IMPACT OF WEATHER VARIABLES ON THE FUNCTIONAL EFFICIENCY OF BENEFICIAL MICROFLORA IN THE RHIZOSPHERE OF BLACK PEPPER (*Piper nigrum* L.)

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Impact of weather variables on the functional efficiency of beneficial microflora in the rhizosphere of black pepper (*Piper nigrum* L.)"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Impact of weather variables on the functional efficiency of beneficial microflora in the rhizosphere of black pepper (*Piper nigrum* L.)"** is a record of research work done independently by **Manju Mohan E.** (2014-11-156) 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.

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ABBREVIATIONS

C	- Carbon
CAS	- Chrome azurole S
cfu/ g	- colony forming unit per gram
CSA	- Competitive Saprophytic Ability
dS m^{-1}	- deci Siemen's per meter
EC	- Electrical Conductivity
Fp	- Fluorescent pseudomonads
GA	- Gibberellic Acid
HA	- Harzianic Acid
HCl	- Hydrochloric acid
HCN	- hydrogen cyanide
hrs	- hours
IAA	- Indole Acetic Acid
K	- Potassium
KAU	- Kerala Agricultural University
kg ha^{-1}	- kilo gram per hectare
LB	- Luria Bertani
M	- Molar
mg g^{-1}	- milli gram/gram
MT	- Metric Tonnes
NBRIP	- National Botanical Research Institute's Phosphate growth medium
NBRY	-National Botanical Research Institute's Phosphate growth medium devoid of yeast extract
Nfb	-Nitrogen free broth
NS	- Non- significant

OC	- Organic Carbon
PDA	- Potato Dextrose Agar
PGPB	- Plant Growth Promoting Bacteria
PSB	- Phosphate Solubilizing Bacteria
SDS	-Sodium Dodecyl Sulphate
TSM	- Trichoderma Selective Medium
WASP	- Web Agri Stat Package

Introduction

1. INTRODUCTION

Black pepper (*Piper nigrum* L.) is an important spice crop of Kerala referred to as the 'King of spices'. It is the most important and one of the oldest of all spices. Malabar region situated in the Western Ghats of South India is reported to be the origin of black pepper. The plant is a climbing perennial vine propagated through cuttings and grown trailed over supports. In India, black pepper is mainly cultivated in Kerala, Karnataka and to lesser extent, in Maharashtra and Andhra Pradesh.

Indian black pepper is more preferred in the international market due to its flavor, aroma, essential oil and piperine content. Various products out of the plant is widely used in Ayurvedic medicines, culinary preparations, food processing even in perfumery. Vietnam is the largest producer of black pepper and India holds third position among world countries. India contributes 11.21 per cent of the world production. Kerala is the largest pepper producing state in India. In 2014-15 alone, Kerala produced 40,700 MT of pepper from an area of 85,400 ha with a productivity of 0.5 MT/ha (SBI, 2015).

Pepper has been reported to be the most vulnerable spice crop to climate change (Rao, 2011). The crop requires a warm and humid climate and an annual rainfall of 250 cm for its proper growth and potential. The optimum required temperature is ranges from 20°-30 °C and it tolerates a maximum of 40 °C and a minimum of 10 °C (KAU, 2011). Heavy rainfall during the monsoon period followed by a prolonged dry period is often detrimental for the growth and yield of black pepper (Rao, 2003). In this context, microorganisms associated with rhizosphere soil of black pepper could play an important role in reducing the impact of abiotic stresses due to climate change in plants. However, the beneficial microflora in rhizosphere are also vulnerable to these stresses which will further affect the growth and development of the plant.

Beneficial microorganisms play an important role in increasing the tolerance to abiotic stresses and adaptation in agricultural crops. Since, they are

associated with plant roots and mitigate the impact of abiotic stresses, they can be utilized to overcome the impact of weather parameters on the plants. The soil microbial community is also influenced by the microclimatic factors such as changes in soil temperature, soil moisture, soil organic carbon etc. Prolonged exposure of soil microorganisms to various stress factors resulted in the variation of their population as well as efficiency (Griffiths *et al.*, 2000). Among the beneficial microorganisms, *Azospirillum,* phosphate solubilizing bacteria, *Pseudomonas fluorescens* and *Trichoderma* sp. are well known plant growth promoters. Changes in the weather variables are not only influencing the growth and yield of black pepper, but also, have an impact on the soil microflora of black pepper.

At present, there is a lack of such reliable information in Kerala on the impact of weather and microclimatic parameters over the population and efficiency of *Azospirillum,* phosphate solubilizing bacteria, *Pseudomonas fluorescens* and *Trichoderma* sp. In this context, the present study was undertaken to assess the impact of weather variables on the growth and efficiency of beneficial microflora in the rhizosphere of black pepper. The objectives of the study were,

- To study the effect of weather and microclimatic variables on the population of *Azospirillum*, phosphorus solubilizing bacteria, *Pseudomonas fluorescens* and *Trichoderma* sp.
- To study the effect of weather and microclimatic variables on the functional efficiency of beneficial microflora present in the rhizosphere of black pepper.

Review of literature

2. REVIEW OF LITERATURE

Black pepper (*Piper nigrum* L.) is a perennial, woody and flowering climber belonging to family Piperaceae. It has been reported to be the most vulnerable spice crop to climate change (Rao, 2011). Microorganisms in the rhizosphere of black pepper also get affected by the climate change. There is no reliable information available on the impact of weather, microclimatic and soil parameters on the population and functional efficiency of beneficial microflora in the rhizosphere of black pepper. In this chapter, an attempt has been made to review the relevance, effect of weather, microclimatic and soil parameters on the population and functional efficiency of beneficial microflora in the rhizosphere of black pepper.

2.1 BLACK PEPPER AND ITS IMPORTANCE

Black pepper (*Piper nigrum*) is one of the most widely used among spices. It is one of the important crops which provide major source of income and employment for rural households in Kerala, where more than 2.5 lakh farm families are involved in pepper cultivation (GOI, 2009). The State Planning Board has compiled commodity composition of the export basket of Kerala (1996) and found the share of Kerala as 97 per cent for pepper in India's export of black pepper.

The global production by 2014 was estimated as 409,000 tons, which is expected to increase to 454,000 tons in 2015. During 2014, Vietnam topped the list with 155,000 tons which is 38.6 per cent share of global production. Due to adverse weather during the early growth stages, the 2015-2016 output of pepper from Vietnam was lower with 145,000 tons. In 2015, India's production was significantly better with 85,000 ton and the main reason for this was favourable weather conditions rather than expansion of area under cultivation (ESA, 2015).

India is the third largest producer of black pepper in the world (11.21 % of the world production). In India, Kerala is the largest producer of black pepper with the production of 40,700 MT in an area of 85,400 ha with a productivity of 0.5 MT/ha during 2014-15 (SBI, 2015).

2.2 GLOBAL CLIMATE CHANGE

Because of increased temperature, soil water content was expected to decrease in some areas (Le Houerou, 1996), leading to enhanced drought in several parts of the world. These climate-changing parameters are known to affect terrestrial macroorganisms such as plants. The global climate is predicted to change drastically over the next century and various parameters will be affected (Houghton *et al.*, 2001).

Joseph *et al.* (2004) analyzed time series of daily rainfall in south Kerala for the summer monsoon season of 95 year (1901-1995) and found that the period of intra-seasonal oscillation did not vary during a monsoon season in most of the years, but, it had large inter-annual variability in the range of 23 to 46 days. They also found that of the 95 years, 11 years had no significant Intra Seasonal Oscillation (ISO).

Atmospheric CO_2 concentration might increase continuously and global surface temperatures were predicted to increase between 1.8 and 3.6 °C by 2100, which will be driven by increased atmospheric CO_2 levels derived from natural and/or anthropogenic sources (IPCC, 2007).

Rao *et al.* (2009a) reported that there exists a cyclic trend in annual rainfall with a declining trend in annual and southwest monsoon rainfall whereas increasing trend in post monsoon rainfall since last 50-60 years.

Rao *et al.* (2009b) reported that there was an increase in maximum temperature over the Kerala state by 0.64 °C during the last 49 years period, (since 1956) while the increase in minimum temperature was 0.23 °C. Overall increase in annual mean temperature was 0.44 °C.

Krishnakumar *et al.* (2007) observed that monthly rainfall in Kerala during June and July decreased while it increased in August and September, indicating that there was a shift in monthly rainfall.

Raj and Azeez (2010) reported that annual rainfall in the Palakkad gap in the Western Ghats showed variation with altitude, revealing that the annual rainfall in the region was comparatively lesser than that of the entire state. A significant decrease in the annual rainfall, winter rainfall and the southwest monsoon were also reported.

The atmospheric concentrations of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) increased since 1750 due to human activity (IPCC, 2013). The concentrations of these greenhouse gases were 391 ppm, 1803 ppb, and 324 ppb, and exceeded the pre-industrial levels by about 40%, 150%, and 20%, respectively during 2011. Greenhouse gases contributed a global mean surface warming in the range of 0.5 °C to 1.3 °C over the period 1951 to 2010, and together, these assessed contributions were consistent with the observed warming of approximately 0.6 °C to 0.7 °C over this period.

2.3 EFFECT OF WEATHER ON GROWTH OF BLACK PEPPER

Black pepper is a weather sensitive crop and the yield is considerably influenced by environment. Menon (1981) observed that extension growth of plagiotrops started in April-May with the receipt of pre-monsoon showers and continued up to August-September. It was found that 82.43% of the total annual growth of the fruiting branches was registered in June-July, coinciding with the peak period of the monsoon and a dry spell before flowering is advantageous for better crop production (Nalini, 1983).

Nalini (1983) also noted a positive correlation of rainfall with flower bud differentiation process which starts during April-May with the receipt of pre-monsoon showers and reached a peak in June-July, synchronizing with maximum rainfall and vegetative growth in the plagiotropes.

Growth of fruit bearing lateral shoots and photosynthetic rate were high during peak monsoon (June-July) in India (Mathai, 1983).

Pillay *et al.* (1987) reported that, after a dry spell, the flowering process in pepper is initiated after the receipt of rainfall equivalent to 70 mm. He also reported that a period of 20 days was required for flower bud to differentiate and rainfall received after a period of stress, induced profuse flowering in pepper.

Pillay *et al.* (1987) compared the rainfall pattern and pepper yields during the two extremely adverse years (1980-81 and 1986-87) with that of a favourable year (1981-82) and observed that during the adverse years, there was a distinct break in the rainfall during at the critical period after flower initiation. It indicated that, stress situation of inadequate rainfall occurring at any time of the critical period can reduce the yield of pepper substantially.

Shyam *et al.* (1988) reported that the percentage degradation of chlorophyll was significantly different in seven varieties during moisture stress.

Lazarus *et al.* (2005) reported that the rainfall and number of rainy days in February and March were negatively correlated to the yield of Panniyur-1 variety of black pepper.

Sujatha *et al.* (2005) reported that no significant correlation was observed between weather parameters studied and yield. However, earliness of summer rains and onset of monsoon showed a positive influence in the earliness of the black pepper.

Thangaselvabal *et al.* (2008) reviewed the different aspects of black pepper cultivation and concluded that the demand for black pepper and its products was getting increased year after year in the world market. However, production was not enough to meet demand for export as the present level of productivity in India was very poor due to non-adoption of good agricultural practices.

2.4 EFFECT OF BENEFICIAL MICROFLORA ON BLACK PEPPER

Kandiannan *et al.* (2000) evaluated the effect of *Azospirillum,* phosphobacteria and vesicular arbuscular mycorrhiza on growth and nutrient

content of black pepper *(Piper nigrum)* cuttings and reported that growth parameters were enhanced significantly over control when applied in combination with biofertilizers. Inoculation with a combination of two / three biofertilizers enhanced plant height, leaf area, biomass, dry matter production and nutrient content significantly over uninoculated control.

In a study conducted at Kerala Agricultural University, it was reported that the average number of roots per cutting and root dry weight were maximum in *Azospirillum* treated black pepper (KAU, 2001).

Thankamani *et al.* (2005) reported that maximum height and leaf area of cuttings were obtained with application of *P. fluorescens* thrice, which was on par with application of *T. harzianum* along with *P. fluorescens* (thrice) under nursery. Number of roots and biomass production were also higher with combined application of *P. fluorescens* (thrice) and *T. harzianum* which was on par with application of *P. fluorescens* (thrice).

A study conducted by Sangeeth *et al.* (2008) to evaluate the effect of indigenous *Azospirillum* spp. in enhancing the growth and nutrient uptake of black pepper cuttings showed 67% increase in the plant height of rooted cuttings which were treated with *Azospirillum* isolates (BPaz4 and BPaz9) than untreated cuttings. The uptake of nitrogen, phosphorus and potassium and total dry weight were also significantly superior in the treated plants.

Maximum fresh yield (2207 g $vine^{-1}$) was recorded with *Azospirillum* sp. + 50% recommended N + Mg followed by application of NPK alone. Significantly higher N, K and Mg content in the soil and N and Ca content in the leaf were observed in the case of 50% recommended N + Mg. Application of *Azospirillum* sp. increased the population of bacteria to 106 x 10^5cfu/g of soil was observed in the treatment with 50% recommended N + Mg (0.2 kg ha^{-1}) compared to uninoculated control (Thankamani *et al.*, 2011).

The microorganisms isolated from the soil were able to dissolve different kinds of rock phosphates in the soil and in the liquid culture medium (Ivanova *et al.*, 2006). Phosphate solubilizing bacteria from the rhizospheric zone of pepper were studied by Ramachandran *et al.* (2003), who reported that *Pseudomonas* and *Azospirillum* can efficiently solubilize phosphorus with more than 20% solubilization.

Anith *et al.* (2003) isolated bacterial antagonists against *Phytophthora capsici* from underground shoot portions of rooted cuttings of black pepper and reported that PN-026 isolate was the most efficient antagonistic bacteria in suppressing the *Phytophthora capsici* wilt of black pepper in the nursery.

Trichoderma harzianum and *Trichoderma viride* have been used as plant growth promoter as well as biological control agents against diseases of black pepper caused by *Phytophthora capsici* both in the main field and in the nursery (Anandaraj and Sarma, 1995).

Only limited attempts have been made on biological control of root rot of black pepper using bacterial antagonists. Jubina and Girija (1998) reported that, in black pepper nursery, bacterial biocontrol agents can be efficiently used for the management of *Phytophthora capsici*.

Rajan *et al.* (2002) reported that *Trichoderma virens* and *Trichoderma harzianum* were the most effective isolates to control the foot rot disease of black pepper.

Anandraj and Sarma (2003) reported the use of biocontrol agents in the prevention and management of soil borne diseases in black pepper, ginger and cardamom, and the role played by PGPRs in suppressing the diseases of foot rot was established.

Trichoderma spp. have been reported as efficient biological control agents against *Phytophthora capsici* induced root rot of black pepper (Sarma *et al.*, 1994).

According to Anith and Manomohandas (2001), application of the biocontrol agents had profound effect on the growth parameters of black pepper cuttings. The increase in root weight with *Alcaligenes, Trichoderma* and the combined application were 44.75, 100 and 156 per cent respectively.

2.5 ROLE OF BENEFICIAL MICROFLORA IN MITIGATING THE EFFECT OF WEATHER VARIABLES

The Extracellular Polymeric substance (EPS) producing *Pseudomonas putida* strain imparts drought tolerance to plant roots by forming biofilm on the root surface (Bensalim, 1998). Vivekananthan *et al.* (2004) reported that fluorescent pseudomonads favoured the plant to withstand different abiotic stress by producing wide range of metabolites and enzymes.

According to Creus *et al.* (2004), under low water condition, inoculation of *Azospirillum brasilense* could improve the water holding capacity of soil. Kasim *et al.* (2013) reported that *Azospirillum brasilense* could significantly mitigate the detrimental effect of drought stress in crops.

2.6 MECHANISM OF PGPR AND BIOCONTROL AGENTS

2.6.1 *Azospirillum*

Azospirillum is a plump, slightly-curved and straight rod, often with pointed ends. They are gram negative to gram variable bacteria and motile in liquid medium by a polar flagellum. Colonies on potato agar is typically light or dark pink, often wrinkled and non-slimy. In complex media such as MPSS broth, *Azospirillum* grow as plump, slightly curved rods and straight cells having a diameter of ~1.0 µm.

In semi-solid nitrogen free malate (Nfb) medium, *Azospirillum lipoferum* develops predominantly into pleomorphic cells within 48 hr in contrast to *A. brasilense,* which retains mainly vibroid form. On BMS (Batata-Malato-Sacarose) agar media, after 1-2 weeks of incubation at 33-35 °C, colonies of *Azospirillum* are pink, opaque, irregular or round, often wrinkled and have umbonate elevation.

Pigmentation is best on BMS agar medium incubated under the light (Tarrand *et al.*, 1978). Cells are motile with a single polar flagellum. Numerous lateral flagella of shorter length are formed in *A. lipoferum* and *A. brasilense* on soft nutrient agar where swarming is observed (Hall and Krieg, 1983).

2.6.1.1Mechanism of plant growth promotion by Azospirillum

2.6.1.1.1 N_2 fixation by *Azospirillum*

Inoculation of *Azospirillum* in wheat and maize indicated that 5-10 per cent and up to 18% of the plant nitrogen were derived from N_2 fixation (Rennie, 1980). In addition, inoculated plants grew normal with only a partial amount of nitrogen fertilizer required for such growth (Kapulnik *et al.*, 1981).

Azospirillum spp. can fix molecular nitrogen and perform assimilatory nitrate reduction and nitrate respiration (Bothe *et al.*, 1981).

Okon (1982) reported that the nitrogen yield of *Azospirillum* inoculated plants exceeded by 77.1 kg N per ha than of uninoculated controls in *Zea mays*.

Neuer *et al.* (1985) found that the association between *Azospirillum* and germinated wheat seeds performed acetylene reduction activity under microaerophilic conditions. According to Gallori and Bazzicalupo (1985), *Azospirillum* fixes atmospheric nitrogen under micro-aerophilic and also N-limiting conditions.

All wild-type *Azospirillum* strains fixed atmospheric nitrogen efficiently either as free-living bacteria or in association with plants and participated in several transformations in the nitrogen cycle (Heulin *et al.,* 1989).

Steenhoudt and Vanderleyden (2000) reported that through the action of the nitrogenase complex, *Azospirillum* can convert atmospheric nitrogen into ammonium under microaerobic conditions at low nitrogen levels.

In a study conducted by Thankamani *et al.* (2011), significantly higher nitrogen content in the soil and black pepper leaf was observed, when it was inoculated with *Azospirillum* along with 50% recommended N and Mg.

An experiment was conducted in the roots of *Setaria viridis*, a C4 grass and roots were effectively colonized by bacterial inoculants which resulted in a significant enhancement of growth under nitrogen-limiting conditions when inoculated with *Azospirillum brasilense* (Pankievicz *et al.*, 2015).

2.6.1.1.2 Phytohormone production by *Azospirillum*

Azospirillum brasilense strains were able to produce phytohormones such as auxins, cytokinins and gibberelins. The effect of *Azospirillum* on root growth and plant weight of pearl millet resembled with same effect as addition of a mixture of auxin, gibberelin, and kinetin (Tien *et al.,* 1979). The excretion of auxin, *i.e.*, 3-indoleacetic acid (IAA) in *A. brasilense* was dependent on the addition of tryptophan.

Hartmann *et al.* (1983) reported that IAA is excreted by the mutants of *Azospirillum brasilense* (Cd) which was resistant to 5-fluorotryptophan (FT) and produced upto 16 pg/mL, which was 30 times greater than the wild-type level.

According to Hernandez *et al.* (1996), a dose-response curve for plant root development with increasing concentrations of an *Azospirillum* strain was similar to the curve obtained with increasing concentrations of indole-3-acetic acid (IAA), suggesting the importance of this phytohormone. Phytohormone production and nitrogen fixation are the processes involved in plant growth promotion by *Azospirillum* (Steenhoudt and Vanderleyden, 2000).

Thuler *et al.* (2003) reported that *Azospirillum* spp. had the ability to produce plant hormones, polyamines and amino acids in the culture. Among these hormones, indole acetic acid (IAA), and gibberellins played an important role.

Among the three phytohormones, IAA was quantitatively most important. It was assumed that bacterial phytohormone production induced changes in root morphology after inoculation with *Azospirillum*. The work with *Azospirillum* mutants altered indole-3- acetic acid production endorsed the hypothesis (Spaepen *et al.*, 2008).

In a study conducted by Lenin and Jayanthi (2012), all the five isolates of *Azospirillum lipoferum* produced IAA and GA_3 and the quantity ranged from 71.64 to 74.24 µg 25 ml^{-1} broth for IAA and 6.45 to 7.10 µg 25 ml^{-1} broth for GA_3.

Masciarelli *et al.* (2013) evaluated the *in vitro* (cell culture supernatants) and *in vivo* (stems and roots of maize seedlings) production of indole-3-acetic acid (IAA) by *Azospirillum brasilense* strains to elucidate the role of phytohormone as a signaling and effector molecule in the symbiotic interaction between maize and *A. brasilense*. All three strains showed IAA production when cultured in NFb medium supplemented with 100 µg/ml L-tryptophan.

2.6.2 Phosphate solubilizing bacteria

Phosphorus is the second important key element after nitrogen as a mineral nutrient in terms of quantitative plant requirement. Although abundant in soils, its availability is restricted as it occurs mostly in insoluble forms. The P content in average soil is about 0.05% (w/w) but only 0.1% of the total P is available to plant because of poor solubility and its fixation in soil (Illmer and Schinner, 1995).

Higher concentration of phosphate solubilizing bacteria was commonly found in the rhizosphere in comparison with the non-rhizosphere soil (Katznelson *et al.*, 1962). Several strains of bacterial and fungal species have been described and investigated in detail for their phosphate-solubilizing capabilities (Glick, 1995). Typically, such microorganisms have been isolated using cultural procedures with species of *Pseudomonas* and *Bacillus* (Illmer and Schinner, 1992).

Mardad *et al.* (2013) screened some bacteria and found that the bacteria were able to solubilize tricalcium phosphate on solid culture state by forming clear halozone, with different degree of solubilization, depending on the type of organism involved.

2.6.2.1 Mechanism of plant growth promotion by phosphate solubilizing bacteria

2.6.2.1.1 Phosphate solubilization by organic acid production

Organic acids produced by PSB solubilize insoluble phosphates by lowering the pH, chelation of cations and competing with phosphate for adsorption sites in the soil (Nahas, 1996). Inorganic acids such as hydrochloric acid can also solubilize phosphate but they are less effective compared to organic acids at the same pH (Kim *et al.*, 1997).

Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms (Sagoe *et al.*, 1998).

Besides organic acids, inorganic acids such as nitric and sulphuric acids produced by the nitrifying bacteria and *Thiobacillus* react with calcium phosphate and convert them into soluble forms (Gaur and Gaind, 1999).

The PSB dissolve the soil P through production of low molecular weight organic acids mainly gluconic and ketogluconic acids (Goldstein, 1995; Deubel *et al.*, 2000) along with lowering the pH of rhizosphere.

Inorganic P is solubilized by a group of heterotrophic microorganisms excreting organic acids that dissolve phosphatic minerals and/or chelate cationic partners of the P ions releasing P into solution (He *et al.*, 2002).

Organic acids such as glycolic acid, oxalic acid, malonic acid, succinic acid, citric acid and propionic acid have also been identified among phosphate

solubilizers. The phosphate solubilizing bacteria have the ability to reduce the pH of the surroundings by the production of organic acids. However, three isolates did not produce any organic acids in detectable amount and phosphate solubilization was minimum (Chen *et al.*, 2006).

2.6.2.1.2 Antagonistic activity against *Phytophthora capsici*

Aravind *et al.* (2008) isolated endophytic bacteria from black pepper which was antagonistic to *Phytophthora capsici*. Among the 74 isolates obtained, many were *Bacillus megaterium* which recorded over 70% disease suppression in green house trials.

Mei *et al.* (2010) worked on 98 isolates from the rhizosphere of healthy black pepper plants with antagonistic activity against *Phytophthora capsici* and two strains of HL-3 and LZ-8 were screened. Those screened isolates were identified as *Paenibacillus polymyxa* and *Bacillus pumilus* respectively. The two strains could inhibit the mycelium growth of *P. capsici* and the inhibitory effect of HL-3 and LZ-8 was 72% and 68%, respectively.

Kumar *et al.* (2012) reported that some strains of PSB were antagonistic to the growth of pathogens such as *Macrophomina phaseolina*, *F. oxysporum*, *F. solani*, *S. sclerotiorum*, *R. solani* and *Colletotrichum* sp. Increase in fungal inhibition corresponded with incubation period.

2.6.2.1.3 Indole acetic acid production

Shahab *et al.* (2009) studied indole acetic acid production by phosphate solubilizing bacteria and found varying levels of IAA production. The range of IAA production in PSBs isolates with tryptophan was 57 to 288 µg/ml, while indole butyric acid was in the range of 22 to 34 µg/ml. These isolates varied greatly in their intrinsic ability to produce IAA.

Inui *et al.* (2012) isolated phosphorus solubilizing and IAA producing microorganisms from the soil. Ten efficient isolates namely *Enterobacter* sp.

(FJ890899), *E. homaechei* sub sp. *Steigerwaltii* (FJ890898), *Labrysportucalensis* (FJ890891), and *Burkholderia* sp. (FJ890895) showed significant results for the production of auxin.

Bacteria from rhizosphere producing auxin may play a key role in plant growth promotion, particularly in the early stages of development and in the process of rooting. It is known that this stimulus is dependent on the doses of the hormone, because an excess can retard, or even inhibit, the growth of the plant (Broek, 1999). The isolates that showed the best values for auxin production were *E. homaechei* subsp. *Verschuerenii* (FJ890898) and *Enterobacter* sp. (FJ890899), obtained from sugarcane crop, with about one year of cultivation, indicated that the hormone may act at different stages of plant development such as tillering and stalk growth.

In a study conducted by Walpola and Yoon (2013), *Pantoea agglomerans* and *Burkholderia anthina* showed positive responses for all the tested plant growth promotion traits including IAA production. But, the IAA production was comparatively lower.

2.6.2.1.4 Siderophore production

Studies have reported the release of siderophores by PSM (Vassilev *et al.*, 2006). However, siderophore production has not been widely implicated as a P-solubilization mechanism. Considering the dominance of mineral dissolution over ligand exchange by organic acid anions as a P-solubilizing mechanism (Parker *et al.*, 2005), the potential role of siderophores in enhancing P availability should be obvious.

According to Caballero-Mellado *et al.* (2007), *Burkholderia* isolates were able to produce siderophores on CAS medium agar plates. In this medium, *B. unamae* isolates formed orange haloes. *B. xenovorans* strains produced the largest halo sizes (13 to 19 mm), followed by *B. tropica* strains (12 to 17 mm), the Bkr group (2 to 14 mm), and *Burkholderia* sp. isolates (3 mm).

Pantoea agglomerans and *Burkholderia anthina* formed orange halos around the colonies on CAS agar thus were considered to be good siderophore producers. High proportion (greater than 80%) of total siderophore produced by *B. anthina* was reported within the first 24 h whereas *P. agglomerans* showed a slow response which took 72 h to exhibit the highest siderophore production. (Walpola and Yoon, 2013).

2.6.2.1.5 Hydrogen Cyanide production

Saha *et al.* (2012) screened 2 isolates of *Bacillus subtilis* for the production of HCN and none of the isolates showed HCN production.

According to Shobha and Kumudini (2012), the isolates of *Bacillus megaterium* produced HCN as their antagonistic mechanism.

Pantoea agglomerans and *Burkholderia anthina* showed positive responses for all the tested plant growth promotion traits including HCN production (Walpola and Yoon, 2013).

2.6.2.1.6 Ammonia production

Shobha and Kumudini (2012) reported that *Bacillus megaterium* isolates could produce ammonia.

Pantoea agglomerans and *Burkholderia anthina* showed positive responses ammonia production (Walpola and Yoon, 2013).

2.6.3 Fluorescent pseudomonads

Fluorescent pseudomonads are important group of plant growth promoting rhizobacteria which play an inevitable role in plant growth promotion as well as disease control. Certain members of the *P. fluorescens* have been shown to be potential agents for the biocontrol which suppress plant diseases by protecting the seeds and roots from fungal infection. They are known to enhance plant growth

promotion and reduce severity of many fungal diseases (Hoffland *et al.,* 1996; Wei *et al.,* 1996).

2.6.3.1 Mechanism of plant growth promotion by Pseudomonas fluorescens

2.6.3.1.1 Phosphate solubilisation

Nautiyal (1999) carried out the quantitative estimation of phosphate solubilization by *Pseudomonas fluorescens*. He reported that the amount of glucose and carbon has an important role in phosphate solubilization.

Park *et al.* (2009) studied the phosphate solubilization by *Pseudomonas fluorescens* RAF15 and found it as an efficient phosphate solubilizer when using glucose was used as the sole carbon source. HPLC analysis showed that gluconic acid, tartaric acid and small amount of 2- ketogluconic, acetic and formic acids are the main acids produced by *Pseudomonas fluorescens* (RAF15). Also, they found that solubilization was proportional to the concentration of gluconic acid.

Oteino *et al.* (2015) studied the phosphate solubilization by different phosphate solubilizing bacteria. Among that, *Pseudomonas fluorescence* showed the highest solubilization efficiency and found reduction in pH correspond to the level of gluconic acid produced.

2.6.3.1.2 Antagonistic activity against *Phytophthora capsici*

The anti-fungal metabolite 2, 4-diacetyl phloroglucinol plays a major role in the biocontrol capabilities of *P. fluorescens* (Delany *et al.,* 2000).

Sang *et al.* (2013) studied the role of some antagonistic bacteria in the control of *Phytophthora capsici* in black pepper. It was reported that *Pseudomonas* (YJR27 and YJR92) protected pepper plants against pathogen infection at similar levels as observed in the case of metalaxyl treatment.

Ozyilmaz and Benlioglu (2013) conducted an experiment to study the biocontrol of *Phytophthora* blight of pepper by *Pseudomonas* sp. The results

showed that the *Pseudomonas fluorescens* strains (6L10, 6ba6 and 3ss9) had biosurfactant producing abilities effective against *P. capsici* on pepper. The enhancement of disease suppression can the achieved by using *Pseudomonas fluorescens* along with olive oil.

2.6.3.1.3 Indole Acetic Acid (IAA) production

Oberhansli *et al.* (1991) reported that *Pseudomonas fluorescens* (strain CHAO) was an effective biocontrol agent against soil-borne fungal plant pathogens.

According to Suzuki *et al.* (2003), the IAA production *by P. fluorescens* HP72 was visually detected by the colour development and found that the amount of IAA produced varied according to the changes in colour development.

Park *et al.* (2009) reported that plant growth promoting hormones such as IAA is produced by *Pseudomonas fluorescence* (RAF15) and quantitatively estimated the production of IAA in the presence of different concentration of L-tryptophan.

2.6.3.1.4 Siderophore production

Loper (1987) investigated the production of fluorescent siderophore by *Pseudomonas fluorescens* in the biocontrol of *Pythium ultimum* in cotton. He reported that fluorescent siderophore produced by *Pseudomonas fluorescens* (3551) contributed but does not include all of its antagonistic mechanism against *P. ultimum*.

In a study conducted by Suzuki *et al.* (2003) the strain *P. fluorescens* (HP72) was checked for the production of siderophore, but the strain did not produce fluorescent siderophores.

Rachid and Ahmed (2005) reported that the ability of *Pseudomonas* to grow and produce siderophore depends upon the iron content and the type of carbon source used in the medium.

Park *et al.* (2009) confirmed the siderophore production by *Pseudomonas fluorescence* (RAF15) on the CAS agar media by the development of a yellow-orange halo around the colony.

According to Vanitha and Ramjegathesh (2014), the mechanism behind the antagonism of *Pseudomonas fluorescens* includes siderophore production.

2.6.3.1.5 Hydrogen Cyanide (HCN) production

Askeland and Morrison (1983) studied 200 isolates and found five strains of *P. fluorescens* and one strain of *P. aeruginosa* as cyanogenic.

Voisard (1989) tested the importance of hydrogen cyanide in the suppression of black root-rot of tobacco and reported that *Pseudomonas fluorescens* (CHA0) produced cyanide and improved the ability of suppression of disease.

Park *et al.* (2009) recorded the HCN production from the *Pseudomonas fluorescence* (RAF15) with the change in the colour of filter paper from yellow to reddish brown.

Vanitha and Ramjegathesh (2014) reported that in *Pseudomonas fluorescens,* the mechanism behind the antagonism includes hydrogen cyanide production.

2.6.3.1.6 Ammonia production

Baligh *et al.* (1996) reported that production of ammonia depends upon the amount of peptone added to the media and varied with the strains. The amount of ammonia produced by bacterial strains ranged from 2.3-17.8µl/ml.

Ramyasmruthi *et al.* (2012) estimated the production of ammonia by *Pseudomonas* sp. They reported that the R isolate produced ammonia. Ammonia production by the PGPB influenced plant growth indirectly.

Kumar *et al.* (2014) isolated 22 *Pseudomonas* sp. and 20 produced ammonia. Ten isolates showed deep brown colour which indicated the higher production of ammonia. Only two isolates showed the negative reaction to ammonia production and the remaining were medium producers.

2.6.4 *Trichoderma* sp.

Trichoderma is an effective bio-inoculant for the control of soil borne pathogen. *Trichoderma* is one of the most commonly used fungi in the biological control of plant diseases (Rosa and Herrera, 2009). The *Trichoderma* strains exert biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism. Activation of each mechanism implies the production of specific compounds and metabolites, such as plant growth factors, hydrolytic enzymes, siderophores, antibiotics, and carbon and nitrogen permeases.

2.6.4.1 Mechanism of plant growth promotion by Trichoderma sp.

2.6.4.1.1 Siderophore production

Anke *et al.* (1991) studied the production of siderophores by nine strains of *Trichoderma* and produced different types of siderophores such as ferricrocin, coprogen B and coprogen under the conditions of iron deficiency. Fusigen type of siderophore was produced by *T. pseudokoningii* and *T. longibrachiatum*.

According to Qi and Zhao (2013) the strain Q1 of *Trichoderma asperellum* produced siderophore on the CAS agar plates and there was 96.6% siderophore production after two days.

Vinale *et al.* (2013) reported a novel siderophore Harzianic acid (HA) from the *Trichoderma harzianum*. They investigated the iron binding capacity of the HA and found that HA could decolorize CAS blue agar and indicated that the HA could form the complex with Fe (III) ions.

2.6.4.1.2 Hydrogen cyanide production

Zhang *et al.* (2012) studied the plant growth promoting activities of the putative mutant of *Trichoderma* sp. (T-E5) and its wild-type (SQR-T037) under *in vitro*. Both showed negative for HCN production in the qualitative detection.

Rinu *et al.* (2014) reported a newly isolated strain, *Trichoderma gamsii* (NFCCI 2177) which was a psychrotolerant plant growth promoting antagonistic fungi. They tested the HCN production by the strain and showed negative reaction to HCN production.

2.6.4.1.3 Ammonia production

Triveni *et al.* (2013) studied the biochemical traits of *Trichoderma* based biofilm for using as plant growth promoting inoculants. The study revealed that *Trichoderma* produced more ammonia when biofilm was formed along with other plant growth promoting rhizobacteria than pure culture of *Trichoderma*.

Rinu *et al.* (2014) reported that *Trichoderma gamsii* (NFCCI 2177) which was a psychrotolerant plant growth promoting antagonistic fungi. They tested the ammonia production and recorded positive reaction for ammonia production.

2.6.4.1.4 Antagonistic activity against *Phytophthora capsici*

Anandaraj and Sarma (1995) reported that *Trichoderma harzianum* and *Trichoderma viride* could be used as biological control agents against diseases of black pepper caused by *Phytophthora capsici* both in the main field as well as in the nursery.

Moayedi and Mostowfizadeh-Ghalamfarsa (2009) isolated *Trichoderma* spp. and recorded the growth and colonization of the isolates on the pathogen mycelia. *Trichoderma virens, Trichoderma atroviride, Trichoderma harzianum* and *Trichoderma asperellum* showed the highest growth rates on the pathogen *Phytophthora* sp. The result of *in planta* experiment showed that when the plants

were inoculated with *Trichoderma* and *Phytophthora* together, the fresh and dry weight were higher in the case of *Trichoderma* sp. than the fresh and dry weight of plant inoculated with the pathogen alone. When the root was inoculated with the pathogen alone, 2-4 weeks after inoculation, internally and externally the root became discoloured and in the inoculation of combination of *Trichoderma* and *Phytophthora* showed less discolouration.

Rinu *et al.* (2014) evaluated the antagonistic activity of newly reported strain *Trichoderma gamsii* (NFCCI 2177) against seven pathogenic fungi. Among the seven phytopathogenic fungi tested, *Trichoderma gamsii* (NFCCI 2177) showed antagonism against six pathogenic fungi.

2.7 EFFECT OF WEATHER VARIABLES ON THE FUNCTIONAL EFFICIENCY OF BENEFICIAL MICROFLORA

Changes in the environment may change the composition and biomass of a microbial community which in turn will affect the plant growth. All microorganisms have a set of optimal environmental conditions, which enhances their optimal growth (Petterson, 2004).

Plants are not only colonized by a wide range of fungi but also by different bacteria and many of them confer beneficial effects to their hosts (Lugtenberg and Kamilova, 2009).

As plant-associated bacteria depends on root exudates (Rangel-Castro *et al.*, 2005) or plant metabolites (Rasche *et al.*, 2009), they are substantially influenced by environmental parameters due to plant physiological changes (Rasche *et al.*, 2006 a, b). It can be expected that environmental conditions associated with climate change will affect these communities.

The change in climate or weather variables will alter the physiology of plant and root exudation. In the root zone, increased C allocation occurs due to elevated carbon dioxide in the atmosphere and alterations might include changes in the

availability of chemoattractants or signal compounds as well as a different C/N ratio or nutrient availability (Kandeler *et al.*, 2006). Drigo *et al.* (2008) reported that elevated CO_2 affects the population of rhizobacterial communities. In addition to bacterial communities colonizing the rhizosphere, endophytic populations may also be impacted.

Prolonged exposure to stress and the impact of recurring stress factors affected the number of microbes in the soil, but not necessarily their metabolic activity (Griffiths *et al.*, 2000). Increased temperature caused microbes to undergo physiological changes that resulted in reduced carbon-use efficiency (Allison *et al.*, 2010). Chooi-Hua *et al.* (2013) reported that change in climate can alter the environmental conditions drastically as a result of which plant-microbe associations are affected. Madhu and Hatfield (2013) reported that elevated CO_2 concentration is likely to increase the C (carbon) allocation to the plant roots and thereby significantly hampers the normal physiological and growth promoting activities of plant root associated microbes. Sharma *et al.* (2014) reported that the climate change leads to a change in the type, distribution and coverage of soil microbial populations.

2.7.1 Effect of weather variables and soil parameters on *Azospirillum*

Kapulnik *et al.* (1981) studied the association between the nitrogen-fixing bacterium *Azospirillum brasilense* (strain cd) and the grass *Setaria italica*, under different environmental and soil conditions. Highest acetylene reduction rates in intact plants were observed at the booting stage of *Setaria* (2350 nmol ethylene produced $hour^{-1}$ $plant^{-1}$) at 27 °C. Higher temperatures, up to 32 °C, enhanced ethylene reduction. At 32/27 °C and 27/22 °C, ethylene production was approximately linear with time during the 1st 24 h of incubation. Lower temperatures, 22/17 and 17/12 °C, decreased the rate of ethylene production. The sharp increase in ethylene production at later stages of incubation at 17/12 °C was probably due to the cold stress, causing the death of cells and leakage of nutrients from the roots, thus enhancing acetylene reduction.

Govindan (1988) reported that the population of *Azospirillum* was considerably reduced with the decrease in soil moisture content in the rhizosphere of black pepper. He also reported that, a soil moisture content of 50.4 to 73.5 per cent was most favourable for the growth of *Azospirillum* and below that the population considerably reduced.

Tripathi and Klingmuller (2011) studied the effect of temperature on the growth and acetylene reduction activity of *Azospirillum* spp. The result showed that *Azospirillum brasilense* and *Azospirillum lipoferum* had optimum acetylene-reducing activity at 25 and 30 °C, respectively, although both the bacteria grew optimally at 35 °C. *Azospirillum halopraeferens* displayed optimum growth and acetylene-reducing activity at 40-41 °C. The experiments indicated that expression of nif genes was generally more sensitive to temperature than was nitrogenase activity. The NifA-dependent activation of a heterologous nifH–lacZ fusion was used to assess the impact of temperature on native NifA activity of *A. brasilense* and *A. lipoferum.* Maximum NifA activity was observed at 25°C in *A. brasilense* and at 30 °C in *A. lipoferum*.

2.7.2 Effect of weather variables and soil parameters on phosphate solubilizing bacteria

Pandey *et al.* (2002) studied the effect of temperature on the phosphate solubilization efficiency of *Pseudomonas corrugate* in a wide range of temperature. The solubilization zone on the Pikovskya's agar plate by the bacteria was minimum (1.9 mm) at 35 °C and maximum (8.2 mm) at 21 °C. No growth of bacteria was obtained at 40 °C. The bacteria were capable of solubilizing tri calcium phosphate at a wide range of temperature from 4 to 35 °C and the pH of the broth decreased due to the bacterial activity.

Maheswar and Sathiyavani (2012) isolated *Bacillus subtilis* and *Bacillus cereus* from the rhizosphere soil of groundnut and optimized the effect of pH, temperature, carbon, nitrogen and potassium sources on phosphate solubilization.

At a temperature of 40 °C, maximum phosphate solubilization was recorded. *Bacillus subtilis* was the most efficient phosphate solubilizer at a pH of 7.

Reena *et al.* (2013) studied the phosphate solubilizing microorganism obtained from different rhizosphere soils of banana. The potential solubilizers obtained were *Aspergillus niger* (234.12 mm) and *Bacillus subtilis* (160.82 mm). They screened the isolates at various pH and temperature. It was found that the maximum solubilization occurred at pH 3 in the case of bacteria and fungi at a temperature of 28 °C and 37 °C respectively.

Nosrati *et al.* (2014) isolated *Azotobacter* sp. which was capable of solubilizing the organic and inorganic phosphates. Under different environmental conditions, phosphate solubilization efficiency and the growth were quantitatively measured. A close association was evident between phosphate solubilizing ability and growth rate as an indicator of active metabolism. All three phosphate solubilizing bacteria (PSB) were able to withstand temperature as high as 45 °C, high concentration of NaCl (up to 5 per cent) and a wide range of initial pH from 5 to 10 while hydrolyzing phosphate compounds actively.

2.7.3 Effect of weather variables and soil parameters on fluorescent pseudomonads

Askeland and Morrison (1983) reported that maximum cyanogenesis by two strains of *P. fluorescens* in a defined growth medium occurred at 25 to 30 °C over a pH range of 6.6 to 8.9.

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

Influences of relative humidity on the air-borne survival of *Pseudomonas fluorescens* were studied. The study was carried out at different levels of relative humidity such as 20, 40, 60 and 80 per cent. According to Handley and Webster (1993), 40 to 60 per cent relative humidity was best for the survival of *Pseudomonas fluorescens* which was suspended in distilled water and the survival was less at 80 per cent relative humidity.

Effect of soil temperature on the colonization of *Pseudomonas fluorescens* in the pea roots were studied by Bowers and Parke (1993). The inoculated seeds were sown in the soil maintained at different soil temperatures such as 16, 20, 24 and 28 °C. The root growth was rapid at 24 °C and the temperature optimum for root colonization of *Pseudomonas fluorescens* was 16 °C.

According to Duffy and Defago (1997), the interaction between a plant pathogen and a biocontrol agent could be influenced by soil properties.

According to O'callaghan *et al.* (2001), establishment of *P. fluorescens* was significantly affected by both soil moisture content and temperature. At 20 °C, population decline was much greater, with populations falling to below the level of detection at all soil moisture levels after 54 days. When sampled at 82 days, *P. fluorescens* could not be recovered from most of the samples incubated at 20 °C.

Persistence of *Pseudomonas* populations in soil has been monitored by several workers as part of studies on strains with biological control potential. Ownley *et al.* (2003) reported 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, sixteen soil properties were correlated with disease suppression. The biocontrol activity was positively correlated with soil pH and negatively correlated with organic matter content, total carbon and total nitrogen. The addition of organic matter as wheat straw, at rates typical of high organic matter soils significantly reduced biocontrol activity of the strain.

Donnarumma *et al.* (2010) reported that the optimum temperature for the growth of *Pseudomonas fluorescens* was 25-30 °C.

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

Guo *et al.* (2013) reported that population of soil bacteria is negatively correlated with soil temperature.

2.7.4 Effect of weather variables and soil parameters on *Trichoderma* sp.

Tronsmo and Dennis (1978) reported that, most isolates of *Trichoderma* sp. tested were antagonistic against the *Botrytis cinerea*. Inhibition of growth by non-volatile metabolites from *Trichoderma* species was most severe at 5 °C, whereas more isolates produced volatile inhibitors at higher temperatures (around 20 °C). No effect of temperature was observed on the hyphal interaction and also carbon dioxide did not account for the inhibition by the volatile component.

According to Ahmad and Baker (1987), when cucumber seeds were treated with the conidia of *Trichoderma harzianum*, the isolate could be detected in rhizosphere soil of pH 5.0, 6.0 and 7.0 at 19, 26 and 33 °C. The population densities were higher in pH 5.0 than in pH 7.0.

Knudsen and Bin (1990) conducted an experiment to study the effect of temperature and soil moisture on the growth of *Trichoderma harzianum* from the alginate pellets. They reported that temperature had a significant positive effect on radial growth rate.

Mukherjee and Raghu (1997) worked on the effect of *Trichoderma* sp. on *S. rolfsii*. Experimental results indicated that *S. rolfsii* and *Trichoderma* sp. had different optimum temperature for growth (30-35 °C) for the pathogen and 25-30°C for the antagonist. In dual culture, *Trichoderma* overgrew *S. rolfsii* at 25 °C and 30 °C, but at 35 °C and 37 °C, *S. rolfsii* overgrew the colony of *Trichoderma.*

Trichoderma produced higher concentration of fungi toxic metabolites in broth culture at higher temperatures.

Jayaraj and Ramabadran (1999) conducted an experiment to know the effect of soil moisture on the growth of *Trichoderma harzianum*. Out of six moisture levels, the highest population (98.3 x 10^3 cfu/g) and competitive saprophytic ability index (0.218) were noticed at 10 per cent moisture level 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 conducive for the survival and multiplication of *Trichoderma* propagules in soil. The least population (8.5 x 10^3cfu/g) and CSA index (0.063) was observed at 80 per cent level of moisture.

The optimum temperature for growth differs among the *Trichoderma* species (Danielson and Davey, 1973). 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.

Reetha *et al.* (2014) studied the effect of temperature and pH on the growth of *Trichoderma harzianum*. They cultured the *Trichoderma* at different temperatures such as 25 °C, 30 °C, 35 °C, 45 °C and also at different pH such as 5,6,7,7.5. The results showed that the faster growth of *Trichoderma harzianum* was at 25- 30 °C and the growth was very slow at above 35 °C and there was no growth at 45 °C. Optimum temperature of *Trichoderma* was found between 25 to 30 °C, approximately 28 °C by radial growth. The maximum growth of *Trichoderma harzianum* was observed at pH 7-7.5 and the minimum growth was observed at the range of pH 5.

Mishra and Khan (2015) reported that *Trichoderma viride* was favoured by acidic pH while poor mycelia development was observed in alkaline medium. Moreover, 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 of the fungus was observed at 28°C with colony diameter of 2.6 cm and optimum pH at 6 with

3.7cm colony diameter and optimum relative humidity for sporulation was obtained at 80-90 per cent.

Chaudhary *et al.* (2016) reported that population of *Trichoderma* was negatively correlated with soil temperature and most of the fungal species were positively correlated with the soil moisture content.

Beneficial microorganisms play an important role in the increase of tolerance to abiotic stresses among agricultural crops. And black pepper has been reported to be the most vulnerable spice crop to the climate change. Changes in the weather and microclimatic variables affect the growth and yield of black pepper and also the rhizosphere microflora. In this scenario correlation data on weather variables and beneficial microflora can be an effective information for future researches to develop stress tolerant beneficial microflora.

Materials and methods

3. MATERIALS AND METHODS

A study on "Impact of weather variables on the functional efficiency of beneficial microflora in the rhizosphere of black pepper (*Piper nigrum* L.)" was carried out in the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara during 2014 to 2016. The materials used and methods followed for the study is given below.

3.1 SELECTION OF EXPERIMENTAL SITE

Black pepper plantation of Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara (N-10.5528°, E- 76.2918°, Altitude-22.25 m) was selected as experimental site. Twelve healthy black pepper plants were randomly selected and used till the end of the study period.

3.2 INSTALLATION OF SOIL THERMOMETERS AND AUTOMATIC WEATHER STATION

Twelve soil thermometers were installed in the respective rhizosphere region of black pepper plants at 20 cm depth. The soil temperatures were recorded at 14.30 IST. From the Automatic Weather Station (Micro-climatic Data Acquisition System- Emcon), temperature and relative humidity were recorded.

3.3 COLLECTION OF SOIL SAMPLES

The rhizosphere soil samples were collected from twelve healthy black pepper plants using standard protocol. Soil samples were collected from three different spots around the plant and mixed together to get a composite sample by quartering technique. The ear marked rhizosphere of plants were subsequently used for soil sample collection at monthly intervals.

3.4 NUTRIENT STATUS OF SOIL

The collected soil samples were analysed for initial and final pH, electrical conductivity, organic carbon (%), total nitrogen, available phosphorus and available

potassium before the experiment (July, 2015) and at 12th month of experiment (June, 2016).

3.4.1 Soil Reaction (pH)

The pH of the soil was determined in 1:2.5 soil-water suspensions. Ten gram of 2 mm sieved air dried soil was taken in a 50 ml beaker with 25 ml distilled water. It was stirred well and kept for half an hour. Again, it was stirred and reading was taken using pH meter (ELICO L1 120).

3.4.2 Electrical conductivity (EC)

The electrical conductivity of 12 different soil samples were determined (Rayment and Higginson, 1992). Ten grams of soil sample was taken in a 50 ml beaker to which 25 ml distilled water (1:2.5 ratio) was added. Suspension was stirred well at regular intervals for 30 minutes and electrical conductivity was recorded using EC meter (Jenway 4510 Conductivity Meter).

3.4.3 Organic Carbon

Soil organic carbon was determined in July, 2015 and June, 2016 by using Walkley and Black wet digestion method (Walkley and Black, 1934). The soil was ground and passed through a 0.5 mm sieve. From this, 1g of sample was taken and transferred into 500 ml conical flask. Exactly 10 ml of 1N $K_2Cr_2O_7$ was added and swirled the flask to disperse the soil in the solution. Then 20 ml of concentrated H_2SO_4 was added rapidly. Immediately, the flask was swirled gently until the soil and the reagents were mixed and kept on the asbestos sheet for 30 minutes. Then, 200 ml of distilled water was added to the flask in order to stop the reaction and 3-4 drops of ferroin indicator was also added to the solution. Titrated the contents in the flask with 0.5 N ferrous ammonium sulphate taken in a burette.

The solution turned to a dark green colour as it approached end point. At this point, the ferrous ammonium sulphate was added drop by drop until the colour

changed from blue to red. A blank was also made in the same manner without soil sample.

$$\text{Organic Carbon (\%)} = \frac{(\text{meq } K_2Cr_2O_7 - \text{meq } Fe(NH_4)_2SO_4) \times 0.003 \times 100 \times 1.3}{\text{weight of soil (g)}}$$

3.4.4 Total Nitrogen

Total nitrogen content of the soil samples were analysed in July, 2015 and June, 2016 (Bremner, 1960). The soil was ground and passed through 0.5 mm sieve. From this, 0.5g of sample was taken and transferred to Kjeldahl's flask. One gram of digestion mixture and 10 ml of conc. H_2SO_4 were added to it. Kept for pre digestion overnight. Then, kept the mixture in digestion unit at 300°C until it becomes a clear solution. After cooling filtered and transferred to a Kjeldahl's distillation unit. To this, 10 ml of 40 per cent NaOH was added and the condensed NH_3 gas was collected in 10 ml of boric acid-indicator mixture in 100 ml conical flask. The colour of boric acid-indicator mixture changed from reddish pink to bluish green as the ammonia entered the flask. When the collected solution became 100 ml, the presence of ammonia is tested by showing wet red litmus paper at the end of the condenser tube. The presence of ammonia turned the litmus paper into blue. At this stage, the distillation was stopped. It is titrated against 0.01 N HCl taken in a burette. The end point was bluish green to reddish pink.

$$\text{Total nitrogen in the soil sample (\%)} = \frac{V \times N \times 14 \times 100}{100 \text{ x W}}$$

where, V= Titre volume

N= Normality of HCl

W= Weight or volume of sample used

3.4.5 Available soil phosphorus

Available 'P' was extracted using Bray No. 1 (Bray and Kurtz, 1945), with 0.03 N NH_4F and 0.025 N HCl. Five grams of soil was added to a 250 ml conical flask with 50 ml of Bray No.1 reagent and shaken for five minutes. Filtering was done though Whatman No. 42 filter paper and to avoid interference of fluoride, 7.5 ml of 0.8 M (10 ml, 4%) boric acid (50 g H_3BO_3 per litre) was added to 5 ml of the extract. 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 and diluted to approximately 20 ml. Four milliliter of reagent B was added and the volume was made up with distilled water and mixed the contents well. After 10 min, the intensity of colour was read at 660 nm. The colour was stable for 24 h and the maximum intensity developed within 10 min. The concentration of P in the sample was computed using standard curve.

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

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

3.4.6 Available soil potassium

Estimation of available potassium was done by flame photometric method (Jackson, 1973). Five gram of soil was mixed with 25 ml of neutral normal potassium acetate for five minutes and filtered immediately through a Whatman No. 42 filter paper. Potassium concentration in the extract was determined using flame photometer after necessary settings and calibration of the instrument.

Standard curve for potassium was prepared using standard solution of potassium chloride. Measured aliquots were diluted from the standard solution using potassium chloride solution to give concentrations of 5 to 20 µg mL^{-1} of K. After attaching the appropriate filter and adjusting the gas and air pressure, the reading was set in the flame photometer as zero for the blank (potassium chloride) and at 100 for 20 µg/mL of K. The curve was obtained by plotting the readings against the different concentrations (5, 10, 15 and 20 µg/mL) of K.

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

3.5 ISOLATION AND ENUMERATION OF SELECTED BENEFICIAL MICROFLORA

The soil samples were used for the isolation of *Azospirillum*, PSB, *Pseudomonas fluorescens* and *Trichoderma* sp. at monthly interval.

3.5.1 Isolation and enumeration of *Azospirillum*

Isolation and enumeration of *Azospirillum* was done at monthly interval for a period of 12 months. Test tubes containing 5.0 ml Nfb (Nitrogen free bromothymol blue) semi-solid medium (Okon *et al.*, 1983) were inoculated with 100 µl of appropriate dilutions (10^{-1} to 10^{-7}) of soil suspension and enumeration was done by MPN method (Doberieneir, 1995). White pellicle formation and blue colour development in the media were taken as positive for *Azospirillum*. After 5-7 days of incubation at 30±2^{0}C, 10 µl of pellicle forming culture was spread on Nfb-solid medium supplemented with ammonium chloride as nitrogen source. Morphologically divergent colonies were picked from the plates and transferred to fresh Nfb semi-solid medium. Colonies were observed for white undulating pellicle formation with blue colour development.

3.5.2 Isolation and enumeration of phosphate solubilizing bacteria (PSB)

Isolation and enumeration of PSB were done at monthly interval for a 12 months period. Ten gram of soil sample was suspended in 90 ml of sterile water and serial dilutions of the suspension were made in sterile water blanks. One millilitre of 10^{-1} to 10^{-8} dilutions were plated on NBRIP (National Botanical Resesarch Institute's Phosphate growth medium) agar medium for obtaining microorganisms capable of dissolving insoluble phosphates (Nautiyal, 1999). The plates were incubated for 4-5 days at 28±2^{0}C. Transparent and clear zone around microbial colonies indicated the extent of phosphate solubilization (Sharma *et al.*, 2011) and such bacterial cultures were purified and maintained on NBRIP agar slants for further studies. Number of colonies on the respective dilution were calculated and expressed as colony forming units per gram of soil ($CFUg^{-1}$).

3.5.3 Isolation and enumeration of *Pseudomonas fluorescens*

The isolation of *Pseudomonas fluorescens* was done at monthly interval. Ten gram of soil sample was suspended in 90 ml of sterile water and serial dilutions of the suspension were prepared in sterile water blanks. One milliliter of 10^{-1} to 10^{-8} dilutions were plated on King's B Agar medium. The plates were incubated for 2-3 days at 28±2^{0}C and observed for fluorescence under UV light at 302 nm (Meyer and Abdallah, 1978).

3.5.4 Isolation and enumeration of *Trichoderma* sp.

The isolation of *Trichoderma* sp. was done at monthly interval for a period of one year. Ten grams of soil sample was suspended in 90 ml of sterile water and serial dilutions of the suspension were made in sterile water blanks. One millilitre of 10^{-1} to 10^{-6} dilutions were plated on Trichoderma Selective Medium (TSM). The plates were incubated for 4-5 days at 28±2 °C and observed for green coloured *Trichoderma* colonies (Samuel *et al.*, 2002).

3.6 SCREENING OF SELECTED BENEFICIAL MICROFLORA FOR FUNCTIONAL EFFICIENCY UNDER *in vitro* CONDITION

The screening of isolated beneficial microflora for functional efficiency was done at monthly intervals for a period of one year under *in vitro* condition.

3.6.1 Screening of *Azospirillum* isolates

3.6.1.1 Nitrogen fixation

Nitrogen fixation by each *Azospirillum* isolate was estimated (Humphries, 1956). To a 250 ml conical flask, 100 ml of the nitrogen free semi-solid malate medium was dispensed and autoclaved at 15 lbs/ sq. inch for 15 min. The *Azospirillum* isolates were grown separately for 24 h in Nfb semi-solid medium and inoculated @ 2 ml/100 ml of the medium. Triplicate samples were kept for each isolate. The flasks were incubated at 32^0C for 14 days. After 14 days of incubation, the culture was homogenized. Five millilitre of the homogenized culture was drawn and digested with 5 ml concentrated H_2SO_4 and 200 mg catalytic mixture (K_2SO_4:$CuSO_4$: Selenium @ 100:10:1) until the contents became clear. After cooling, the volume was made upto 25 ml with distilled water. Then, 5 ml of aliquot was transferred to microkjeldhal distillation unit. An aliquot of 10 ml of 40% NaOH was added and steam distilled. Ammonia evolved was collected over 2% boric acid (20 ml) containing 2 drops of mixed indicator (83.3 mg bromocresol green+16.6 mg methyl red indicator dissolved in 10 ml of 95% ethanol) and back titrated against 0.05 N H_2SO_4. Total nitrogen content of the culture was determined and the results were expressed as mg of N fixed per gram of malate.

$$\text{Per cent nitrogen} = \frac{(TV \times N \times 0.014)}{W} \times \frac{25}{5} \times 100$$

Where,

TV = Titre volume

N = Normality of H_2SO_4

W = Weight or volume of sample used

3.6.1.2 IAA production

The isolates were screened for the production of IAA with a modified procedure of Brick *et al.* (1991). Luria Bertani (LB) broth supplemented with 0.06 per cent Sodium Dodecyl Sulphate (SDS) and glycerol (one per cent) was transferred to test tubes and sterilized. Overnight growth cultures of each isolates were inoculated in the broth. Tubes were incubated for 4 days at 30±2 °C and Salkowski reagent was added to each test tube. Bacteria producing IAA were identified by the formation of characteristic pink to red color in the broth.

3.6.1.3 Gibberellic Acid production

Hundred millilitres of nutrient broth was dispensed in 250 ml conical flasks and inoculated with the *Azospirillum* cultures. The culture flasks were incubated at 35°C for 48 hrs. After incubation, bacterial culture was centrifuged at 10,000 rpm for 15-20 minutes. The pH of culture supernatant was adjusted to 2.5 using stock 3.75 N HCl. The culture supernatants were extracted using liquid-liquid (ethyl acetate/$NaHCO_3$) extraction method. The amount of GA in the ethyl acetate was measured by UV spectrophotometer at 254 nm against control blank (Pandya and Desai, 2014)

3.6.2 Screening of PSB and *Pseudomonas fluorescens* isolates

3.6.2.1 Phosphate solubilization efficiency

The isolates were spotted on Pikovskaya's agar plate and incubated at 28°C for seven days. The plates were then examined and the diameter of halozone and colony diameter were recorded (Pikovskaya, 1948).

$$\text{Solubilization Efficiency (\%)} = \frac{\text{Solubilization diameter (mm)}}{\text{Colony diameter (mm)}} \times 100$$

3.6.2.2 Antagonistic activity against Phytophthora capsici

Bacterial antagonists were evaluated for antagonistic activity against *Phytophthora capsici* by dual culture technique (Utkhde, 1984). *P. capsici* disc (8 mm) was placed on PDA (Potato Dextrose Agar) at 2 cm from the periphery and incubated at 28±2°C for 48 hours. After incubation, bacterial antagonists were inoculated at opposite side of the same plate at 3-4 cm away from the pathogen. Three replicates were maintained for each treatment. Plate with pathogen alone served as control.

After 7 days of incubation, when the growth of pathogen in control plates reached maximum, the radial growth of the pathogen was measured. Percent inhibition over control was worked out according to the equation.

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

I= Inhibition percentage

C= growth of pathogen in control (mm)

T= growth of pathogen in dual culture (mm)

3.6.2.3 IAA production

The isolates were screened for the production of IAA with a modified procedure of Brick *et al.* (1991) as given in ***3.6.1.2***.

3.6.2.4 Siderophore production

Chrome azurole S (CAS) agar medium described by Schwyn and Neilands (1987) was used for the determination of siderophore production. Test organisms were spot inoculated on CAS agar and incubated at 28±2°C for seven days. Development of yellow orange halo around the growth was considered as positive for siderophore production. The control plates of CAS agar (uninoculated) were incubated under the same conditions as described above and no colour change observed.

3.6.2.5 HCN production

The antagonistic bacteria were streaked on Kings' B agar media amended with glycine at $4.4gl^{-1}$ (Bakker and Schipper, 1987). Sterile filter paper (Whatman No:1) saturated with picric acid solution (2.5 g of picric acid; 12.5 g of Na_2CO_3, 1000 ml distilled water) was placed in the upper lid of the Petri plate. The plates 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 were recorded as weak (+), moderate (++) or high (+++) reaction respectively.

3.6.2.6 Ammonia production

Freshly grown bacterial culture were inoculated in a test tube containing 4 per cent peptone water and incubated at 28±2°C. Bacterial isolates were incubated for 2 days and fungi for 4 days. After incubation, Nessler's reagent (0.5 ml) was added to each tube. Development of yellow to brown colour was positive for ammonia production (Cappuccino and Sherman, 1992). Based on the intensity of color produced the reaction was rated as follows. Yellow weak: +, Orange moderate: ++ and Brown high: +++

3.6.3 Screening of *Trichoderma* sp. isolates

3.6.3.1 Siderophore production

Chrome azurole S (CAS) agar medium described by Schwyn and Neilands (1987) was used for the determination of siderophore production. Test organisms were spot inoculated on CAS agar and incubated at 28±2°C for seven days. Development of yellow-orange halozone around the colonies were considered as positive for siderophore production. The control plates of CAS agar (uninoculated) were incubated under the same conditions as described above and no colour change was observed.

3.6.3.2 HCN production

The fungal antagonists were inoculated on Kings' B agar media amended with glycine at 4.4 gl^{-1} (Bakker and Schipper, 1987). Sterile filter paper (Whatman No: 1) saturated with picric acid solution (2.5 g of picric acid; 12.5 g of Na_2CO_3, 1000 ml 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.

3.6.3.3 Ammonia production

Freshly grown fungal culture were inoculated in a test tube containing 4 per cent peptone water and incubated at 28±2 °C. Fungal isolates were incubated for 4 days. After incubation, Nessler's reagent (0.5 ml) was added to each tube. Development of yellow to brown colour was a positive test for ammonia production (Cappuccino and Sherman, 1992). Based on the intensity of color produced the reaction was rated as Yellow weak: (+), Orange moderate (++) and Brown high (+++).

3.6.3.4 Antagonistic activity against Phytophthora capsici

Trichoderma sp. isolates were tested for their antagonistic activity using dual culture plate method (Skidmore and Dickinson, 1976). Mycelial disc (8mm) of the pathogen from four-day-old culture was inoculated aseptically on one side of Petri plate at 3 cm from the periphery and incubated at 28±2 °C for 48 hours. After this, 5 mm mycelial disc of fungal isolate was placed in the same PDA plate at 3 cm away from the pathogen disc and incubated at 28±2 °C. Three replications were maintained for each isolate. Petri plate inoculated with mycelial disc of pathogen alone served as control. The growth measurements were recorded at regular interval after 24 hrs of antagonist's inoculation of the up to 7 days. The per cent inhibition of mycelial growth of pathogen (I) was calculated as given in 3.6.2.2.

3.7 MICROCLIMATIC PARAMETERS

3.7.1 Soil respiration

Soil respiration (Carbon dioxide evolution) of 12 different soil samples were determined at monthly interval for a period of one year by alkali trap method (Chhonkar *et al.*, 2007). Moisture content of the samples was determined by gravimetric method for which 40 g moist soil was taken in a beaker of 50 ml capacity. Water was added to bring its moisture content to field capacity and kept the beaker inside the glass jar. Five milliliters of 0.5 N NaOH was taken in a scintillation vial and placed inside the jar beside the beaker. Then, 2-3 ml of water was added at the bottom of the jar and closed the lid to make it air tight. One blank was also kept without soil. The jars were kept in the incubator at 37 °C. After 7 days of incubation, the jars were taken out from the incubator and 0.5 N NaOH solution from the vial was transferred to a conical flask after several washings of the vials to ensure complete transfer. Few drops of saturated $BaCl_2$ solution was added along with phenolphthalein indicator. These contents in the conical flasks were titrated against 0.5 N HCl, slowly until the pink colour disappeared and recorded the titre value. The CO_2 evolved was calculated by using the following equation.

$mgCO_2/g$ soil= (Blank value-Titre value) x (Normality of acid x 22)

3.7.2 Soil moisture content

Soil moisture content of the 12 different samples was determined (Black, 1965) at monthly interval for a period of one year. An empty clean moisture can was weighed and 10 g of fresh soil sample collected from the field was added into it. Again, the can was weighed with sample. The sample cans were placed in an oven at 105°C till a constant weight was obtained. After cooling, the dry weight of sample was taken. From the differences in the weight, the moisture content was expressed as percentage on oven dry basis.

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

3.8 WEATHER PARAMETERS

Weather parameters such as temperature, rainfall, sunshine hours and relative humidity were recorded at the Automatic Weather Station (AWS) installed at the Pepper Unit, Department of Plantation crops and spices, College of Horticulture, Vellanikkara for a period of one year.

3.9 STATISTICAL ANALYSIS

Population and microclimatic parameters were analysed using the software WASP.2. To study the effect of weather and microclimatic 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. Data were analysed using SPSS package.

Results

4. RESULTS

The study was conducted with a main objective of assessing the effect of weather and micro-climatic parameters on the population and efficiency of *Azospirillum*, PSB, fluorescent pseudomonads and *Trichoderma* sp. The black pepper unit of Department of Plantation crops and spices, COH at Vellanikkara was used as the experimental site and 12 randomly selected black pepper plants were selected. The rhizosphere soil samples were collected at monthly interval for a period of one year and the isolation and screening of *Azospirillum*, phosphate solubilizing bacteria (PSB), fluorescent pseudomonads and *Trichoderma* were carried out. The experimental results obtained are presented below.

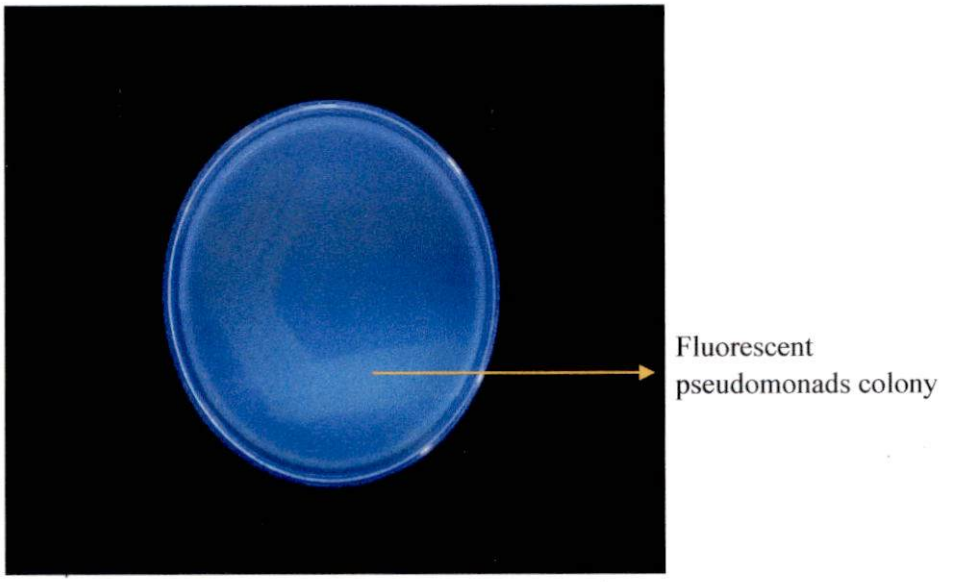
4.1 ENUMERATION OF SELECTED BENEFICIAL MICROFLORA FROM RHIZOSPHERE SOIL OF BLACK PEPPER

The rhizosphere soil samples were subjected to the isolation and enumeration of *Azospirillum,* PSB, *Pseudomonas fluorescens* and *Trichoderma* sp. (Plate 1) at monthly interval (July, 2015 to June, 2016) in order to determine the impact of weather variables and micro-climatic parameters on the population and efficiency of beneficial microflora.

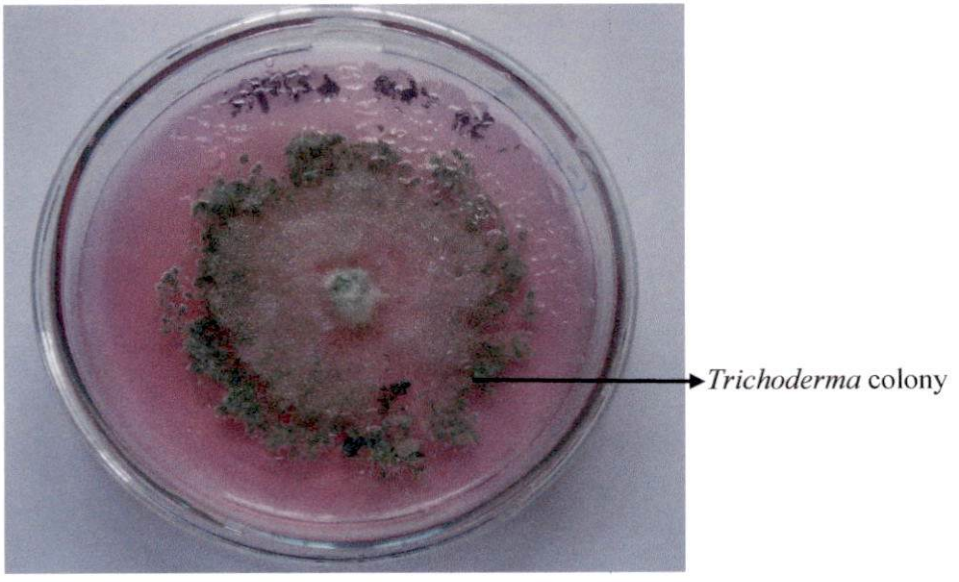
4.1.1 Enumeration of *Azospirillum* sp., PSB, fluorescent pseudomonads and *Trichoderma* sp.

The *Azospirillum* sp. and PSB population were not found during the period from July, 2015 to June, 2016 (Table 1).

The population of fluorescent pseudomonads (Fp) were significantly different at 1% and 5% level of significance. The population was highest in September, 2015 (Table 1). However the populations of Fp in December 2015, August 2015, November 2015, July 2015, January 2016 and October 2015 were on par with the population of September, 2015. The least population was recorded in the month of



a) Fluorescent pseudomonads



b) *Trichoderma* sp.

Plate 1: Beneficial microflora obtained from rhizosphere of black pepper

February, 2016 and May, 2016. A total of thirty one isolates of fluorescent pseudomonads were obtained during twelve months of study period (Table 2).

The *Trichoderma* isolates were obtained only in the months of July, 2015, August, 2015 and June, 2016. The population was highest in July, 2015, and the populations in August, 2015 was on par with the population of July, 2015 (Table 1). The lowest population was recorded in June, 2016. A total of only three isolates (Table 2) were obtained during the entire twelve months period.

Table 1: Enumeration of *Azospirillum* sp., PSB, fluorescent pseudomonads and *Trichoderma* sp. in the rhizosphere soil of black pepper during the period (July, 2015 to June, 2016)

Months	*Azospirillum* sp. ($x10^1$ Cfu g^{-1})	PSB ($x10^1$ Cfu g^{-1})	Fluorescent pseudomonads ($x10^1$ Cfu g^{-1})	*Trichoderma* sp. ($x10^1$ Cfu g^{-1})
July, 2015	a	a	14.4 (2.120^{ab})**	36.6 (2.534^{a})
August, 2015	a	a	15.0 (2.130^{ab})	2.6.6 (2.360^{a})
September, 2015	a	a	17.5 (2.195^{a})	a
October, 2015	a	a	11.2 (2.038^{ab})	a
November, 2015	a	a	14.2 (2.129^{ab})	a
December, 2015	a	a	15.0 (2.135^{ab})	a
January, 2016	a	a	14.4 (2.120^{ab})	a
February, 2016	a	a	4.4 (1.559^{c})	a
March, 2016	a	a	a	a
April, 2016	a	a	a	a
May, 2016	a	a	3.7 (1.484^{c})	a
June, 2016	a	a	6.0 (1.774^{bc})	6.3 (1.778^{b})

** Logarithmic transformed values are given in the parentheses

a: absent

Table 2: Number of isolates obtained from the rhizosphere soil of black pepper during twelve months (July, 2015 to June, 2016)

Month	*Azospirillum* sp.	Phosphate solubilizing bacteria	Fluorescent pseudomonads	*Trichoderma* sp.
July, 2015	0	0	3	1
August, 2015	0	0	2	1
September,	0	0	5	0
October, 2015	0	0	4	0
November,	0	0	3	0
December,	0	0	3	0
January, 2016	0	0	3	0
February, 2016	0	0	3	0
March, 2016	0	0	0	0
April, 2016	0	0	0	0
May, 2016	0	0	4	0
June, 2016	0	0	1	1
Total	0	0	31	3

4.2 SCREENING OF DIFFERENT ISOLATES FOR THE PRODUCTION OF IAA, AMMONIA, HYDROGEN CYANIDE, SIDEROPHORE PRODUCTION, PHOSPHATE SOLUBILIZATION AND ANTAGONISTIC ACTIVITY UNDER *in vitro*

The screening of fluorescent pseudomonads and *Trichoderma* sp. for functional efficiency was done at monthly interval from July, 2015 to June, 2016 under *in vitro.*

4.2.1 Screening of fluorescent pseudomonads for IAA, ammonia, HCN and siderophore production

A total of 31 isolates of fluorescent pseudomonads were obtained during the twelve months period. These isolates were screened for IAA, ammonia, HCN and siderophore production at a monthly interval to know the effect of weather variables and microclimatic parameters on the efficiency of fluorescent pseudomonads.

4.2.1.1 IAA production

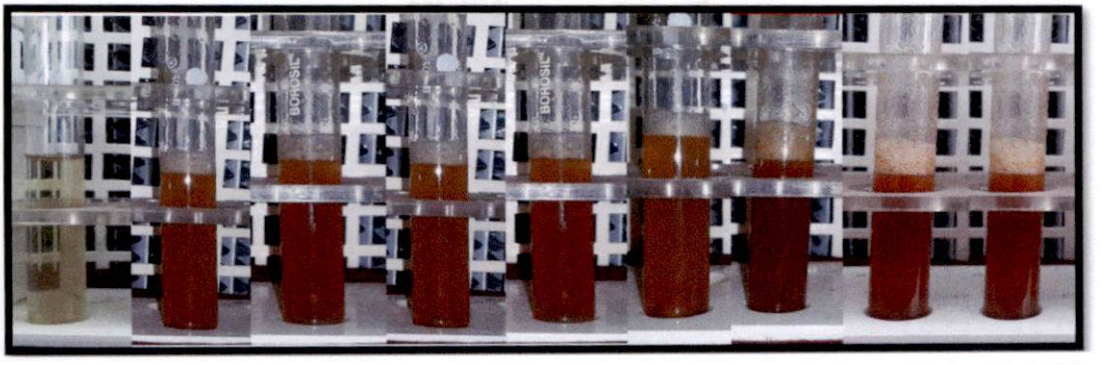
None of the thirty one isolates of fluorescent pseudomonads produced indole acetic acid.

4.2.1.2 Ammonia production

Among 31 isolates, 29 isolates produced ammonia with varied intensity. Eight isolates were high producers of ammonia (Plate 2), nineteen were medium producers and two were low producers of ammonia (Table 3). Highest ammonia production was recorded in September, 2015.

4.2.1.3 HCN production

Fifteen isolates produced HCN (Table 3) and out of which, four isolates were high producers of HCN (Plate 3) whereas, four were medium and seven were low producers of HCN. Maximum HCN production was recorded in October, 2015.



Control Fp3 (I) Fp 2(III) Fp 3(III) Fp 5(III) Fp 3 (V) Fp 3(VI) Fp 3 (XI) Fp 4 (XI)

Plate 2: Screening of fluorescent pseudomonads for ammonia production

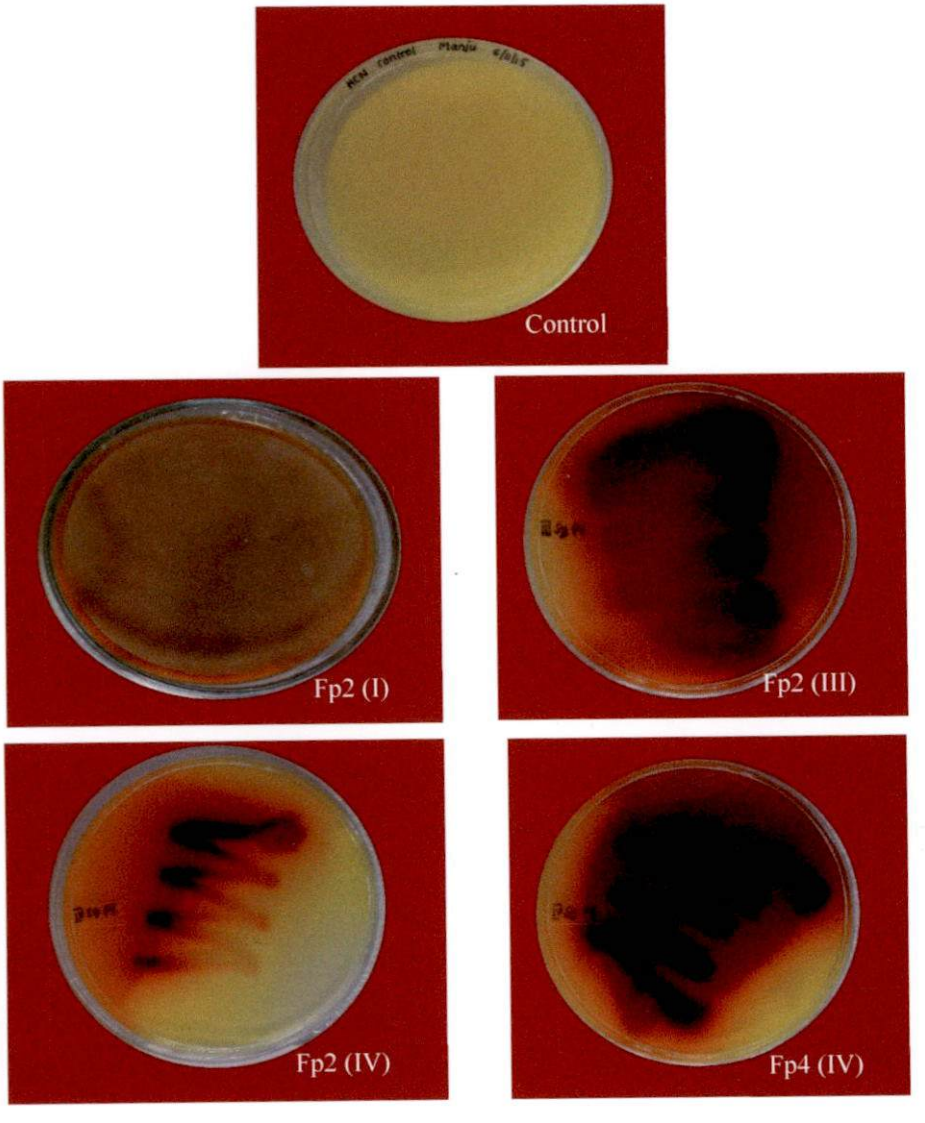


Plate 3: Screening of fluorescent pseudomonads for HCN production

4.2.1.4 Siderophore production

Among the thirty one isolates obtained, only ten isolates produced siderophore (Table 3, Plate 4) during the twelve month period with highest in July, 2015.

4.2.1.5 Phosphate solubilization

All the thirty one isolates were screened for phosphate solubilization activity (Table 4). Only five isolates (Plate 5) were the phosphate solubilizers. The highest phosphate solubilization was recorded in Fp2 (II) during August, 2015 (192%) which was on par with Fp1 (II) in the same month (189%). The lowest solubilization zone was observed in Fp1 (I) during July, 2015 (152%).

4.2.1.6 Antagonistic activity against Phytophthora capsici

All the thirty one isolates were screened for their antagonistic activity against *Phytophthora capsici* (Table 4). Only four isolates showed (Plate 6) antagonistic activity against *P. capsici*. The Fp2 (I) (July, 2015) isolate showed maximum percentage inhibition and minimum was showed by Fp1 (II) (August, 2015).

Among all the parameters studied, maximum number of fluorescent pseudomonads isolates produced ammonia (29 isolates) and the antagonism was recorded by minimum number of isolates (4 isolates).

Table 3: Screening of fluorescent pseudomonads for ammonia, HCN and siderophore production

Isolates	Ammonia production	HCN production	Siderophore production
Fp1 (I)	++	++	+
Fp2(I)	++	+++	+
Fp3(I)	+++	+	+
Fp1(II)	+	+	+
Fp2(II)	++	++	+
Fp1(III)	-	++	-
Fp2(III)	+++	+++	-
Fp3(III)	+++	-	-
Fp.4(III)	+	-	-
Fp5(III)	+++	+	+
Fp1(IV)	++	+	-
Fp2(IV)	++	+++	-
Fp3(IV)	++	-	-
Fp4(IV)	-	+++	-
Fp1(V)	++	-	-
Fp2(V)	++	-	-
Fp3(V)	+++	+	-
Fp1(VI)	++	-	-
Fp2(VI)	++	-	-
Fp3(VI)	+++	-	-
Fp1(VII)	++	-	-
Fp2(VII)	++	-	-
Fp3(VII)	++	-	-
Fp1(VIII)	++	-	-
Fp2(VIII)	++	-	-
Fp3(VIII)	++	-	-
Fp1(XI)	++	-	+
Fp2(XI)	++	-	-
Fp3(XI)	+++	++	+
Fp4(XI)	+++	+	+
Fp1(XII)	++	+	+

Ammonia and HCN: -negative, + low, ++ medium, +++ high; siderophore: - negative, + positive

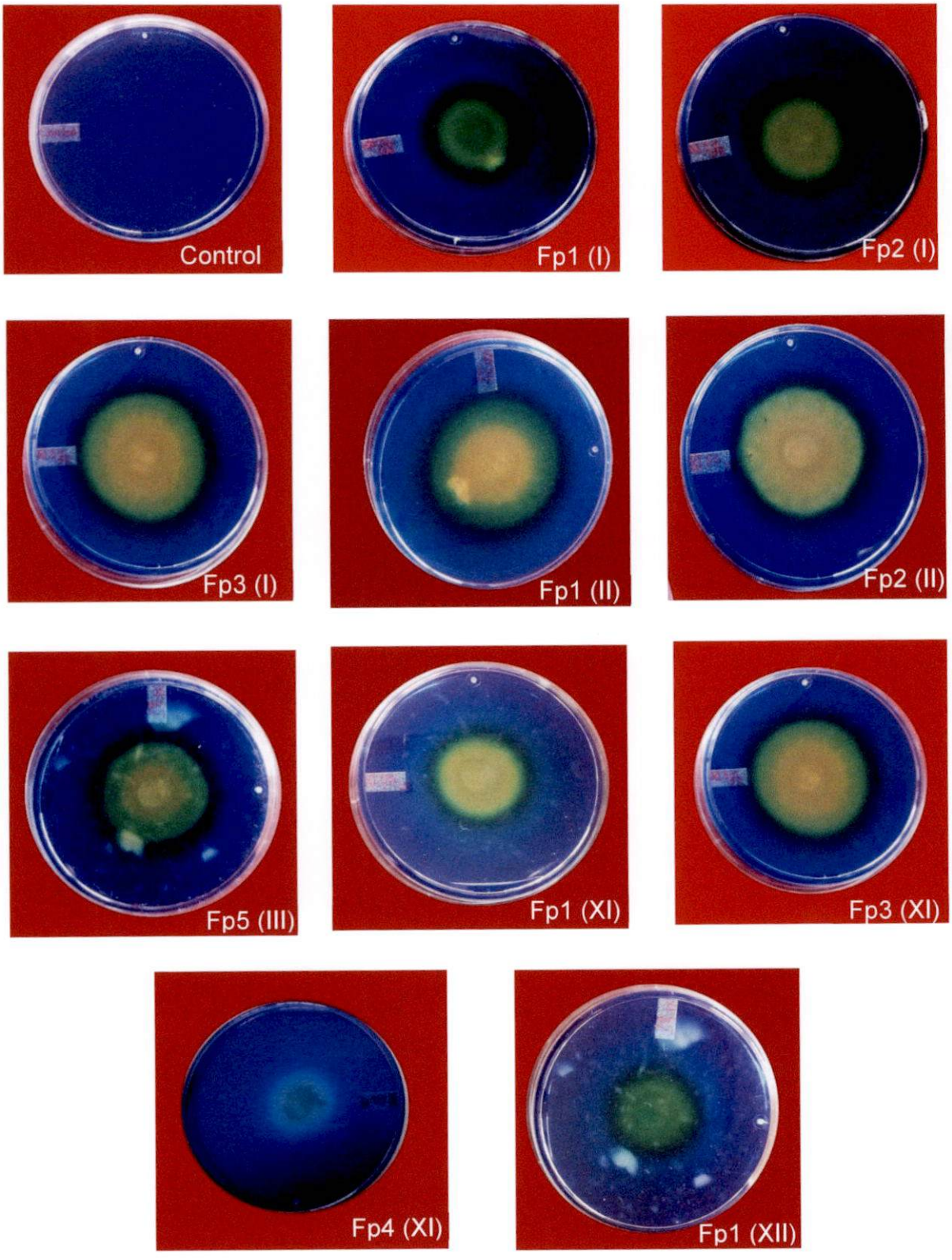


Plate 4: Screening of fluorescent pseudomonads for siderophore production

Table 4: Screening of fluorescent pseudomonads for phosphate solubilization and antagonistic activity against *Phytophthora capsici*

Isolates	Solubilization index (%)	Percent inhibition (%)
Fp1 (I)	152[c]	40[a]
Fp2 (I)	160[b]	44.4[a]
Fp3 (I)	158[b]	42.2[a]
Fp1 (II)	189[a]	33.3[b]
Fp2 (II)	192[a]	-
Fp1 (III)	-	-
Fp2 (III)	-	-
Fp3 (III)	-	-
Fp4 (III)	-	-
Fp5 (III)	-	-
Fp1 (IV)	-	-
Fp2 (IV)	-	-
Fp3 (IV)	-	-
Fp4 (IV)	-	-
Fp1 (V)	-	-
Fp2 (V)	-	-
Fp3 (V)	-	-
Fp1 (VI)	-	-
Fp2 (VI)	-	-
Fp3 (VI)	-	-
Fp1 (VII)	-	-
Fp2 (VII)	-	-
Fp3 (VII)	-	-
Fp1 (VIII)	-	-
Fp2 (VIII)	-	-
Fp3 (VIII)	-	-
Fp1 (XI)	-	-
Fp2 (XI)	-	-
Fp3 (XI)	-	-
Fp4 (XI)	-	-
Fp1 (XII)	-	-

(Each value represents mean of three replications)

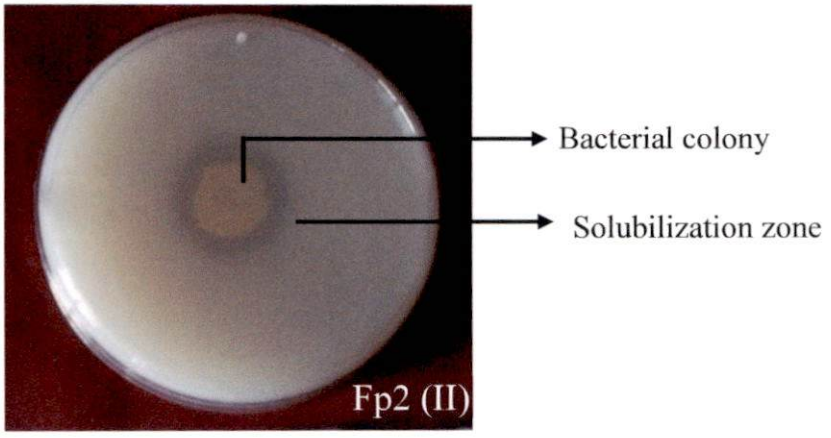


Plate 5: Phosphate solubilization by fluorescent pseudomonads

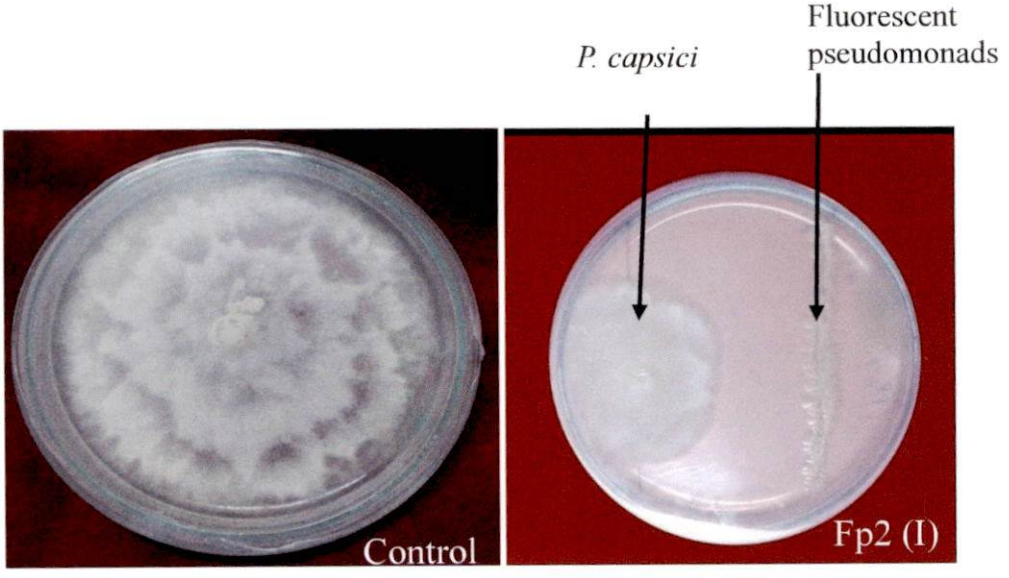


Plate 6: Antagonistic activity of fluorescent pseudomonads against *Phytophthora capsici*

4.2.2 Screening of *Trichoderma* sp. isolates for ammonia, HCN and siderophore production

All the three isolates of *Trichoderma* sp. were screened for ammonia, hydrogen cyanide (HCN), siderophore production and their antagonistic activity against *Phytophthora capsici* at monthly intervals for a period from July, 2015 to June, 2016 (12 months).

4.2.2.1 Ammonia production

Among the three isolates, two isolates produced ammonia with varied intensity (Table 5). One isolate was medium producer (June, 2016) of ammonia and one was low producer (August, 2015) (Plate 7a).

4.2.2.2 HCN production

Among the three isolates, only one isolate produced HCN (Table 5) at a low intensity (June, 2016) (Plate 7b).

4.2.2.3 Siderophore production

Among the three isolates obtained, only two isolates (July, 2015 and August, 2015) produced siderophore as their antagonistic mechanism (Table 5) during twelve months

4.2.2.4 Antagonistic activity against Phytophthora capsici

All the three isolates were screened for their antagonistic activity against *Phytophthora capsici* (Table 5). Only two isolates showed antagonistic activity with maximum percent inhibition in the case of Tricho (I) during July, 2015 (Plate 7c).

Table 5: Screening of *Trichoderma* sp. for ammonia, HCN, siderophore production and antagonistic activity against *Phytophthora capsici*

Isolates	Ammonia production	HCN production	Siderophore production	Percent inhibition (%)
Tricho (I)	-	-	+	72.2
Tricho (II)	+	-	+	66.7
Tricho (XII)	++	+	-	-

Ammonia and HCN: -Negative, + Low, ++ Medium, +++ High; siderophore: - Negative, + Positive

(Each value represents mean of three replications)

4.3 WEATHER PARAMETERS AT THE EXPERIMENTAL SITE

Monthly mean of temperature, rainfall, sunshine hours and relative humidity were recorded at the experimental site.

4.3.1 Temperature (°C)

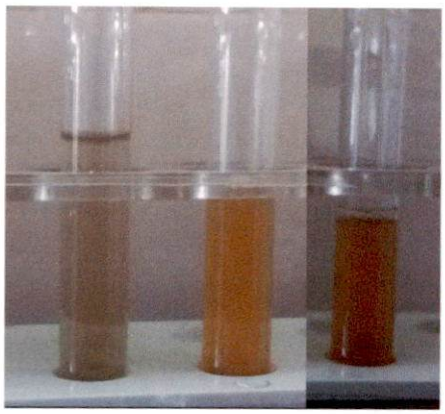
The temperature ranged from 25.75 to 31°C. The maximum temperature recorded was 31°C during April, 2016 and minimum was 25.75°C recorded in June, 2016 (Table 6).

4.3.2 Rainfall (mm)

The rainfall from July, 2015 to June, 2016 ranged from 9.8 to 654.7 mm. The maximum rainfall was recorded in June, 2016 (654.7 mm) and the minimum was recorded in March, 2016 (9.8 mm) (Table 6).

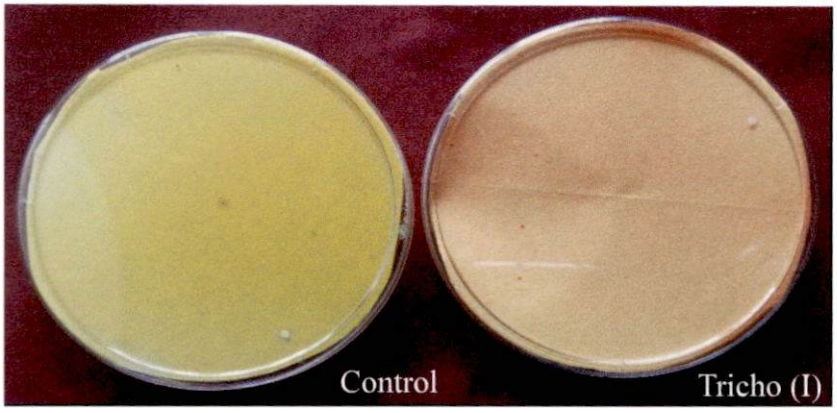
4.3.3 Sunshine hours (hrs)

The maximum sunshine hours was recorded (8.6 hours) in January, 2016 and the minimum was 1.6 hours in June, 2016 (Table 6).

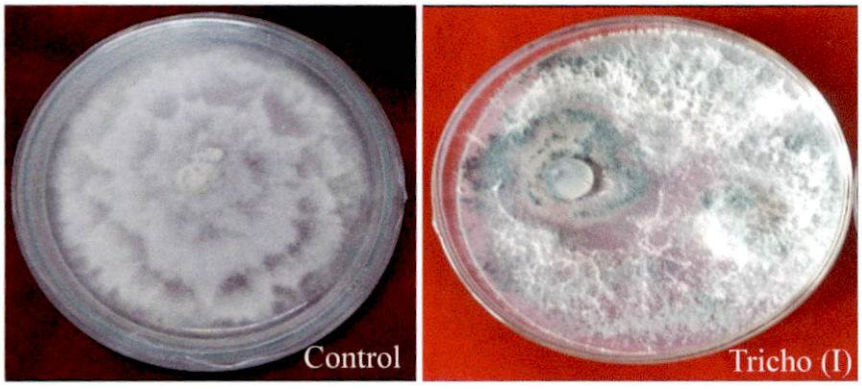


Control Tricho (II) Tricho (XII)

a) Ammonia production



b) HCN production



c) Antagonistic activity against *Phytophthora capsici*

Plate 7: Screening of *Trichoderma* isolates for ammonia, HCN production and antagonistic activity against *Phytophthora capsici*

4.3.4 Relative humidity (%)

The relative humidity of twelve months ranged from 58.8 to 92.8 per cent. The highest relative humidity was recorded in June, 2016 and the lowest was in January, 2016 (Table 6).

Table 6: Mean of temperature, rainfall, sunshine hours and relative humidity at monthly interval (July, 2015 to June, 2016)

Month	Temperature (°C)	Rainfall (mm)	Sunshine hours (hrs)	Relative humidity (%)
July, 2015	26.90	510.1	3.80	86.16
August, 2015	27.35	320.8	5.80	85.14
September, 2015	27.80	248.2	5.60	84.11
October, 2015	28.30	203.8	5.60	82.35
November, 2015	27.70	151.2	4.60	77.32
December, 2015	27.80	88.3	6.90	67.40
January, 2016	28.10	23.8	8.60	58.80
February, 2016	29.40	11.4	8.20	61.10
March, 2016	30.75	9.8	8.00	69.40
April, 2016	31.00	25.8	7.90	73.20
May, 2016	29.10	270.7	5.90	86.90
June, 2016	25.75	654.7	1.60	92.80

4.4 MICRO-CLIMATIC AND SOIL PARAMETERS IN THE RHIZOSPHERE OF BLACK PEPPER

Microclimatic parameters such as soil temperature, soil moisture and soil respiration were recorded at monthly intervals for a period of 12 months from July, 2015 to June, 2016.

4.4.1 Soil temperature (°C)

The soil temperature was found to be significant. It ranged from 28.1 to 33.51°C. The maximum soil temperature was recorded in April, 2016 followed by March, 2016 (Table 7). The minimum soil temperature was recorded in November, 2015.

4.4.4 Soil moisture (%)

The soil moisture content ranged from 2.28 to 20.87 per cent (Table 7). The highest soil moisture content was recorded in the 12th month (June, 2016) which was on par with the moisture during July, 2015. The lowest moisture content was recorded in the month of March, 2016.

4.4.5 Soil respiration (mg CO_2/g/day)

The soil respiration of rhizosphere soil were estimated for a period of the twelve months and found significantly different in each month (Table 7). The highest soil respiration was recorded in October, 2015 (4.72 mg CO_2/g/day) and the lowest in the month of February, 2016(1.03 mg CO_2/g/day).

4.4.6 Soil pH

The soil pH of rhizosphere soil was determined for twelve months from July, 2015 to June, 2016. The mean pH was found to be non-significant. (Table 7). The maximum mean pH was recorded in June, 2016 (5.56) and the minimum was recorded in April, 2016 (5.13).

Table 7: Microclimatic and soil parameters in the rhizosphere of black pepper at monthly interval (July, 2015 to June, 2016)

Month	Soil temperature (°C)	Soil moisture (%)	Soil respiration ($mgCO_2/g/day$)	Soil pH
July, 2015	29.37^{f}	20.54^{a}	2.60^{cde}	5.22
August, 2015	28.81^{g}	15.04^{b}	1.44^{gh}	5.21
September,	29.24^{f}	15.37^{b}	2.27^{def}	5.24
October, 2015	31.26^{d}	14.83^{b}	4.72^{a}	5.27
November,	28.1^{h}	10.12^{cd}	2.77^{cd}	5.23
December,	28.2^{h}	12.47^{bc}	1.92^{efg}	5.25
January, 2016	29.8^{e}	7.67^{de}	1.68^{fgh}	5.23
February, 2016	31.0^{d}	6.54^{de}	1.03^{h}	5.20
March, 2016	32.8^{b}	2.28^{f}	3.67^{b}	5.19
April, 2016	33.5^{a}	5.86^{ef}	3.04^{bc}	5.13
May, 2016	31.7^{c}	14.80^{b}	1.52^{gh}	5.33
June, 2016	29.89^{e}	20.87^{a}	1.89^{fg}	5.56

4.5 EFFECT OF WEATHER AND MICRO-CLIMATIC PARAMETERS ON THE POPULATION OF SELECTED BENEFICIAL MICROFLORA

4.5.1 Effect of weather and micro-climatic parameters on the population of fluorescent pseudomonads

The effect of weather and micro-climatic variables such as temperature, rainfall, sunshine hours, relative humidity, soil temperature, soil moisture, soil respiration and soil pH were correlated with the population of the fluorescent pseudomonads. It was found that the population of fluorescent pseudomonads was positively correlated with rainfall and soil moisture and negatively correlated with temperature, sunshine hours and soil temperature (Table 8).

4.5.2 Effect of weather and micro-climatic parameters on the population of *Trichoderma* sp.

The population of *Trichoderma* sp. was positively correlated with rainfall, relative humidity and soil moisture whereas it was negatively correlated with temperature, sunshine hours and soil temperature (Table 8).

Table 8: Correlation of population of fluorescent pseudomonads and *Trichoderma* sp. in the rhizosphere of black pepper with weather and micro-climatic parameters

Parameters	Correlation coefficient	
	Population of fluorescent pseudomonads	Population of *Trichoderma* sp.
Ambient temperature	-0.683*	-0.458*
Rainfall	0.225**	0.594*
Sunshine hours	-0.256*	-0.416*
Relative humidity	-	0.434*
Soil temperature	-0.880*	-0.322*
Soil moisture	0.474*	0.550*
Soil respiration	-	-
Soil pH	-	-

* Correlation significant at 1% level

** Correlation significant at 5% significant level

4.6 EFFECT OF WEATHER AND MICRO-CLIMATIC PARAMETERS ON THE FUNCTIONAL EFFICIENCY OF SELECTED BENEFICIAL MICROFLORA

The isolates of fluorescent pseudomonads and *Trichoderma* sp. were screened for their functional efficiency under *in vitro* condition. The observations

recorded were cross tabulated with each of the weather and micro climatic 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 computed.

4.6.1 Effect of weather, micro-climatic and soil parameters on ammonia, HCN and siderophore production by fluorescent pseudomonads

4.6.1.1 Effect of temperature on ammonia, HCN and siderophore production by fluorescent pseudomonads

The temperature during 12 months period ranged from 25.75 °C (June 2016) to 31.0 °C (April, 2016). The effect of temperature on ammonia production did not show any significant differences. In this temperature range, most of the isolates produced ammonia at a moderate level. The maximum ammonia production was recorded at a temperature of 27.80 °C (September, 2015).

The effect of temperature on HCN production also did not show any significant differences. At this temperature range, most of the isolates did not produce HCN. The maximum HCN production was observed when the temperature was 28.30 °C (October, 2015).

The temperature showed a significant differences on siderophore production by fluorescent pseudomonads. Out of thirty one isolates obtained, only ten isolates were siderophore producers. All the isolates showed siderophore production in July, 2015, August 2015 and June 2016, when the temperatures were 26.9, 27.35 and 25.75 °C respectively. Hence, the optimum temperature range for the siderophore production was 25 to 28 °C.

Among the ammonia, HCN and siderophore production the temperature had a significant effect only on the siderophore production (Table 9).

4.6.1.2 Effect of rainfall on ammonia, HCN and siderophore production by fluorescent pseudomonads

The rainfall during 12 months period ranged from 9.8 mm (March 2016) to 654.7 mm (June, 2016). In this range of rainfall, maximum number of isolates were medium level producer of ammonia. The maximum ammonia production was recorded at a rainfall of 248.2 mm (September, 2015).

The effect of rainfall on HCN production also did not show any significant differences. A maximum number of isolates did not produce HCN in this rainfall range. The maximum HCN production was observed with a rainfall of 203.8 mm (October, 2015).

The rainfall showed a significant differences on siderophore production by fluorescent pseudomonads. Siderophore production occurred at a rainfall range of 248.2 to 654.7 mm. All the isolates showed the siderophore production in July, 2015, August 2015 and June 2016, when the rainfall were 510.1, 320.8 and 654.7 mm respectively. The optimum rainfall for the maximum production of siderophore was found to be 300 to 650 mm.

Among the ammonia, HCN and siderophore production, the mean rainfall had a significant effect only on siderophore production (Table 10).

4.6.1.3 Effect of sunshine hours on ammonia, HCN and siderophore production by fluorescent pseudomonads

The sunshine hours during 12 months period ranged from 1.60 (June 2016) to 8.60 (January, 2016). The effect of sunshine hours on ammonia production did not show any significant differences. Most of the isolates were found to be medium level producers of ammonia. The maximum ammonia production was recorded in September, 2015, when the sunshine hours was 5.60.

The effect of sunshine hours on HCN production did not show any significant differences. The HCN was produced, when the sunshine hours were 3.80

to 5.90. The maximum HCN production recorded was in October, 2015, when the sunshine hours was 5.60.

The sunshine hours showed a significant effect on siderophore production by fluorescent pseudomonads. Siderophore production occurred at a sunshine hours range of 1.60 to 5.90. All isolates showed the siderophore production in July, 2015, August 2015 and June, 2016, when the duration of sunshine were recorded 3.80, 5.80 and 1.60 hours respectively. The optimum sunshine hours for maximum siderophore production ranged from 1.60 to 5.80.

The sunshine hours had a significant effect on siderophore production, but not on ammonia and HCN production (Table 11).

4.6.1.4 Effect of relative humidity on ammonia, HCN and siderophore production by fluorescent pseudomonads

The relative humidity during 12 months period ranged from 58.8 January, 2016) to 92.8 per cent (June, 2016). The effect of relative humidity on ammonia production did not show any significant differences. The maximum ammonia production was recorded when the relative humidity was 84.11 per cent (September, 2015).

The effect of relative humidity on HCN production did not show any significant differences. The relative humidity in which HCN production occurred was in the range of 77.32 to 92.80 per cent. The maximum HCN production was recorded when the relative humidity was 82.35 per cent (October, 2015).

The relative humidity showed a significant effect on siderophore production by fluorescent pseudomonads. Siderophore production occurred at relative humidity range of 84.11 to 92.80 per cent. All the isolates showed the siderophore production in July, 2015, August 2015 and June, 2016 when the relative humidity were 86.16, 85.14 and 92.80 per cent respectively. The optimum relative humidity

for the maximum production of siderophore was found to be 86.16 to 92.80 per cent.

Among the ammonia, HCN and siderophore production, the relative humidity had a significant effect only on siderophore production (Table 12).

Table 9: Effect of temperature on ammonia, HCN and siderophore production by fluorescent pseudomonads

		*Mean temperature (°C)												Chi-square
		26.90 July'15	27.35 Aug'15	27.80 Sept'15	28.30 Oct'15	27.70 Nov'15	27.80 Dec'15	28.10 Jan'16	29.40 Feb'16	30.75 Mar'16	31.00 Apr'16	29.10 May'16	25.75 June'16	Total	
Ammonia production	Negative (-)	0	0	1	1	0	0	0	0	0	0	0	0	2	25.62^{NS}
	Low (+)	0	1	1	0	0	0	0	0	0	0	0	0	2	
	Medium (++)	2	1	0	3	2	2	3	3	0	0	2	1	19	
	High (+++)	1	0	3	0	1	1	0	0	0	0	2	0	8	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
HCN production	Negative (-)	0	0	2	1	2	3	3	3	0	0	2	0	16	27.52^{NS}
	Low (+)	1	1	1	1	1	0	0	0	0	0	1	1	7	
	Medium(++)	1	1	1	0	0	0	0	0	0	0	1	0	4	
	High (+++)	1	0	1	2	0	0	0	0	0	0	0	0	4	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
Siderophore production	Negative (-)	0	0	4	4	3	3	3	3	0	0	1	0	21	23.90^{**}
	Positive (+)	3	2	1	0	0	0	0	0	0	0	3	1	10	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
** Significant at 1% significant level
* Average value for the month

Table 10: Effect of rainfall on ammonia, HCN and siderophore production by fluorescent pseudomonads

		*Mean rainfall (mm)												Chi-square
		510.1 July'15	320.8 Aug'15	248.2 Sept'15	203.8 Oct'15	151.2 Nov'15	88.3 Dec'15	23.8 Jan'16	11.4 Feb'16	9.8 Mar'16	25.8 Apr'16	270.7 May'16	654.7 June'16	Total	
Ammonia production	Negative (-)	0	0	1	1	0	0	0	0	0	0	0	0	2	25.62^{NS}
	Low (+)	0	1	1	0	0	0	0	0	0	0	0	0	2	
	Medium (++)	2	1	0	3	2	2	3	3	0	0	2	1	19	
	High (+++)	1	0	3	0	1	1	0	0	0	0	2	0	8	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
HCN production	Negative (-)	0	0	2	1	2	3	3	3	0	0	2	0	16	27.52^{NS}
	Low (+)	1	1	1	1	1	0	0	0	0	0	1	1	7	
	Medium(++)	1	1	1	0	0	0	0	0	0	0	1	0	4	
	High (+++)	1	0	1	2	0	0	0	0	0	0	0	0	4	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
Siderophore production	Negative (-)	0	0	4	4	3	3	3	3	0	0	1	0	21	23.90^{**}
	Positive (+)	3	2	1	0	0	0	0	0	0	0	3	1	10	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
** Significant at 1% significant level
* Average value for the month

Table 11: Effect of sunshine hours on ammonia, HCN and siderophore production by fluorescent pseudomonads

		*Mean sunshine hours (hrs)												Chi-square
		3.80 July'15	5.80 Aug'15	5.60 Sept'15	5.60 Oct'15	4.60 Nov'15	6.90 Dec'15	8.60 Jan'16	8.20 Feb'16	8.00 Mar'16	7.90 Apr'16	5.90 May'16	1.60 June'16	Total	
Ammonia production	Negative (-)	0	0	1	1	0	0	0	0	0	0	0	0	2	19.01^{NS}
	Low (+)	0	1	1	0	0	0	0	0	0	0	0	0	2	
	Medium (++)	2	1	0	3	2	2	3	3	0	0	2	1	19	
	High (+++)	1	0	3	0	1	1	0	0	0	0	2	0	8	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
HCN production	Negative (-)	0	0	2	1	2	3	3	3	0	0	2	0	16	25.16^{NS}
	Low (+)	1	1	1	1	1	0	0	0	0	0	1	1	7	
	Medium(++)	1	1	1	0	0	0	0	0	0	0	1	0	4	
	High (+++)	1	0	1	2	0	0	0	0	0	0	0	0	4	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
Siderophore production	Negative (-)	0	0	4	4	3	3	3	3	0	0	1	0	21	23.50^{**}
	Positive (+)	3	2	1	0	0	0	0	0	0	0	3	1	10	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
** Significant at 1% significant level
* Average value for the month

Table 12: Effect of relative humidity on ammonia, HCN and siderophore production by fluorescent pseudomonads

		*Mean relative humidity (%)												Chi-square
		86.16 July'15	85.14 Aug'15	84.11 Sept'15	82.35 Oct'15	77.32 Nov'15	67.40 Dec'15	58.80 Jan'16	61.10 Feb'16	69.40 Mar'16	73.20 Apr'16	86.90 May'16	92.80 June'16	Total	
Ammonia production	Negative (-)	0	0	1	1	0	0	0	0	0	0	0	0	2	25.62^{NS}
	Low (+)	0	1	1	0	0	0	0	0	0	0	0	0	2	
	Medium (++)	2	1	0	3	2	2	3	3	0	0	2	1	19	
	High (+++)	1	0	3	0	1	1	0	0	0	0	2	0	8	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
HCN production	Negative (-)	0	0	2	1	2	3	3	3	0	0	2	0	16	27.52^{NS}
	Low (+)	1	1	1	1	1	0	0	0	0	0	1	1	7	
	Medium(++)	1	1	1	0	0	0	0	0	0	0	1	0	4	
	High (+++)	1	0	1	2	0	0	0	0	0	0	0	0	4	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
Siderophore production	Negative (-)	0	0	4	4	3	3	3	3	0	0	1	0	21	23.90^{**}
	Positive (+)	3	2	1	0	0	0	0	0	0	0	3	1	10	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
** Significant at 1% significant level
* Average value for the month

4.6.1.5 Effect of soil temperature on ammonia, HCN and siderophore production by fluorescent pseudomonads

The soil temperature during 12 months period ranged from 28.1 °C (November, 2015) to 33.51 °C (April, 2016). The soil temperature did not show any significant differences on ammonia production. In this temperature range, a moderate ammonia production was observed by maximum number of isolates. The maximum ammonia production was recorded with a soil temperature of 29.24 °C (September, 2015).

The effect of soil temperature on HCN production did not show any significant differences. The maximum HCN production recorded was in October, 2015 when the soil temperature was 31.26 °C.

The soil temperature had significant differences on siderophore production by fluorescent pseudomonads. Siderophore production occurred at a temperature range of 28.81 to 31.7 °C. All the isolates showed siderophore production in July, 2015, August, 2015 and June, 2016, when the soil temperatures were 29.37, 28.81 and 29.89 °C respectively. The optimum soil temperature range for the maximum production of siderophore was found to be 29 °C.

Soil temperature had a significant effect on siderophore production, but not on ammonia and HCN production (Table 13).

4.6.1.6 Effect of soil moisture on ammonia, HCN and siderophore production by fluorescent pseudomonads

The soil moisture percent during 12 months period ranged from 2.28 (March 2016) to 20.87 per cent (June, 2016). The effect of soil moisture content on ammonia production did not show any significant differences. The maximum ammonia production was recorded in September, 2015 when the soil moisture content was 15.37 per cent.

The effect of soil moisture content on HCN production did not show any significant differences. The soil moisture content in which HCN production occurred was found to be 10.12 to 20.87 per cent. The maximum HCN production recorded when the soil moisture content was 14.83 per cent (October, 2015).

The soil moisture content showed a significant effect on siderophore production by fluorescent pseudomonads. Siderophore production occurred at a soil moisture content range of 14.80 to 20.87 per cent. All the isolates showed the siderophore production in July, 2015, August 2015 and June, 2016, when the soil moisture contents were 20.54, 15.14 and 20.87 per cent respectively. The optimum soil moisture content for the maximum production of siderophore was found to be 15 to 21 per cent.

Among ammonia, HCN and siderophore production, only siderophore production was significantly affected by soil moisture content (Table 14).

4.6.1.7 Effect of soil respiration on ammonia, HCN and siderophore production by fluorescent pseudomonads

The soil respiration during 12 months period ranged from 1.03 mg CO_2/g/day (February, 2016) to 4.72 mg CO_2/g/day (October, 2015). The effect of soil respiration on ammonia production did not show any significant differences. The maximum ammonia production was recorded when the soil respiration was 2.27 mg CO_2/g/day (September, 2015).

The effect of soil respiration on HCN production did not show any significant differences. The maximum HCN production recorded was in the month of October, 2015 when the soil respiration was 4.72 mg CO_2/g/day.

The soil respiration showed a significant effect on siderophore production by fluorescent pseudomonads. Siderophore production occurred at a soil respiration range of 1.44 to 2.60 mg CO_2/g/day. All the isolates showed the siderophore production in July, 2015, August 2015 and June, 2016, when the soil respiration

were recorded 2.60, 1.44 and 1.89 mg CO_2/g/day respectively. The optimum soil respiration for siderophore production was found to be 1.44 to 2.60 mg CO_2/g/day.

The soil respiration had a significant effect only on siderophore production (Table 15).

4.6.1.8 Effect of soil pH on ammonia, HCN and siderophore production by fluorescent pseudomonads

The soil pH during 12 months period ranged from 5.13 (April, 2016) to 5.56 (June, 2016). The effect of soil pH on ammonia production did not show any significant differences. The maximum ammonia production was recorded when the soil pH was 5.24 (September, 2015).

The effect of soil pH on HCN production did not show any significant differences. The maximum HCN production was recorded in the month of October, 2015 when the soil pH was 5.27.

The soil pH showed a significant effect on siderophore production by fluorescent pseudomonads. Siderophore production occurred at a soil pH range of 5.21 to 5.56. All isolates showed the siderophore production in July, 2015, August, 2015 and June, 2016, when the soil pH were 5.22, 5.21 and 5.56 respectively. The optimum pH range for the maximum production of siderophore was found to be 5.21 to 5.56.

The soil pH had a significant effect only on siderophore production, not on ammonia and HCN production (Table 16).

Table 13: Effect of soil temperature on ammonia, HCN and siderophore production by fluorescent pseudomonads

		*Mean soil temperature (°C)												Chi-square
		29.37 July'15	28.81 Aug'15	29.24 Sept'15	31.26 Oct'15	28.10 Nov'15	28.20 Dec'15	29.80 Jan'16	31.00 Feb'16	32.80 Mar'16	33.51 Apr'16	31.70 May'16	29.89 June'16	Total	
Ammonia production	Negative (-)	0	0	1	1	0	0	0	0	0	0	0	0	2	25.62^{NS}
	Low (+)	0	1	1	0	0	0	0	0	0	0	0	0	2	
	Medium (++)	2	1	0	3	2	2	3	3	0	0	2	1	19	
	High (+++)	1	0	3	0	1	1	0	0	0	0	2	0	8	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
HCN production	Negative (-)	0	0	2	1	2	3	3	3	0	0	2	0	16	27.52^{NS}
	Low (+)	1	1	1	1	1	0	0	0	0	0	1	1	7	
	Medium(++)	1	1	1	0	0	0	0	0	0	0	1	0	4	
	High (+++)	1	0	1	2	0	0	0	0	0	0	0	0	4	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
Siderophore production	Negative (-)	0	0	4	4	3	3	3	3	0	0	1	0	21	23.90^{**}
	Positive (+)	3	2	1	0	0	0	0	0	0	0	3	1	10	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
** Significant at 1% significant level
* Average value for the month

Table 14: Effect of soil moisture content on ammonia, HCN and siderophore production by fluorescent pseudomonads

		*Mean soil moisture (%)												Chi-square
		20.54 July'15	15.04 Aug'15	15.37 Sept'15	14.83 Oct'15	10.12 Nov'15	12.47 Dec'15	7.67 Jan'16	6.54 Feb'16	2.28 Mar'16	5.86 Apr'16	14.80 May'16	20.87 June'16	Total	
Ammonia Production	Negative (-)	0	0	1	1	0	0	0	0	0	0	0	0	2	25.62^{NS}
	Low (+)	0	1	1	0	0	0	0	0	0	0	0	0	2	
	Medium (++)	2	1	0	3	2	2	3	3	0	0	2	1	19	
	High (+++)	1	0	3	0	1	1	0	0	0	0	2	0	8	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
HCN production	Negative (-)	0	0	2	1	2	3	3	3	0	0	2	0	16	27.52^{NS}
	Low (+)	1	1	1	1	1	0	0	0	0	0	1	1	7	
	Medium(++)	1	1	1	0	0	0	0	0	0	0	1	0	4	
	High (+++)	1	0	1	2	0	0	0	0	0	0	0	0	4	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
Siderophore production	Negative (-)	0	0	4	4	3	3	3	3	0	0	1	0	21	23.90^{**}
	Positive (+)	3	2	1	0	0	0	0	0	0	0	3	1	10	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
** Significant at 1% significant level
* Average value for the month

Table 15: Effect of soil respiration on ammonia, HCN and siderophore production by fluorescent pseudomonads

		*Mean soil respiration (mg CO_2/g/day)													Chi-square
		2.60 July'15	1.44 Aug'15	2.27 Sept'15	4.72 Oct'15	2.77 Nov'15	1.92 Dec'15	1.68 Jan'16	1.03 Feb'16	3.67 Mar'16	3.04 Apr'16	1.52 May'16	1.89 June'16	Total	
Ammonia production	Negative (-)	0	0	1	1	0	0	0	0	0	0	0	0	2	25.62^{NS}
	Low (+)	0	1	1	0	0	0	0	0	0	0	0	0	2	
	Medium (++)	2	1	0	3	2	2	3	3	0	0	2	1	19	
	High (+++)	1	0	3	0	1	1	0	0	0	0	2	0	8	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
HCN production	Negative (-)	0	0	2	1	2	3	3	3	0	0	2	0	16	27.52^{NS}
	Low (+)	1	1	1	1	1	0	0	0	0	0	1	1	7	
	Medium(++)	1	1	1	0	0	0	0	0	0	0	1	0	4	
	High (+++)	1	0	1	2	0	0	0	0	0	0	0	0	4	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
Siderophore production	Negative (-)	0	0	4	4	3	3	3	3	0	0	1	0	21	23.90^{**}
	Positive (+)	3	2	1	0	0	0	0	0	0	0	3	1	10	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
** Significant at 1% significant level
* Average value for the month

Table 16: Effect of soil pH on ammonia, HCN and siderophore production by fluorescent pseudomonads

		*Mean soil pH													Chi-square
		5.22 July'15	5.21 Aug'15	5.24 Sept'15	5.27 Oct'15	5.23 Nov'15	5.25 Dec'15	5.23 Jan'16	5.20 Feb'16	5.19 Mar'16	5.13 Apr'16	5.33 May'16	5.56 June'16	Total	
Ammonia production	Negative (-)	0	0	1	1	0	0	0	0	0	0	0	0	2	24.698^{NS}
	Low (+)	0	1	1	0	0	0	0	0	0	0	0	0	2	
	Medium (++)	2	1	0	3	2	2	3	3	0	0	2	1	19	
	High (+++)	1	0	3	0	1	1	0	0	0	0	2	0	8	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
HCN production	Negative (-)	0	0	2	1	2	3	3	3	0	0	2	0	16	26.456^{NS}
	Low (+)	1	1	1	1	1	0	0	0	0	0	1	1	7	
	Medium(++)	1	1	1	0	0	0	0	0	0	0	1	0	4	
	High (+++)	1	0	1	2	0	0	0	0	0	0	0	0	4	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	
Siderophore production	Negative (-)	0	0	4	4	3	3	3	3	0	0	1	0	21	23.907^{**}
	Positive (+)	3	2	1	0	0	0	0	0	0	0	3	1	10	
Total		3	2	5	4	3	3	3	3	0	0	4	1	31	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
** Significant at 1% significant level
* Average value for the month

4.6.4 Effect of weather, micro-climatic and soil parameters on ammonia, HCN and siderophore production by *Trichoderma* sp.

4.6.4.1 Effect of temperature on ammonia, HCN and siderophore production by Trichoderma sp.

The effect of temperature on ammonia production did not show any significant differences. The maximum ammonia production was recorded when the temperature was 25.75 °C (June, 2016).

The effect of temperature on HCN production did not show any significant differences. The HCN was produced when the temperature was 25.75 °C (June, 2016).

The temperature did not show any significant effect on siderophore production also by *Trichoderma* sp. All the isolates showed the siderophore production in July, 2015 and August 2015, when the temperature were 26.9 and 27.35 °C respectively.

Mean temperature had no significant effect on ammonia, HCN and siderophore production (Table 17).

4.6.4.2 Effect of rainfall on ammonia, HCN and siderophore production by Trichoderma sp.

The effect of rainfall on ammonia production did not show any significant differences. The maximum ammonia production was recorded when the rainfall was 654.7 mm (June, 2016).

The effect of rainfall on HCN production did not show any significant differences. It is found that HCN is produced when the rainfall was 654.7 mm (June, 2016).

The rainfall did not show any significant effect on siderophore production by *Trichoderma* sp. All isolates showed the siderophore production in July, 2015 and August 2015, when the rainfall were 510.1 and 320.8 mm respectively.

Mean rainfall had no significant effect on ammonia, HCN and siderophore production by *Trichoderma* sp. (Table 18).

4.6.4.3 Effect of sunshine hours on ammonia, HCN and siderophore production by Trichoderma sp.

The effect of sunshine hours on ammonia production did not show any significant differences. The *Trichoderma* sp. isolates produced ammonia when the sunshine hours were 1.60 and 5.80. The maximum ammonia production was recorded when the sunshine hours was 1.60 (June, 2016).

The effect of sunshine hours on HCN production did not show any significant differences. The isolate showed HCN production when the sunshine hours was 1.60 (June, 2016).

The sunshine hours did not show any significant effect on siderophore production by *Trichoderma* sp. All isolates showed siderophore production in July, 2015 and August, 2015, when the sunshine hours were 3.80 and 5.80 respectively.

The mean sunshine hours had no significant effect on ammonia, HCN and siderophore production by *Trichoderma* sp. (Table 19).

4.6.4.4 Effect of relative humidity on ammonia, HCN and siderophore production by Trichoderma sp.

The effect of relative humidity on ammonia production did not show any significant differences. The isolates showed ammonia production when the mean relative humidity were 85.14 and 92.8 per cent. The maximum ammonia production recorded was in June, 2016, when the relative humidity was 92.8 per cent.

The effect of relative humidity on HCN production did not show any significant differences. The isolates produced HCN when the mean relative humidity was 92.8 per cent (June, 2016).

The relative humidity did not show any significant effect on siderophore production by *Trichoderma* sp. All isolates showed the siderophore production in July, 2015 and August 2015, when the relative humidity were 86.19 and 85.14 per cent respectively.

The mean relative humidity had no significant effect on ammonia, HCN and siderophore production by *Trichoderma* sp. (Table 20).

Table 17: Effect of temperature on ammonia, HCN and siderophore production by *Trichoderma* sp.

		*Mean temperature (°C)												Chi-square
		26.90 July'15	27.35 Aug'15	27.80 Sept'15	28.30 Oct'15	27.70 Nov'15	27.80 Dec'15	28.10 Jan'16	29.40 Feb'16	30.75 Mar'16	31.00 Apr'16	29.10 May'16	25.75 June'16	Total	
Ammonia production	Negative (-)	1	0	0	0	0	0	0	0	0	0	0	0	1	6.0^{NS}
	Low (+)	0	1	0	0	0	0	0	0	0	0	0	0	1	
	Medium (++)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
HCN production	Negative (-)	1	1	0	0	0	0	0	0	0	0	0	0	2	3.0^{NS}
	Low (+)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
Siderophore production	Negative (-)	0	0	0	0	0	0	0	0	0	0	0	1	1	3.0^{NS}
	Positive (+)	1	1	0	0	0	0	0	0	0	0	0	0	2	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
* Average value for the month

Table 18: Effect of rainfall on ammonia, HCN and siderophore production by *Trichoderma* sp.

		*Mean rainfall (mm)												Chi-square value
		510.1 July'15	320.8 Aug'15	248.2 Sept'15	203.8 Oct'15	151.2 Nov'15	88.3 Dec'15	23.8 Jan'16	11.4 Feb'16	9.8 Mar'16	25.8 Apr'16	270.7 May'16	654.7 June'16	Total	
Ammonia production	Negative (-)	1	0	0	0	0	0	0	0	0	0	0	0	1	6.0^{NS}
	Low (+)	0	1	0	0	0	0	0	0	0	0	0	0	1	
	Medium (++)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
HCN production	Negative (-)	1	1	0	0	0	0	0	0	0	0	0	0	2	3.0^{NS}
	Low (+)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
Siderophore production	Negative (-)	0	0	0	0	0	0	0	0	0	0	0	1	1	3.0^{NS}
	Positive (+)	1	1	0	0	0	0	0	0	0	0	0	0	2	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
* Average value for the month

Table 19: Effect of sunshine hours on ammonia, HCN and siderophore production by *Trichoderma* sp.

		*Mean sunshine hours (hrs)												Chi-square value
		3.80 July'15	5.80 Aug'15	5.60 Sept'15	5.60 Oct'15	4.60 Nov'15	6.90 Dec'15	8.60 Jan'16	8.20 Feb'16	8.00 Mar'16	7.90 Apr'16	5.90 May'16	1.60 June'16	Total	
Ammonia production	Negative (-)	1	0	0	0	0	0	0	0	0	0	0	0	1	6.0^{NS}
	Low (+)	0	1	0	0	0	0	0	0	0	0	0	0	1	
	Medium (++)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
HCN production	Negative (-)	1	1	0	0	0	0	0	0	0	0	0	0	2	3.0^{NS}
	Low (+)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
Siderophore production	Negative (-)	0	0	0	0	0	0	0	0	0	0	0	1	1	3.0^{NS}
	Positive (+)	1	1	0	0	0	0	0	0	0	0	0	0	2	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
* Average value for the month

Table 20: Effect of relative humidity on ammonia, HCN and siderophore production by *Trichoderma* sp.

		*Mean relative humidity (%)												Chi-square value
		86.16 July'15	85.14 Aug'15	84.11 Sept'15	82.35 Oct'15	77.32 Nov'15	67.40 Dec'15	58.80 Jan'16	61.10 Feb'16	69.40 Mar'16	73.20 Apr'16	86.90 May'16	92.80 June'16	Total	
Ammonia production	Negative (-)	1	0	0	0	0	0	0	0	0	0	0	0	1	6.0^{NS}
	Low (+)	0	1	0	0	0	0	0	0	0	0	0	0	1	
	Medium (++)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
HCN production	Negative (-)	1	1	0	0	0	0	0	0	0	0	0	0	2	3.0^{NS}
	Low (+)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
Siderophore production	Negative (-)	0	0	0	0	0	0	0	0	0	0	0	1	1	3.0^{NS}
	Positive (+)	1	1	0	0	0	0	0	0	0	0	0	0	2	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
* Average value for the month

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

The effect of soil temperature on ammonia production did not show any significant differences. The isolates produced ammonia when the soil temperature was 28.81 to 29.89 °C. The maximum ammonia production was recorded when the soil temperature was 29.89 °C (June, 2016).

The effect of soil temperature on HCN production did not show any significant differences. The isolate showed HCN production when the soil temperature was 29.89 °C (June, 2016).

The soil temperature did not show any significant effect on siderophore production by *Trichoderma* sp. All isolates showed the siderophore production in July, 2015 and August, 2015, when the soil temperature were 29.37 and 28.81 °C respectively.

The soil temperature did not show any significant effects on ammonia, HCN and siderophore production by *Trichoderma* sp. (Table 21).

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

The effect of soil moisture content on ammonia production did not show any significant differences. The isolates showed ammonia production when the soil moisture content were 15.04 and 20.87 per cent. The maximum ammonia production was recorded when the soil moisture content was 20.87 per cent (June, 2016).

The effect of soil moisture content on HCN production did not show any significant differences. The HCN production by the isolates was recorded when the soil moisture content was 20.87 per cent (June, 2016).

The soil moisture content did not show any significant effect on siderophore production by *Trichoderma* sp. All isolates showed the siderophore production in July, 2015 and August 2015, when the soil moisture content were 20.54 and 15.04 per cent respectively.

The soil moisture content had no significant effect on the ammonia, HCN and siderophore production by *Trichoderma* sp. (Table 22).

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

The effect of soil respiration on ammonia production did not show any significant differences. The isolates showed ammonia production when the soil respiration was in a range of 1.44 to 1.89 mg CO_2/g/day. The maximum ammonia production was recorded when the soil respiration was 1.89 mg CO_2/g/day (June, 2016).

The effect of soil respiration on HCN production did not show any significant differences. The HCN production by the isolates was recorded when the soil respiration was 1.89 mg CO_2/g/day (June, 2016).

The soil respiration did not show any significant effect on siderophore production by *Trichoderma* sp. All isolates showed siderophore production in July, 2015 and August, 2015, when the soil respiration were 2.60 and 1.44 mg CO_2/g/day respectively.

Mean soil respiration had no significant effect on ammonia, HCN and siderophore production by *Trichoderma* sp. (Table 23).

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

The effect of soil pH on ammonia production did not show any significant differences. The isolates showed ammonia production when the soil pH ranged

from 5.21 to 5.56. The maximum ammonia production was recorded when the soil pH was 5.56 (June, 2016).

The effect of soil pH on HCN production did not show any significant differences. The HCN was produced by the isolates when the soil pH was 5.56 (June, 2016).

The soil pH did not show any significant effect on siderophore production by *Trichoderma* sp. All isolates showed the siderophore production in July, 2015 and August 2015, when the soil pH were 5.22 and 5.21 respectively.

The soil pH had no significant effect on ammonia, HCN and siderophore production by *Trichoderma* sp. (Table 24).

Table 21: Effect of soil temperature on ammonia, HCN and siderophore production by *Trichoderma* sp.

		*Mean soil temperature (°C)												Chi-square Value
		29.27 July'15	28.81 Aug'15	29.24 Sept'15	31.26 Oct'15	28.10 Nov'15	28.20 Dec'15	29.80 Jan'16	31.00 Feb'16	32.80 Mar'16	33.51 Apr'16	31.70 May'16	29.89 June'16	Total	
Ammonia production	Negative (-)	1	0	0	0	0	0	0	0	0	0	0	0	1	6.0^{NS}
	Low (+)	0	1	0	0	0	0	0	0	0	0	0	0	1	
	Medium (++)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
HCN production	Negative (-)	1	1	0	0	0	0	0	0	0	0	0	0	2	3.0^{NS}
	Low (+)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
Siderophore production	Negative (-)	0	0	0	0	0	0	0	0	0	0	0	1	1	3.0^{NS}
	Positive (+)	1	1	0	0	0	0	0	0	0	0	0	0	2	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
* Average value for the month

Table 22: Effect of soil moisture on ammonia, HCN and siderophore production by *Trichoderma* sp.

		*Mean soil moisture (%)												Chi-square
		20.54 July'15	15.04 Aug'15	15.37 Sept'15	14.83 Oct'15	10.12 Nov'15	12.47 Dec'15	7.67 Jan'16	6.54 Feb'16	2.28 Mar'16	5.86 Apr'16	14.80 May'16	20.87 June'16	Total	
Ammonia production	Negative (-)	1	0	0	0	0	0	0	0	0	0	0	0	1	6.0^{NS}
	Low (+)	0	1	0	0	0	0	0	0	0	0	0	0	1	
	Medium (++)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
HCN production	Negative (-)	1	1	0	0	0	0	0	0	0	0	0	0	2	3.0^{NS}
	Low (+)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
Siderophore production	Negative (-)	0	0	0	0	0	0	0	0	0	0	0	1	1	3.0^{NS}
	Positive (+)	1	1	0	0	0	0	0	0	0	0	0	0	2	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
* Average value for the month

Table 23: Effect of soil respiration on ammonia, HCN and siderophore production by *Trichoderma* sp.

		*Mean soil respiration ($mgCO_2/g/day$)												Chi-square value
		2.60 July'15	1.44 Aug'15	2.27 Sept'15	4.72 Oct'15	2.77 Nov'15	1.92 Dec'15	1.68 Jan'16	1.03 Feb'16	3.67 Mar'16	3.04 Apr'16	1.52 May'16	1.89 June'16	Total	
Ammonia production	Negative (-)	1	0	0	0	0	0	0	0	0	0	0	0	1	6.0^{NS}
	Low (+)	0	1	0	0	0	0	0	0	0	0	0	0	1	
	Medium (++)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
HCN production	Negative (-)	1	1	0	0	0	0	0	0	0	0	0	0	2	3.0^{NS}
	Low (+)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
Siderophore production	Negative (-)	0	0	0	0	0	0	0	0	0	0	0	1	1	3.0^{NS}
	Positive (+)	1	1	0	0	0	0	0	0	0	0	0	0	2	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
* Average value for the month

Table 24: Effect of soil pH on ammonia, HCN and siderophore production by *Trichoderma* sp.

		*Mean soil pH												Chi-square value
		5.22 July'15	5.21 Aug'15	5.24 Sept'15	5.27 Oct'15	5.23 Nov'15	5.25 Dec'15	5.23 Jan'16	5.20 Feb'16	5.19 Mar'16	5.13 Apr'16	5.33 May'16	5.56 June'16	Total	
Ammonia production	Negative (-)	1	0	0	0	0	0	0	0	0	0	0	0	1	6.0^{NS}
	Low (+)	0	1	0	0	0	0	0	0	0	0	0	0	1	
	Medium (++)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
HCN production	Negative (-)	1	1	0	0	0	0	0	0	0	0	0	0	2	3.0^{NS}
	Low (+)	0	0	0	0	0	0	0	0	0	0	0	1	1	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	
Siderophore production	Negative (-)	0	0	0	0	0	0	0	0	0	0	0	1	1	3.0^{NS}
	Positive (+)	1	1	0	0	0	0	0	0	0	0	0	0	2	
Total		1	1	0	0	0	0	0	0	0	0	0	1	3	

Each value in the table represents the total number of isolates during the month
NS- Non-Significant
* Average value for the month

4.7 NUTRIENT STATUS OF SOIL

Soil nutrient parameters such as soil pH, Electrical conductivity, organic carbon, total nitrogen, available phosphorus and available potassium were estimated in the initial (July, 2015) and the final month of study (June, 2016).

4.7.1 Soil reaction (pH)

The pH of the soil samples were highly acidic in nature. The initial pH of the soil sample was 5.22 and the final pH after the study was 5.56. A slight increase in the pH was observed (Table 25).

4.7.2 Electrical conductivity (EC)

The electrical conductivity of the soil samples initially recorded was 0.09 and the final EC was 0.08. There was a slight decrease in EC (Table 25).

4.7.3 Organic carbon (OC)

The initial organic carbon content was high in initial and final samples. The initial organic carbon content was 2.09 and the final organic carbon content was 2.05 % (Table 25).

4.7.4 Total soil nitrogen

The total nitrogen content of the initial and the final soil sample was 0.20% (Table 25).

4.7.5 Available soil phosphorus

Both the initial and final soil samples were analysed for available phosphorus in the initial (July 2015) and in the final month of the study (June, 2016). The initial and final available soil phosphorus (Table 25) was found to be high in rhizosphere soils of the black pepper. The initial available P was 97.75 kg ha $^{-1}$ and the final P content was 98.28 kg ha $^{-1}$.

4.7.6 Available soil potassium

Available soil potassium was estimated in the initial (July, 2015) and in the final month of the study (June, 2016). The initial and final available soil potassium (Table 25) was found to be high. The initial K content was 294.12 kg ha $^{-1}$ and the final K content was 292.04 kg ha $^{-1}$.

Table 25: Initial and final nutrient status of rhizosphere soils of black pepper in July, 2015 and June, 2016

Soil parameters	Initial (July, 2015)	Final (June, 2016)
pH	5.22	5.56
EC (dS m^{-1})	0.09 (Low)	0.08 (Low)
Organic carbon (%)	2.09 (High)	2.05 (High)
Total N (%)	0.20	0.20
Available P (kg ha $^{-1}$)	97.75 (High)	98.28 (High)
Available K (kg ha^{-1})	294.12 (High)	292.04 (High)

Discussion

5. DISCUSSION

Black pepper is an important spice crop of Kerala and is a major source of income and employment for rural households in Kerala. Kerala is the largest producer of black pepper in the country with 40,700 MT production (IPC, 2010). However, in the recent years there has been a change in the climatic parameters which has affected many crops in Kerala. Black pepper is one of the weather sensitive crop and the changing weather and climatic parameters have influenced the yield considerably (Rao, 2011).

In order to overcome the effect of weather and climatic parameters the microbial inoculants serves as an alternative. Even though microbial inoculants can help the black pepper to survive under the influence of climatic change, microflora themselves gets affected due to change in weather, microclimatic and soil parameters. As there are no studies conducted on the impact of weather variables on the functional efficiency of beneficial microflora in black pepper under sub-humid tropical region, a study was undertaken to determine the impact of weather variables and microclimatic parameters on the population as well as its efficiency of selected bioinoculants.

5.1 ENUMERATION OF SELECTED BENEFICIAL MICROFLORA FROM RHIZOSPHERE SOIL OF BLACK PEPPER

A black pepper plantation under Kerala Agricultural University at Vellanikkara, Thrissur district was selected for the study. A total of 12 plants were selected and the same plants were used for the rhizosphere soil sample collection at monthly interval from July, 2015 to June, 2016. Among the different isolates of beneficial microflora obtained, *Azospirillum* and PSB isolates were not found during the entire period of the present study. In a similar study, Govindan (1988) reported that *Azospirillum* population increased with more than 50 per cent moisture content. In the present study, the maximum mean moisture content recorded during the entire 12 months period was 20 per cent. This might be the reason for the poor survivability of *Azospirillum* in the soil. In a similar study, Athulya (2014) and

Haritha (2015) also reported that *Azospirillum* was absent in rhizosphere of ginger grown in lateritic soils of Vellanikkara.

Similarly, the absence of PSB in the rhizosphere soil of black pepper might be due to unfavourable composition of root exudates. The important role played by plant in selecting and enriching the types of bacteria depends on the constituents of their root exudates (Dakora, 2003). Thus, the bacterial community in rhizosphere develops depending on the nature and concentration of organic constituents of exudates and the corresponding ability of the bacteria to utilize these as source of energy (Curl and Truelove, 1986). In another study, Wright *et al.* (1995) reported reduction of the population size of bacterial inoculants due to predation by protozoa in soil which have been confirmed in number of recent studies. The present studies clearly indicated the need for inoculation of *Azospirillum* and PSB for black pepper.

However, 31 isolates of fluorescent pseudomonads and 3 isolates of *Trichoderma* were obtained during the period from July, 2015 to June, 2016. The highest population of fluorescent pseudomonads was obtained in the month of September, 2015 (Fig. 1). During this period, the ambient temperature (27.8 °C), soil temperature (29.24 °C) and per cent moisture (15.37%) were recorded. Since the optimum temperature for the growth of fluorescent pseudomonads 28-30 °C and the moisture was less, the fluorescent pseudomonads recorded the highest population. These results are in agreement with the earlier studies where Donnarumma *et al.* (2010) reported that the optimum temperature for the growth of *Pseudomonas fluorescens* was 25-30 °C.

In case of *Trichoderma*, the highest population was recorded during July, 2015 (Fig. 2) when the ambient temperature was 26.9 °C, soil temperature was 29.37 °C and moisture percentage was 20.54%. It is apparently clear that 27 to 30°C was the favourable temperature for the growth of *Trichoderma* sp. As there was less rainfall during the July, 2015 (510.1 mm) and the moisture was 20.54 per cent, the growth of *Trichoderma* sp. was good which might be due to its aerobic nature. Moreover, Gupta and Sharma (2013) also reported that the optimum temperature

Fig. 1: Population of fluorescent pseudomonads from July, 2015 to June, 2016 (12 months)

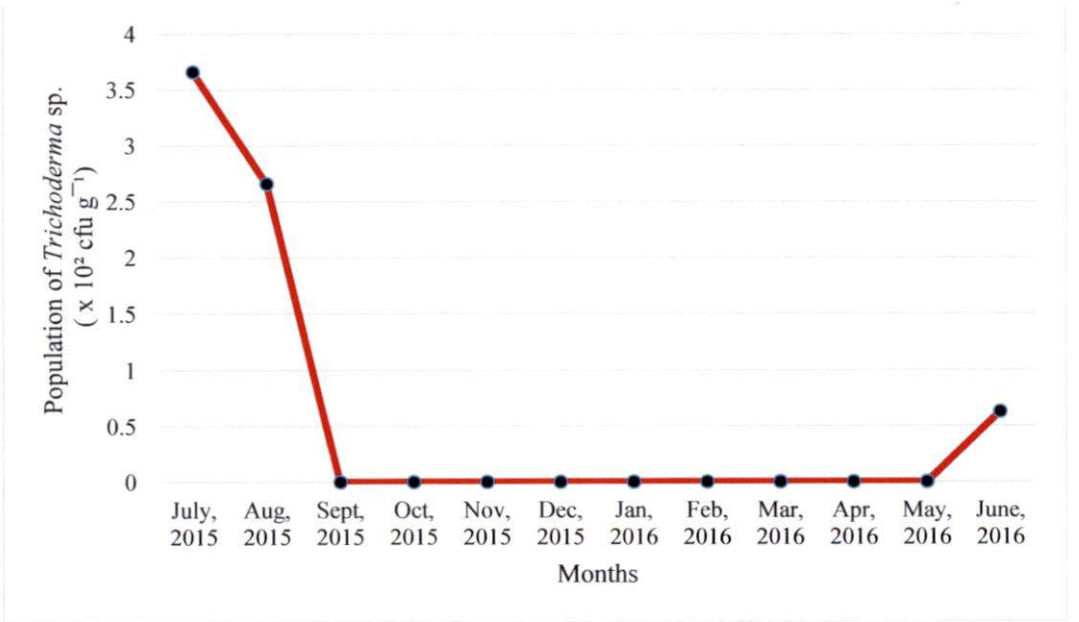


Fig. 2: Population of *Trichoderma* sp. from July, 2015 to June, 2016 (12 months)

for *Trichoderma harzianum* was between 25 to 30 °C. In the present studies, the pH 5.2 also might have favoured the growth of *Trichoderma,* since *Trichoderma* grows well under acidic pH. In a similar study, Ahmad and Baker (1987) reported that the population of *Trichoderma harzianum* were higher in acidic pH (5.0) than at neutral pH (7.0).

5.2 SCREENING OF DIFFERENT ISOLATES FOR THE PRODUCTION OF IAA, AMMONIA, HYDROGEN CYANIDE, SIDEROPHORE PRODUCTION, PHOSPHATE SOLUBILIZATION AND ANTAGONISTIC ACTIVITY UNDER *in vitro*

One of the stress faced by the soil microorganisms is the changes in the weather variables and microclimatic parameters. The changes in the environment might change the composition and biomass of the microbial community, which in turn will affect the plant growth. Therefore, all the microorganism needs optimum environmental condition, which maintains the microbial population as well as their metabolic activity (Pettersson, 2004). Since, the microbial community are influenced by the environmental parameters, the isolates obtained in the present studies were evaluated for the efficiency due to the influence of weather and microclimatic parameters.

Out of 31 isolates of fluorescent pseudomonads, 29 isolates produced ammonia (Fig. 3) under *in vitro* condition, 15 isolates produced HCN (Fig. 4) and 10 isolates produced siderophore (Fig. 5) as their antagonistic mechanism. This might be due to the variation in the metabolic activity and rate of the isolates due to changes in the weather and micro-climatic parameters. In a similar study, Park *et al.* (2009) reported the HCN and siderophore production from *Pseudomonas fluorescens* (RAF 15) which was a mechanism for antagonism against plant pathogens. However, Suzuki *et al.* (2003) reported that *Pseudomonas fluorescens* (HP72) did not produce fluorescent siderophores, which is in agreement with the results of present studies in which some of the isolates did not produce fluorescent siderophores. During the period of study, ammonia, HCN and siderophore

production were not recorded during every months of the study. However, it was highest during July, 2015 to Oct, 2015 when the temperature ranged from 26.9-28.3 °C, soil temperature 29.24-31.26 °C, relative humidity 82.35-86.16 per cent, soil moisture 14.83- 20.54 per cent and the soil respiration were 2.6- 4.77 mg CO_2/g/day. It indicated that these parameters are favourable for the highest antagonism by fluorescent pseudomonads. Tailor and Joshi (2012) reported *Pseudomonas fluorescens* strain from sugarcane rhizosphere as highest siderophore producer at pH 7 and 29 °C. Since, the soil temperature and ambient temperature was in the range of 26.9-31.26°C, it favoured the siderophore production by fluorescent pseudomonads in the present studies.

Phosphate solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids which will convert the unavailable form of phosphorus in to available phosphorus. In the present studies, out of 31 isolates of fluorescent pseudomonads, only 5 isolates were found to be P- solubilizer (Fig. 6). During the month of August, 2015, the few number of isolates were obtained as P- solubilizer, which might be due to less rainfall (320.8 mm) and moisture content (15.04%). However, the highest phosphate solubilization by fluorescent pseudomonads was found in the month of August, 2015, which might be due to high production of organic acid by the isolates. In a similar studies, Oteino *et al.* (2015) reported highest solubilization efficiency of *Pseudomonas fluorescens* due to drop in the pH corresponding to the gluconic acid production level.

The fluorescent pseudomonads were also screened for antagonistic activity against *Phytophthora capsici* under *in vitro* condition. Out of 31 isolates of fluorescent pseudomonads, only 4 isolates were antagonistic to *Phytophthora capsici* (Fig. 7). In a similar study, Anith *et al.* (2003) reported that *Pseudomonas fluorescens* (PN-026) was the most efficient antagonistic bacteria suppressing the *Phytophthora capsici* of black pepper under *in vitro* condition as well as nursery. In another study, Jubina and Girija (1998) reported that bacterial biocontrol agent could be efficiently used for the management of *P. capsici* in black pepper nursery. The present studies revealed that the isolates of fluorescent pseudomonads were

effective against *P. capsici*. The fluorescent pseudomonads recorded maximum percent inhibition (July, 2015) against *P. capsici* when the temperature was 26.9°C, rainfall (510.1 mm), soil temperature (29.27 °C), relative humidity (86.16%), soil moisture percentage (20.54%) and soil respiration (2.6 mg CO_2/g/day). These parameters were found to be optimum condition for the antagonistic activities by fluorescent pseudomonads.

Trichoderma is one of the biocontrol agent which is an effective inoculant for the management of phytopathogens either directly or indirectly. The biocontrol process by *Trichoderma* depends on its efficiency, host plant and the environmental conditions. It produced specific chemical compounds as well as metabolites (Zeilinger *et al.*, 2016) and these metabolites can be either over produced or produced in less quantity. Therefore, the *Trichoderma* isolates were screened for efficiency at monthly interval, so as to assess the impact of weather and microclimatic parameters on the *Trichoderma* sp. Out of three isolates of *Trichoderma*, two isolates produced ammonia, one isolate produced HCN and two isolates produced siderophore.

In the present studies, the number of *Trichoderma* isolates obtained were less in number. Moreover, ammonia was the only metabolite which was produced in a moderate level (Fig. 8) whereas, three isolates recorded less production of HCN (Fig. 9) and siderophore (Fig. 10). During the period in which the *Trichoderma* isolates were obtained, the ambient temperature of 25.75-27.35^{0}C, soil temperature of 28.81-29.89 °C, relative humidity of 85.14-92.8 per cent, soil moisture content of 15.04-20.87 per cent and soil pH of 5.20-5.56 were recorded. It indicated that these parameters were favourable for the growth and ammonia production by *Trichoderma* sp. According to Ahmad and Baker (1987), *Trichoderma harzianum* was found in the rhizosphere soil of cucumber with pH range 5-7 and a temperature range of 19-33 °C. However, in case of HCN and siderophore production, the less production by *Trichoderma* might be due to less moisture per cent during the period of study.

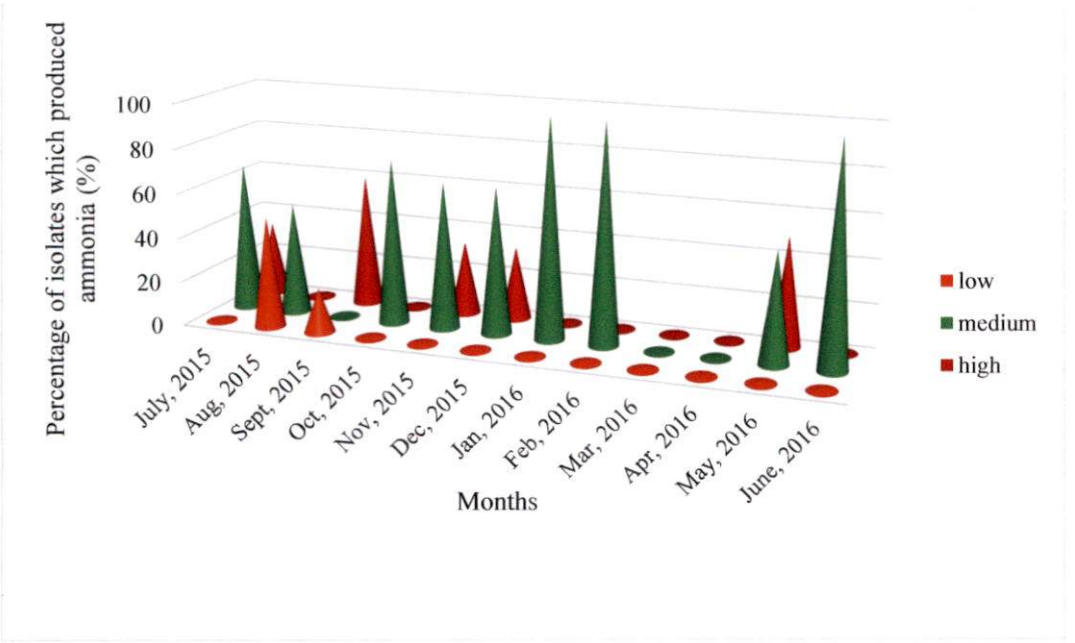


Fig. 3: Ammonia production by fluorescent pseudomonads (12 months)

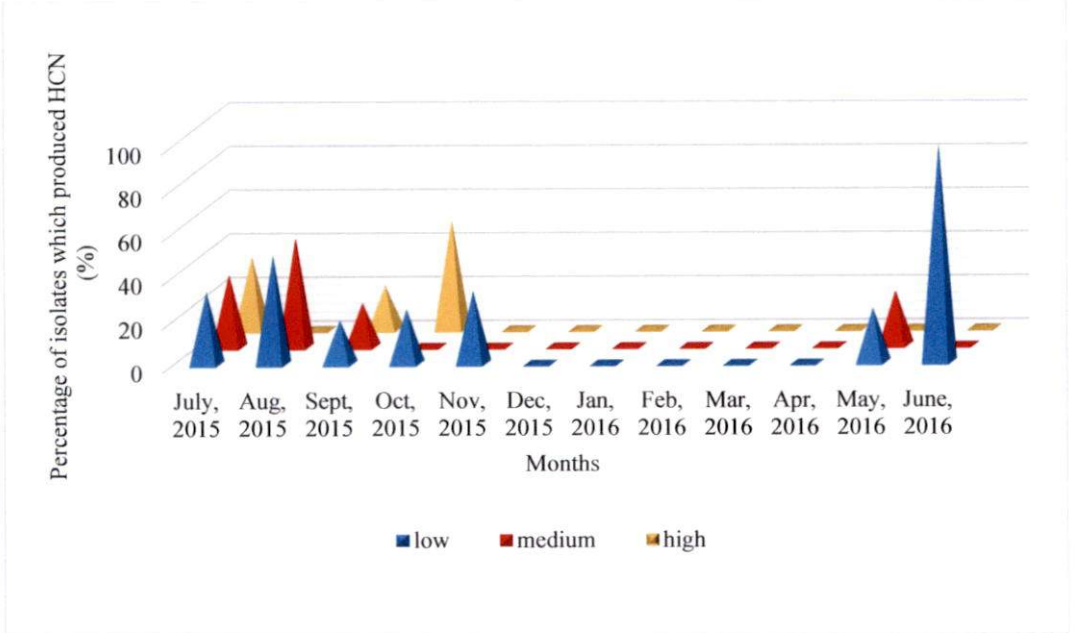


Fig. 4: HCN production by fluorescent pseudomonads (12 months)

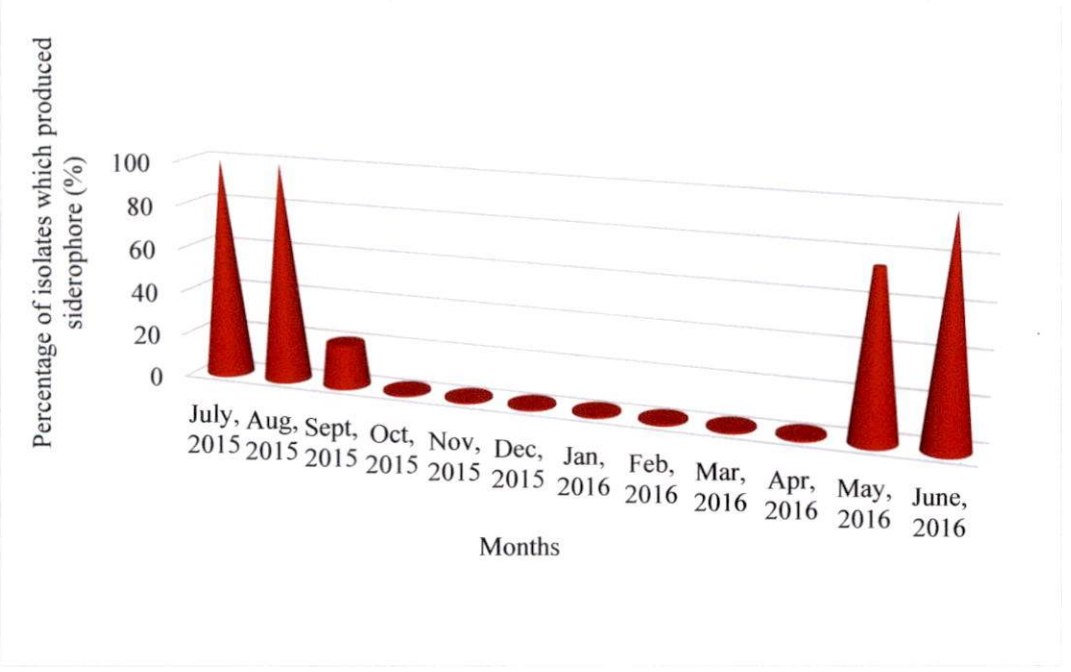


Fig. 5: Siderophore production by fluorescent pseudomonads (12 months)

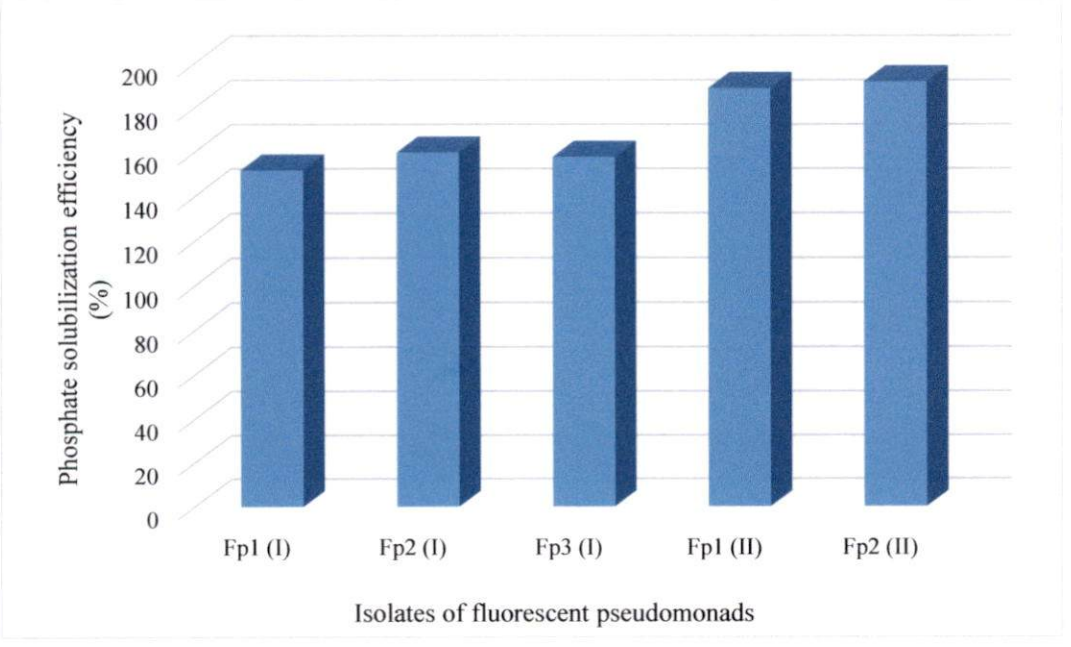


Fig. 6: Phosphate solubilization by fluorescent pseudomonads

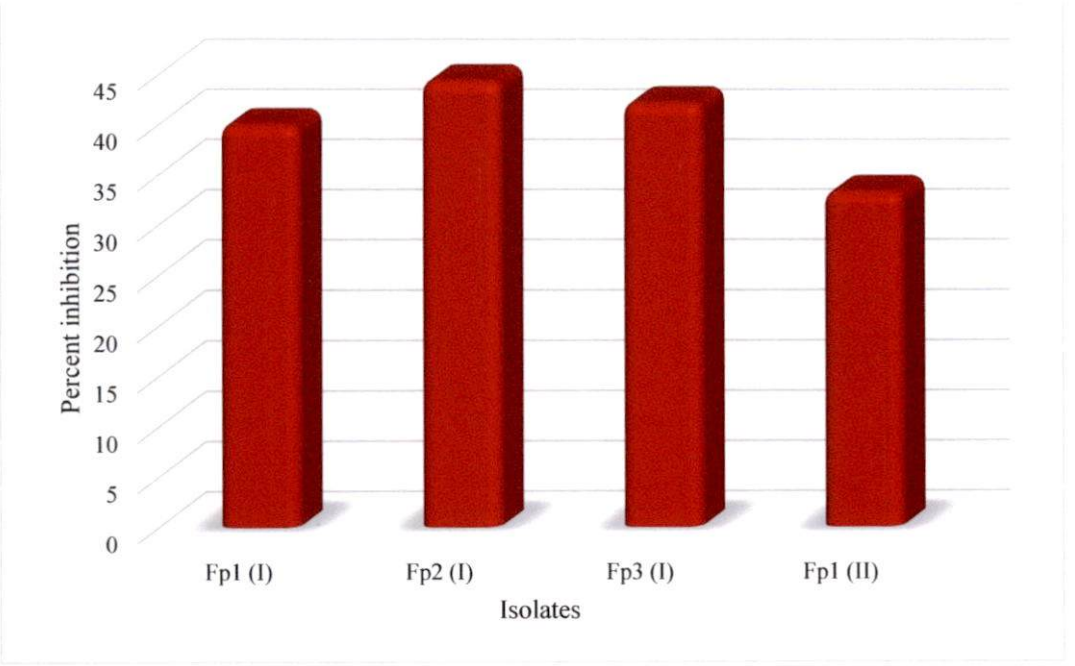


Fig. 7: Antagonistic activity by fluorescent pseudomonads against *Phytophthora capsici*

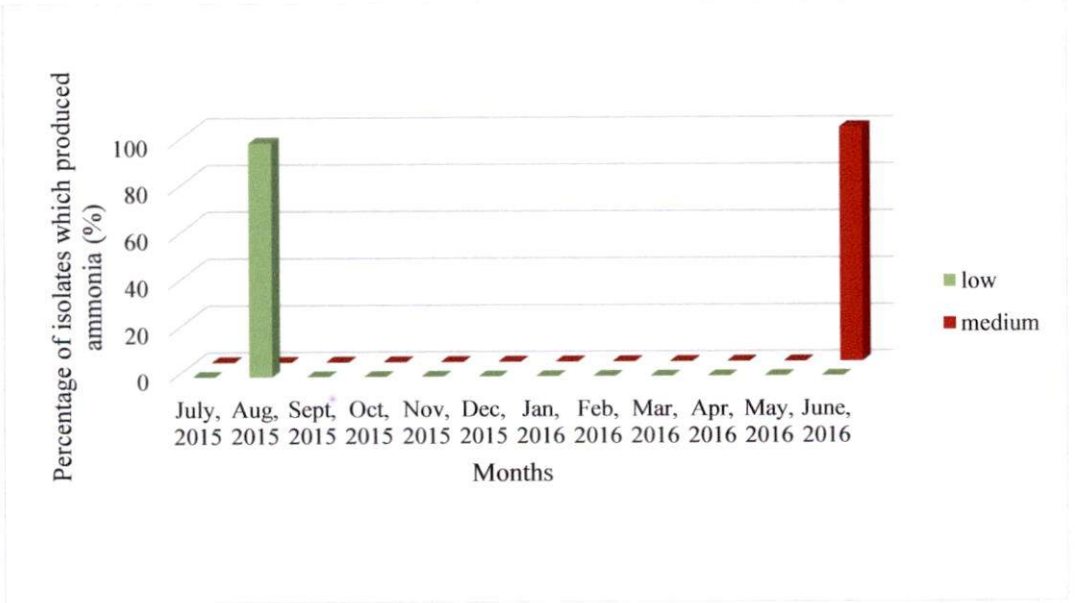


Fig. 8: Ammonia production by *Trichoderma* sp. (12 months)

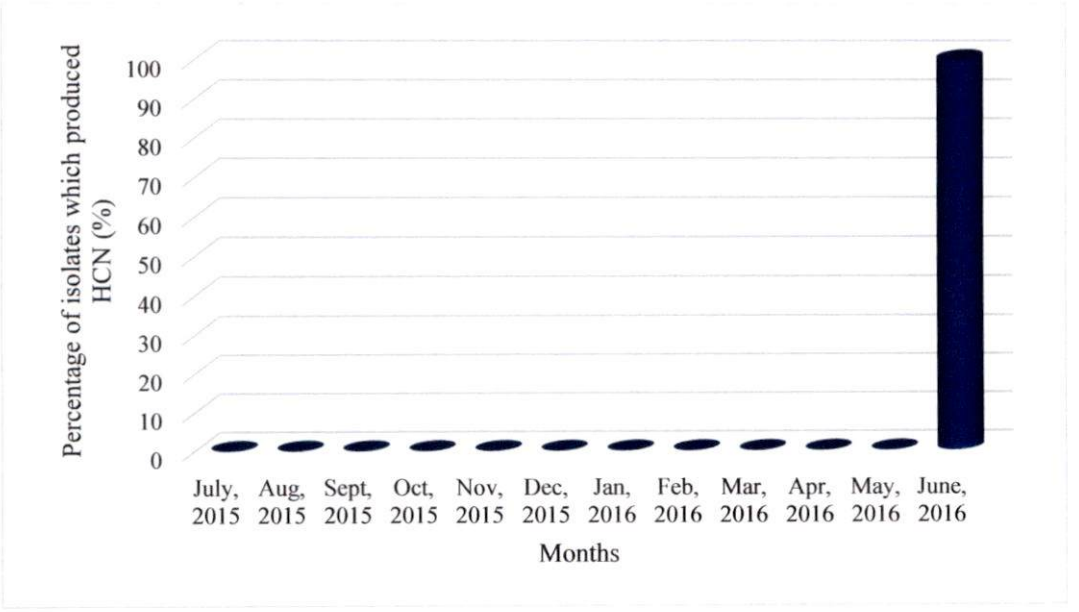


Fig. 9: HCN production by *Trichoderma* sp. (12 months)

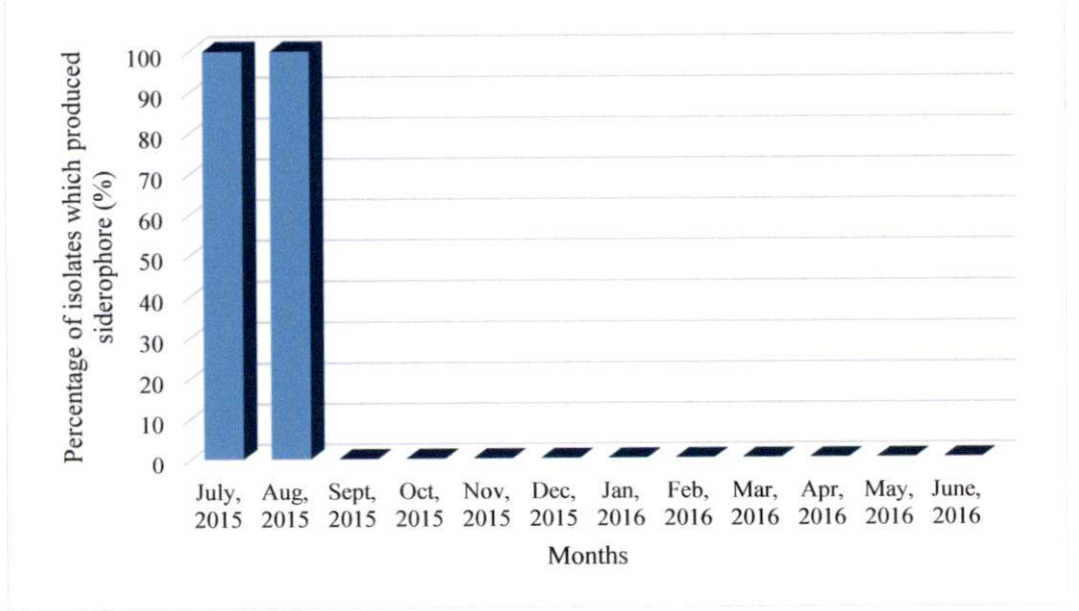


Fig. 10: Siderophore production by *Trichoderma* sp. (12 months)

The *Trichoderma* sp. were also screened for antagonistic activity against *Phytophthora capsici* under *in vitro* condition. Out of three isolates of *Trichoderma* sp., two isolates of *Trichoderma* were antagonistic against the *Phytophthora capsici* (Fig. 11). The present studies revealed that *Trichoderma* isolates were effective against *P. capsici*. However, in case of *Trichoderma,* the per cent inhibition was 72.2 per cent which might be due to optimum soil temperature (29.27 °C) and relative humidity (86.16%) during July, 2015. These results are in agreement with earlier report that the antifungal activity of gliotoxin towards plant pathogens is greater at 32 °C (Lumsden *et al.*, 1992).

The present studies also dealt with the impact of weather variables, micro-climatic and soil parameters in the rhizosphere of black pepper. The maximum rainfall and relative humidity was recorded during June, 2016, whereas, maximum temperature and sunshine hours were recorded during April, 2016 and January, 2016 respectively (Fig. 12). With the increased rainfall, the soil moisture was maximum in June, 2016 which is in agreement with the result of Dermody *et al.*, (2007), who reported that, soil moisture increases with the increase in rainfall. The soil temperature was maximum in April, 2016 which might be due to increase in temperature which led to increase in soil temperature which is in agreement with the results of Cruz-Martinez *et al.* (2012). The soil respiration is exponentially increased with the soil temperature. In a similar study, Karhu *et al.* (2014) also reported the same. There was no significant difference with respect to change in soil pH (Fig. 13).

One of the impacts of weather variables and microclimatic parameters is on the microbial population and its functional efficiency. In the present studies, an attempt was made to assess the changes in the weather and micro-climatic parameters, so as to determine about the parameter or variable affecting the population and efficiency.

In case of fluorescent pseudomonads, the population were positively correlated with rainfall and soil moisture. In a similar study, Cruz-Martinez *et al.*,

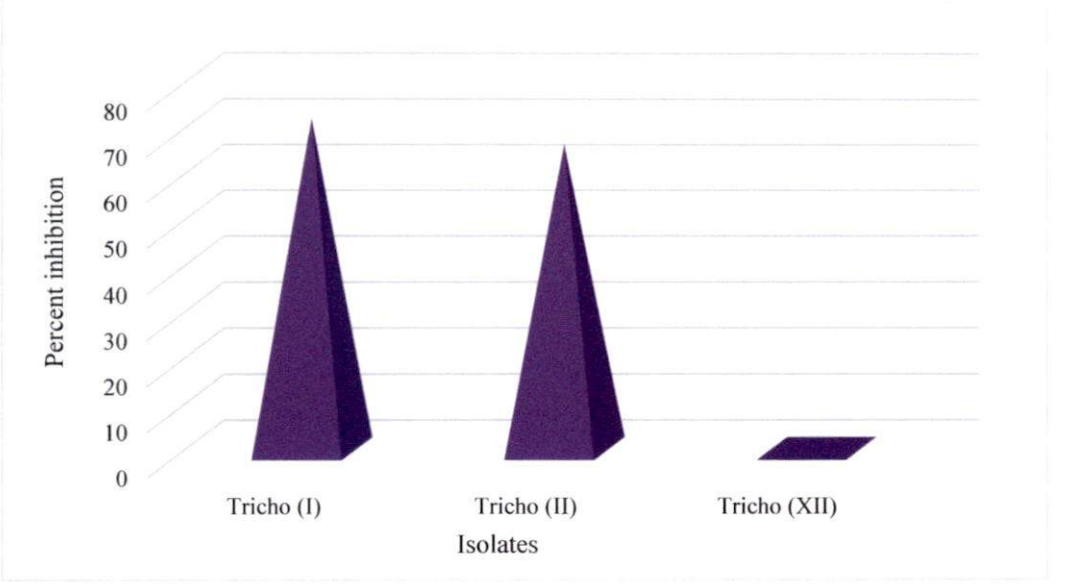


Fig. 11: Antagonistic activity of *Trichoderma* sp. against *Phytophthora capsici*

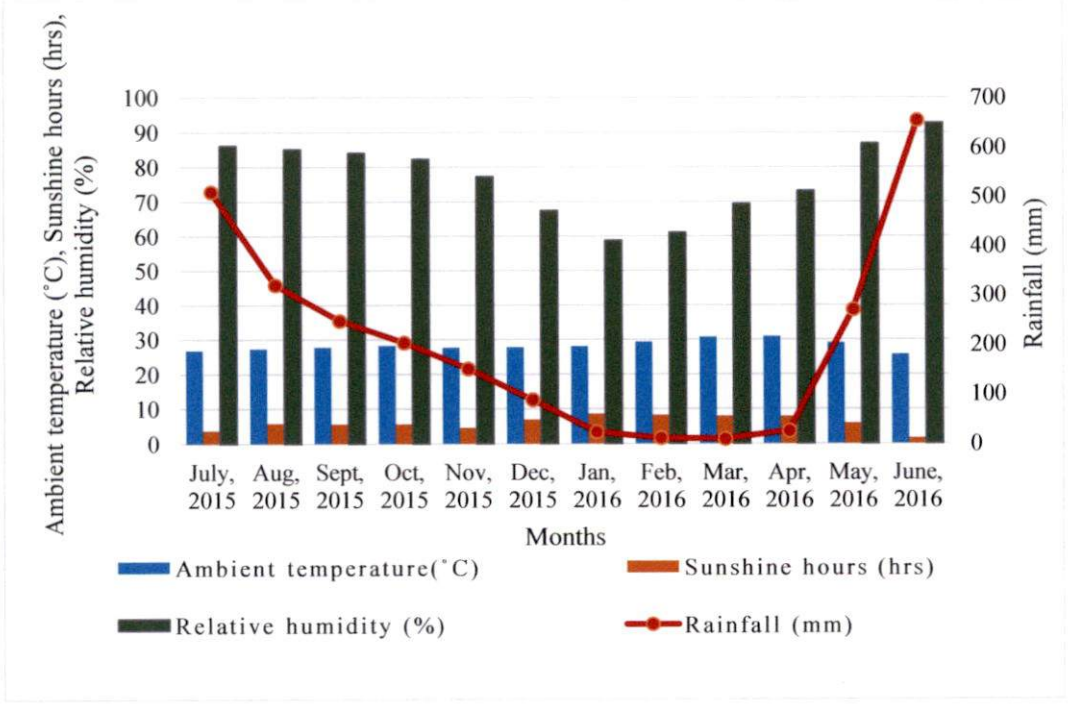


Fig. 12: Weather variables recorded from July, 2015 to June, 2016

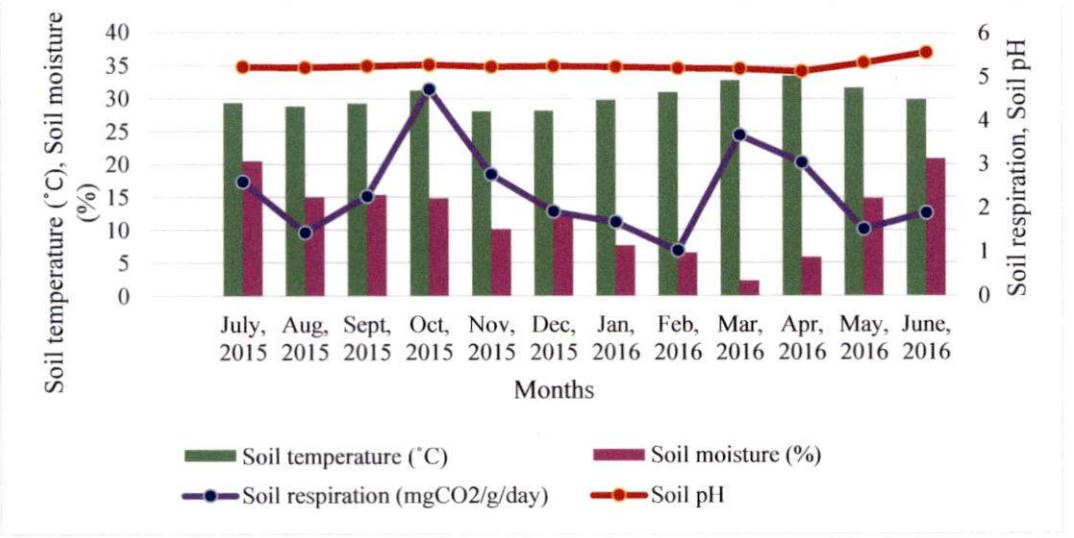
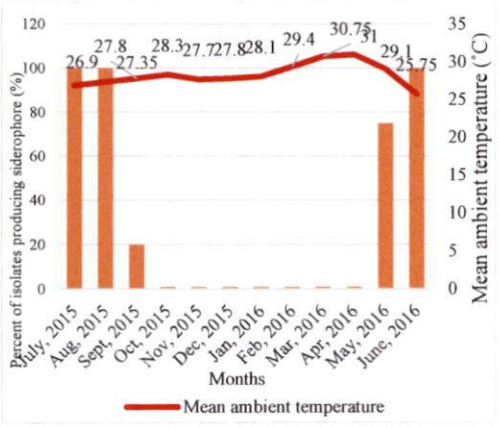


Fig. 13: Micro-climatic parameters recorded from July, 2015 to June, 2016

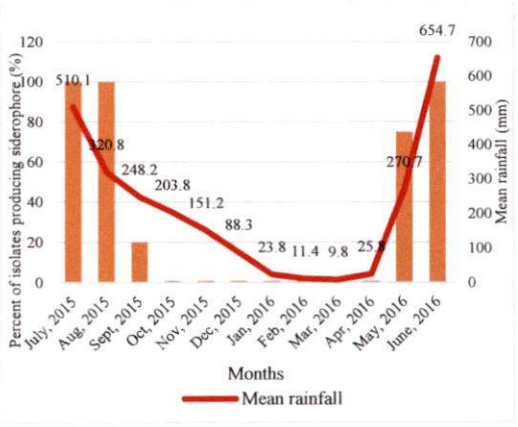
(2012) reported that most of the bacteria have positive correlation with soil moisture. However, the soil microbial population was negatively correlated with the temperature, sunshine hours and soil temperature. In a similar study, Guo *et al.* (2013) reported that soil bacteria is negatively correlated with soil temperature which is in agreement with the present studies. When the efficiency was correlated with the weather and micro-climatic parameters, the siderophore production was significantly affected by the parameters (Fig. 14, Fig. 15).

Trichoderma was also positively correlated with rainfall, relative humidity and soil moisture whereas, it was negatively correlated with ambient temperature, sunshine hours and soil temperature. In a similar study, Chaudhary *et al.* (2016) reported that population of *Trichoderma* was negatively correlated with soil temperature and most of the fungal species were positively correlated with the soil moisture content. When the efficiency of *Trichoderma* sp. was statistically cross tabulated with the weather and micro-climatic parameters, there was no significant difference on the effect of weather and micro-climatic parameters on the efficiency.

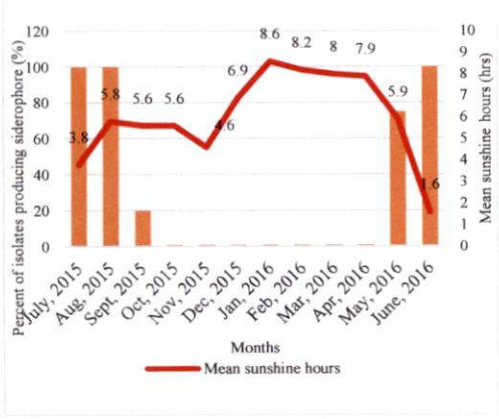
The present studies clearly indicated that weather and micro-climatic parameters influenced the functional efficiency of beneficial microflora. The siderophore production by fluorescent pseudomonads was affected by the weather and micro-climatic parameters. But, in the case of *Trichoderma,* ammonia, HCN and siderophore production were not affected by weather and microclimatic parameters. Hence, *Trichoderma* sp. could be a potential PGPR and biocontrol agent for black pepper as it tolerated the weather and micro-climatic parameters. This study has identified the weather variables such as temperature, rainfall, sunshine hours and relive humidity, and microclimatic and soil parameters such as soil temperature, soil moisture, soil respiration and soil pH affecting the population as well as efficiency of *Trichoderma* and fluorescent pseudomonads. This information will help to develop tolerant PGPR/ *Trichoderma* for black pepper in order to overcome the effect of weather, microclimatic and soil parameters on the *Trichoderma* sp. and fluorescent pseudomonas.



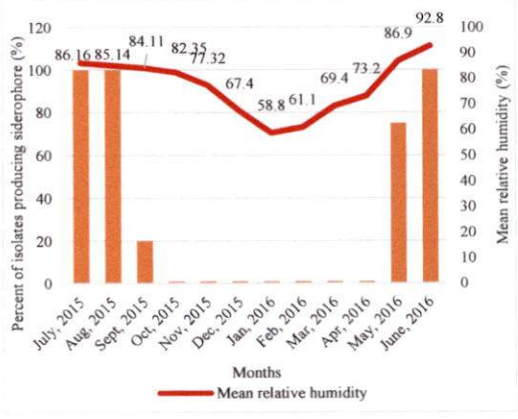
a) Effect of mean ambient temperature



b) Effect of mean rainfall

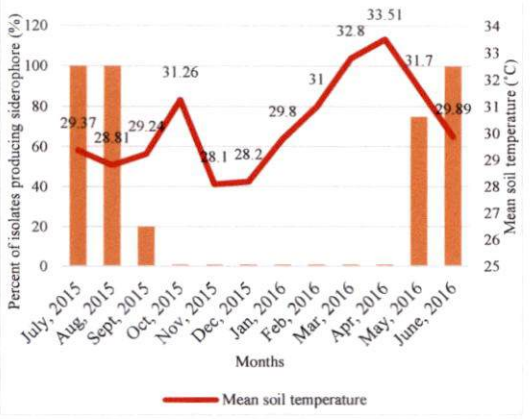


c) Effect of mean sunshine hours

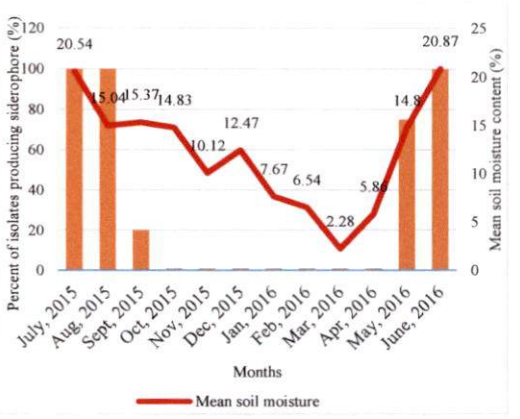


d) Effect of mean relative humidity

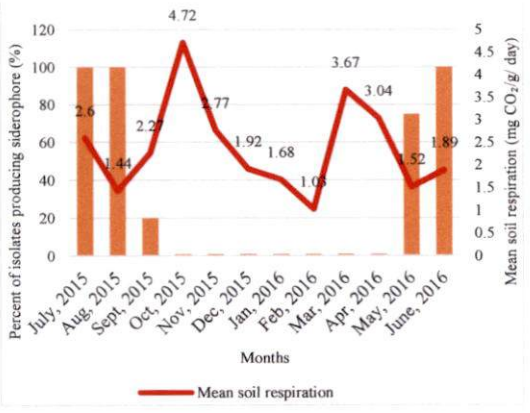
Fig. 14: Effect of weather variables on siderophore production by fluorescent pseudomonads



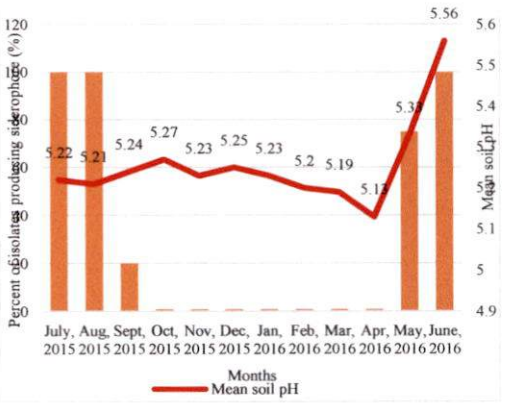
a) Effect of mean soil temperature



b) Effect of mean soil moisture



c) Effect of mean soil respiration



d) Effect of mean soil pH

Fig. 15: Effect of micro-climatic parameters on siderophore production by fluorescent pseudomonads

Summary

6. SUMMARY

The present study on "Impact of weather variables on the functional efficiency of beneficial microflora in the rhizosphere of black pepper" was carried out in the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara during 2014-2016. The major objectives were to study the effect of weather and micro-climatic variables on the population of *Azospirillum*, phosphorus solubilizing bacteria, *Pseudomonas fluorescens* and *Trichoderma* sp. and to study their effects on the functional efficiency of beneficial microflora. The important findings of the study are summarized below:

- Twelve soil samples were collected from the black pepper rhizosphere at the Pepper Unit maintained by Department of Plantation crops and spices, College of Horticulture, Kerala Agricultural University at monthly interval.
- In all the 12 samples, *Azospirillum* and PSB were absent during the period of study. The population of fluorescent pseudomonads and *Trichoderma* were very less.
- A total of 31 isolates of fluorescent pseudomonads and three isolates of *Trichoderma* were obtained.
- The highest population of fluorescent pseudomonads was obtained in September, 2015 (17.5×10^1). The least population was observed in May, 2016 (3.7×10^1). The highest population of *Trichoderma* was in July, 2015 (36.6×10^1) and the least population was found in June, 2016 (6.3×10^1)
- Out of 31 isolates of fluorescent pseudomonas 29 isolates were found to produce ammonia at different intensity, fifteen isolates were found to be producing HCN and only ten isolates produced siderophore as their antagonistic mechanism. Only five isolates were found to be phosphate solubilizers and only four isolates were antagonistic to *Phytophthora capsici.*
- Out of three isolates of *Trichoderma* sp. two were producing ammonia, one isolate was HCN producer and two isolates produced siderophore. Only two isolates were showed antagonism against *Phytophthora capsici.*

- The maximum ambient temperature was recorded in April, 2016 (31°C) and minimum was recorded in June, 2016 (25.75°C). The rainfall was maximum in June, 2016 (654.7 mm) and the minimum was in March, 2016 (9.8 mm). The maximum sunshine hours recorded was 8.6 hours in January, 2016 and the minimum was 1.6 hours in June, 2016. The relative humidity was maximum in June, 2016 (92.8%) and the minimum was in January, 2016 (58.8%).
- The maximum soil temperature was recorded in April, 2016 (33.51°C) and the minimum soil temperature was recorded in November, 2015 (28.1°C). The highest soil moisture content was recorded in the June, 2016 (20.87%) and the lowest moisture content was recorded in the month of March, 2016 (2.28%). The highest soil respiration was recorded in October, 2015 (4.72 $mgCO_2$/g/day) and the lowest was recorded in February (1.03 $mgCO_2$/ g/ day). The monthly observation of pH did not show any significant differences.
- The population of fluorescent pseudomonads was positively correlated with rainfall and soil moisture and negatively correlated with ambient temperature, sunshine hours and soil temperature and the population of *Trichoderma* sp. was positively correlated with rainfall, relative humidity and soil moisture whereas it was negatively correlated with ambient temperature, sunshine hours and soil temperature.
- In fluorescent pseudomonads, weather and microclimatic parameters did not show any significant effect on ammonia and HCN production, but had significant effect on siderophore production.
- The optimum weather parameters for the siderophore production by fluorescent pseudomonads were found to be, temperature in a range of 25.75 to 27.35°C, mean rainfall of 300 to 650 mm, sunshine hours in the range of 1.60 to 5.80 and relative humidity in the range of 86.16 to 92.80 per cent.
- The optimum micro climatic parameters for the production of siderophore by fluorescent pseudomonads were found to be, soil temperature in a range

of 28 to 29°C, soil moisture content of 15 to 21 per cent, soil respiration range of 1.44 to 2.60 mgCO_2/g/day and a soil pH of 5.21 to 5.56.

- In *Trichoderma* sp., the weather and microclimatic parameters had no significant effects on the ammonia, HCN and siderophore production.

The future line of work includes conducting the experiments at different locations, where the black pepper is grown. The experiment can be done repeatedly for several years. This study can be extended to other beneficial microorganisms also.

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Appendix

APPENDIX

1. Nfb semi-solid medium

Malic acid	– 5g
K_2HPO_4	– 0.5g
KOH	– 4g
Mg $SO_4.7H_2O$	– 0.1g
NaCl	– 0.02g
$CaCl_2$	– 0.01g
$FeSO_4.\ 7H_2O$	– 0.05g
Na_2MoO_4	– 0.002g
$MnSO_4$	– 0.01g
Bromothymol blue (BTB)	– 2 ml (0.5% alchohol solution)
Agar	– 2g
Distilled water	– 1000 ml
pH	– 6.8-7.2

2. NBRIP medium

Glucose	– 10g
$Ca_3\ (PO_4)_2$	– 5g
$MgCl_2.\ 6\ H_2O$	– 5g
Mg $SO_4.\ 7H_2O$	– 0.25g
KCl	– 0.2g
$(NH_4)_2\ SO_4$	–0.1 g
Distilled water	– 1000 ml
Agar	– 20g
pH	– 7

3. Kings' B media

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

4. Trichoderma Selective Media

K_2HPO_4	– 0.9g
$MgSO_4$. 7 H_2O	– 0.2g
KCl	– 0.15g
NH_4NO_3	– 1g
Glucose	– 3g

p-dimethylamino benzene diazo sodium sulfonate- 0.3g

Pentachloronitrobenzene – 0.2g

Rose Bengal	– 0.15g
Distilled water	– 1000 ml
Agar	– 20g

5. IAA production

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

6. HCN production

LB	– 6.25g
Glycine	– 1.1g
Distilled water	– 250 ml
Agar	– 4g

7. CAS agar media

Chrome azurole S	– 60.5 mg in 50 ml water
Iron (III) solution	– 10 ml (1 mM $FeC1_3.6H20$, 10 mM HCl)
HDTMA	– 72.9 mg in 40 ml water
Kings' B medium	– 900 ml
Agar	– 20g

8. Potato Dextrose Agar media

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

9. Peptone water

Peptone	– 4 g
Distilled water	– 100 ml

10. Pikovskya's media

Glucose	– 10g

$Ca_3(PO_4)_2$	– 5 g
$(NH_4)_2SO_4$	– 0.5 g
NaCl	– 0.2 g
$MgSO_4.7H_2O$	– 0.1 g
KCl	– 0.2 g
Yeast extract	– 0.5 g
$MnSO_4.H_2O$	–0.002g
$FeSO_4.7H_2O$	– 0.002 g
Distilled water	– 1000 ml
Agar	– 20g
pH	– 7

11. Salkowski reagent

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

IMPACT OF WEATHER VARIABLES ON THE FUNCTIONAL EFFICIENCY OF BENEFICIAL MICROFLORA IN THE RHIZOSPHERE OF BLACK PEPPER (*Piper nigrum* L.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Black pepper has been reported to be the most vulnerable spice crop to climate change. The beneficial microorganisms in the rhizosphere of black pepper can reduce the impact of abiotic stress due to changes in the weather variables. However, the soil microbial community are also influenced by changes in weather and microclimatic parameters.

A study was undertaken on the "Impact of weather variables on the functional efficiency of beneficial microflora in the rhizosphere of black pepper". The main objectives were to study the effect of weather and microclimatic parameters on the population and functional efficiency of beneficial microflora namely; *Azospirillum*, phosphate solubilizing bacteria (PSB), *Pseudomonas fluorescens* and *Trichoderma* sp.

The rhizosphere soil samples from black pepper were collected at monthly interval for a period of one year from Pepper Unit, Kerala Agricultural University (KAU), Vellanikkara. The selected beneficial microorganisms were enumerated and *in vitro* screening was done at monthly interval for IAA, ammonia, HCN, siderophore production, phosphate solubilization and antagonistic activity against *Phytophthora capsici*. Simultaneously, the weather and microclimatic parameters were also recorded.

The *Azospirillum* and PSB were not obtained throughout the study period from July, 2015 to June, 2016. The population of fluorescent pseudomonads was highest in September, 2015 and was absent in March, 2016 and April, 2016. *Trichoderma* sp. recorded the highest population in July, 2015 and lowest in June, 2016. A total of 31 isolates of fluorescent pseudomonads and 3 isolates of *Trichoderma* sp. were obtained during the entire study period.

Out of 31 isolates of fluorescent pseudomonads, 29 isolates produced ammonia with different concentrations. Fifteen isolates produced HCN and only ten isolates showed siderophore production as their antagonistic mechanism. Only 5 isolates were phosphate solubilizers and 4 isolates were antagonistic to *Phytophthora capsici.* However, in the case of *Trichoderma* sp. two isolates

produced ammonia, one isolate was HCN producer, two produced siderophore and two isolates showed antagonistic activity against *Phytophthora capsici.*

Considering the correlation studies between weather, microclimatic parameters and population of isolates obtained, it was found that the population of fluorescent pseudomonads were positively correlated with rainfall and soil moisture whereas negatively correlated with air temperature, sunshine hours and soil temperature. However, the population of *Trichoderma* sp. was positively correlated with rainfall, relative humidity and soil moisture whereas it was negatively correlated with air temperature, sunshine hours and soil temperature. The study indicated that rainfall (200 to 500 mm) and soil moisture (15 to 20%) favoured fluorescent pseudomonads and *Trichoderma* population.

The functional efficiency of the isolates were also correlated with the weather and microclimatic parameters. In case of fluorescent pseudomonads, the weather and microclimatic parameters had no significant effect on its ammonia and HCN production. However, significant effect on the siderophore production was noticed. In the case of *Trichoderma*, weather and microclimatic parameters had no significant effect on ammonia, HCN and siderophore production.

The present studies clearly indicated that the weather and microclimatic parameters affected the siderophore production in the case of fluorescent pseudomonads but, there was no effect on functional efficiency of *Trichoderma* sp. However, mitigation strategies have to be studied in the case of fluorescent pseudomonads to overcome the effect of weather and microclimatic variables on functional efficiency.