU POP

P. fluorescens (KAU ref. culture) + KAU POP

T. harzianum (CKT)

P. fluorescens (PAP)

T. viride (KAU reference culture)

P. fluorescens (KAU reference culture)

Control

Plate 7 : Screening for IAA production

Table 19: Screening of *Trichoderma* sp. and *Pseudomonas fluorescens* for hydrogen cyanide production

HCN production						
Isolates	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 : *Trichoderma harzianum* (CKT) + KAU POP 2016	++	-	-	-	-	-
T_2 : *Pseudomonas fluorescens* (PAP) + KAU POP 2016	++	-	-	-	-	-
T_3 : *Trichoderma viride* (KAU reference culture) + KAU POP 2016	++	-	-	-	-	a
T_4 : *Pseudomonas fluorescens* (KAU reference culture) + KAU POP 2016	++	-	-	-	-	-
T_5 : *Trichoderma harzianum* (CKT)	++	++	-	-	-	-
T_6 : *Pseudomonas fluorescens* (PAP)	++	-	-	-	-	-
T_7 : *Trichoderma viride* (KAU reference culture)	++	-	-	-	-	-
T_8 : *Pseudomonas fluorescens* (KAU reference culture)	++	++	-	-	-	-
T_9 : Package of Practices Recommendations (KAU, 2016)	a	a	a	a	a	a
T_{10}: Organic Package of Practices (KAU,2009)	a	a	a	a	a	a
T_{11}: Chemical treatment (Kocide 2g L^{-1})	a	a	a	a	a	a
T_{12}: Absolute control	a	a	a	a	a	a

a: No isolate obtained

- Negative, + Low, ++ Medium, +++ High

Control

T. harzianum (CKT) + KAU POP

P. fluorescens (PAP) + KAU POP

T. viride (KAU ref. culture) + KAU POP

P. fluorescens (KAU ref. culture) + KAU POP

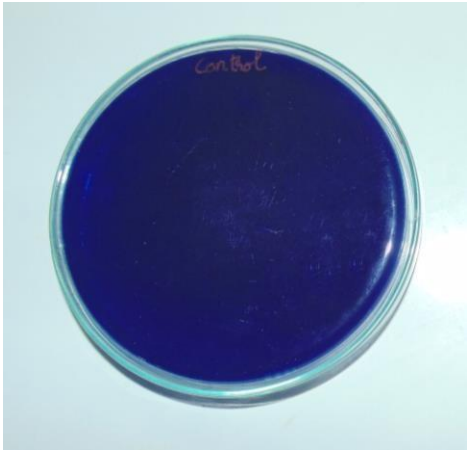
Plate 8 : Screening for HCN production

Table 20: Screening of *Trichoderma* sp. and *Pseudomonas fluorescens* for siderophore production

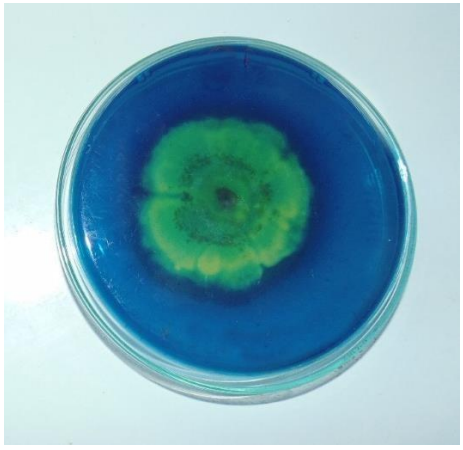
Siderophore production						
Isolates	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 : *Trichoderma harzianum* (CKT) + KAU POP 2016	-	-	-	-	-	-
T_2 : *Pseudomonas fluorescens* (PAP) + KAU POP 2016	-	-	-	-	-	-
T_3 : *Trichoderma viride* (KAU reference culture) + KAU POP 2016	-	-	-	-	-	a
T_4 : *Pseudomonas fluorescens* (KAU reference culture) + KAU POP 2016	-	-	-	-	-	-
T_5 : *Trichoderma harzianum* (CKT)	+	-	-	-	-	-
T_6 : *Pseudomonas fluorescens* (PAP)	+	+	-	-	-	-
T_7 : *Trichoderma viride* (KAU reference culture)	-	-	-	-	-	-
T_8 : *Pseudomonas fluorescens* (KAU reference culture	+	+	-	-	-	-
T_9 : Package of Practices Recommendations (KAU, 2016)	a	a	a	a	a	a
T_{10}: Organic Package of Practices (KAU,2009)	a	a	a	a	a	a
T_{11}: Chemical treatment (Kocide 2g L^{-1})	a	a	a	a	a	a
T_{12}: Absolute control	a	a	a	a	a	a

a: No isolate obtained

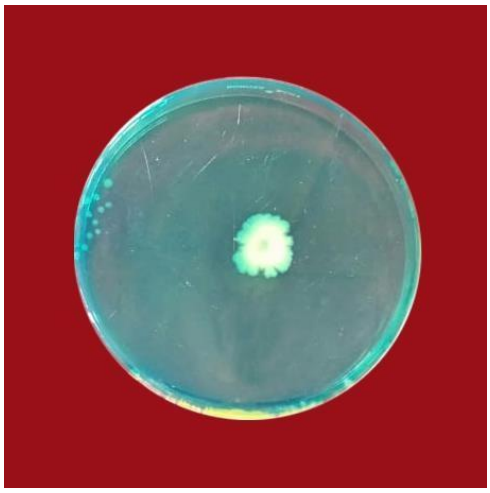
+ Present, - Absent



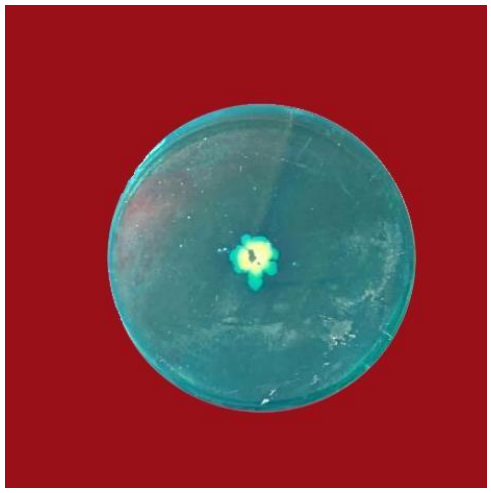
Control



Trichoderma harzianum (CKT)

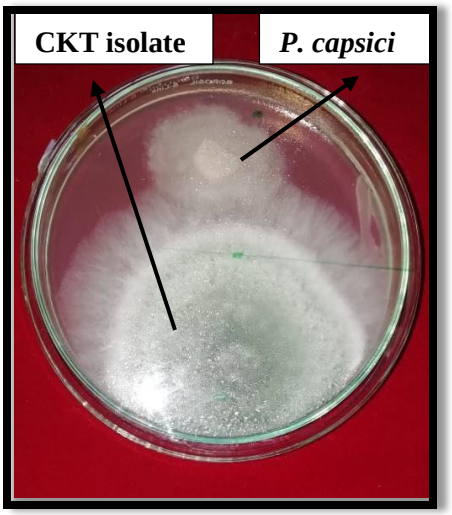


P. fluorescens (PAP)

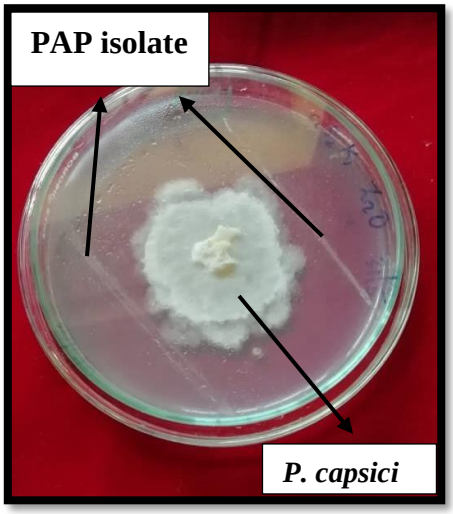


P. fluorescens (KAU reference culture)

Plate 9 : Screening for siderophore production



Trichoderma harzianum (CKT)



Pseudomonas fluorescens (PAP)

Control

Plate 10 : Antagonistic activity of *Trichoderma harzianum* (CKT) *and Pseudomonas fluorescens* (PAP) against *Phytophthora capsici*

Table 21: Effect of different treatments on soil temperature at monthly intervals under field condition

Soil temperature (°C)						
Treatments	**December**	**January**	**February**	**March**	**April**	**May**
T_1 : *Trichoderma harzianum* (CKT isolate) + KAU POP 2016	27.4	28.1	29.6	31.6	30.7	28.4
T_2 : *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016	27.3	28.2	29.7	31.5	30.3	28.0
T_3 : *Trichoderma viride* (KAU ref. culture) + KAU POP 2016	27.0	28.4	29.5	31.8	30.5	28.5
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016	27.2	28.3	29.6	31.7	30.4	28.7
T_5 : *Trichoderma harzianum* (CKT isolate)	26.6	27.5	28.4	30.4	29.4	27.6
T_6 : *Pseudomonas fluorescens* (PAP isolate)	26.2	27.4	28.2	30.3	29.2	27.4
T_7 : *Trichoderma viride (*KAU ref. culture)	26.5	27.3	28.3	29.6	29.1	27.5
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)	26.3	27.2	28.5	29.7	29.5	27.2
T_9 : Package of Practices Recommendations (KAU, 2016)	27.8	28.7	29.6	31.8	30.9	28.7
T_{10}: Organic Package of Practices (KAU,2009)	27.5	28.3	29.2	31.1	30.3	28.1
T_{11}: Chemical treatment (Kocide 2g L^{-1})	26.1	28.0	29.4	29.3	28.4	26.6
T_{12}: Absolute control	25.8	27.7	29.3	29.4	28.2	26.4
$CD_{(0.05)}$	NS	NS	NS	NS	NS	NS

Initial soil temperature: 25.4°C

Each value represents an average of four replications

NS- Non-significant

DAP – Days After Planting

4.2.3.2 Effect of different treatments on soil moisture under field condition

Soil moisture recorded at monthly intervals under field condition are presented in Table 22. Treatments were significantly different among each other. Initial field moisture content was 13.4 per cent.

Highest soil moisture content (19.4 %) was recorded in the treatment T_{10} [Organic Package of Practices (KAU, 2009)] followed by treatments T_1, T_2, and T_4 (18.6, 18.3, and 18.1 % respectively) which were on par with treatment T_{10} at 30 DAP. Lowest moisture content (13.6 %) was observed in treatment T_{11}.

At 180 DAP, maximum soil moisture content was recorded in the treatments T_{10} (19.6 %) and T_1 (19.4 %) which were on par with treatments T_2, T_4, T_3, T_5 and T_6 (18.9, 18.6, 18.4, 18.3 and 18.0 % respectively). Minimum moisture content (14.4 %) was observed in treatment T_{11}.

4.2.3.3 Effect of different treatments on soil pH under field condition

Soil pH was recorded at monthly intervals under field condition (Table 23). Soil pH was found to be decreasing gradually from 30 DAP to 180 DAP. It ranged from 6.4 to 5.6. Initial soil pH in the field soil was 5.7. However, there were no significant differences among the treatments.

4.2.3.4 Effect of different treatments on soil respiration (CO_2 evolution) under field condition

Soil respiration recorded at monthly intervals under field condition are presented in Table 24. Initial soil respiration in the field soil was 4.3 mg CO_2 g^{-1} day^{-1}. Treatments were significantly different among each other.

Highest CO_2 evolution was recorded in the treatments Organic Package of Practices (KAU, 2009) and *Trichoderma harzianum* (CKT isolate) + KAU POP 2016 (12.6 mg CO_2 g^{-1} day^{-1} and 12.2 mg CO_2 g^{-1} day^{-1} respectively) which were on par with

Table 22: Effect of soil moisture on different treatments at monthly intervals under field condition

Soil moisture (%)						
Treatments	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1:*Trichoderma harzianum* (CKT isolate) + KAU POP 2016	18.6^{ab}	17.7^{a}	17.5^{ab}	15.6^{a}	16.8^{a}	19.4^{a}
T_2 :*Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016	18.3^{ab}	17.4^{ab}	17.2^{ab}	15.3^{ab}	16.0^{ab}	18.9^{ab}
T_3 :*Trichoderma viride (*KAU ref. culture) + KAU POP 2016	17.9^{bc}	17.0^{ab}	16.6^{bc}	14.4^{bc}	15.6^{bc}	18.4^{ab}
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016	18.1^{ab}	17.2^{ab}	16.8^{bc}	14.8^{bc}	15.8^{bc}	18.6^{ab}
T_5 : *Trichoderma harzianum* (CKT isolate)	17.8^{bc}	16.6^{bc}	15.6^{cd}	14.5^{bc}	15.4^{bc}	18.3^{ab}
T_6 : *Pseudomonas fluorescens* (PAP isolate)	17.3^{bc}	16.2^{bc}	14.8^{de}	14.4^{bc}	14.9^{cd}	18.0^{ab}
T_7 : *Trichoderma viride (*KAU ref. culture)	16.4^{cd}	15.7^{cd}	14.3^{de}	13.2^{cd}	14.2^{cd}	17.7^{bc}
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)	16.7^{cd}	15.5^{cd}	15.2^{cd}	13.7^{cd}	14.6^{cd}	17.4^{bc}
T_9 : Package of Practices Recommendations (KAU, 2016)	15.8^{de}	14.8^{de}	13.8^{e}	12.5^{de}	13.1^{de}	15.8^{cd}
T_{10}: Organic Package of Practices (KAU,2009)	19.4^{a}	17.9^{a}	18.6^{a}	15.8^{a}	16.6^{a}	19.6^{a}
T_{11}: Chemical treatment (Kocide 2g L^{-1})	13.6^{f}	13.5^{e}	12.8^{f}	11.3^{e}	12.3^{e}	14.4^{d}
T_{12}: Absolute control	14.9^{e}	14.2^{de}	13.2^{ef}	11.9^{e}	12.5^{e}	14.8^{d}
$CD_{(0.05)}$	1.06	0.94	0.76	0.79	0.83	0.86

Initial soil moisture: 13.4 %

Each value represents an average of four replications

DAP – Days After Planting

Table 23: Effect of different treatments on soil pH at monthly intervals under field condition

Soil pH						
Treatments	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 : *Trichoderma harzianum* (CKT isolate) + KAU POP 2016	6.2	6.2	6.1	6.1	6.0	5.9
T_2 : *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016	6.3	6.3	6.2	6.2	6.1	6.0
T_3 : *Trichoderma viride (*KAU ref. culture) + KAU POP 2016	6.3	6.3	6.2	6.2	6.1	6.0
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016	6.3	6.2	6.2	6.1	6.0	5.9
T_5 : *Trichoderma harzianum* (CKT isolate)	5.7	5.7	5.6	5.6	5.6	5.5
T_6 : *Pseudomonas fluorescens* (PAPisolate)	5.8	5.7	5.7	5.7	5.6	5.5
T_7 : *Trichoderma viride (*KAU ref. culture)	5.8	5.8	5.8	5.7	5.7	5.6
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)	5.7	5.8	5.7	5.7	5.7	5.6
T_9 : Package of Practices Recommendations (KAU, 2016)	6.4	6.3	6.2	6.2	6.1	6.0
T_{10}: Organic Package of Practices (KAU,2009)	6.3	6.2	6.1	6.1	6.0	6.0
T_{11}: Chemical treatment (Kocide 2g L^{-1})	6.0	5.9	5.8	5.8	5.7	5.7
T_{12}: Absolute control	5.8	5.8	5.7	5.7	5.6	5.6
$CD_{(0.05)}$	NS	NS	NS	NS	NS	NS

Initial soil pH: 5.7

Each value represents an average of four replications

DAP – Days After Planting

Table 24: Effect of different treatments on soil respiration (CO_2 evolution) at monthly intervals under field condition

Soil respiration (mg CO_2 g^{-1} day^{-1})						
Treatments	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 : *Trichoderma harzianum* (CKT isolate) + KAU POP 2016	12.2^{a}	11.9^{a}	11.7^{ab}	11.3^{a}	10.7^{ab}	10.4^{ab}
T_2 : *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016	11.8^{ab}	11.5^{ab}	11.4^{ab}	10.8^{ab}	10.3^{ab}	10.1^{ab}
T_3 : *Trichoderma viride (*KAU ref. culture) + KAU POP 2016	11.3^{ab}	11.0^{ab}	10.8^{bc}	10.2^{ab}	9.6^{bc}	9.5^{bc}
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016	11.6^{ab}	11.2^{ab}	10.6^{bc}	10.4^{ab}	9.9^{bc}	9.8^{bc}
T_5 : *Trichoderma harzianum* (CKT isolate)	10.7^{bc}	10.4^{bc}	9.9^{cd}	9.5^{bc}	9.0^{bc}	8.9^{cd}
T_6 : *Pseudomonas fluorescens* (PAP isolate)	10.4^{bc}	10.1^{bc}	9.7^{cd}	9.1^{bc}	8.7^{cd}	8.3^{cd}
T_7 : *Trichoderma viride (*KAU ref. culture)	9.3^{cd}	9.8^{cd}	9.2^{cd}	8.6^{cd}	8.2^{cd}	7.6^{de}
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)	9.5^{cd}	9.2^{cd}	9.0^{cd}	8.8^{cd}	7.5^{de}	7.8^{de}
T_9 : Package of Practices Recommendations (KAU, 2016)	7.8^{de}	7.5^{de}	7.3^{de}	7.1^{de}	5.9^{ef}	5.7^{ef}
T_{10}: Organic Package of Practices (KAU,2009)	12.6^{a}	12.3^{a}	11.9^{a}	11.6^{a}	11.2^{a}	10.8^{a}
T_{11}: Chemical treatment (Kocide 2g L^{-1})	3.7^{f}	3.5^{f}	3.0^{f}	3.2^{f}	3.9^{g}	4.1^{g}
T_{12}: Absolute control	6.4^{ef}	6.2^{ef}	5.8^{ef}	5.3^{ef}	4.2^{fg}	4.6^{fg}
$CD_{(0.05)}$	0.96	0.93	1.09	0.79	0.98	0.81

Initial soil respiration: 4.3 mg CO_2 g^{-1} day^{-1}

Each value represents an average of four replications

DAP – Days After Planting

treatments *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016, *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016 and *Trichoderma viride (*KAU ref. culture) + KAU POP 2016 (11.8, 11.6, and 11.3 mg CO_2 g^{-1} day^{-1} respectively) at 30 DAP. Lowest soil respiration (3.7 mg CO_2 g^{-1} day^{-1}) was observed in treatment T_{11}.

At 180 DAP, maximum soil respiration (10.8 mg CO_2 g^{-1} day^{-1}) was recorded in the treatment Organic Package of Practices (KAU, 2009) followed by treatments *Trichoderma harzianum* (CKT isolate) + KAU POP 2016 and *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016 (10.4 and 10.1 CO_2 g^{-1} day^{-1} respectively) which were on par with treatment Organic Package of Practices (KAU, 2009). Lowest soil respiration (4.1 mg CO_2 g^{-1} day^{-1}) was observed in treatment T_{11}.

4.2.4 Effect of micro-climatic and soil parameters on the population of *Trichoderma* sp. and *Pseudomonas fluorescens* under field condition

Micro-climatic and soil parameters such as soil temperature, soil moisture, soil pH and soil respiration were correlated (spearman's correlation) with the population of *Trichoderma harzianum* (CKT), *Trichoderma viride (*KAU ref. culture), *Pseudomonas fluorescens* (PAP isolate) and *Pseudomonas fluorescens* (KAU ref. culture). It was found that soil temperature was negatively correlated with the population of all the four isolates and soil moisture was positively correlated with all the four isolates. Soil pH and soil respiration were not having any significant correlation between the population of those isolates (Table 25).

4.2.5 Effect of micro-climatic and soil parameters on functional efficiency of *Trichoderma* sp. and *Pseudomonas fluorescens*

Effect of micro-climatic and soil parameters on functional efficiency of *Trichoderma* sp. and *Pseudomonas fluorescens* are represented in Table 26 – Table 33. The observations of functional efficiency of the isolates were cross tabulated with each of the micro-climatic and soil parameters and the number of isolates with

Table 25: Correlation of micro-climatic and soil parameters with population of *Trichoderma* sp. and *Pseudomonas fluorescens* under field condition

Parameters	Correlation coefficient			
	Population of *Trichoderma harzianum* (CKT)	**Population of *Trichoderma viride* (KAU ref. culture)**	**Population of *Pseudomonas fluorescens* (PAP)**	**Population of *Pseudomonas fluorescens* (KAU ref. culture)**
Soil temperature	- 0.416*	- 0.207*	- 0.633*	- 0.450*
Soil moisture	0.668**	0.264*	0.899**	0.349**
Soil pH	0.099NS	0.404NS	0.250NS	0.054NS
Soil respiration	0.030NS	0.343NS	0.407NS	0.029NS

* Correlation significant at 1 % level of significance

** Correlation significant at 5 % level level of significance

different efficiency were recorded. The dependency of one character on the other was measured through significance of chi-square statistics computed.

4.2.5.1 Effect of soil temperature on IAA, ammonia, HCN and siderophore production by Trichoderma sp.

The soil temperature during six months period ranged from 26.8°C (December 2017) to 30.6°C (March 2018). The effect of soil temperature on ammonia, HCN and siderophore production did not show any significant differences (Table 26). However, soil temperature showed significant effect on IAA production at 1% significant level. Out of sixteen isolates obtained during six months period, fourteen produced IAA at higher level and two isolates produced IAA at low level. Optimum temperature range for IAA production was from 25 to 30°C.

4.2.5.2 Effect of soil moisture on IAA, ammonia, HCN and siderophore production by Trichoderma sp.

The soil moisture content during six months period ranged from 14.0 % (March 2018) to 17.6 % (May 2018). The effect of soil moisture on ammonia, HCN and siderophore production did not show any significant differences (Table 27). Soil moisture showed significant effect on IAA production at 1% level of significance. Out of sixteen isolates obtained during six months period, fourteen produced IAA at higher level and two isolates produced IAA at low level.

4.2.5.3 Effect of soil pH on IAA, ammonia, HCN and siderophore production by Trichoderma sp.

The soil pH during six months period ranged from 6.1 (December 2017) to 5.7 (May 2018). The effect of soil pH on ammonia, HCN and siderophore production did not show any significant differences (Table 28). However, soil pH showed significant differences on IAA production at 1% significant level. Out of

sixteen isolates obtained during six months period, fourteen produced IAA at higher level and two isolates produced IAA at low level.

4.2.5.4 Effect of soil respiration on IAA, ammonia, HCN and siderophore production by Trichoderma sp.

The soil respiration during six months period ranged from 9.8 mg CO_2 g^{-1} day^{-1} (December 2017) to 8.1 mg CO_2 g^{-1} day^{-1} (May 2018). The effect of soil respiration on ammonia, HCN and siderophore production did not show any significant differences (Table 29). Soil respiration showed significant differences on IAA production at 1% significant level. Out of sixteen isolates obtained during six months period, fourteen produced IAA at higher level and two isolates produced IAA at low level.

4.2.5.5 Effect of micro-climatic and soil parameters on IAA, ammonia, HCN and siderophore production by Pseudomonas fluorescens

Effect soil temperature, soil moisture, soil pH and soil respiration on ammonia, HCN and siderophore production did not show any significant differences. However, out of total twelve isolates obtained ten isolates produced IAA at higher level. Two isolates showed IAA production at a low level. Eight isolates produced ammonia at a high level while four isolates produced ammonia at a medium level. Four isolates produced HCN at a medium level while eight isolates did not show HCN production. Only three isolates showed siderophore production and nine did not produced siderophore (Tables 30, 31, 32 and 33).

4.2.6 Effect of different treatments on biometric parameters under field condition

4.2.6.1 Effect of different treatments on number of leaves under field condition

Number of leaves recorded under field condition at monthly intervals were presented in Table 34. Treatments did not show significant differences up to 60 DAP.

Table 26: Effect of soil temperature on number of isolates of *Trichoderma* sp. producing ammonia, IAA, HCN and siderophore

Parameter and level of production	Month	Number of isolates obtained						Total number of isolates	Chi – square value
		Dec' 17	Jan' 18	Feb' 18	March' 18	April' 18	May' 18		
	Mean monthly soil temperature (°C)	26.8	27.9	29.1	30.6	29.7	27.8		
	Negative (-)	0	0	0	0	4	3	7	
Ammonia	Medium (++)	2	2	4	4	0	0	12	4.51^{NS}
Production	High (+++)	2	2	0	0	0	0	4	
Total		4	4	4	4	4	3	23	
	Negative (-)	0	0	0	0	0	2	2	
IAA	Low (+)	0	0	1	4	4	1	10	16^{**}
Production	High (+++)	4	4	3	0	0	0	11	
Total		4	4	4	4	4	3	23	
HCN	Negative (-)	0	3	4	4	4	3	18	
Production	Medium (++)	4	1	0	0	0	0	5	6.86^{NS}
Total		4	4	4	4	4	3	23	
Siderophore	Negative (-)	3	4	4	4	4	3	22	
Production	Positive (+)	1	0	0	0	0	0	1	3.20^{NS}
Total		4	4	4	4	4	3	23	

Each value in the table represents the total number of isolates during the month

NS – Non-Significant

** Significant at 1 % significant level

Table 27: Effect of soil moisture on number of isolates of *Trichoderma* sp. producing ammonia, IAA, HCN and siderophore

Parameter and level of production	Month	Number of isolates obtained						Total number of isolates	Chi – square value
		Dec' 17	Jan' 18	Feb' 18	March' 18	April' 18	May' 18		
	Soil moisture (%)	17.1	16.2	15.5	14.0	14.8	17.6		
	Negative (-)	0	0	0	0	4	3	7	
Ammonia	Medium (++)	2	2	4	4	0	0	12	4.51^{NS}
Production	High (+++)	2	2	0	0	0	0	4	
Total		4	4	4	4	4	3	23	
	Negative (-)	0	0	0	0	0	2	2	
IAA	Low (+)	0	0	1	4	4	1	10	16^{**}
Production	High (+++)	4	4	3	0	0	0	11	
Total		4	4	4	4	4	3	23	
HCN	Negative (-)	0	3	4	4	4	3	18	
Production	Medium (++)	4	1	0	0	0	0	5	6.86^{NS}
Total		4	4	4	4	4	3	23	
Siderophore	Negative (-)	3	4	4	4	4	3	22	
Production	Positive (+)	1	0	0	0	0	0	1	3.20^{NS}
Total		4	4	4	4	4	3	23	

Each value in the table represents the total number of isolates during the month

NS – Non-Significant

** Significant at 1 % significant level

Table 28: Effect of soil pH on number of isolates of *Trichoderma* sp. producing ammonia, IAA, HCN and siderophore

Parameter and level of production	Month	Number of isolates obtained						Total number of isolates	Chi – square value
		Dec' 17	Jan' 18	Feb' 18	March' 18	April' 18	May' 18		
	Soil pH	6.1	6.0	5.9	5.9	5.8	5.7		
	Negative (-)	0	0	0	0	4	3	7	
Ammonia	Medium (++)	2	2	4	4	0	0	12	4.51^{NS}
Production	High (+++)	2	2	0	0	0	0	4	
Total		4	4	4	4	4	3	23	
	Negative (-)	0	0	0	0	0	2	2	
IAA	Low (+)	0	0	1	4	4	1	10	16^{**}
Production	High (+++)	4	4	3	0	0	0	11	
Total		4	4	4	4	4	3	23	
HCN	Negative (-)	0	3	4	4	4	3	18	
Production	Medium (++)	4	1	0	0	0	0	5	6.86^{NS}
Total		4	4	4	4	4	3	23	
Siderophore	Negative (-)	3	4	4	4	4	3	22	
Production	Positive (+)	1	0	0	0	0	0	1	3.20^{NS}
Total		4	4	4	4	4	3	23	

Each value in the table represents the total number of isolates during the month

NS – Non-Significant

** Significant at 1 % significant level

Table 29: Effect of soil respiration on number of isolates of *Trichoderma* sp. producing ammonia, IAA, HCN and siderophore

Parameter and level of production	Month	Number of isolates obtained						Total number of isolates	Chi – square value
		Dec' 17	Jan' 18	Feb' 18	March' 18	April' 18	May' 18		
	Soil respiration (mg CO_2 g^{-1} day^{-1})	9.8	9.5	9.2	8.8	8.3	8.1		
	Negative (-)	0	0	0	0	4	3	7	
Ammonia	Medium (++)	2	2	4	4	0	0	12	4.51^{NS}
Production	High (+++)	2	2	0	0	0	0	4	
Total		4	4	4	4	4	3	23	
	Negative (-)	0	0	0	0	0	2	2	
IAA	Low (+)	0	0	1	4	4	1	10	16^{**}
Production	High (+++)	4	4	3	0	0	0	11	
Total		4	4	4	4	4	3	23	
HCN	Negative (-)	0	3	4	4	4	3	18	
Production	Medium (++)	4	1	0	0	0	0	5	6.86^{NS}
Total		4	4	4	4	4	3	23	
Siderophore	Negative (-)	3	4	4	4	4	3	22	
Production	Positive (+)	1	0	0	0	0	0	1	3.20^{NS}
Total		4	4	4	4	4	3	23	

Each value in the table represents the total number of isolates during the month

NS – Non-Significant

** Significant at 1 % significant level

Table 30: Effect of soil temperature on number of isolates of *Pseudomonas fluorescens* producing ammonia, IAA, HCN and siderophore

Parameter and level of production	Month	Number of isolates obtained						Total number of isolates	Chi – square value
		Dec' 17	Jan' 18	Feb' 18	March' 18	April' 18	May' 18		
	Mean monthly soil temperature (°C)	26.8	27.9	29.1	30.6	29.7	27.8		
	Negative (-)	0	0	0	0	4	4	8	7.45^{NS}
Ammonia	Medium (++)	2	2	4	4	0	0	12	
Production	High (+++)	2	2	0	0	0	0	4	
Total		4	4	4	4	4	4	24	
	Negative (-)	0	0	0	0	0	3	3	18.60^{**}
IAA	Low (+)	0	0	1	4	4	1	10	
Production	High (+++)	4	4	3	0	0	0	11	
Total		4	4	4	4	4	4	24	
HCN	Negative (-)	0	3	4	4	4	4	19	8.62^{NS}
Production	Medium (++)	4	1	0	0	0	0	5	
Total		4	4	4	4	4	4	24	
Siderophore	Negative (-)	2	2	4	4	4	4	20	5.34^{NS}
Production	Positive (+)	2	2	0	0	0	0	4	
Total		4	4	4	4	4	4	24	

Each value in the table represents the total number of isolates during the month

NS – Non-Significant, ** Significant at 1 % significant level

Table 31: Effect of soil moisture on number of isolates of *Pseudomonas fluorescens* producing ammonia, IAA, HCN and siderophore

Parameter and level of production	Month	Number of isolates obtained						Total number of isolates	Chi – square value
		Dec' 17	Jan' 18	Feb' 18	March' 18	April' 18	May' 18		
	Soil moisture (%)	17.1	16.2	15.5	14.0	14.8	17.6		
	Negative (-)	0	0	0	0	4	4	8	7.45^{NS}
Ammonia	Medium (++)	2	2	4	4	0	0	12	
Production	High (+++)	2	2	0	0	0	0	4	
Total		4	4	4	4	4	4	24	
	Negative (-)	0	0	0	0	0	3	3	18.60^{**}
IAA	Low (+)	0	0	1	4	4	1	10	
Production	High (+++)	4	4	3	0	0	0	11	
Total		4	4	4	4	4	4	24	
HCN	Negative (-)	0	3	4	4	4	4	19	8.62^{NS}
Production	Medium (++)	4	1	0	0	0	0	5	
Total		4	4	4	4	4	4	24	
Siderophore	Negative (-)	2	2	4	4	4	4	20	5.34^{NS}
Production	Positive (+)	2	2	0	0	0	0	4	
Total		4	4	4	4	4	4	24	

Each value in the table represents the total number of isolates during the month

NS – Non-Significant, ** Significant at 1 % significant level

Table 32: Effect of soil pH on number of isolates of *Pseudomonas fluorescens* producing ammonia, IAA, HCN and siderophore

Parameter and level of production	Month	Number of isolates obtained						Total number of isolates	Chi – square value
		Dec' 17	Jan' 18	Feb' 18	March' 18	April' 18	May' 18		
	Soil pH	6.1	6.0	5.9	5.9	5.8	5.7		
	Negative (-)	0	0	0	0	4	4	8	
Ammonia	Medium (++)	2	2	4	4	0	0	12	7.45^{NS}
Production	High (+++)	2	2	0	0	0	0	4	
Total		4	4	4	4	4	4	24	
	Negative (-)	0	0	0	0	0	3	3	
IAA	Low (+)	0	0	1	4	4	1	10	18.60^{**}
Production	High (+++)	4	4	3	0	0	0	11	
Total		4	4	4	4	4	4	24	
HCN	Negative (-)	0	3	4	4	4	4	19	
Production	Medium (++)	4	1	0	0	0	0	5	8.62^{NS}
Total		4	4	4	4	4	4	24	
Siderophore	Negative (-)	2	2	4	4	4	4	20	
Production	Positive (+)	2	2	0	0	0	0	4	5.34^{NS}
Total		4	4	4	4	4	4	24	

Each value in the table represents the total number of isolates during the month

NS – Non-Significant, ** Significant at 1 % significant level

Table 33: Effect of soil respiration on number of isolates of *Pseudomonas fluorescens* producing ammonia, IAA, HCN and siderophore

Parameter and level of production	Month	Number of isolates obtained						Total number of isolates	Chi – square value
		Dec' 17	Jan' 18	Feb' 18	March' 18	April' 18	May' 18		
	Soil respiration (mg CO_2 g^{-1} day^{-1})	9.8	9.5	9.2	8.8	8.3	8.1		
	Negative (-)	0	0	0	0	4	4	8	7.45^{NS}
Ammonia	Medium (++)	2	2	4	4	0	0	12	
Production	High (+++)	2	2	0	0	0	0	4	
Total		4	4	4	4	4	4	24	
	Negative (-)	0	0	0	0	0	3	3	18.60^{**}
IAA	Low (+)	0	0	1	4	4	1	10	
Production	High (+++)	4	4	3	0	0	0	11	
Total		4	4	4	4	4	4	24	
HCN	Negative (-)	0	3	4	4	4	4	19	8.62^{NS}
Production	Medium (++)	4	1	0	0	0	0	5	
Total		4	4	4	4	4	4	24	
Siderophore	Negative (-)	2	2	4	4	4	4	20	5.34^{NS}
Production	Positive (+)	2	2	0	0	0	0	4	
Total		4	4	4	4	4	4	24	

Each value in the table represents the total number of isolates during the month

NS – Non-Significant, ** Significant at 1 % significant level

At 90 DAP, treatment T_1 (CKT+ KAU POP) recorded maximum number of leaves (21.75) followed by T_2 (PAP + KAU POP) (17.35) which was on par with T_1. Minimum number of leaves (11.35 and 11.75) was recorded in treatments T_{12} (Absolute control) and T_{11} (Chemical treatment).

At 180 DAP, highest number of leaves (35.25) was recorded in T_1 followed by T_2 (31.35) which was on par with T_1. Lowest number of leaves (16.15 and 16.85) was recorded in treatments T_{12} and T_{11}.

4.2.6.2 Effect of different treatments on plant height under field condition

Plant height was recorded under field condition at monthly intervals (Table 35). Treatments did not show significant differences up to 30 DAP. Treatment T_1 (CKT+ KAU POP) recorded maximum plant height (74.83 cm) at 60 DAP followed by T_2 (PAP + KAU POP) (68.16 cm) which was on par with T_1. Minimum plant height (47.35 cm) was recorded in treatment T_{12}.

At 120 DAP, highest plant height (125.72 cm) was recorded by T_1 followed by T_2 (118.94 cm) which was on par with T_1. Lowest plant height (62.75 cm) was recorded in treatment T_{12}.

At 180 DAP, highest plant height (165.23 cm) was recorded by T_1 followed by T_2 (156.64 cm) which was on par with T_1. Lowest plant height (81.25 cm) was recorded in treatment T_{12}.

4.2.6.3 Effect of different treatments on number of lateral branches under field condition

Number of lateral branches recorded at monthly intervals under field condition are presented in Table 36. Treatments did not show any significant differences during the six months of experiment.

Table 34: Effect of different treatments on number of leaves at monthly intervals under field condition

Number of leaves							
Treatments	**Initial**	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 : *Trichoderma harzianum* (CKT isolate) + KAU POP 2016	8.00	12.50	16.85	21.75^{a}	26.35^{a}	30.85^{a}	35.25^{a}
T_2 : *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016	7.50	11.25	15.25	17.35^{ab}	21.35^{ab}	26.75^{ab}	31.35^{ab}
T_3 : *Trichoderma viride (*KAU ref. culture) + KAU POP 2016	7.25	10.50	11.75	13.45^{bc}	15.85^{bc}	19.35^{cd}	24.15^{cd}
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016	7.50	10.55	12.35	14.55^{bc}	18.45^{bc}	23.15^{bc}	28.65^{bc}
T_5 : *Trichoderma harzianum* (CKT isolate)	7.50	10.25	11.55	12.75^{bc}	14.85^{bc}	18.25^{de}	20.50^{def}
T_6 : *Pseudomonas fluorescens* (PAP isolate)	7.25	9.65	11.25	12.55^{bc}	14.50^{bc}	17.35^{def}	19.45^{def}
T_7 : *Trichoderma viride (*KAU ref. culture)	7.50	9.35	10.65	12.25^{bc}	14.15^{bc}	16.25^{ef}	18.15^{ef}
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)	7.25	9.55	10.85	12.45^{bc}	14.45^{bc}	16.55^{ef}	18.45^{ef}
T_9 : Package of Practices Recommendations (KAU, 2016)	7.55	10.25	11.55	12.95^{bc}	15.65^{bc}	18.65^{de}	22.50^{de}
T_{10}: Organic Package of Practices (KAU,2009)	7.50	10.15	11.25	12.75^{bc}	15.25^{bc}	18.35^{de}	21.45^{def}
T_{11}: Chemical treatment (Kocide 2g L^{-1})	7.45	8.35	10.65	11.75^{c}	13.85^{c}	14.75^{ef}	16.85^{ef}
T_{12}: Absolute control	7.25	8.25	10.55	11.35^{c}	13.45^{c}	14.35^{f}	16.15^{f}
$CD_{(0.05)}$	NS	NS	NS	5.69	7.23	6.26	5.95

Each value represents an average of four replications, DAP – Days After Planting, NS- Non-significant

Table 35: Effect of different treatments on plant height at monthly intervals under field condition

Plant height (cm)							
Treatments	**Initial**	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 : *Trichoderma harzianum* (CKT isolate) + KAU POP 2016	34.28	57.65	74.83^{a}	96.25^{a}	125.72^{a}	146.38^{a}	165.23^{a}
T_2 : *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016	32.65	54.28	68.16^{ab}	89.53^{ab}	118.94^{a}	134.33^{ab}	156.64^{a}
T_3 : *Trichoderma viride (*KAU ref. culture) + KAU POP 2016	33.42	50.26	60.48^{bcd}	78.85^{bc}	96.67^{bc}	118.45^{bc}	125.62^{c}
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016	33.67	52.34	63.65^{bc}	80.98^{bc}	105.53^{b}	123.16^{b}	141.57^{b}
T_5 : *Trichoderma harzianum* (CKT isolate)	34.21	47.56	54.42^{cde}	70.56^{cde}	84.67^{def}	99.38^{cd}	108.26^{de}
T_6 : *Pseudomonas fluorescens* (PAP isolate)	33.52	46.43	52.37^{cde}	69.48^{cde}	78.25^{ef}	91.47^{de}	101.34^{e}
T_7 : *Trichoderma viride (*KAU ref. culture)	33.57	45.27	51.83^{de}	62.37^{ef}	74.63^{fg}	82.26^{de}	95.25^{ef}
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)	32.46	45.82	53.24^{cde}	65.48^{def}	76.29^{fg}	88.14^{de}	97.83^{e}
T_9 : Package of Practices Recommendations (KAU, 2016)	34.33	48.63	57.82^{bcde}	74.62^{cd}	91.52^{cd}	114.78^{bc}	120.37^{cd}
T_{10}: Organic Package of Practices (KAU,2009)	35.16	48.74	56.36^{cde}	72.39^{cde}	88.43^{de}	100.36^{cd}	117.85^{cd}
T_{11}: Chemical treatment (Kocide 2g L^{-1})	35.34	43.58	50.24^{de}	57.35^{f}	65.46^{gh}	73.58^{e}	84.66^{fg}
T_{12}: Absolute control	32.18	41.36	47.35^{e}	55.35^{g}	62.75^{h}	70.34^{e}	81.25^{g}
$CD_{(0.05)}$	NS	NS	10.49	9.59	10.92	21.71	13.19

Each value represents an average of four replications
DAP – Days After Planting, NS- Non-significant

Table 36: Effect of different treatments on number of lateral branches at monthly intervals under field condition

Number of lateral branches					
Treatments	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 : *Trichoderma harzianum* (CKT isolate) + KAU POP 2016	0.25	0.75	1.50	1.75	2.5
T_2 : *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016	0.25	0.50	1.00	1.50	2.25
T_3 : *Trichoderma viride (*KAU ref. culture) + KAU POP 2016	0.25	0.5	1.00	1.25	1.75
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016	0.25	0.50	1.00	1.50	2.00
T_5 : *Trichoderma harzianum* (CKT isolate)	0	0.50	0.75	1.00	1.50
T_6 : *Pseudomonas fluorescens* (PAP isolate)	0	0.25	0.50	0.75	1.25
T_7 : *Trichoderma viride (*KAU ref. culture)	0	0.5	0.50	1.00	1.25
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)	0	0.25	0.50	1.50	1.25
T_9 : Package of Practices Recommendations (KAU, 2016)	0	0.50	0.75	1.00	1.75
T_{10}: Organic Package of Practices (KAU,2009)	0	0.25	0.50	1.00	1.50
T_{11}: Chemical treatment (Kocide 2g L^{-1})	0	0	0.50	0.75	1.00
T_{12}: Absolute control	0	0	0.25	0.50	0.75
$CD_{(0.05)}$	NS	NS	NS	NS	NS

Each value represents an average of four replications
DAP – Days After Planting, NS- Non-significant

4.2.6.4 Effect of different treatments on number of nodes under field condition

Number of nodes recorded under field condition at monthly intervals were presented in Table 37. Treatments did not show significant differences up to 60 DAP. Treatment T_1 recorded maximum number of nodes (22) at 90 DAP followed by T_2 (17.85) which was on par with T_1. Minimum number of nodes (11.50) was recorded in treatment T_{12}.

At 180 DAP, highest number of nodes (35.75) was recorded in treatment T_1 followed by T_2 (31.85) which was on par with T_1. Lowest number of nodes (16.85 and 17.15) was recorded in treatments T_{12} and T_{11}.

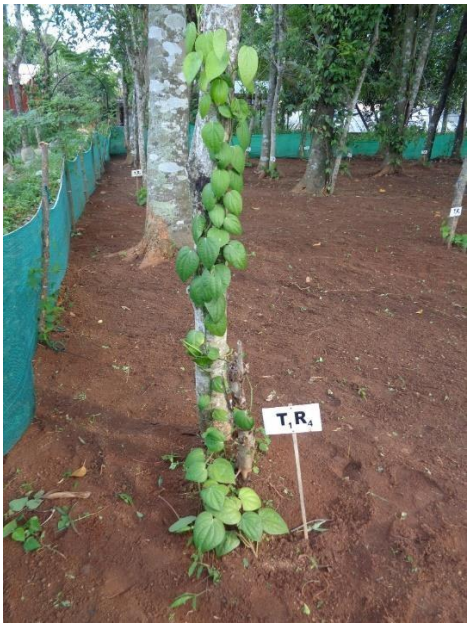
4.2.6.5 Effect of different treatments on internodal length under field condition

Internodal length was recorded under field condition at monthly intervals (Table 38). Treatments did not show significant differences up to 120 DAP. Treatment T_1 recorded maximum internodal length (7.28 cm) at 150 DAP followed by T_2 (7.05 cm) which was on par with T_1. Minimum internodal length (4.20 cm and 4.27 cm) was recorded in treatments T_{12} and T_{11}.

At 180 DAP, highest internodal length (7.62 cm) was recorded by T_1 followed by T_2 (7.34 cm) which was on par with T_1. Lowest internodal length (4.28 cm and 4.36 cm) was recorded in treatments T_{12} and T_{11}.

4.2.7 Effect of different treatments on nutrient status under field studies

Organic carbon, available nutrients (Nitrogen, phosphorus and potassium) and pH before planting and six months after planting was recorded (Table 39). Initially, the field soil contained high level of organic carbon (1.8%), medium level of available nitrogen (403.21 kg ha^{-1}), high level of available phosphorus (27.84 kg ha^{-1}), medium level of available potassium (208.21 kg ha^{-1}) and acidic pH (5.7).



T_1 : CKT + KAU POP

T_9 : KAU POP (2016)

T_{12}: Absolute control

Plate 11 : Effect of bio-inoculants on plant growth

Table 37: Effect of different treatments on number of nodes at monthly intervals under field condition

Number of nodes							
Treatments	**Initial**	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 : *Trichoderma harzianum* (CKT isolate) + KAU POP 2016	8.00	12.50	17.15	22.00^{a}	26.65^{a}	31.05^{a}	35.75^{a}
T_2 : *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016	7.65	11.45	15.50	17.85^{ab}	21.75^{ab}	27.15^{ab}	31.85^{ab}
T_3 : *Trichoderma viride (*KAU ref. culture) + KAU POP 2016	7.55	10.50	11.85	13.75^{cd}	16.15^{cd}	19.85^{cd}	24.65^{cd}
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016	7.50	10.65	12.55	14.95^{bc}	18.75^{bc}	23.45^{bc}	28.00^{bc}
T_5 : *Trichoderma harzianum* (CKT isolate)	7.55	10.35	11.75	13.05^{cd}	15.15^{cd}	18.75^{cd}	21.15^{def}
T_6 : *Pseudomonas fluorescens* (PAP isolate)	7.50	975	11.45	12.85^{cd}	14.85^{de}	17.65^{cd}	20.00^{def}
T_7 : *Trichoderma viride (*KAU ref. culture)	7.65	9.45	10.75	12.50^{cd}	14.65^{de}	16.75^{de}	18.45^{ef}
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)	7.30	9.55	10.95	12.75^{cd}	14.75^{de}	16.85^{de}	19.15^{ef}
T_9 : Package of Practices Recommendations (KAU, 2016)	7.55	10.35	12.15	13.15^{cd}	16.00^{cd}	18.95^{cd}	22.95^{cde}
T_{10}: Organic Package of Practices (KAU,2009)	7.35	10.25	11.35	12.95^{cd}	15.85^{cd}	18.75^{cd}	22.75^{de}
T_{11}: Chemical treatment (Kocide 2g L^{-1})	7.45	8.55	10.85	11.95^{de}	14.05^{ef}	15.00^{ef}	17.15^{f}
T_{12}: Absolute control	7.25	8.35	10.75	11.50^{e}	13.85^{f}	14.75^{f}	16.85^{f}
$CD_{(0.05)}$	NS	NS	NS	4.12	4.91	6.47	5.06

Each value represents an average of four replications

DAP – Days After Planting, NS- Non-significant

Table 38: Effect of different treatments on internodal length at monthly intervals under field condition

Internodal length (cm)							
Treatments	**Initial**	**30 DAP**	**60 DAP**	**90 DAP**	**120 DAP**	**150 DAP**	**180 DAP**
T_1 : *Trichoderma harzianum* (CKT isolate) + KAU POP 2016	5.73	5.98	6.26	6.42	6.64	7.28^{a}	7.62^{a}
T_2 : *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016	5.84	5.95	6.17	6.38	6.56	7.05^{ab}	7.34^{ab}
T_3 : *Trichoderma viride (*KAU ref. culture) + KAU POP 2016	5.46	5.58	5.73	5.98	6.19	6.38^{bc}	6.53^{bc}
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016	5.80	5.91	6.06	6.18	6.23	6.42^{bc}	6.87^{bc}
T_5 : *Trichoderma harzianum* (CKT isolate)	5.36	5.42	5.58	5.64	5.86	6.03^{bc}	6.29^{bc}
T_6 : *Pseudomonas fluorescens* (PAP isolate)	4.82	4.98	5.10	5.16	5.20	5.26^{cd}	5.68^{cd}
T_7 : *Trichoderma viride (*KAU ref. culture)	4.86	4.92	5.05	5.10	5.14	5.18^{cd}	5.32^{cd}
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)	4.88	4.96	5.02	5.08	512	5.16^{cd}	5.47^{cd}
T_9 : Package of Practices Recommendations (KAU, 2016)	5.36	5.42	5.64	5.76	6.17	6.26^{bc}	6.38^{bc}
T_{10}: Organic Package of Practices (KAU,2009)	5.44	5.53	5.68	5.84	6.08	6.14^{bc}	6.27^{bc}
T_{11}: Chemical treatment (Kocide 2g L^{-1})	3.84	3.96	4.08	4.14	4.16	4.27^{d}	4.36^{d}
T_{12}: Absolute control	3.80	3.92	4.05	4.10	4.12	4.20^{d}	4.28^{d}
C.D	NS	NS	NS	NS	NS	0.37	0.49

Each value represents an average of four replications

DAP – Days After Planting, NS- Non-significant

After six months of planting, highest organic carbon (2.71 % - high) was observed in the treatment T_{10} (Organic Package of Practices). Lowest OC (1.82 % - high) was recorded in the treatment T_{11} (Chemical treatment). Maximum available nitrogen was observed in the treatment T_1 [*Trichoderma harzianum* (CKT isolate) + KAU POP 2016] and it was 548.56 kg ha^{-1} which is medium. Lowest N was found in treatment T_{12} (Absolute control) and which was 336.28 kg ha^{-1} (medium).

Highest available phosphorus (P) and available potassium (K) were recorded in the case of treatment T_9 [Package of Practices Recommendations (KAU, 2016)]. P and K are found to be 62.36 kg ha^{-1} (high) and 265.38 kg ha^{-1} (medium) respectively in T_9. Lowest P and K are estimated in treatment T_{12} (Absolute control). They were 28.92 kg ha^{-1} (high) and 211.01 kg ha^{-1} (high) respectively.

4.2.8 Per cent disease incidence under field condition

No natural *Phytophthora* disease incidence was recorded under field condition.

4.2.9 Selection of best treatment based on biometric and biological parameters under field condition

On the basis of biometric parameters (number of leaves, plant height, number of lateral branches, number of nodes and internodal length) and biological parameters (soil respiration), best treatment under field condition was found out using pooled ranking method in statistics (Table 40). Accordingly, treatment *Trichoderma harzianum* (CKT) + KAU POP is the best treatment followed by *Pseudomonas fluorescens* (PAP) + KAU POP under field condition.

Table 39: Initial and final nutrient status under field condition

INITIAL NUTRIENT STATUS										
Item	**Organic Carbon (%)**		**Available Nitrogen (kg ha^{-1})**		**AvailablePhosphorus (kg ha^{-1})**		**Available Potassium (kg ha^{-1})**		**pH**	
Initial field soil sample	1.8	High	403.21	Medium	27.84	High	208.21	Medium	5.7	Acidic
FINAL NUTRIENT STATUS										
Treatments	**Organic Carbon (%)**		**Available Nitrogen (kg ha^{-1})**		**Available Phosphorus (kg ha^{-1})**		**Available Potassium (kg ha^{-1})**		**pH**	
T_1 : *Trichoderma harzianum* (CKT) + KAU POP 2016	2.56	High	548.56	Medium	57.11	High	263.26	Medium	5.9	Acidic
T_2 : *Pseudomonas fluorescens* (PAP) + KAU POP 2016	2.51	High	539.84	Medium	56.42	High	260.89	Medium	6.0	Slightly acidic
T_3 : *Trichoderma viride* (KAU ref. culture) + KAU POP 2016	2.48	High	526.12	Medium	54.04	High	258.75	Medium	6.0	Slightly acidic
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016	2.42	High	519.68	Medium	55.20	High	252.40	Medium	5.9	Acidic
T_5 : *Trichoderma harzianum* (CKT)	2.26	High	506.24	Medium	45.42	High	238.91	Medium	5.5	Acidic
T_6 : *Pseudomonas fluorescens* (PAP)	2.22	High	497.28	Medium	43.87	High	236.64	Medium	5.5	Acidic
T_7 : *Trichoderma viride* (KAU ref. culture)	2.19	High	490.56	Medium	40.59	High	227.56	Medium	5.6	Acidic
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)	2.14	High	478.32	Medium	45.42	High	238.97	Medium	5.6	Acidic
T_9 : KAU POP (2016)	2.53	High	534.19	Medium	62.36	High	265.38	Medium	6.0	Neutral
T_{10}: Organic POP (2009)	2.71	High	514.67	Medium	51.34	High	256.12	Medium	6.0	Neutral
T_{11}: Chemical treatment (Kocide 2g L^{-1})	1.82	High	339.46	Medium	30.78	High	217.73	Medium	5.7	Acidic
T_{12}: Absolute control	1.84	High	336.28	Medium	28.92	High	211.01	Medium	5.6	Acidic

Table 40: Selection of best treatment based on biometric and biological parameters under field condition

Treatments	Number of leaves	Plant height (cm)	Number of branches	Number of nodes	Internodal length (cm)	Soil respiration (mg CO_2 $g^{-1}day^{-1}$)	Ranking by pooled ranking method
	180 DAP	180 DAP	180 DAP	180 DAP	180 DAP	180 DAP	
T_1 : *Trichoderma harzianum* (CKT isolate) + KAU POP 2016	35.25^{a}	165.23^{a}	2.50	35.75^{a}	7.62^{a}	8.80^{bc}	1
T_2 : *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016	31.35^{ab}	156.64^{a}	2.25	31.85^{ab}	7.34^{ab}	8.10^{bc}	2
T_3 : *Trichoderma viride* (KAU ref. culture) + KAU POP 2016	24.15^{cd}	125.62^{c}	1.75	24.65^{cd}	6.53^{bc}	7.50^{cd}	4
T_4 : *Pseudomonas fluorescens* (KAU ref. culture) + KAU POP 2016	28.65^{bc}	141.57^{b}	2.00	28.00^{bc}	6.87^{bc}	7.20^{cd}	3
T_5 : *Trichoderma harzianum* (CKT isolate)	20.50^{def}	108.26^{de}	1.50	21.15^{def}	6.29^{bc}	10.70^{a}	6
T_6 : *Pseudomonas fluorescens* (PAP isolate)	19.45^{def}	101.34^{e}	1.25	20.00^{def}	5.68^{cd}	10.30^{a}	8
T_7 : *Trichoderma viride* (KAU ref. culture)	18.15^{ef}	95.25^{ef}	125	18.45^{ef}	5.32^{cd}	9.50^{ab}	10
T_8 : *Pseudomonas fluorescens* (KAU ref. culture)	18.45^{def}	97.83^{e}	1.25	19.15^{ef}	5.47^{cd}	9.80^{ab}	9
T_9 : Package of Practices Recommendations (KAU, 2016)	22.50^{de}	120.37^{cd}	1.75	22.95^{cde}	6.38^{bc}	5.20^{de}	5
T_{10}: Organic Package of Practices (KAU,2009)	21.45^{def}	117.85^{cd}	1.50	22.75^{de}	6.27^{d}	10.90^{a}	7
T_{11}: Chemical treatment (Kocide 2g L^{-1})	16.85^{ef}	84.66^{fg}	1.00	17.15^{f}	4.36^{d}	4.40^{e}	11
T_{12}: Absolute control	16.15^{f}	81.25^{g}	0.75	16.85^{f}	4.28^{d}	5.60^{de}	12

Discussion

5. DISCUSSION

Black pepper (*Piper nigrum* L.), known as “the king of spices” is an important spice commodity of commerce and trade in India. It is highly sensitive to abiotic stresses like climate changes. The abiotic stresses in plants can be overcome by the use of beneficial microorganisms. However, the microorganisms themselves are vulnerable to abiotic and biotic stresses. Therefore, abiotic stress tolerant strains of beneficial microorganisms have to be developed for black pepper to overcome changes due to micro-climatic variables and soil parameters.

In an earlier study conducted in the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara, *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) were found to be tolerant against abiotic stresses like high temperature, acidic pH and osmotic stress (drought). The present study was carried out to evaluate abiotic stress tolerant strains of *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) for plant growth promotion and *Phytophthora* disease management in black pepper and to assess the effect of micro-climatic and soil parameters on those strains under field condition. Bae *et al.* (2009) proved that the *T. hamatum* increased tolerance of cocoa plants to water deficit through increasing root growth that provided greater water resources to treated plants and delayed the onset of water deficit in those plants.

Abiotic stress tolerant strains of *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) were evaluated for plant growth promotion and *Phytophthora* disease management under pot culture experiment based on chemical analysis of soil (organic carbon, available nitrogen, available phosphorus and available potassium), enumeration of *Trichoderma* sp. and *Pseudomonas fluorescens*, micro-climatic and soil parameters (soil temperature, soil moisture, soil pH and soil respiration), per cent disease incidence and biometric parameters (number

of leaves, plant height, number of lateral branches, number of nodes and internodal length).

Population of *Trichoderma* sp. and *Pseudomonas fluorescens* in potting mixture was recorded at monthly intervals (Fig. 1) in order to know the fate and survivability of microbial inoculants in the potting mixture. Initially, population of *Trichoderma* sp. and *Pseudomonas* sp. was absent in the potting mixture. It might be because the potting mixture was sterilized initially, before planting. After planting, population was present only in treatments *Trichoderma harzianum* (CKT), *Pseudomonas fluorescens* (PAP), *Trichoderma viride* (KAU reference culture) and *Pseudomonas fluorescens* (KAU reference culture). Population of *Trichoderma* sp. and *Pseudomonas* sp. declined gradually from 30 DAP to 180 DAP in the potting mixture. Population decreased from 10^5 cfu ml^{-1} to 10^3 cfu ml^{-1} in the case of *Trichoderma* sp. and from 10^6 cfu ml^{-1} to 10^4 cfu ml^{-1} in the case of *Pseudomonas fluorescens*. Haritha (2015) reported that population of the inoculated microorganisms showed a decreasing trend in the soil from planting to harvest of the crop. This might be due to various soil abiotic factors such as texture, pH, temperature, moisture content, and substrate availability need critical assessment, since these highly determine the survival and activity of the introduced microorganisms (Gray, 1975). Population of *Pseudomonas fluorescens* (PAP isolate) and *Pseudomonas fluorescens* (KAU reference culture) was higher than that of *Trichoderma* sp. throughout the experiment.

Effect of different treatments on micro-climatic and soil parameters such as soil temperature, soil moisture, soil pH and soil respiration was found out. Treatments did not show any significant differences in soil temperature and soil pH but showed significant differences in the case of soil moisture and soil respiration (Fig. 2 and Fig. 3 respectively). According to Petterson (2004), environmental changes may alter the composition and biomass of a microbial community which in

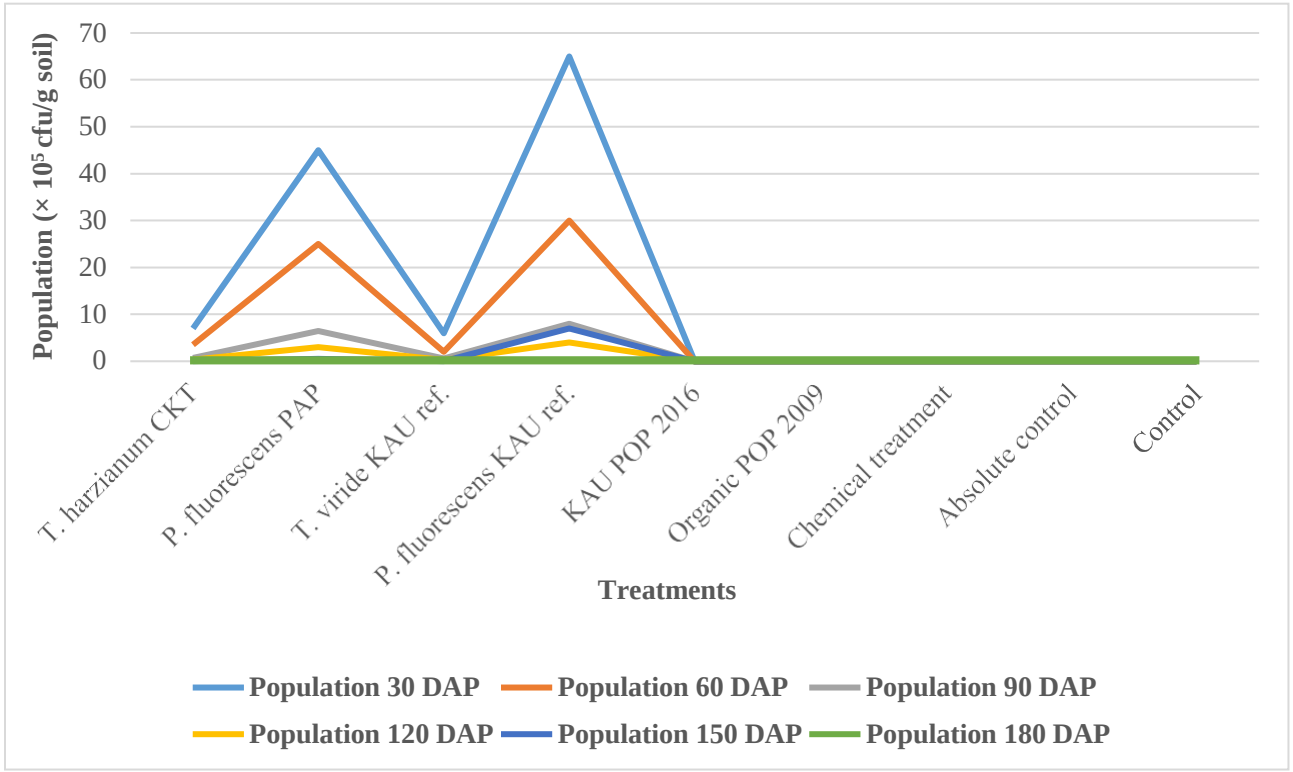


Fig.1: Population of *Trichoderma* sp. and *Pseudomonas fluorescens* at monthly intervals (cfu g^{-1} soil) under pot culture (sterile)

turn will affect the growth of plants. All microorganisms have a set of optimal environmental conditions, which enhances their optimal growth.

Effect of soil temperature, soil moisture, soil pH and soil respiration on population of *Trichoderma* sp. and *Pseudomonas fluorescens* were found out using Spearman's correlation method statistically. Population of all the four microbial isolates such as *Trichoderma harzianum* (CKT), *Trichoderma viride* (KAU ref. culture), *Pseudomonas fluorescens* (PAP isolate) and *Pseudomonas fluorescens* (KAU ref. culture) was positively correlated with soil moisture and negatively correlated with soil temperature. There was no significant correlation between the population of microbes and the parameters such as soil pH and soil respiration. Manju (2017), showed that population of *Trichoderma* sp. and fluorescent pseudomonads was negatively correlated with soil temperature and positively correlated with soil moisture which is in agreement with the results of current study. In another study, Chaudhary *et al.* (2016) revealed that the population of *Trichoderma* was negatively correlated with soil temperature and positively correlated with the soil moisture which is also supporting the results of the present study.

All plants except the treatment absolute control were challenge inoculated with *Phytophthora capsici*. Per cent disease incidence and per cent disease index were found out at 7 and 14 DAI and is represented in Fig. 4 and Fig. 5 respectively. Maximum disease incidence (100%) at 7 DAI was observed with the treatment T_9 (Control) followed by treatments T_5 (KAU POP 2016) and T_6 (Organic POP) (50% each). Minimum disease incidence (0%) was observed in the case of treatments T_9 (Absolute control) and T_7 (chemical treatment). At 7 DAI, maximum foot rot disease index (85%) was recorded in the control treatment followed by treatments KAU POP 2016 and organic POP (45, 40% respectively). Least per cent disease index (0%) was recorded in the case of treatments absolute control and chemical

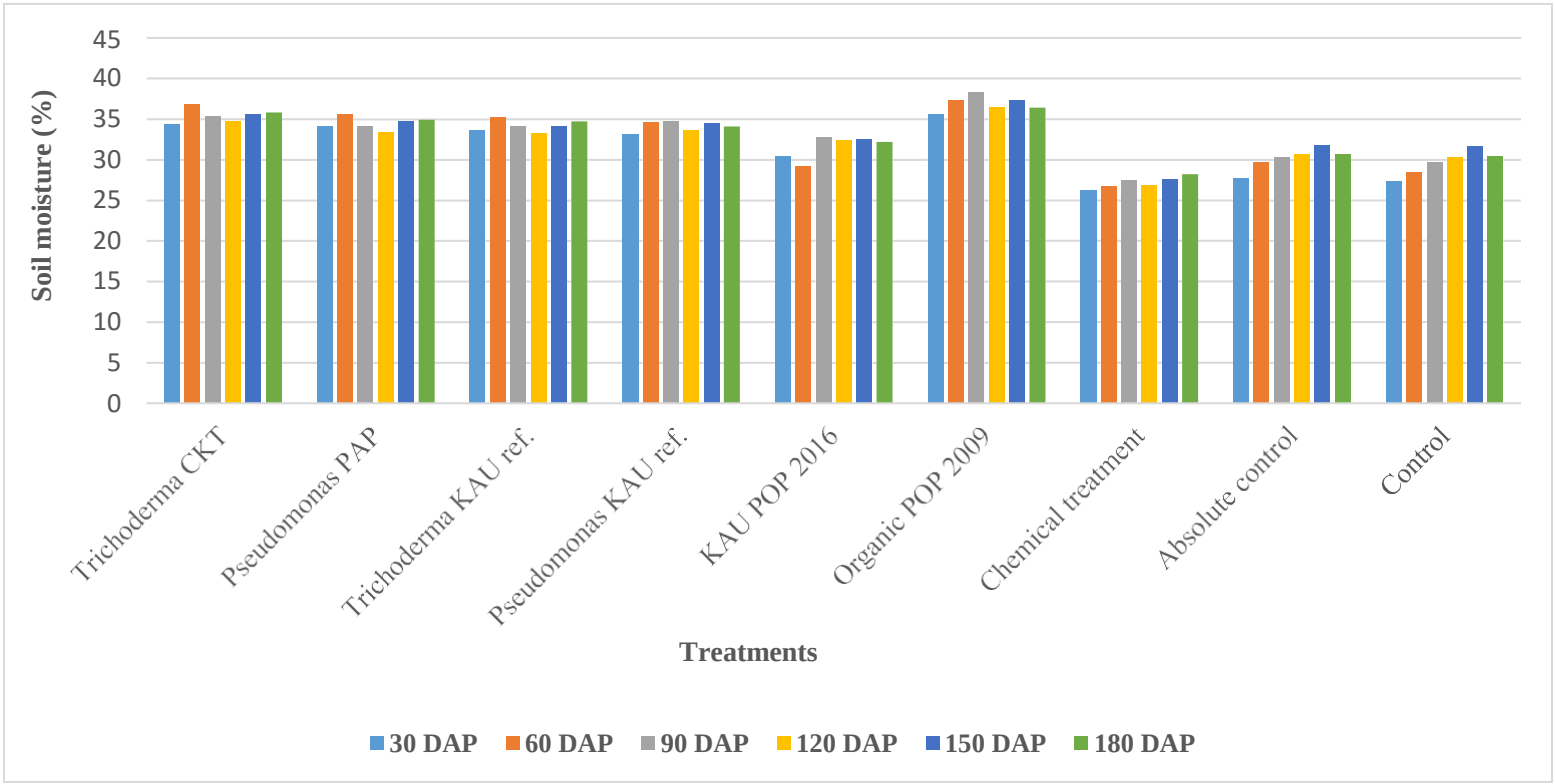


Fig. 2: Effect of different treatments on soil moisture at monthly intervals under pot culture studies

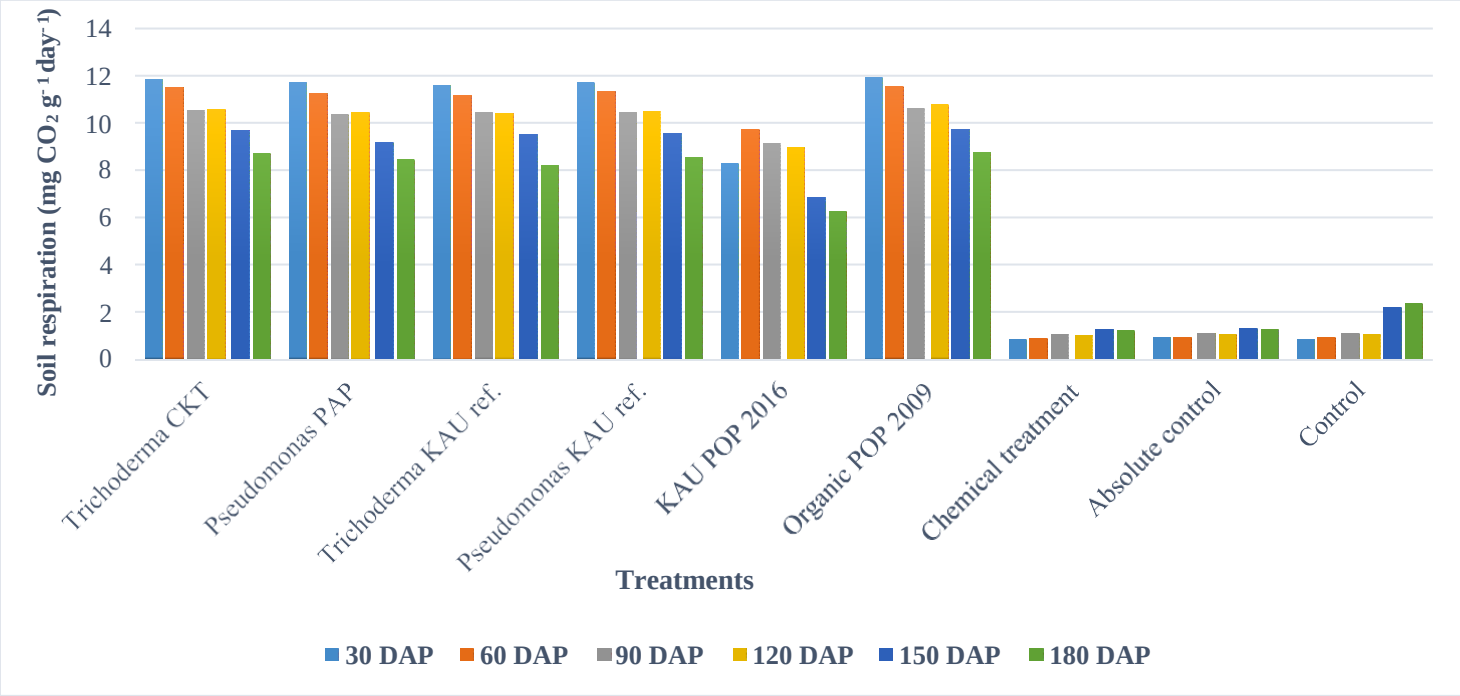


Fig. 3: Effect of different treatments on soil respiration (CO_2 evolution) at monthly intervals under pot culture studies

treatment. Treatments T_1 [*Trichoderma harzianum* (CKT)] and T_2 [*Pseudomonas fluorescens* (PAP)] showed low per cent disease incidence (16.67% each) and per cent disease index (10% and 15 % respectively) at 7 DAI.

At 14 DAI, highest per cent disease incidence (100%) was shown by the treatment control followed by treatments KAU POP 2016 and organic POP 2009 (66.67 % each). Minimum disease incidence (0 %) was observed in the case of treatment absolute control. Maximum per cent disease index was recorded in the treatment control (100%) followed by treatments KAU POP 2016 and organic POP (60 and 50 %) and minimum in the case of treatment absolute control (0%). Treatments T_1 [*Trichoderma harzianum* (CKT)] and T_2 [*Pseudomonas fluorescens* (PAP)] showed low per cent disease incidence (16.67% each) and per cent disease index (20% each) at 14 DAI. Also, population of *Phytophthora capsici* in the potting mixture in every treatments were recorded. Treatments *Trichoderma harzianum* (CKT) and chemical treatment (Copper hydroxide) recorded least population of *P. capsici* (0.1×10^2 cfu g^{-1} soil) followed by *Pseudomonas fluorescens* (PAP) (0.2×10^2 cfu g^{-1} soil). Treatment T_9 (Control) recorded highest population of *P. capsici* (1.3×10^2 cfu g^{-1} soil). However, *P. capsici* population was absent in the case of treatment T_8 (Absolute control), and it might be due to that *P. capsici* was not inoculated in this treatment. Altogether, isolate *Trichoderma harzianum* (CKT) performed well in the case of *Phytophthora* disease management followed by *Pseudomonas fluorescens* (PAP).

Effect of different treatments on biometric characters of black pepper (plant growth promotion) such as number of leaves, plant height, number of lateral branches, number of nodes and internodal length is represented in Fig. 6 to Fig. 10. In the case of biometric characters, best performance was shown by treatment T_5 (KAU POP 2016) followed by treatments T_1 [*Trichoderma harzianum* (CKT)] and T_2 [*Pseudomonas fluorescens* (PAP)] which were on par with T_5. Poor performance

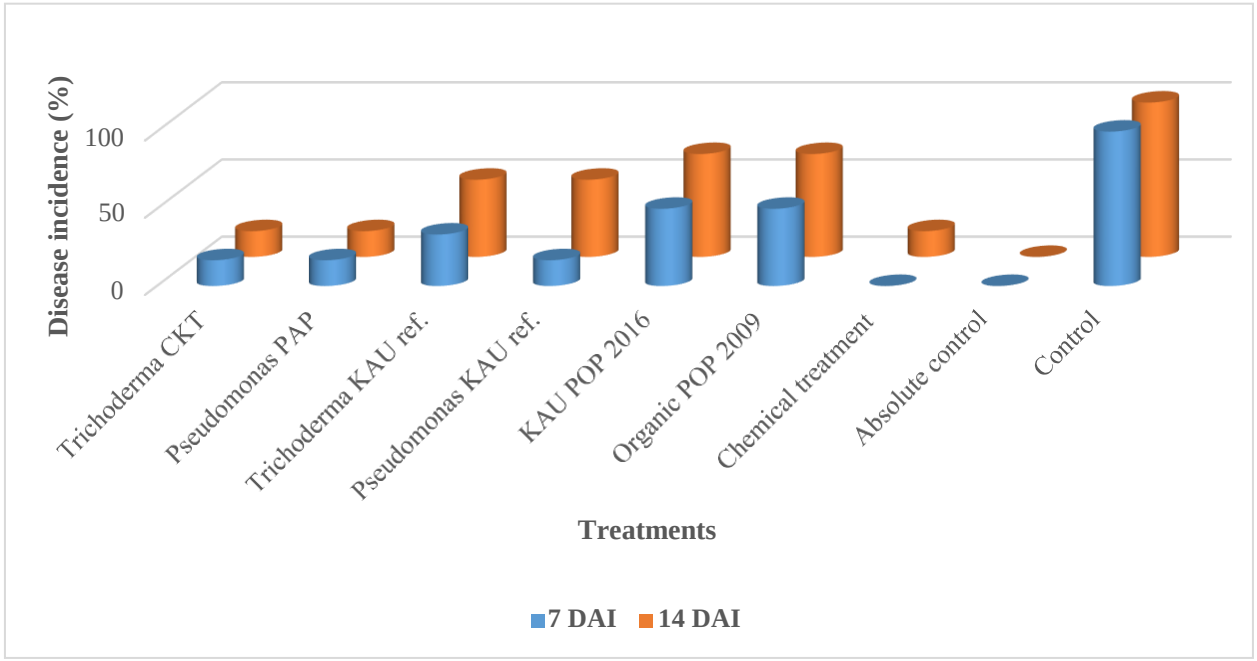


Fig. 4: Per cent disease incidence in different treatments at monthly intervals under pot culture studies

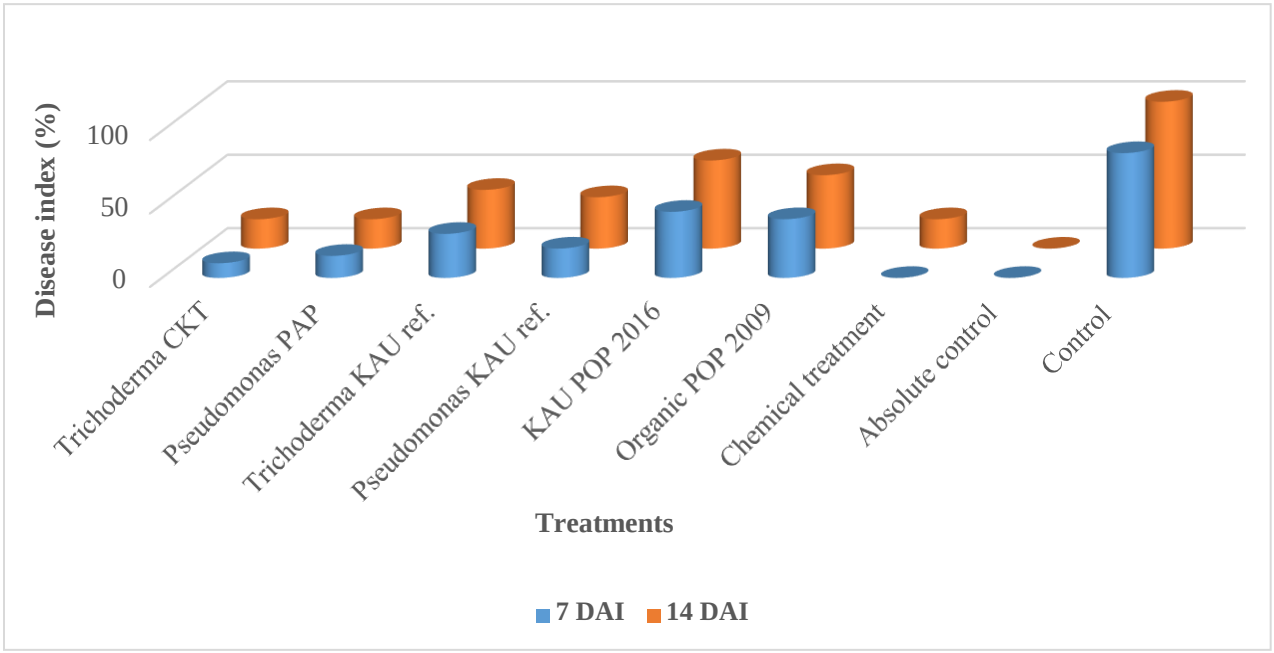


Fig. 5: Per cent disease index in different treatments at monthly intervals under pot culture studies

was shown by T_9 (Control). It might be because of no inoculants were given to it except pathogen.

On the basis of biometric and biological parameters (soil respiration), the best treatment was found out by using pooled ranking method statistically. Accordingly, the most promising treatment for growth promotion under pot culture studies was T_1 [*Trichoderma harzianum* (CKT)] followed by T_2 [*Pseudomonas fluorescens* (PAP)]. Also, based on disease management against *Phytophthora capsici*, treatments *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) were the best treatments. Considering over all parameters, *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) were the most promising isolates under pot culture studies. *Trichoderma* strains have long been accepted as biological agents, for the control of plant diseases and for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, and uptake and use of nutrients. *Trichoderma* sp. are among the most studied fungal biocontrol agents and commercially marketed as biopesticides, biofertilizers and soil amendments (Harman, 2000; Harman *et al.*, 2004; Lorito *et al.*, 2004). It is well reported that some strains of *Trichoderma* promote plant growth, increase nutrient availability, improve crop production and enhance disease resistance (Harman *et al.*, 2004). In a similar study, Wahyuno *et al*. (2016) reported that an application of *Trichoderma* sp. and *Pseudomonas fluorescens* individually or in combination was advantageous for plant protection and plant growth. In another study, Mastouri *et al.* (2010), documented that tomato seedlings treated with *Trichoderma harzianum* had several beneficial effects, from accelerating seed germination and enhancing seedling vigour to amelioration of abiotic stresses such as water, salinity and heat. Rajan *et al*. (2002) demonstrated *Trichoderma harzianum* for its biocontrol activity against *Phytophthora capsici* causing foot rot disease of black pepper. Drenching with *P. fluorescens* in soil before the planting of black pepper reduced wilt disease caused by *P. capsici* under greenhouse conditions (Anith, 2002). Similarly, fluorescent

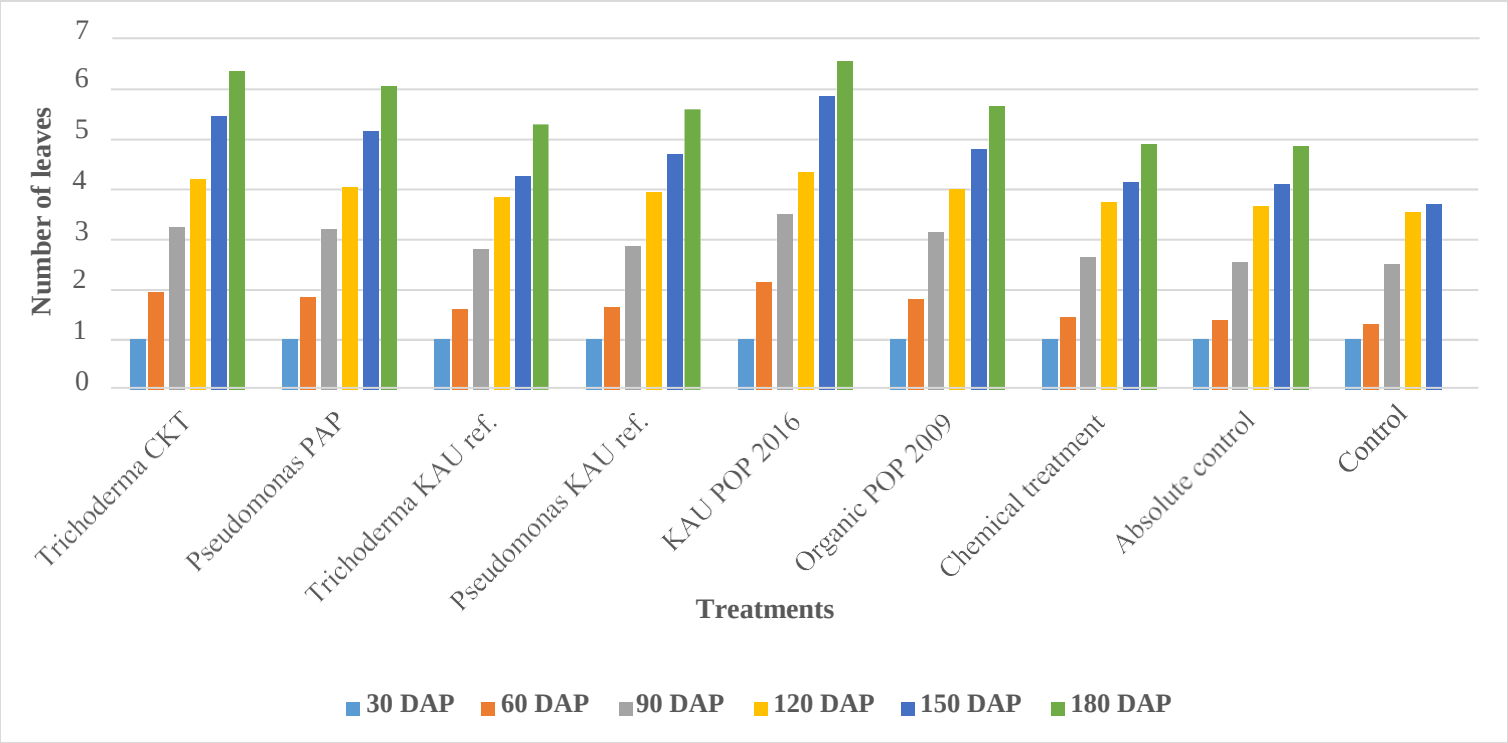


Fig. 6: Effect of different treatments on number of leaves at monthly intervals under pot culture studies

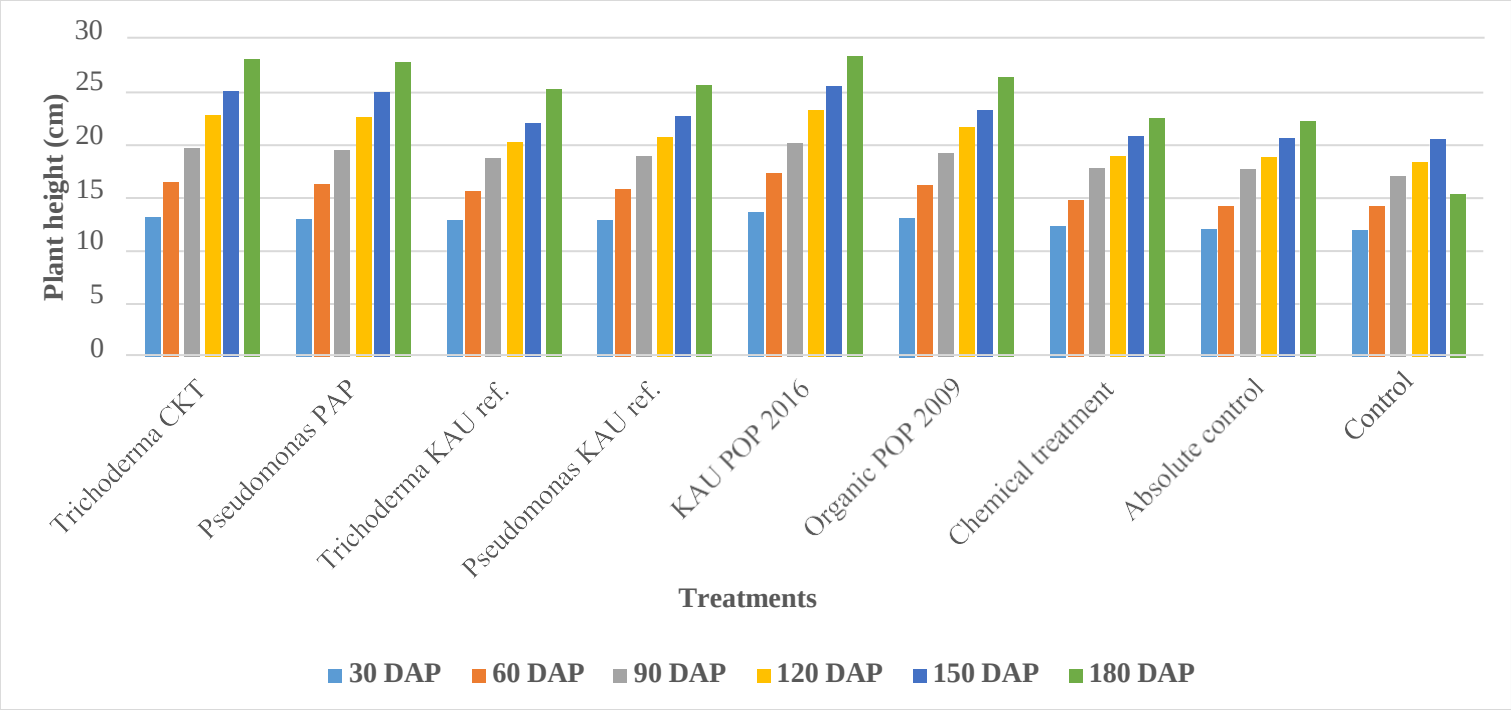


Fig. 7: Effect of different treatments on plant height at monthly intervals under pot culture studies

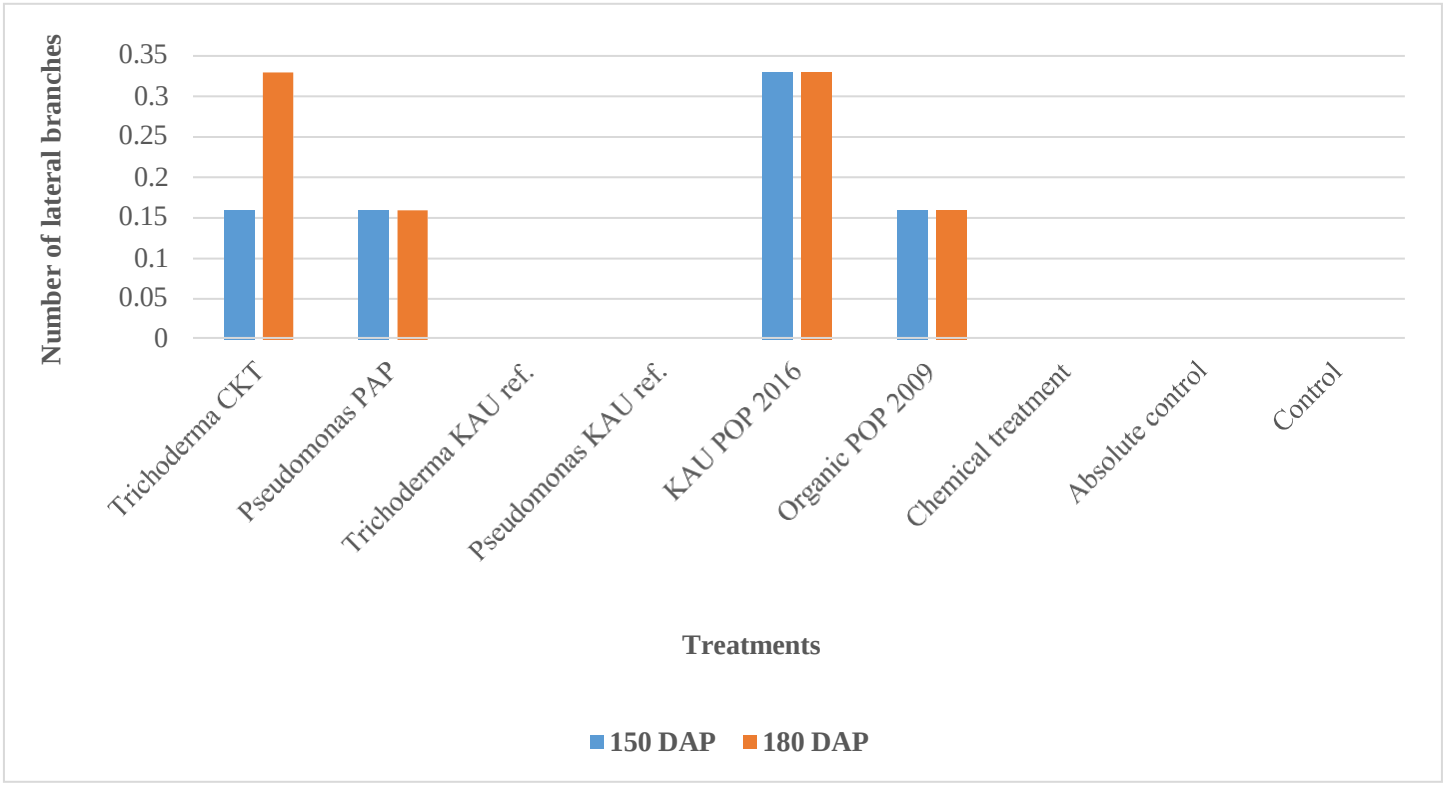


Fig. 8: Effect of different treatments on number of lateral branches at monthly intervals under pot culture studies

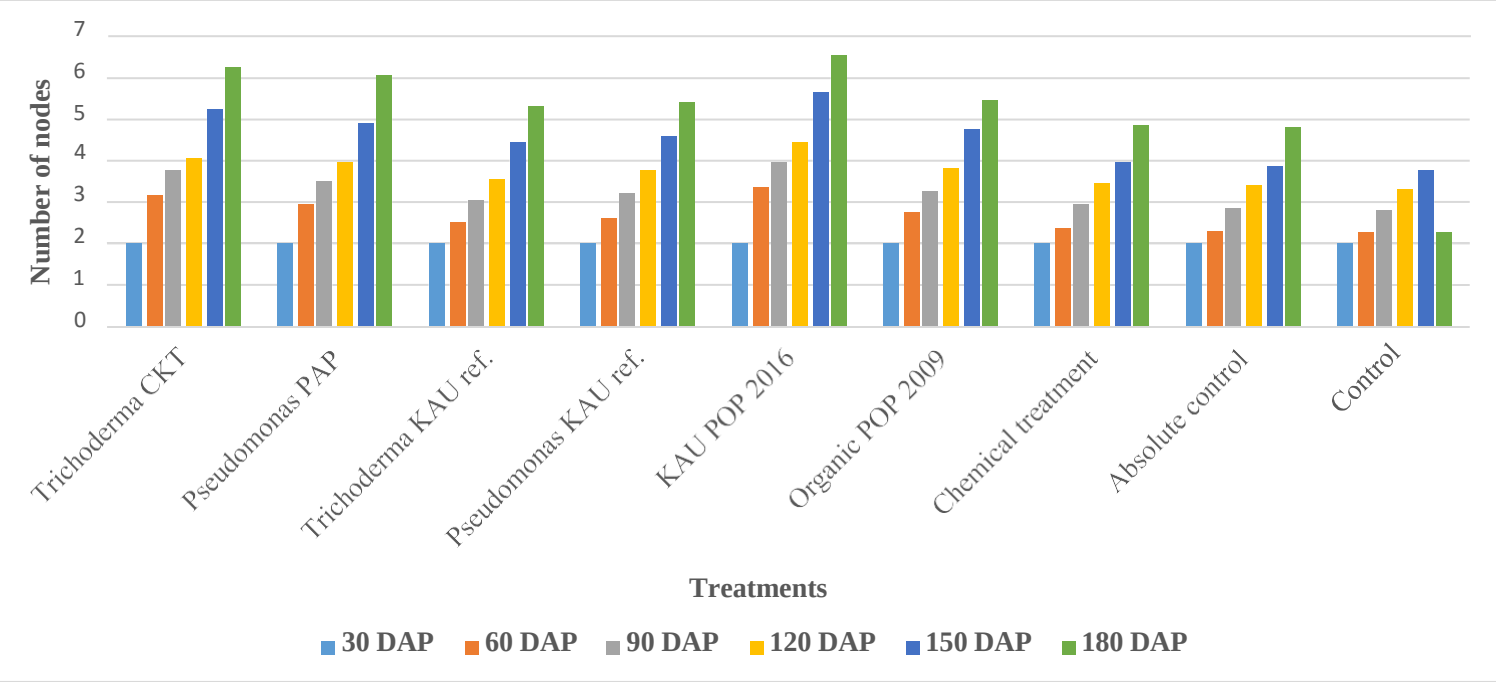


Fig. 9: Effect of different treatments on number of nodes at monthly intervals under pot culture studies

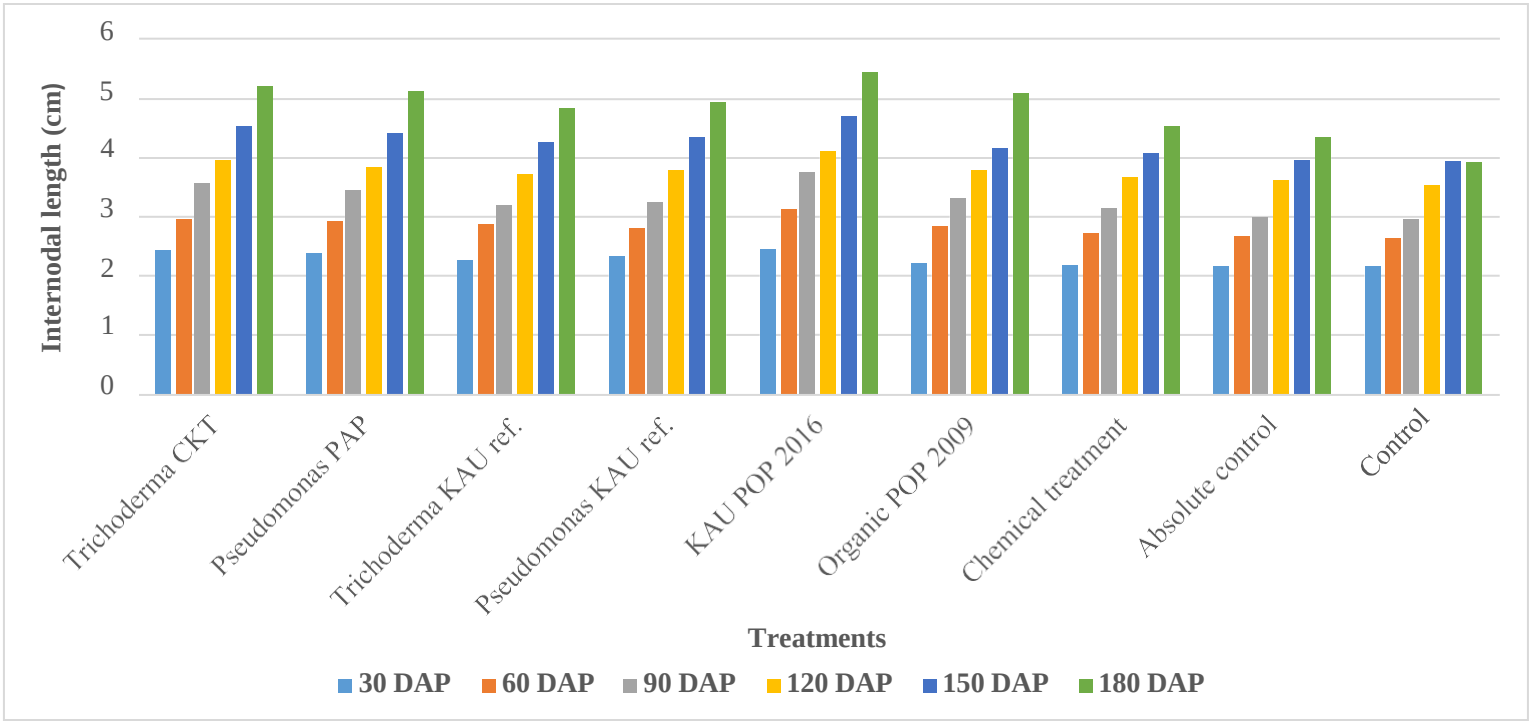


Fig. 10: Effect of different treatments on internodal length at monthly intervals under pot culture studies

pseudomonads is found to be largest and potentially most promising group of plant growth promoting rhizobacteria involved in the biocontrol of plant diseases (Kloepper *et al.,* 1988). Paul *et al.* (2005) isolated *Pseudomonas fluorescens* from black pepper roots and reported that they produced volatile and non-volatile metabolites and inhibited the growth of *Phytophthora capsici. Pseudomonas fluorescens* can produce a wide range of enzymes and metabolites helping the plants to withstand varied biotic and abiotic stresses (Saravanakumar *et al*., 2011).

Organic carbon, available nutrients (nitrogen, phosphorus and potassium) and pH of potting mixture before planting and six months after planting were recorded and is represented in fig. 11 to fig. 15. Initial sterilized potting mixture contained high level of organic carbon (2.10%), medium level of available nitrogen (470.42 kg ha^{-1}), high level of available phosphorus (52.46 kg ha^{-1}), high level of available potassium (295.18 kg ha^{-1}) and neutral pH (7.2).

At six months after planting, highest organic carbon (2.66 % - high) was observed in the treatment T_6 (Organic Package of Practices) and lowest OC (1.97 % - high) in the treatment T_7 (Chemical treatment). Maximum available nitrogen (608.24 kg ha^{-1} - high) was observed in the treatment T_5 (KAU POP, 2016). Lowest N was found in the treatment T_7 (Chemical treatment) and which is 441.38 kg ha^{-1} (medium). Highest available phosphorus (78.32 kg ha^{-1} - high) and available potassium (357.68 kg ha^{-1} - high) were recorded in the case of treatment T_5 (KAU POP, 2016). Lowest P and K were estimated in treatment T_7 (Chemical treatment) and they were 49.67 kg ha^{-1} (high) and 295.87 kg ha^{-1} (high) respectively.

Under field condition, evaluation of abiotic stress tolerant strains of *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) for plant growth promotion and disease management in black pepper was conducted. For that, six months old black pepper plants were planted. Field evaluation was carried out based on chemical analysis of soil (organic carbon and available nitrogen,

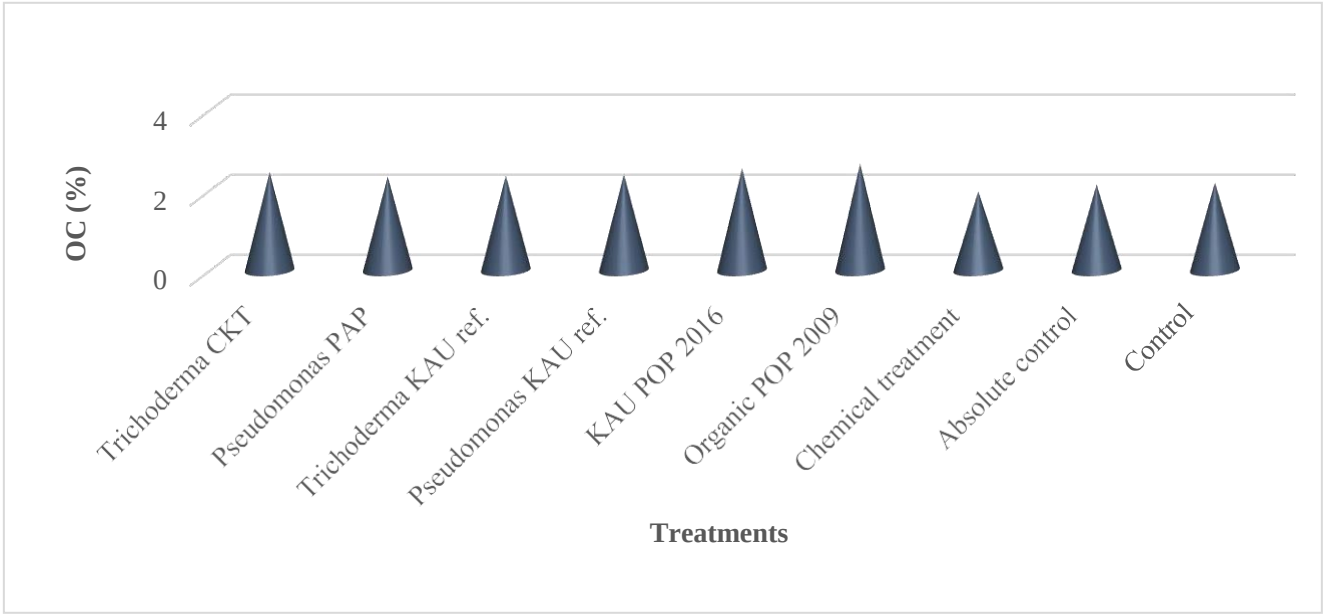


Fig. 11: Effect of different treatments on organic carbon content six months after planting under pot culture studies

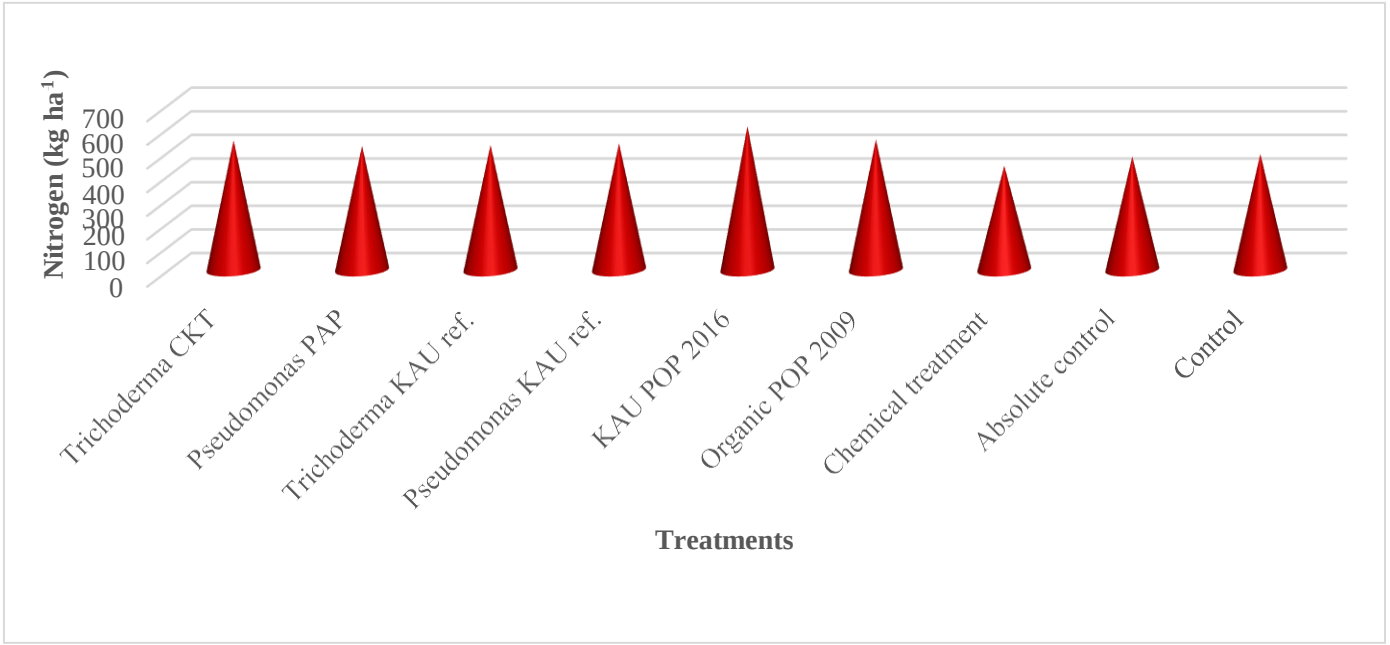


Fig. 12: Effect of different treatments on available nitrogen six months after planting under pot culture studies

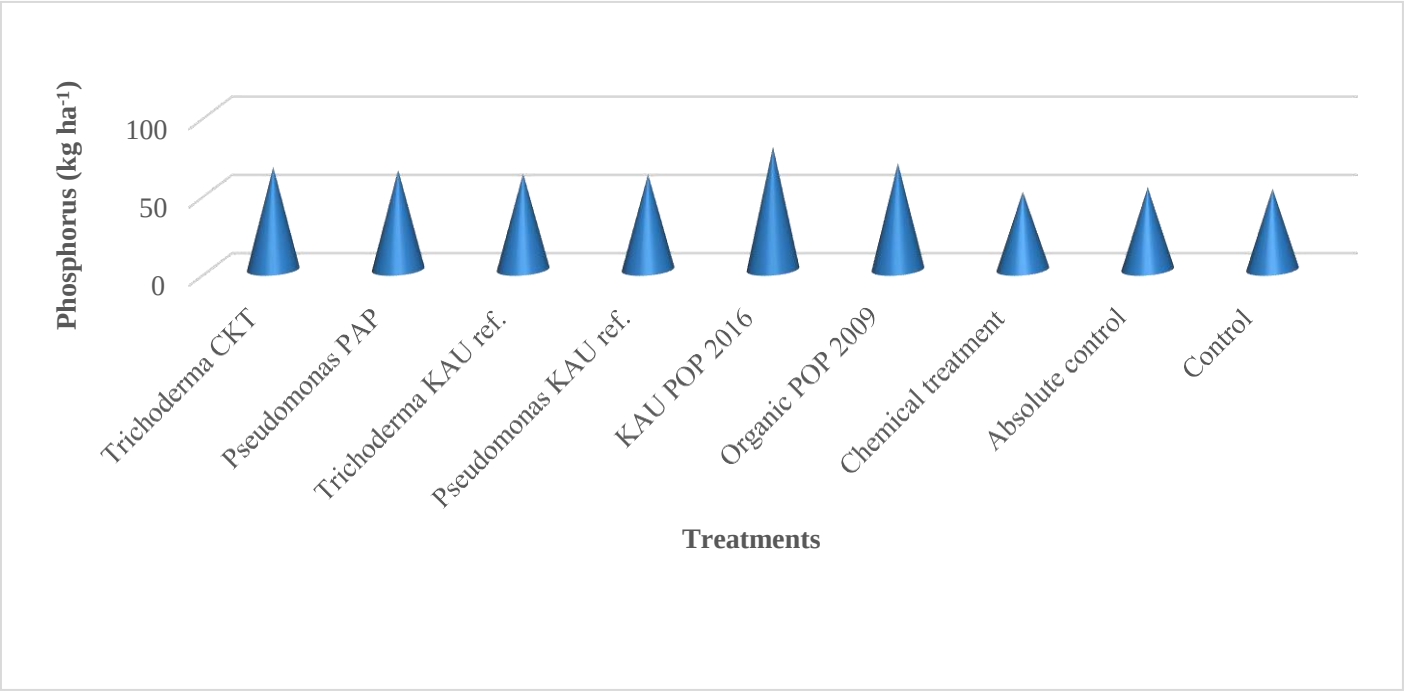


Fig. 13: Effect of different treatments on available phosphorus six months after planting under pot culture studies

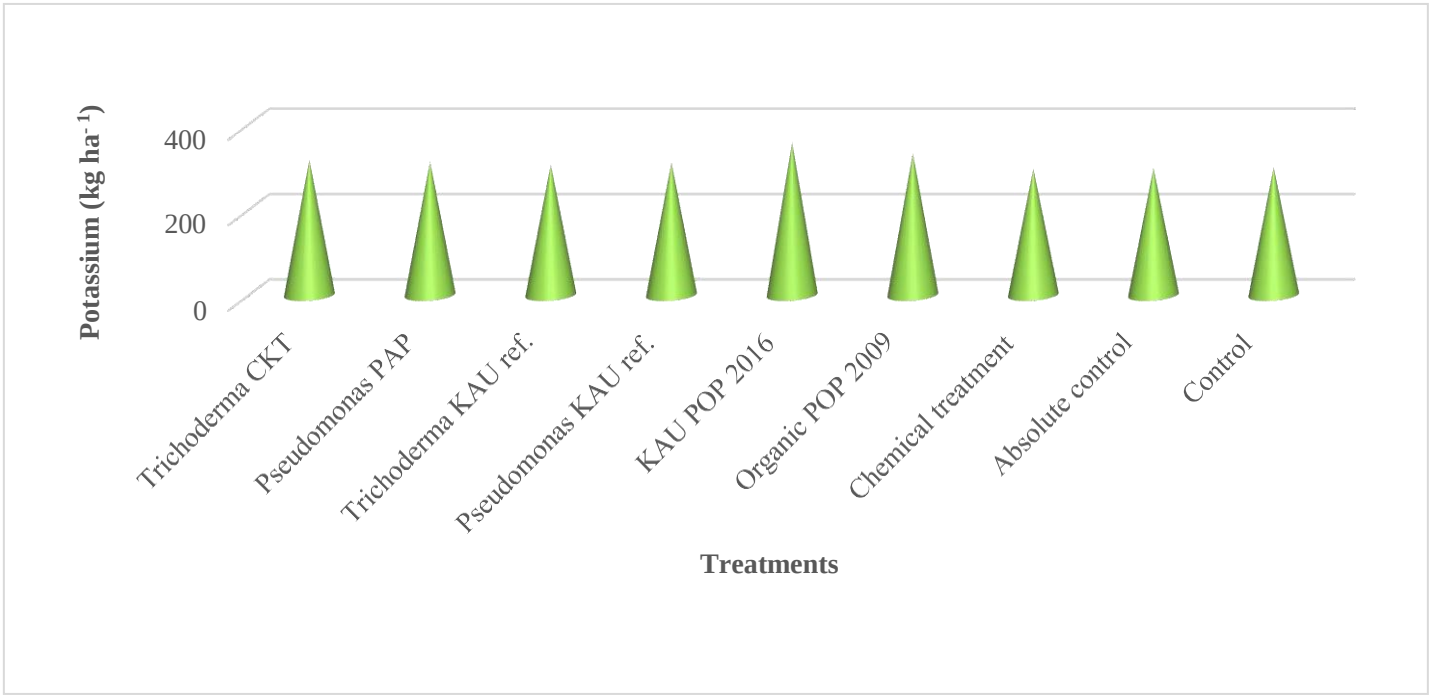


Fig. 14: Effect of different treatments on available potassium six months after planting under pot culture studies

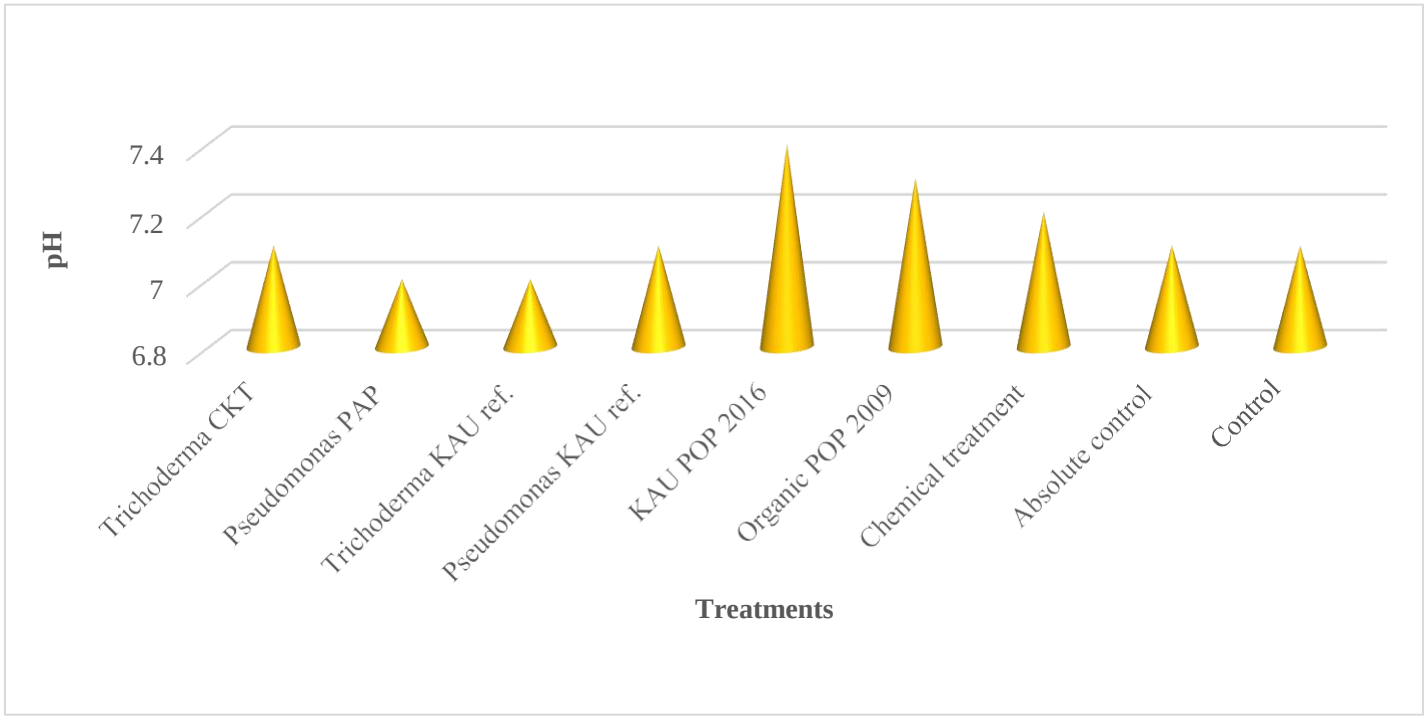


Fig. 15: Effect of different treatments on soil pH six months after planting under pot culture studies

phosphorus and potassium), population of *Trichoderma* sp. and *Pseudomonas fluorescens*, functional efficiency of *Trichoderma* sp. and *Pseudomonas fluorescens*, micro-climatic and soil parameters (soil temperature, soil moisture, soil pH and soil respiration), per cent disease incidence and biometric parameters (number of leaves, plant height, number of lateral branches, number of nodes and internodal length).

Population of *Trichoderma* sp. and *Pseudomonas fluorescens* at monthly intervals was recorded to know the fate and survivability of microbial inoculants in the soil (Fig. 16). There was no population of *Trichoderma* sp. and *Pseudomonas fluorescens* in the field soil before start of the experiment. After planting, population was present in only treatments T_1 [*Trichoderma harzianum* (CKT) + KAU POP 2016], T_2 [*Pseudomonas fluorescens* (PAP) + KAU POP 2016], T_3 [*Trichoderma viride* (KAU reference culture) + KAU POP 2016], T_4 [*Pseudomonas fluorescens* (KAU reference culture) + KAU POP 2016], T_5 [*Trichoderma harzianum* (CKT)], T_6 [*Pseudomonas fluorescens* (PAP)], T_7 [*Trichoderma viride* (KAU reference culture)] and T_8 [*Pseudomonas fluorescens* (KAU reference culture)]. Population of *Trichoderma* sp. and *Pseudomonas fluorescens* declined gradually from 30 DAP to 180 DAP in the soil. According to Bashan *et al.* (1995), population size of microorganisms decrease more or less rapidly following introduction into a natural soil, and growth of introduced population in microbiologically undisturbed soil is a rare phenomenon. Population decreased from 10^4 cfu ml^{-1} to 10^2 cfu ml^{-1} in the case of *Trichoderma* sp. and from 10^5 cfu ml^{-1} to 10^2 cfu ml^{-1} in the case of *Pseudomonas fluorescens*. Haritha (2015) documented that population of the inoculated microorganisms showed a decreasing trend in the soil from planting to harvest of the crop. This might be due to different abiotic factors in soil such as texture, pH, temperature, moisture content, and substrate availability need critical assessment, since these highly determine the survival and activity of the introduced microorganisms (Gray, 1975). Population of *Pseudomonas fluorescens* (PAP isolate) and *Pseudomonas fluorescens* (KAU reference culture) was higher than *Trichoderma*

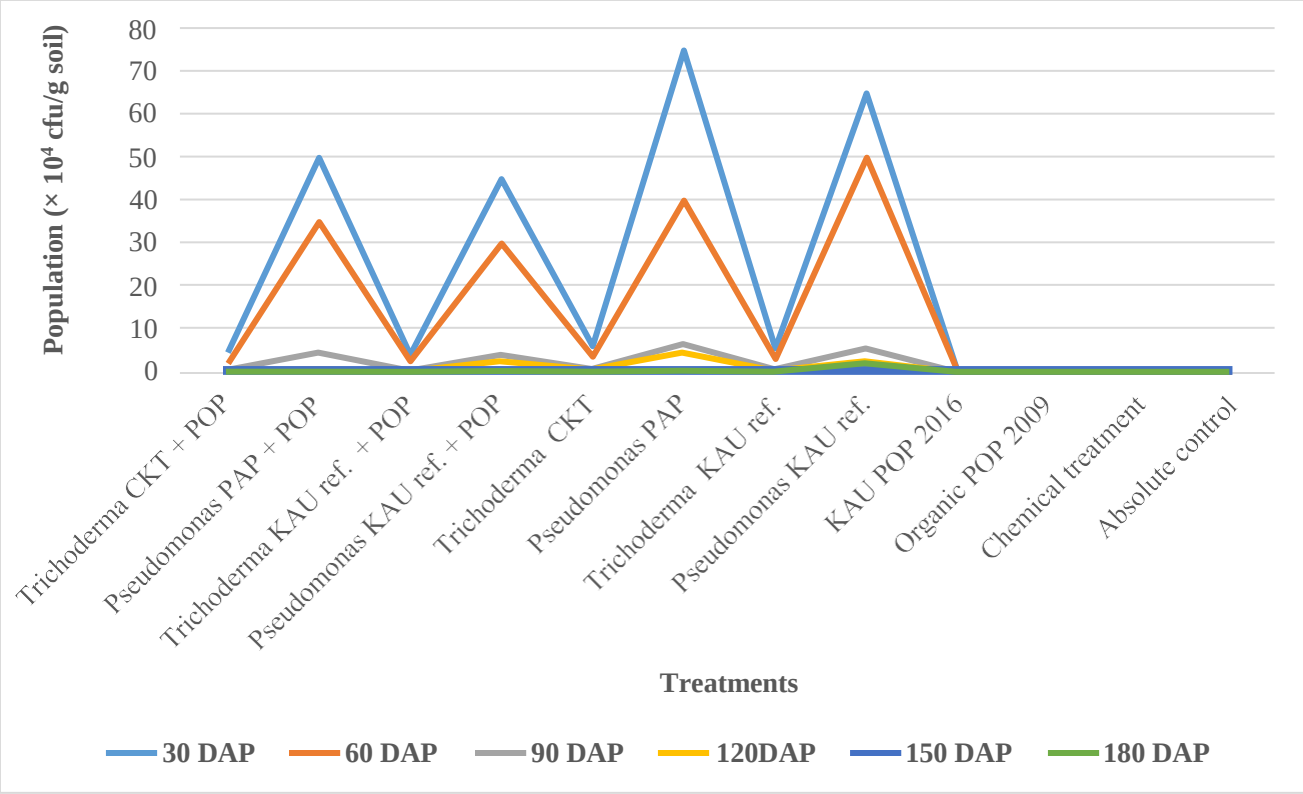


Fig. 16: Population of *Trichoderma* sp. and *Pseudomonas fluorescens* in field soil at monthly intervals

sp. throughout the experiment. Also, population of microorganisms was found to be less in treatments in which microorganisms were treated along with KAU POP than those treated without KAU POP. According to Wright *et al.* (1995), reduction of the population size of bacterial inoculants was due to predation by protozoa in soil which have been confirmed in a lot of recent studies.

All the isolates obtained were screened for ammonia, IAA, HCN and siderophore production at monthly intervals for a period from December 2017 to May 2018 and the isolates *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP) were tested for antagonistic activity against *Phytophthora capsici in vitro*. According to Petterson (2004), variations in the environment might change the composition and biomass of the microbial community, which might further affect the plant growth. Hence, every microorganism needs optimum environmental conditions, which upholds microbial population as well as their metabolic activities. The isolates obtained in the present study are evaluated for the functional efficiency due to the influence of micro-climatic and soil parameters.

Isolates obtained from the treatments T_1 (CKT+ KAU POP 2016), T_4 [*Pseudomonas fluorescens* (KAU ref. culture) + KAU POP], T_5 (CKT) and T_8 [*Pseudomonas fluorescens* (KAU ref. culture)] produced ammonia at a high intensity at 30 DAP (December, 2017) and 60 DAP (January, 2018). Treatments T_2 (PAP + KAU POP), T_3 [*Trichoderma viride* (KAU ref. culture) + KAU POP)], T_6 (PAP) and T_7 [*Trichoderma viride* (KAU reference culture)] recorded medium ammonia production. Thereafter, production of ammonia was declined gradually. At 150 and 180 DAP, no ammonia was produced.

All the eight isolates obtained in the experiment recorded high production of IAA at 30 DAP (December, 2017) and 60 DAP (January, 2018). Later, IAA production was decreased gradually. At 180 DAP (May, 2018), only two isolates T_5

(CKT) and T_6 (PAP) showed IAA production and with a low intensity. No IAA was produced by the other isolates at 180 DAP.

All the eight isolates (T_1, T_2, T_3, T_4, T_5, T_6, T_7 and T_8) obtained at 30 DAP (December, 2017), recorded HCN production at a medium intensity. At 60 DAP, only two isolates (T_5, $T_{6)}$ produced HCN and at a medium level. No HCN production was recorded from 90 DAP (February, 2018) to 180 DAP (May, 2018).

Among the eight isolates obtained at 30 DAP (December, 2017), only three isolates T_5 (CKT), T_6 (PAP) and T_8 [*Pseudomonas fluorescens* (KAU ref. culture)] produced siderophore. At 60 DAP, siderophore production was recorded by the isolates obtained from treatments T_6 and T_8. Thereafter, siderophore production was found to be negative from 90 DAP to 180 DAP.

The isolates *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP) were found to exhibit antagonistic activity against *Phytophthora capsici in vitro* with per cent inhibition of 64.3% and 57.1%.

Effect of different treatments on micro-climatic and soil parameters such as soil temperature, soil moisture, soil pH and soil respiration under field conditions were found out. Treatments showed significant differences in the case of soil moisture and soil respiration (Fig. 17 and Fig. 18) but did not show any significant differences in the case of soil temperature and soil pH. Petterson (2004) outlined that environmental changes may alter the composition and biomass of a microbial community which in turn will affect the growth of plants and that all microorganisms have a set of optimal environmental conditions, which enhances their optimal growth.

Population of *Trichoderma harzianum* (CKT), *Trichoderma viride (*KAU ref. culture), *Pseudomonas fluorescens* (PAP isolate) and *Pseudomonas fluorescens* (KAU ref. culture) was correlated with micro-climatic and soil parameters such as soil temperature, soil moisture, soil pH and soil respiration using spearman's

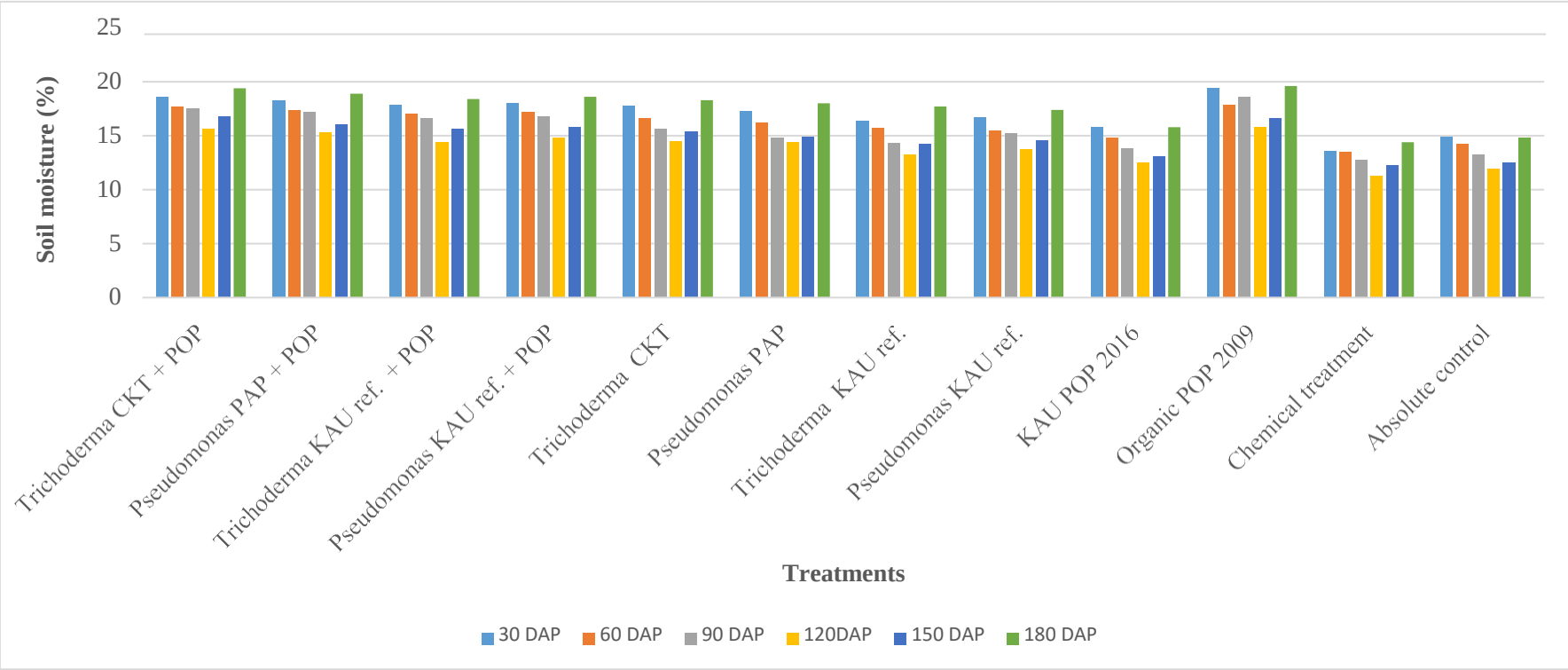


Fig. 17: Effect of different treatments on soil moisture at monthly intervals under field condition

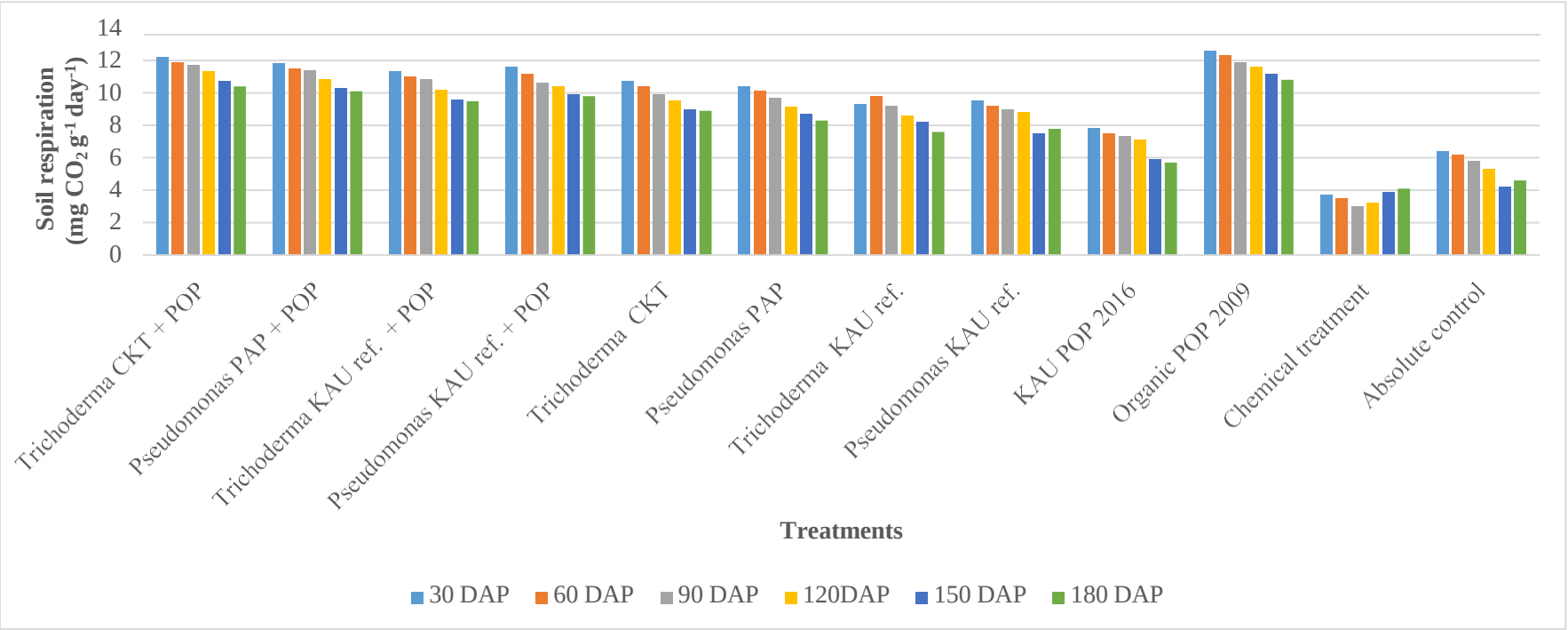


Fig. 18: Effect of different treatments on soil respiration at monthly intervals under field condition

correlation method statistically (Table 25). It was found that soil temperature was negatively correlated with the population of all the four isolates and soil moisture was positively correlated with the population of all the four isolates. However, soil pH and soil respiration did not show any effect on the population of those isolates which is in agreement with earlier reports. Manju (2017), showed that population of *Trichoderma* sp. and fluorescent pseudomonads was negatively correlated with soil temperature and positively correlated with soil moisture. In another study, Chaudhary *et al.* (2016) revealed that the population of *Trichoderma* was negatively correlated with soil temperature and positively correlated with the soil moisture. Similarly, Knudsen and Bin (1990) conducted a study on the effect of soil moisture and temperature on the growth of *Trichoderma harzianum* from the alginate pellets. They showed that temperature had a significant positive effect on radial growth rate. In another study, Guo *et al*. (2013) concluded that population of soil bacteria was negatively correlated with soil temperature. Gamliel and Khan (1991) reported an inverse relationship between soil pH and population densities of *Pseudomonas fluorescens* which is contradictary to the present study where there is no effect for soil pH on the population of *Pseudomonas fluorescens.* In another study, Francis (2018) reported that soil respiration had no significant effect on population of fluorescent pseudomonads which is in agreement with the results of current study where soil respiration had no significant correlation with the population of *Pseudomonas fluorescens.*

Effect of micro-climatic and soil parameters on functional efficiency of *Trichoderma* sp. and *Pseudomonas fluorescens* was found out statistically. The observations of functional efficiency of the isolates were cross tabulated with each of the micro-climatic and soil parameters and the number of isolates with different efficiency were recorded. The dependency of one character on the other was measured through significance of chi-square statistics.

In the case of *Trichoderma* isolates, the soil temperature during six months period ranged from 26.8°C (December 2017) to 30.6°C (March 2018). The effect of soil temperature on ammonia, HCN and siderophore production did not show any significant differences. However, soil temperature showed significant differences on IAA production at 1 per cent significant level. Out of sixteen isolates obtained during six months period, fourteen produced IAA at higher level and two isolates produced IAA at low level. Optimum temperature range for IAA production was from 25 to 30°C. The soil moisture content during six months period ranged from 14 per cent (March 2018) to 17.6 per cent (May 2018). The effect of soil moisture on ammonia, HCN and siderophore production by *Trichoderma* isolates did not show any significant differences. However, soil moisture showed significant differences on IAA production at 1 per cent significant level. Out of sixteen isolates obtained during six months period, fourteen produced IAA at higher level and two isolates produced IAA at low level. The soil pH during six months period ranged from 6.1 (December 2017) to 5.7 (May 2018). The effect of soil pH on ammonia, HCN and siderophore production did not show any significant differences. Soil pH showed significant differences on IAA production at 1 per cent significant level. Out of sixteen isolates obtained during six months period, fourteen produced IAA at higher level and two isolates produced IAA at low level. The soil respiration during six months period ranged from 9.8 mg CO_2 g^{-1} day^{-1} (December 2017) to 8.1 mg CO_2 g^{-1} day^{-1} (May 2018). The effect of soil respiration on ammonia, HCN and siderophore production did not show any significant differences. Soil respiration showed significant differences on IAA production at 1 per cent significant level. Out of sixteen isolates obtained during six months period, fourteen produced IAA at higher level and two isolates produced IAA at low level.

In the case of *Pseudomonas fluorescens*, effect of soil temperature, soil moisture, soil pH and soil respiration on ammonia, HCN and siderophore production did not show any significant differences. However, out of total twelve

isolates obtained, ten isolates produced IAA at higher level. Two isolates showed IAA production at a low level. Eight isolates produced ammonia at a high level while four isolates produced ammonia at a medium level. Four isolates produced HCN at a medium level while eight isolates did not show HCN production. Only three isolates showed siderophore production and nine did not produced siderophore. However, among ammonia, IAA, HCN, and siderophore production by *Pseudomonas fluorescens* isolates, micro-climatic and soil parameters had a significant effect on the IAA production.

Effect of different treatments on biometric characters of black pepper (plant growth promotion) such as number of leaves, plant height, number of lateral branches, number of nodes and internodal length under field condition was recorded at monthly intervals. It is represented graphically in Fig. 19 to Fig. 22. On the basis of all the biometric parameters, T_1 [*Trichoderma harzianum* (CKT) + KAU POP] performed as the best treatment which was followed by T_2 [*Pseudomonas fluorescens* (PAP) + KAU POP]. It can be found that the treatments where microbial inoculants supplied along with chemical fertilizers (KAU POP) showed better performance than the treatments where microbial inoculants are given alone without fertilizers. Among those treatments, *Trichoderma harzianum* (CKT) + KAU POP (T_1) was the best treatment followed by *Pseudomonas fluorescens* (PAP) + KAU POP. Also, the treatments where microbial inoculants given along with KAU POP showed much better performance than the treatment where KAU POP was supplied alone (T_9). Treatment T_{12} (Absolute control) showed poor performance in the case of biometric parameters. It might be because no inoculants were given to it. There was no natural incidence of *Phytophthora* foot rot disease in the field. Based on biometric and biological parameters, best treatment was found out using pooled ranking method statistically. Accordingly, treatment *Trichoderma harzianum* (CKT) + KAU POP was the best treatment followed by *Pseudomonas fluorescens* (PAP) + KAU POP under field conditions. These results are in

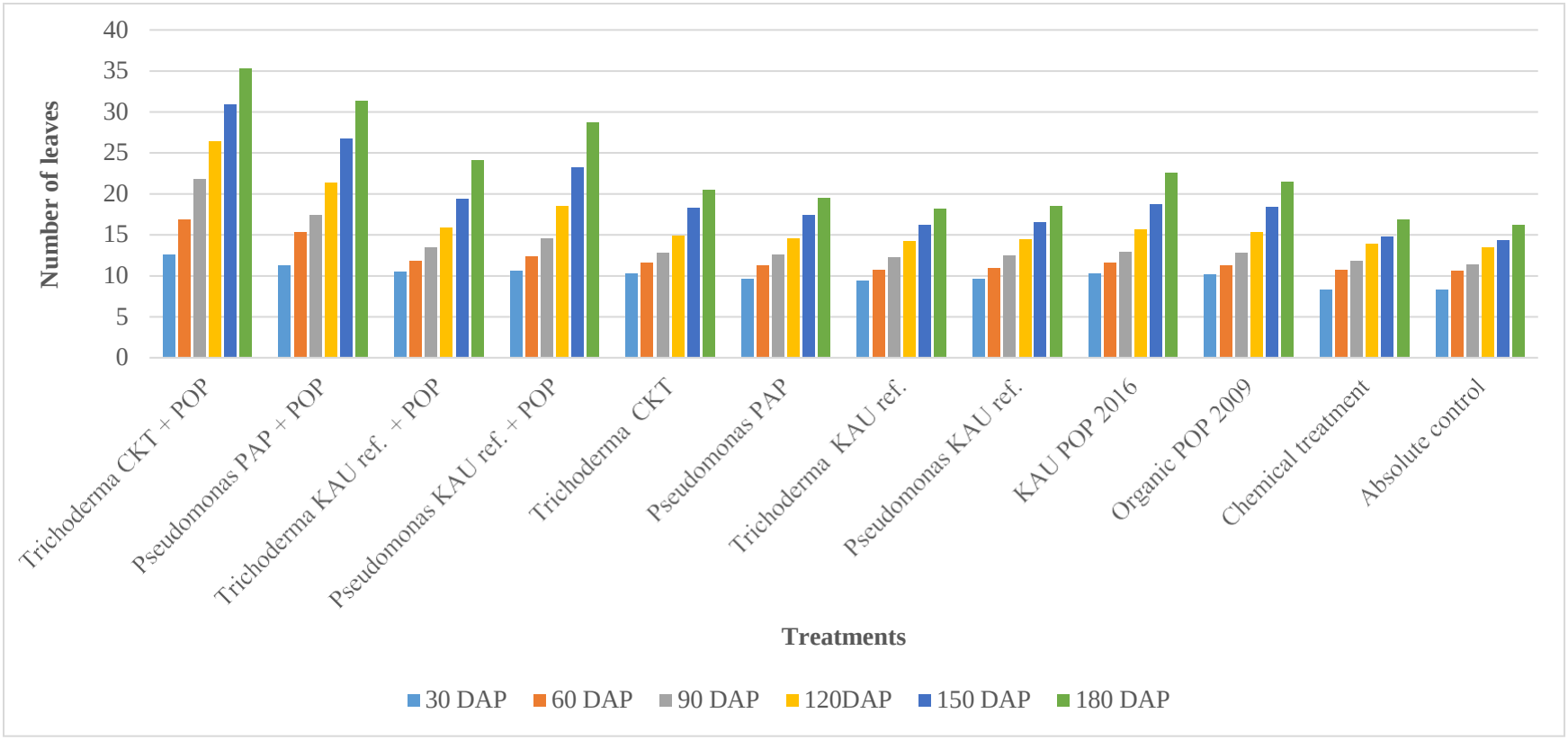


Fig. 19: Effect of different treatments on number of leaves at monthly intervals under field condition

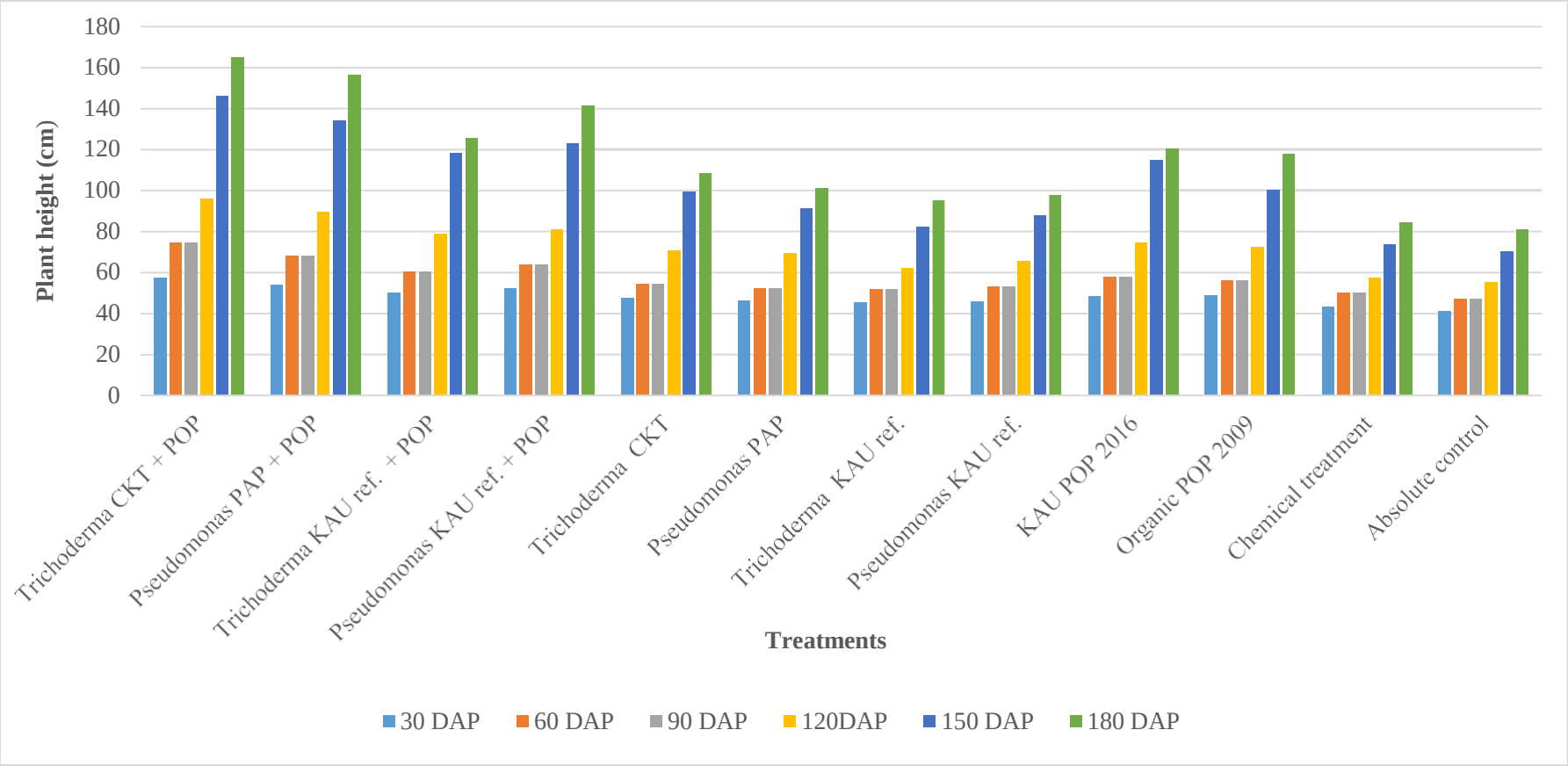


Fig. 20: Effect of different treatments on plant height at monthly intervals under field condition

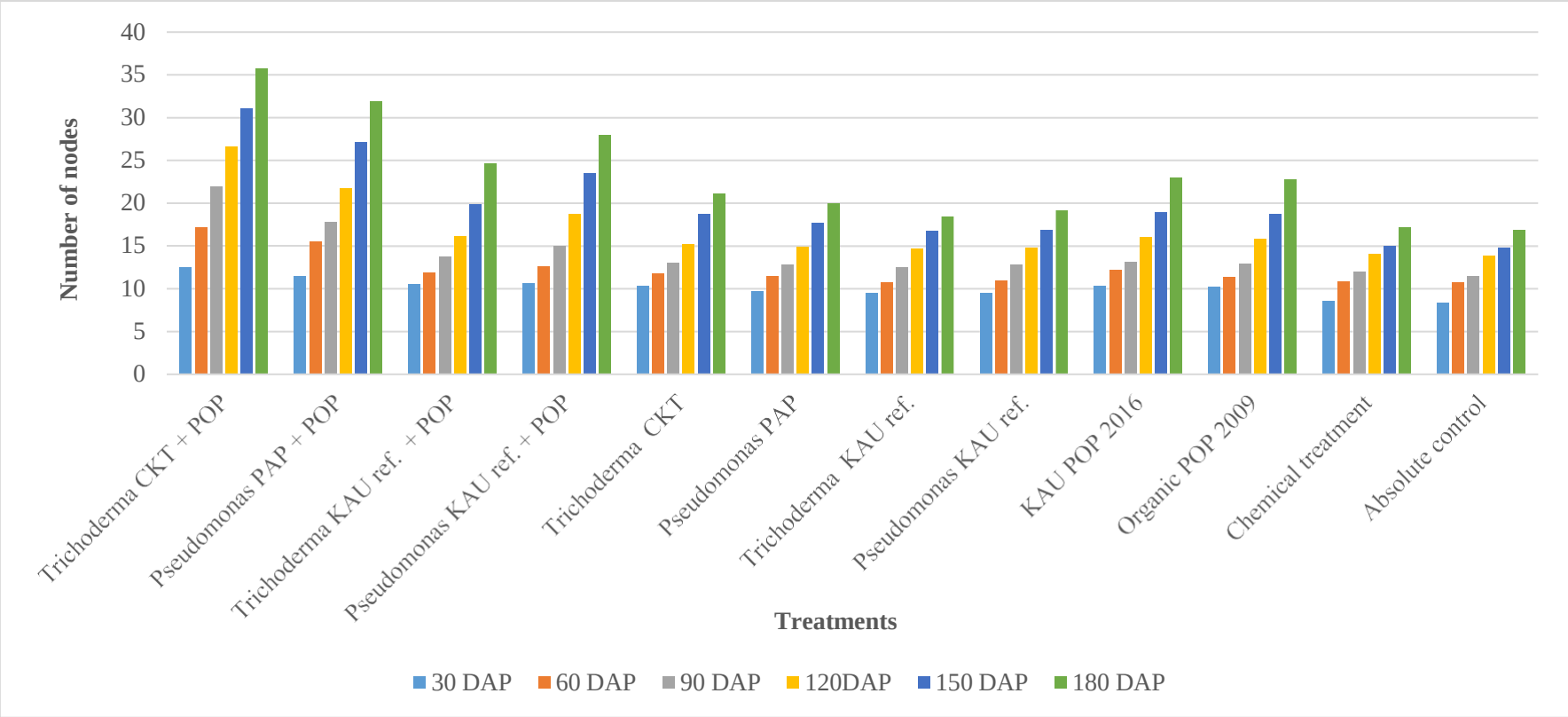


Fig. 21: Effect of different treatments on number of nodes at monthly intervals under field condition

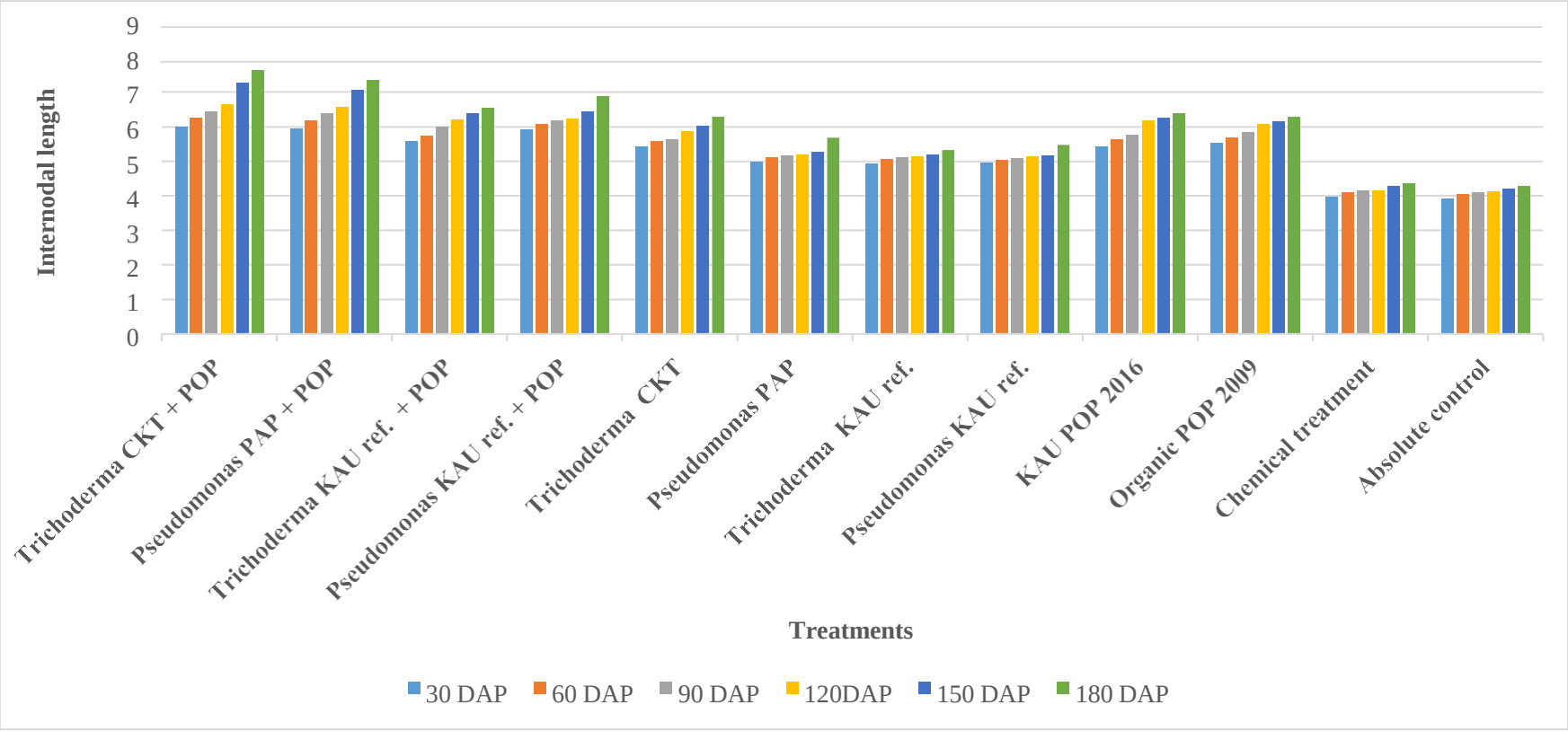


Fig. 22: Effect of different treatments on internodal length at monthly intervals under field condition

agreement with some earlier reports. In a similar study, Jisha *et al.* (2002) reported that *T. harzianum* and *Pseudomonas fluorescens* (IISR-6) promoted growth and vigour of black pepper, ginger and cardamom and suppressed soil-borne fungal pathogens in field condition. It is well reported that some strains of *Trichoderma* sp. promote plant growth, increase nutrient availability, improve crop production and enhance disease resistance (Harman *et al..,* 2004). In another study, Mastouri *et al.* (2010) documented that tomato seedlings treated with *Trichoderma harzianum* had several beneficial effects, from accelerating seed germination and enhancing seedling vigour to amelioration of abiotic stresses such as water, salinity and heat. In a similar study, Haque *et al.* (2012) reported that sole application of *Trichoderma* sp. did not show remarkable contribution in plant growth. However, combined application of *Trichoderma* sp. along with NPK fertilizers boosted up the growth in mustard and tomato plants. These are in agreement with the results of present study. In another study, Rushda *et al.* (2014), concluded that combined application of *Trichoderma* sp. and chemical fertilizers could be utilized for improving crop growth and yield under field conditions.

Organic carbon, available nutrients (nitrogen, phosphorus and potassium) and pH before planting and six months after planting were recorded and are represented graphically in fig. 23 to fig. 27. Initially, the field soil contained high level of organic carbon (1.8 %), medium level of available nitrogen (403.21 kg ha^{-1}), high level of available phosphorus (27.84 kg ha^{-1}), medium level of available potassium (208.21 kg ha^{-1}) and acidic pH (5.7).

After six months of planting, highest organic carbon (2.71 % - high) was observed in the treatment T_{10} (Organic Package of Practices). Lowest OC (1.82 % - high) was recorded in the treatment T_{11} (Chemical treatment). However, the organic carbon per cent increased at the end of the experiment. It might be due to higher organic matter addition through manures and microbial inoculants (Ferrini *et al*., 2008). Maximum available nitrogen was observed in the treatment T_1 [*Trichoderma*

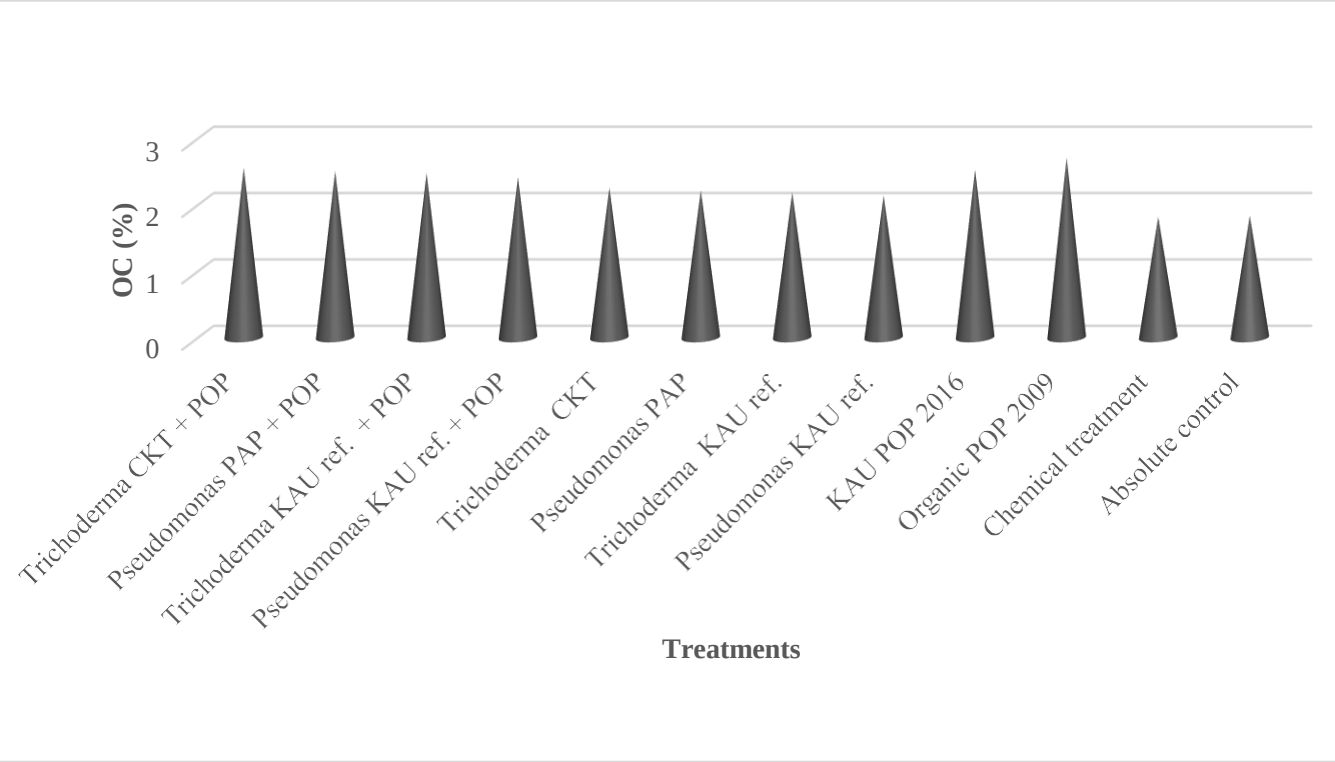


Fig. 23: Effect of different treatments on organic carbon content six months after planting under field condition

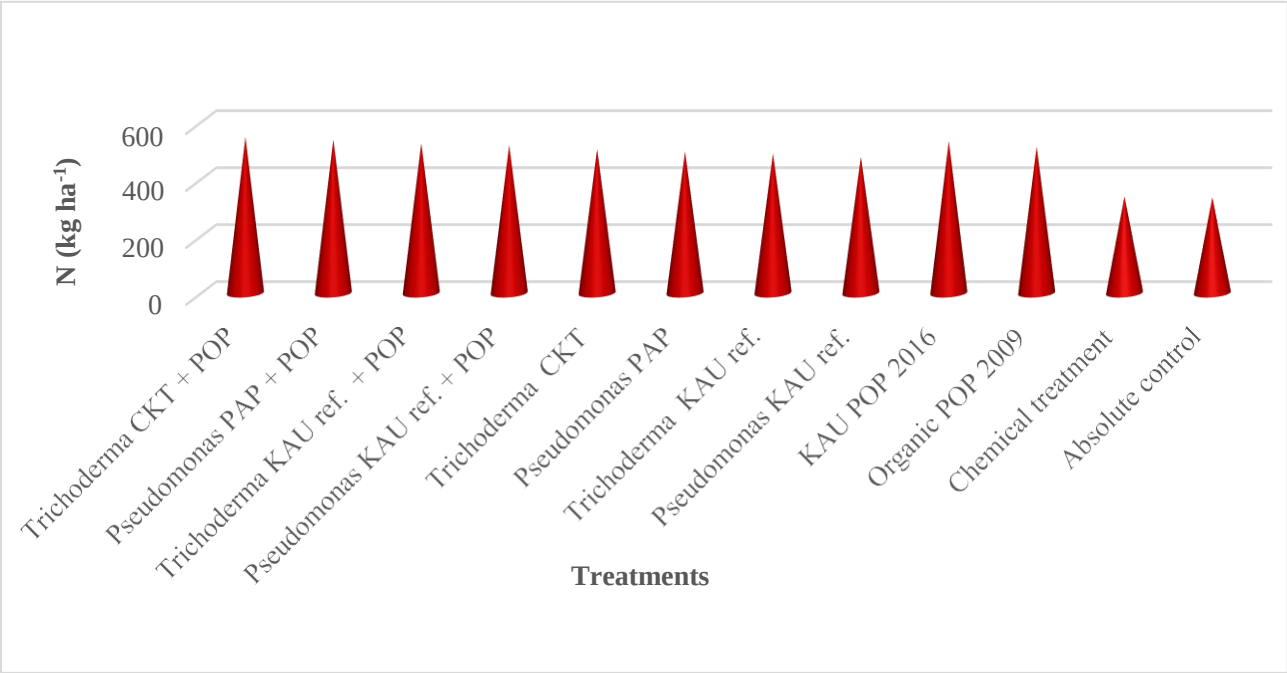


Fig. 24: Effect of different treatments on available nitrogen six months after planting under field condition

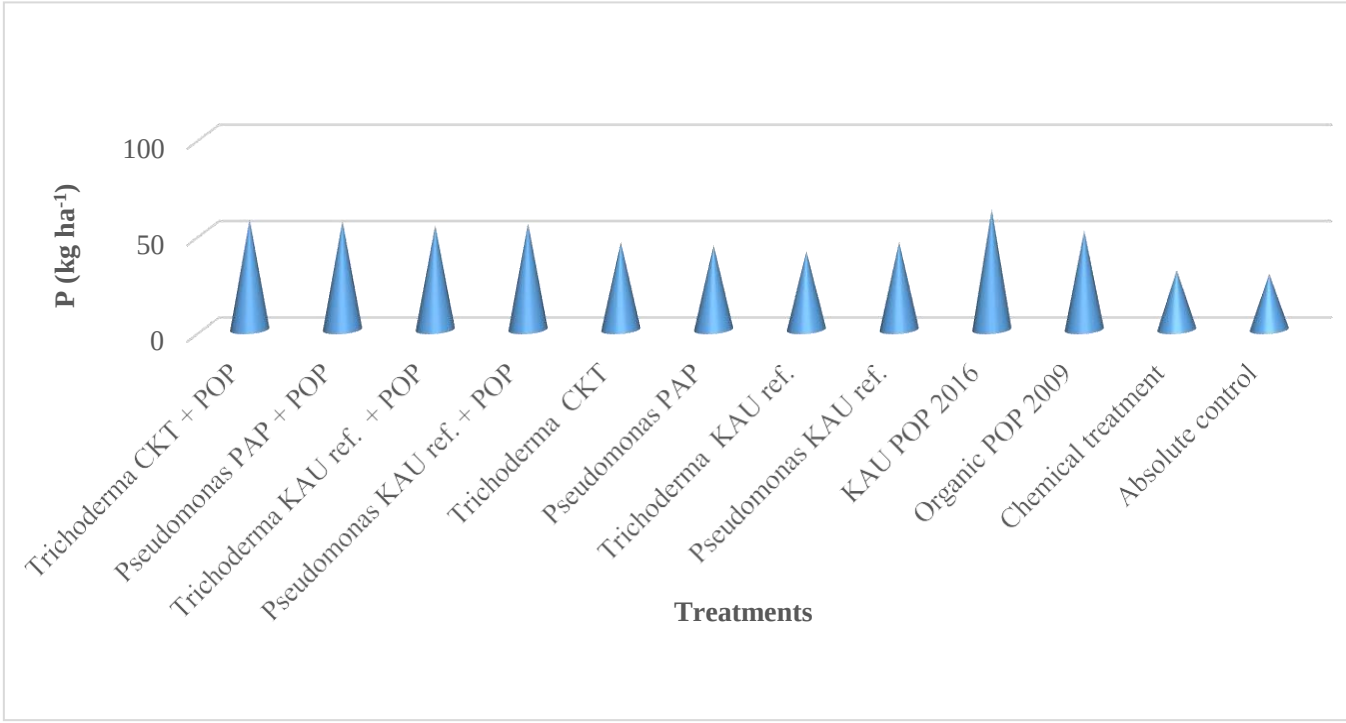


Fig. 25: Effect of different treatments on available phosphorus six months after planting under field condition

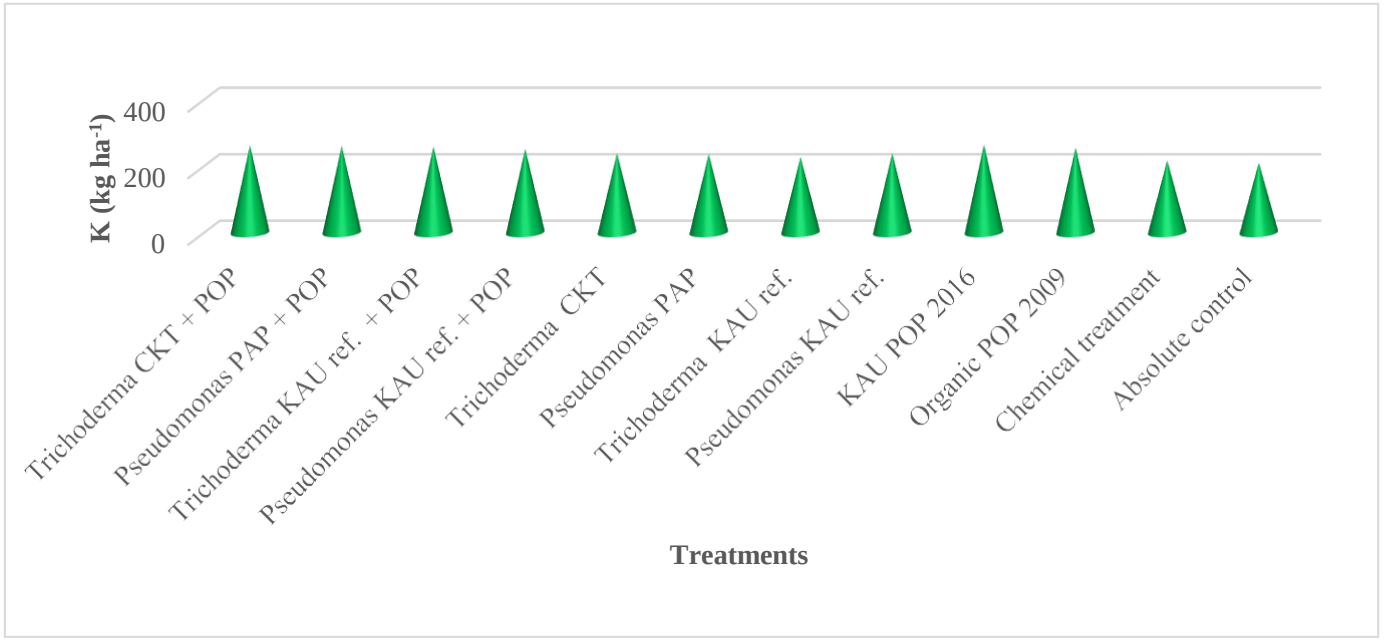


Fig. 26: Effect of different treatments on available potassium six months after planting under field condition

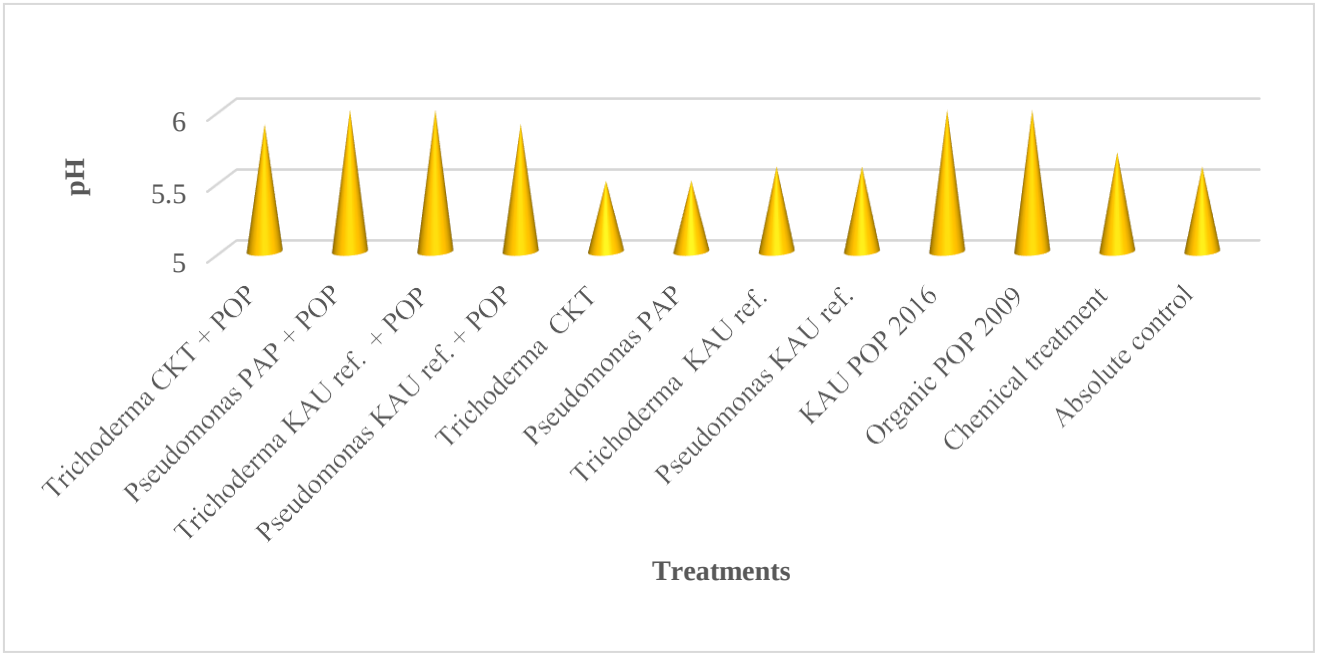


Fig. 27: Effect of different treatments on soil pH six months after planting under field condition

harzianum (CKT isolate) + KAU POP 2016] and it was 548.56 kg ha^{-1} which is high. Lowest N was found in treatment T_{12} (Absolute control) and which was 336.28 kg ha^{-1} (medium). Highest available phosphorus (P) and available potassium (K) were recorded in the case of treatment T_9 [Package of Practices Recommendations (KAU, 2016)]. P and K are found to be 62.36 kg ha^{-1} (high) and 265.38 kg ha^{-1} (medium) respectively in T_9. Lowest P and K are estimated in treatment T_{12} (Absolute control). They were 28.92 kg ha^{-1} (high) and 211.01 kg ha^{-1} (high) respectively. Highest available phosphorus recorded in treatment KAU POP might be due to the availability of easily available phosphorus due to the addition of chemical phosphatic fertilizers (Srilatha and Sharma, 2015). Highest potassium was also recorded in treatment KAU POP. It might be due to application of potash fertilizers (IISR, 1998).

The present study indicated that *Trichoderma harzianum* (CKT isolate) was the most promising isolate for growth promotion and *Phytophthora* disease management based on biometric, biological and disease management characters in black pepper under pot culture studies which was followed by the isolate *Pseudomonas fluorescens* (PAP isolate). *Trichoderma harzianum* (CKT isolate) was the most promising isolate for growth promotion in black pepper on the basis of biometric and biological parameters under field conditions.

Future line of work

- Multi-locational field trials for evaluation of the promising isolate under different agro-ecological units of Kerala need to be carried out.
- Mass production of *Trichoderma harzianum* (CKT) for distribution to farmers.

Summary

6. SUMMARY

The present study on "Field evaluation of abiotic stress tolerant strains of *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) for *Phytophthora* disease management in black pepper (*Piper nigrum* L.)" was carried out in the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara. The major objective was to study the effect of micro-climatic and soil parameters on abiotic stress tolerant strains of *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) under field conditions. The important findings of the study are summarized below:

Pot culture experiment:

- Population of *Pseudomonas fluorescens* (KAU reference culture) and *Pseudomonas fluorescens* (PAP) was found to be higher from 30 DAP (6.5×10^6 cfu g^{-1} and 4.5×10^6 cfu g^{-1} respectively) to 180 DAP (4.5×10^4 cfu g^{-1} and 2.5×10^4 cfu g^{-1} respectively).
- Population of *Trichoderma harzianum* (CKT) and *Trichoderma viride* (KAU reference culture) was found to be less from 30 DAP (7.0×10^5cfu g^{-1} and 6.0×10^5 cfu g^{-1} respectively) to 180 DAP (3.0×10^3 cfu g^{-1} and 2.0×10^3 cfu g^{-1} respectively).
- There were no significant differences between the treatments in the case of soil temperature and soil pH throughout the entire experiment.
- Soil moisture content was higher in Organic Package of Practices (KAU, 2009) followed by *Trichoderma harzianum* (CKT).
- Soil respiration was higher in Organic Package of Practices (KAU, 2009) followed by *Trichoderma harzianum* (CKT), *Pseudomonas fluorescens* (KAU reference culture), *Pseudomonas fluorescens* (PAP) and *Trichoderma viride* (KAU reference culture).

- Soil temperature was negatively correlated with the population of *Trichoderma harzianum* (CKT) and *Trichoderma viride* (KAU reference culture), *Pseudomonas fluorescens* (PAP) and *Pseudomonas fluorescens* (KAU reference culture).
- Soil moisture content was positively correlated with the population of *Trichoderma harzianum* (CKT) and *Trichoderma viride* (KAU reference culture), *Pseudomonas fluorescens* (PAP) and *Pseudomonas fluorescens* (KAU reference culture).
- However, soil pH and soil respiration did not have any correlation with the population of *Trichoderma* sp. and *Pseudomonas fluorescens.*
- Treatment Package of Practices Recommendations (KAU, 2016) showed higher performance in biometric parameters such as number of leaves, plant height, number of lateral branches, number of nodes and internodal length of black pepper plants followed by the treatments *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP).
- Chemical treatment (copper hydroxide @ 2g L^{-1}), *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) showed better performance based on disease management characters.
- In the case of initial nutrient status of the potting mixture (before the experiment), organic carbon was 2.1 per cent (high), available nitrogen was 470.42 kg ha^{-1} (medium), available phosphorus was 52.46 kg ha^{-1} (high) and available potassium was 295.18 kg ha^{-1} (high).
- Final nutrient status (six months after the experiment) revealed that the treatment Organic Package of Practices showed highest organic carbon content (2.66 %), treatment Package of Practices Recommendations (KAU, 2016) showed highest available nitrogen (608.24 kg ha^{-1} - high), highest available phosphorus (78.32 kg ha^{-1} – high) and highest available potassium (357.68 kg ha^{-1} -high).

- *Trichoderma harzianum* (CKT isolate) was the most promising isolate for growth promotion and *Phytophthora* disease management based on biometric, biological and disease management characters in black pepper under pot culture studies which was followed by the isolate *Pseudomonas fluorescens* (PAP isolate).

Field experiment:

- Population of *Pseudomonas fluorescens* (PAP) and *Pseudomonas fluorescens* (KAU reference culture) was found to be higher from 30 DAP (7.5×10^5 cfu g^{-1} and 6.5×10^6 cfu g^{-1} respectively) to 180 DAP (2.5×10^3 cfu g^{-1} and 2×10^3 cfu g^{-1} respectively).
- Population of *Trichoderma harzianum* (CKT) and *Trichoderma viride* (KAU reference culture) was found to be less from 30 DAP (4.5×10^4 cfu g^{-1} and 4×10^4 cfu g^{-1} respectively) to 180 DAP (3.5×10^2 cfu g^{-1} and 0×10^2 cfu g^{-1} respectively).
- *Trichoderma* sp. and *Pseudomonas fluorescens* were screened for growth promoting and antagonistic characters such as indole acetic acid production and production of ammonia, hydrogen cyanide, siderophore *in vitro*.
- IAA production was high at 30 and 60 days after planting by all the microbial inoculants (*Trichoderma* sp. and *Pseudomonas fluorescens*) which were applied alone and along with KAU POP 2016. Later, IAA production was gradually decreased. Finally, at 180 DAP, IAA was produced only by the treatments *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) and at low level.
- Ammonia was produced at high level at 30 and 60 DAP by *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (KAU reference culture) which were applied alone and along with KAU POP 2016. Ammonia production gradually decreases from 30 DAP to 180 DAP. There were no production of ammonia at 150 and 180 DAP.
- HCN production was at medium level at 30 DAP by all the microbial inoculants (*Trichoderma* sp. and *Pseudomonas fluorescens*) which were applied alone and

along with KAU POP 2016. At 60 DAP, HCN was produced only by the treatments *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (KAU reference culture). Later onwards, the production was stopped completely.

- Production of siderophore was shown only by the treatments *Trichoderma harzianum* (CKT), *Pseudomonas fluorescens* (PAP) and *Pseudomonas fluorescens* (KAU reference culture) at 30 DAP. From 90 DAP onwards, there was no siderophore production up to the end of the experiment.
- Isolates *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP) were tested and found to be antagonistic to *Phytophthora capsici in vitro.*
- Treatments were not significantly different among each other in the case of soil temperature and soil pH throughout the experiment.
- Soil moisture content was high in the treatments Organic Package of Practices (KAU, 2009) and *Trichoderma harzianum* (CKT isolate).
- Treatment Organic Package of Practices (KAU, 2009) showed higher soil respiration followed by treatments *Trichoderma harzianum* (CKT isolate) + KAU POP 2016 and *Pseudomonas fluorescens* (PAP isolate) + KAU POP 2016.
- Soil temperature was negatively correlated with the population of *Trichoderma harzianum* (CKT) and *Trichoderma viride* (KAU reference culture), *Pseudomonas fluorescens* (PAP) and *Pseudomonas fluorescens* (KAU reference culture).
- Soil moisture content was positively correlated with the population of *Trichoderma harzianum* (CKT) and *Trichoderma viride* (KAU reference culture), *Pseudomonas fluorescens* (PAP) and *Pseudomonas fluorescens* (KAU reference culture).
- Micro-climatic and soil parameters had significant effect on IAA production at 1 per cent level of significance but did not show any significant effect on production of ammonia, HCN and siderophore.

- Soil temperature in a range of 26 to 31°C, soil moisture content of 14 to 17.1 per cent, soil pH of 5.9 to 6.1 and soil respiration of 8.8 to 9.8 mg CO_2 g^{-1} day^{-1} were the optimum micro-climatic and soil parameters for IAA production.
- Best treatment in the field experiment was *Trichoderma harzianum* (CKT) + KAU POP based on biometric characters such as number of leaves, plant height, number of lateral branches, number of nodes and internodal length of black pepper plants.
- There was no natural *Phytophthora* disease incidence in the field.
- Initial nutrient status in the field soil (before the experiment) showed 1.8 per cent organic carbon content (high), 403.21 kg ha^{-1} available nitrogen (medium), 27.84 kg ha^{-1} available phosphorus (high), and 208.21 kg ha^{-1} available potassium.

- In the case of final nutrient status in the field soil (six months after the experiment), highest organic carbon content (2.71 % - high) was recorded in the treatment Organic Package of Practices (KAU, 2009), highest available nitrogen (548.56 kg ha^{-1}) was recorded in the treatment *Trichoderma harzianum* (CKT isolate) + KAU POP 2016, highest available phosphorus and available potassium (62.36 kg $ha^{-1}$and 265.38 kg ha^{-1}) were recorded in the treatment Package of Practices Recommendations (KAU, 2016).

- The present study indicated that *Trichoderma harzianum* (CKT isolate) was the most promising isolate for growth promotion in black pepper on the basis of biometric and biological parameters under field conditions.

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Appendices

APPENDIX - I

a. Potato Dextrose Agar medium

Potato	- 200 g
Dextrose	- 20 g
Distilled water	- 1000 ml
Agar	- 20 g

b. King's B medium

Peptone	- 20 g
Glycerol	- 10 ml
K_2HPO_4	- 1.5 g
$MgSO_4$	- 1.5 g
Distilled water	- 1000 ml
Agar	- 20 g
pH	- 7.2 – 7.4

c. Nutrient Agar medium

Peptone	- 5 g
Yeast/ beef extract	- 3 g
NaCl	- 5 g
Distilled water	- 1000 ml
Agar	- 20 g
pH	- 6.8 – 7.2

d. *Trichoderma* Selective medium

K_2HPO_4	- 0.9 g
$MgSO_4$	- 0.2 g
KCl	- 0.15 g
NH_4NO_3	- 1 g
Glucose	- 3 g
Chloramphenicol	- 0.25 g

p – dimethyl amino benzene diazo sodium sulfonate – 0.3 g

Pentachloronitrobenzene	- 0.2 g
Rose Bengal	- 0.15 g
Distilled water	- 1000 ml
Agar	- 20 g

e. Luria Bertani broth (LB)

Tryptone	- 10 g
Yeast extract	- 5 g
NaCl	- 10 g
pH	- 7.5 g
Distilled water	- 1000 ml

f. IAA production

LB	- 7.5 g
SDS	- 0.18 g
Glycerol	- 3 ml
Distilled water	- 300 ml

g. HCN production

LB	- 6.25 g
Glycine	- 1.1 g
KCl	- 0.15 g
NH_4NO_3	- 1 g

h. Peptone water

Peptone	- 4 g
Distilled water	- 100 ml

i. CAS agar medium

Chrome azurole S	- 60.5 mg in 50 ml water
Iron (III) solution	- 10 ml (1 mM $FeCl_3.6H_2O$, 10 mM HCl)
HDTMA	- 72.9 mg in 40 ml water
King's B medium	- 900 ml
Agar	- 20 g

j. Czapek dox agar medium

Sucrose	- 30 g
Dipotassium phosphate	- 1 g
Potassium chloride	- 0.50 g
Sodium nitrate	- 3 g
Magnesium sulphate	- 0.50 g
Ferrous sulphate	- 0.01 g
Agar	- 20 g
Distilled water	- 1000 ml

pH	- 7.3 ± 0.2

k. Salkowski reagent

$FeCl_3$ (0.5 M)	- 2 g in 100 ml water
Perchloric acid	- 35 %

l. Picric acid solution

Picric acid	- 2.5 g
Na_2CO_3	- 12.5 g
Distilled water	- 1000 ml

APPENDIX – II

Nucleotide sequence of CKT isolate

AAACTGCGTCGACGTGATCCGAGGTCACATTTCAGAAGTTTGGGGTGT
TTAACGGCTGTGGACGCGCCGCGCTCCCGATGCGAGTGTGCAAACTAC
TGCGCAGGAGAGGCTGCGGCGAGACCGCCACTGTATTTCGGGGCCGG
CCCCGTAAAGGGCCGATCCCCAACGCCGACCCCCCGGAGGGGTTCGA
GGGTTGAAATGACGCTCGGACAGGCATGCCCGCCAGAATACTGGCGG
GCGCAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTC
ACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCCAGAACCAAG
AGATCCGTTGTTGAAAGTTTTGATTCATTTTCGAAACGCCCACGAGGG
GCGCCGAGATGGCTCAGATAGTAAAAAACCCGCGAGGGGGTATACAA
TAAGAGTTTTGGTTGGTCCTCCGGCGGGCGCCTTGGTCCGGGGCTGCG
ACGCACCCGGGGCAGAGATCCCGCCGAGGCAACAGTTTGGTAACGTTC
ACATTGGGTTTGGGAGTTGTAAACTCGGTAATGATCCCTCCGCAGGTT
CACCTACGGAATACCGAGTTTATACTCCAAACCAATGTGAACGTTACC
AAACTGTTGCCTCAGCGGGATCTAGCCCGGTGCGTCGACCCGAACAAG
GGCCGCCGGATGAACAACAAACTCTTAATGTATACCCCTCGCGGGTTT
TTTTCTATCTGAGCCTCTGGCGCCTCTGGGTGTTCTCAAAATGATCAAA
ACTTTCACAAGGTAATCCTTGCTTTCTTGCATGCCTGAACAAACGCCAC
GAAATGCGATAAGTAATGGTGAATTGCAAACTCTGGTGAATCATCGTA
TCTTTGAACGCCATTGCGCCCGCCGATATTCTGGCGGGATGGCTGTCC
GAAGCTCTTGAACTTCCATCCTCCCGGGGGGGTCGCGATGGGATCGGC
CTTAACGGGGCGGCCCGAAATCTGGGGGTTCGCCCGACCCTCTCTGGA
CAGGAGGTGCCAACTCCACTCGGGAGCGCGGCGGCTCATGCTTTAACC
CCCCACCTCGATTTAACTGAACGGAGAATACCTGCCGAATTACCATAC
TAACCAGGGGCAATCT

Nucleotide sequence of PAP isolate

CGGGCCTGGCGCTATCAGAGGAGCTAGGTCGAATAAGCTAGTGGTGG
GTAAGGCTCACCAAGGCAGATCCGTAACTGGTCGAGAGGATGATCAG
TCACATGGACTGAGACACGTCAGACCTACGGAGGCAGCAGTGGGGAA
TATGACATGGCGAAAGCTGATCAGCATGCCGCGTGTGGAAGAGTCTCG
ATGTAAAGCACTTTAAGTGGGAGGAAGGGCATAACCTAATACGTTAGT
GTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCA
GCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAATCCCCGGGCT
CAACCTGGGAACTGCATTCAAAACTGACTGACTAGAGTATGGTAGAGG
GTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGA
ACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGT
GCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG
CCGTAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCG
CAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTA
AAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG
GTTTAATTCGAAGCACGCGAGACCTTACCAGGCCTTGACATCCAATGA
ACTTTCTAGAGATAGATGGTGCCTTCGGGACATTGAGACAGGTGCTGC
ATGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAGTCCCGTACGA
GCGCACCCTGTCTAGTTACAGCACGTATGGTGGCACTCTAGGAGACTG
CCGGTGACACGAGGAAGGTGGGATGACGTCAAGTCATCATGACCTAC
GGCTGGCTACCACGTGCTACATGTCGTACGAGGTGCCAGCCGCCGGAG
CTAATACCCAAACCGATCGTATCGGATCGCAGTCGCACTCGACTGCGT
GAAGCCGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATC
GTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTG
CACCAGAAGTAGCTAGTCTAACCTTCGGGAGGACGGTTACCACGGTGT
GATTCATGACTGGGGGAAGTCGTACATGAGCTATGCAC

FIELD EVALUATION OF ABIOTIC STRESS TOLERANT STRAINS OF *Trichoderma harzianum* AND *Pseudomonas fluorescens* FOR *Phytophthora* DISEASE MANAGEMENT IN BLACK PEPPER

(*Piper nigrum* L.)

By

RIMA K. R.

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ABSTRACT OF THE THESIS

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Faculty of Agriculture

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DEPARTMENT OF AGRICULTURAL MICROBIOLOGY

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ABSTRACT

Black pepper (*Piper nigrum* L.), known as "the king of spices" is an important spice commodity of commerce and trade in India since pre-historic period. It is highly sensitive to abiotic stresses like climate changes. The abiotic stresses in plants can be overcome by the use of beneficial microorganisms. However, the microorganisms themselves are vulnerable to abiotic and biotic stresses. Therefore, abiotic stress tolerant strains of beneficial microorganisms have to be developed for black pepper to overcome changes due to micro-climatic variables and soil parameters.

In an earlier study in the Department of Agricultural Microbiology, College of Horticulture, native abiotic stress tolerant isolates of *Trichoderma harzianum* (CKT isolate) and *Pseudomonas fluorescens* (PAP isolate) were identified for growth promotion and disease management in black pepper (*Piper nigrum* L.) under pot culture studies. As the performance of these strains varies under field conditions, a study on "Field evaluation of abiotic stress tolerant strains of *Trichoderma harzianum* and *Pseudomonas fluorescens* for *Phytophthora* disease management in black pepper (*Piper nigrum* L.)" was undertaken with an objective to study the effect of micro-climatic and soil parameters on abiotic stress tolerant strains under field conditions.

Before field evaluation, pot culture experiment was conducted to evaluate the efficiency of the abiotic stress tolerant *Trichoderma harzianum* (CKT) and *Pseudomonas fluorescens* (PAP) for growth promotion and *Phytophthora* disease management in black pepper during April – October, 2017. *T. harzianum* (CKT) was the most promising isolate for growth promotion and disease management based on biometric (number of leaves, plant height, number of lateral branches, number of nodes, intermodal length), biological and disease management characters under pot culture studies. It was found that population of *Trichoderma harzianum* (CKT), *Pseudomonas fluorescens* (PAP), *Trichoderma viride* (KAU reference culture) and *Pseudomonas fluorescens* (KAU reference culture) was negatively correlated with soil temperature and positively correlated with soil moisture. There was no

significant correlation between the population of microbes and soil pH and soil respiration.

Field evaluation revealed that *Trichoderma harzianum* (CKT) + KAU POP was the most promising treatment for growth promotion under field conditions based on biometric and biological characters.

The population of *Trichoderma harzianum* (CKT), *Pseudomonas fluorescens* (PAP), *Trichoderma viride* (KAU reference culture) and *Pseudomonas fluorescens* (KAU reference culture) were negatively correlated with soil temperature and positively correlated with soil moisture content. However, soil pH and soil respiration did not have any correlation with the population of *Trichoderma* sp. and *Pseudomonas fluorescens.*

Micro-climatic and soil parameters did not show any effect on the production of ammonia, HCN and siderophore but had significant effect on production of IAA in *Trichoderma* sp. and *Pseudomonas fluorescens.* Soil temperature (26.8 to 30.6 °C), soil moisture content (14 to 17.6 %), soil pH (5.7 to 6.1) and soil respiration (8.1 to 9.8 mg CO_2 g^{-1} day^{-1}) were found to be the optimum micro-climatic and soil parameters for IAA production.

Trichoderma harzianum (CKT) was the most promising isolate for growth promotion under field conditions. However, multilocational field trials at different agro- ecological zones of Kerala need to be conducted before commercialization of *Trichoderma harzianum* (CKT).