Biofilm based consortia for growth promotion and soil-borne disease management in cowpea (*Vigna unguiculata* L. Walp)

Vinaykumar, B. and Surendra Gopal,K.*

ABSTRACT

Biofilm based antagonists were evaluated for growth promotion and soil borne disease management in cowpea (*Vigna unguiculata* L. Walp). Nine *Trichoderma* sp. and five *Bacillus* sp. were obtained from ten rhizosphere soils of cowpea growing areas of Thrissur district (Kerala). The highest population of *Trichoderma* sp. was recorded in Chellakara (4.8 x 10^3 cfu g^{-1}) and *Bacillus* sp. in Pananchery (4.48 x 10^3 cfu g^{-1}). Among *Trichoderma* sp., TCH (Chellakara) isolate recorded maximum inhibition (51.1 %) against *Rhizoctonia solani* and *Pythium aphanidermatum* (57.7 %). None of the *Bacillus* sp. showed antagonistic activity against *Rhizoctonia solani* and *Pythium aphanidermatum. Bacillus* sp. were screened for biofilm production, and BCH (Chellakara) isolate (0.0600) was the most efficient followed by BPN (Pananchery)(0.058) and BML (Mala) (0.056). Based on the plant growth promoting traits and antagonistic activities, three most efficient *Trichoderma* sp. (TCH, TPZ and TMT) and *Bacillus* sp. (BCH, BPN and BML) were selected for further studies. Three best *Trichoderma* based *Bacillus* sp. biofilms (TPZ+BPN, TCH+BCH and TMT+BML) were selected based on the growth promotion, antagonistic activity, biofilm production and compatibility under *in vitro*. Population of inoculated *Trichoderma* sp., *Bacillus* sp. and *Rhizobium* sp. in the potting mixture showed declining trend till the final harvest of the crop. Among the biofilm based formulations, TCH (*Trichoderma* sp.) + BCH (*Bacillus* sp.) (T2) was the most promising treatment for growth promotion and disease management in cowpea under pot culture studies. However, further studies are needed to evaluate under field conditions.

Keywords: Biofilm, Cowpea, *Trichoderma, Bacillus*

MS History: 18.03.2019 (Received)- 14.09.2019 (Revised)- 04.11.2019 (Accepted).

Citation: Vinaykumar, B. and Surendra Gopal, K. 2019. Biofilm based consortia for growth promotion and soil -borne disease management in cowpea (*Vigna unguiculata* L. Walp) . *Journal of Biopesticides,* **12**(2): 177-185.

INTRODUCTION

The microbial inoculants play an important role as eco-friendly, non-bulky and low cost agricultural inputs. *Trichoderma* sp. and *Bacillus* sp. are the most successful bioagents, which are commercially exploited in India. *Trichoderma* sp. can parasitize the fungal pathogens by several mechanisms. It can induce systemic and localized resistance to plants against many plant pathogens. *Trichoderma* spp are also involved in promoting plant growth by stimulating many enzymes and pathogenesis related protein in plants. *Bacillus* sp. is the most consistent plant growth promoter. It protects the plants from pathogenic microorganisms through various mechanisms (Cherif *et al*., 2016) .Some strains of *Bacillus* sp. enhance plant growth by releasing phytohormones (IAA), acids (HCN) and have the ability to solubilize P from soil reserves and improve the P- uptake in plants.

Inconsistent field performance often restricts the use of many bioagents and plant growth promoting rhizobacteria (PGPR). Biotic and abiotic factors could affect bioagents under laboratory and field conditions. It could be attributed to adaptability to a non-native soil, negative effects of interaction with existing microbes in crop environment and incompatibility in colonizing different crop plants (Elsas *et al*., 1986). All these reasons affect the survivability of the inoculated bioagents. Failure of bio agents to survive in soil results in the development of plant diseases, causing huge crop loss. Improvement in the survivability of biocontrol agents have become a major area of concern. In this context, biofilm based bioinoculant is a novel approach which has the ability to protect the bioinoculants from various environmental stress such as UV radiation, extreme pH, osmotic shock, dehydration, antimicrobial substances and predators . A biofilm comprises microbial cells and sticky extracellular polymeric substance (EPS) which provide structure and protection in natural environment. Biofilm based bioinoculants are known to take part in soil fertility management, nutrient uptake, higher rate of biological nitrogen fixation, release of organic acids, phosphate solubilisation and help in successful management of plant diseases (Jayasinghearachchi *et al*., 2004) . Cowpea is one of the important legume crops which is ranked among the top- five legume crops in the world. Fungal diseases like collar-rot (*Rhizoctonia solani*), root rot (*Pythium aphanidermatum*), anthracnose (*Colletotrichum lindemuthianum)*, powdery mildew (*Erysipheae polygoni*) and other soil borne diseases have become a major concern in cowpea (Sathish *et al*., 2000). There is a need to increase the emphasis on use of eco-friendly approach, such as bioinoculants, to provide a long lasting solution. Bioinculants applied to soil, shows good results but survivability in the soil for a long period is affected due to many biotic and abiotic factors. Survival and functioning of commercial biofertilizers are inconsistent under field conditions due to heterogeneity of biotic and abiotic stress factors and competition with indigenous organisms. PGP traits of novel biofilms were developed using *Trichoderma*, *Pseudomonas fluorescens* and *Bacillus subtilis* as partners (Triveni *et al*., 2012). Such biofilms exhibited higher biochemical attributes like enhanced antifungal activity, ammonia, indole acetic acid (IAA) and siderophore production as compared to the monocultures and dual cultures. Earlier studies have indicated that biofilm based microbial inoculants perform better than carrier-based inoculants. Hence, a study was carried out to evaluate the biofilm based bioagents for growth promotion and management of *Rhizoctonia solani* and *Pythium aphanidermatum* diseases in cowpea and increase the survivability of inoculated biogents in soil.

MATERIALS AND METHODS

A survey was conducted on cowpea growing areas of Thrissur district in Kerala and rhizosphere soil samples were collected from ten different locations. Six rhizosphere soils from healthy cowpea plants and four samples from collar rot infected plants were collected. *Trichoderma* and *Bacillus* were isolated and enumerated from rhizosphere soils by serial dilution plate technique (Chen *et al*., 2016).

Cultural and morphological characters of nine *Trichoderma* isolates were studied. The bacterial isolates were identified by16S rDNA sequencing. Using micropipette, single colony of the isolate was mixed with 10 µL of sterile water. 2µl of this suspension was used as template for amplification of 16S rRNA gene. The quality of isolated DNA was evaluated though agarose gel electrophoresis. The PCR product was purified and sequenced at Vision Scientific Services Angamaly, Kerala using the primers 8F and 1522r. They were screened for IAA production, HCN production, siderophore production, ammonia production and phosphate solubilisation ability. *Trichoderma* and *Bacillus* isolates were screened for antagonistic activity against *Rhizoctonia solani* and *Pythium aphanidermatum* by dual culture method and mutual compatibility was also tested. *Bacillus isolates* were screened for biofilm production (Mathur *et al*., 2006; Deka 2014). Based on the growth promotion traits, antagonistic activities, biofilm production and compatibility studies, three most efficient *Trichoderma* based *Bacillus* biofilm were evaluated for growth promotion and management of *Rhizoctonia solani* and *Pythium aphanidermatum* in cowpea under pot culture with three replications (5 plants / replication) with CRD design. Treatment details are as follows:

T_1: Biofilm based *Trichoderma* sp. (TPZ) + *Bacillus* sp. (BPN); T_2: Biofilm based *Trichoderma* sp. (TCH) + *Bacillus* sp. (BCH); T_3: Biofilm based *Trichoderma* sp. (TMT) + *Bacillus* sp. (BML); T_4: *Bacillus* sp. (KAU ref. culture); T*5*: *Trichoderma* sp. (KAU ref. culture); T**6**: *Bacillus* sp. + *Trichoderma* sp.; T_7: Carbendazim + Mancozeb (2 g l^{-1} as soil drenching); T_8 : Package of Practices, recommendations of KAU (KAU, 2011); T_9 : PGPR Mix –II (@ 2.5 kg ha^{-1}) and T_{10} : absolute control

Organic package was applied to all the treatments except T8 and T10, which included seed treatment with *Rhizobium* @ 0.5 kg 10 kg^{-1} of seeds, manuring with FYM @ 20 t ha^{-1} and lime application @ 250 kg ha^{-1}. All the treatments were supplemented with FYM orcowdung @ 2 t ha^{-1} along with rock phosphate @ 100 kg ha^{-1}. *Bacillus* sp. @ 4 g kg^{-1} seed and *Trichoderma* sp. @ 4 g kg^{-1} seed were applied at the time of seed treatment. The plants were challenge inoculated with each pathogen in two separate sets of experiments. Challenge inoculation of *Rhizoctonia solani* and *Pythium aphanidermatum* @ 20 g plant-1 (@ 8 × 10^5 cfu g^{-1}) were done one month after the application of antagonists. *Pythium aphanidermatum* was grown on sterilized carrot bits for 5 days and the fully grown *Pythium aphanidermatum* was used for artificial inoculation.

RESULTS AND DISCUSSION

Population of native *Trichoderma* sp. and *Bacillus sp.* in the rhizosphere soil of cowpea

Among the isolates, *Trichoderma* sp. (TCH) from Chellakara recorded the highest population (4.8 × 10^3 cfu g^{-1}) followed by TPZ isolate (3.7 × 10^3 cfu g^{-1}) from Pazhayanur (Table 1). The lowest *Trichoderma* sp. (TCK) population (1.8 × 10^3 cfu g^{-1}) was recorded in Chalakudy. However, population of the *Bacillus* sp. (BPN) was the highest (4.48 x 10^5 cfu g^{-1}) in Pananchery followed by B MT isolate (3.24 × 10^5 cfu g^{-1}) from Mattathur. The lowest *Bacillus* sp. (BPN) population (2.17 × 10^5 cfu g^{-1}) was recorded in Pananchery. In the present studies, the population of *Trichoderma* sp. varied among the location. The highest population of *Trichoderma* sp. (4.8 × 10^3 cfu g^{-1}) (TCH) and *Bacillus* sp. (4.48 × 10^5 cfu g^{-1}) (BPN) were recorded in healthy cowpea of Chellakkara and Pananchery respectively.

Table 1. Native population of *Trichoderma* sp. and *Bacillus* sp. in cowpea rhizosphere soil

Locations	***Trichoderma* sp. (x10^3 cfu g^{-1} of soil)**	***Bacillus* sp. (x10^5 cfu g^{-1} of soil)**
Chellakara (CH)	4.8^a(3.68)	2.66(3.42)
Pazhayanur PZ)	3.7^{ab} (3.56)	0.00 (0.71
Chalakudy (CK)	1.8^c (3.25)	0.00 (0.71)
Mattathur (MT)	3.23^{abc} (3.50)	3.24 (3.51)
Mala (ML)	2.56^{bc} (3.40)	2.17 (3.33)
Mullasery (MS)	3.68^{ab} (3.56)	2.84 (3.45)
Elanad (EL)	0.00 (0.71)	0.00 (0.71)
Nadathara (NT)	0.00 (0.71)	0.00 (0.71)
Pananchery (PN)	0.00 (0.71)	4.48 (3.65)
Vellanikara (VL)	0.00 (0.71)	0.00 (0.71)
CD (0.05)	1.819	NS

NS- Non significant; Values in the parenthesis indicate log transformed values

The results indicated that the healthy plants of cowpea harbored more population of antagonists compared to the rhizosphere of infected plants. Soil microorganisms were found to vary in their population due to the influence by high temperature, dryness/heavy rainfall in tropical countries (Mota *et al.*, 2008). However, healthy plants also favour the growth of microorganism which might be due to the more root exudates released of the plant.

Screening of *Trichoderma* sp. and *Bacillus* sp. for antagonistic activity against *Rhizoctonia solani* and *Pythium aphanidermatum*

In order to develop an efficient microbial antagonist, *Trichoderma* sp. and *Bacillus* sp. were screened for antagonistic activity against *Rhizoctonia solani* and *Pythium aphanidermatum*. *Trichoderma* sp. (TCH-1) recorded the highest per cent inhibition (51.1 %) against *Rhizoctonia solani* under *in vitro*. The lowest per cent inhibition (44.6 %) was recorded by *Trichoderma* sp. (TCK-2) isolate. Among the *Bacillus* isolates, none of the isolates showed inhibition against *Rhizoctonia solani* (Table 2). .Similarly, the highest per cent inhibition (57.7 %) against *Pythium aphanidermatum* was recorded by TCH-1 followed by TML isolate of *Trichoderma* sp.(53.3 %). The lowest inhibition (47.7 %) was recorded by TCH-2. However, none of the *Bacillus* sp. isolates showed antagonism against *Pythium aphanidermatum* under *in vitro*. The antagonistic activity of *Trichoderma* sp. is due to the production of various secondary metabolites which act as inhibitors to various plant pathogens. In the present studies, *Trichoderma* sp. were found to be effective against *Rhizoctonia solani* and *Pythium aphanidermatum*. These results are in agreement with earlier reports that the mutant strain of *Trichoderma viride* (1433) showed significant antagonistic activity against *Pythium aphanidermatum* by the production of volatile and non-volatile metabolites (Khare *et al.*, 2010). Similarly, it was reported that different isolates of *Trichoderma* sp. were effective against *Rhizoctonia solani, Sclerotium rolfsii, Fusarium ciceri* and *Machrophomina phaseolina* under *in vitro* conditions due to the production of both volatile and non-volatile inhibitors of *Trichoderma* sp. (Pan, *et al.2013*).

Table 2. Antagonistic activity of *Trichoderma* sp. against *Rhizoctonia solani* and *Pythium aphanidermatum* under *in vitro*

Bioagent	Isolates	Per cent inhibition against	
		Rhizoctonia solani	***Pythium aphanidermatum***
Trichoderma sp.	TCH1 (Chellakara)	51.1	57.7
	TCH2 (Chellakara)	45.5	47.7
	TCH3 (Chellakara)	47.7	51.1
	TPZ (Pazhayanur)	50.0	51.1
	TCK1 (Chalakudy)	46.6	52.2
	TCK2(Chalakudy)	44.4	49.9
	TMT (Mattathur)	44.6	48.8
	TML (Mala)	48.8	53.3
	TMS (Mullassery)	47.7	51.1
Bacillus sp.	BCH (Chellakara)	-	-
	BMS (Mullassery)	-	-
	BML (Mala)	-	-
	BPN (Pananchery)	-	-
	BMT (Mattathur)	-	-

Each value represents mean of three replications, (–): No inhibition

Screening of *Bacillus sp.* for biofilm production

Five isolates of *Bacillus* sp. were screened for biofilm production under *in vitro*. The BCH isolate recorded the maximum OD value (0.060) followed by BPN (0.056) isolate (Table 3).

Table 3. Screening of *Bacillus sp.* isolates for biofilm production under *in vitro* condition

Isolates	O.D values (570 nm)
BCH (Chellakara)	0.060
BMS (Mullassery)	0.056
BML (Mala)	0.056
BPN (Pananchery)	0.058
BMT (Mattathur)	0.055

The least OD value (0.0556) was recorded in the case of BMT isolate. Three most efficient biofilm producers (BCH, BPN, BMT) based on qualitative and quantitative results were selected for further studies. The applications of microbial antagonists in soil often do not reproduce their beneficial effect consistently because the survival and establishment of these organisms in rhizosphere soil are influenced by various environmental stresses. Therefore, there is a need to develop a n alternate approach so as to improve the survivability and efficiency of inoculated microbial antagonists. Microbial biofilms are the communities of microorganisms adhering to abiotic and biotic surfaces and they are embedded in an organic matrix of biological origin which provides structure and stability to the community (Webb, *et.al*., 2003). Since, biofilms comprise layers of microbial cells, they play a key role in plant microbe interactions. Microbes in biofilm are sessile and encased in extra cellular polysaccharide matrix which provides protection from environmental stress (Flemming and Wingender 2001). So, there is a scope for microbial biofilm production to overcome the poor survival of microbial inoculants under harsh environmental conditions. Novel microhabitats were formed in soil which enhanced the movement and survival of bacteria in soil especially as biofilms (Warmink *et al.,* 2011). In the present studies, the *Bacillus* sp. were found to be the potential biofilm producers. These results are in agreement with the studies (Cavaglieri *et al*., 2005) who reported that *Bacillus subtilis* protects roots from plant pathogenic bacteria by biofilm formation, antibiotic and surfactin production which possesses antimicrobial activity against pathogens. Similarly, *Paenibacillus polymyxa* provided protection from pathogens, when it formed biofilms by colonizing *Arabidopsis thaliana* (Timmusk *et al.,* 2005).

Table 4. Effect of different treatments on yield of cowpea after challenge inoculation with *Rhizoctonia solani* (30 DAS) under pot culture experiment

Treatments	Yield at harvest (g plant^{-1})	Projected yield (t ha^{-1})
T2: Biofilm based *Trichoderma* sp. (TCH) + *Bacillus* sp. (BCH)	48.00^{bc}	3.55
T3 : Biofilm based *Trichoderma* sp. (TMT) + *Bacillus* sp. (BML)	44.85^{cd}	3.32
T4: *Bacillus* sp. (KAU ref.culture)	43.42^{d}	3.21
T5: *Trichoderma* sp. (KAU ref.culture)	44.85^{cd}	3.32
T6: *Bacillus* sp. + *Trichoderma* sp.	48.49^{b}	3.59
T7: Carbendazim + Mancozeb (2 g / l as soil drenching)	45.14^{bcd}	3.34
T8: Package of practices (KAU, 2011)	44.57^{d}	3.30
T9: PGPR Mix – II @ 2.5 kg / ha	52.28^{a}	3.87
T10: Absolute control	39.85^{e}	2.95
CD (5 %)	3.31	-

Effect of different treatments on growth, disease incidence and yield of cowpea after challenge inoculation with Rhizoctonia solani and Pythium aphanidermatum

Three most promising *Trichoderma* based *Bacillus* sp. (TCH+BCH, TPZ+BPN and TMT+BML) biofilm inoculants with talc powder as carrier material were evaluated for their efficiency in growth promotion and disease management in cowpea under pot culture. The experiment was conducted as two separate studies with challenge inoculation of *Rhizoctonia solani* and *Pythium aphanidermatum*. Biofilm based TCH (*Trichoderma* sp. + BCH *Bacillus* sp. (T2) treatment performed better with respect to early germination (Table 4), plant height, number of leaves, minimum days taken for flowering, fresh weight and dry weight of plants . After the artificial inoculation of *Rhizoctonia solani*, per cent collar-rot disease was recorded at fortnightly interval (Table 5).

Table 5. Effect of different treatments on per cent collar rot disease caused by *Rhizoctonia solani* after challenge inoculation (30 DAS) under pot culture experiment

Treatments	Percent disease incidence (40)	Percent Disease incidence (55)	Percent disease incidence	Percent disease incidence
T1 : Biofilm based *Trichoderma* sp. (TPZ) + *Bacillus* sp.	14.28	14.28	14.28	28.57
T2 : Biofilm based *Trichoderma* sp. (TCH) + *Bacillus* sp.	0	0	0	14.28
T3 : Biofilm based *Trichoderma* sp. (TMT) + *Bacillus* sp	14.28	14.28	28.57	28.57
T4 : *Bacillus* sp. (KAU ref.culture)	28.57	28.57	28.57	28.57
T5 : *Trichoderma* sp. (KAU ref.culture)	14.28	14.28	14.28	14.28
T6 : *Bacillus* sp. + *Trichoderma* sp.	42.85	42.85	42.85	42.85
T7 : Carbendazim + Mancozeb (2 g / l as soil drenching)	14.28	14.28	14.28	14.28
T8 : Package of practices (KAU, 2011)	42.85	42.85	42.85	42.85
T9 : PGPR Mix – II @ 2.5 kg / ha	0	0	0	14.28
T10 : Absolute control	57.14	57.14	57.14	57.14

Each value represents mean of seven replications; DAS: Days after sowing; T1 –T7: Organic Package (KAU, 2009); T8: Package of practices (KAU, 2011) T9: Organic Package (KAU, 2009) + PGPR Mix – II @ 2.5 kg / ha

Overall, T2 and T9 recorded lowest disease incidence in cowpea and yield as influenced by artificial inoculation of *Rhizoctonia solani,* treatments did not show any significant differences. In the case of *Pythium aphanidermatum* inoculated plants, biofilm based TCH (*Trichoderma* sp.) + BCH (*Bacillus* sp.) (T2) performed better with respect to early germination , plant height, number of leaves , minimum days taken for flowering (52.71), fresh weight (63.71 g plant^{-1}) and dry weight of plants. The artificial inoculation with *Pythium aphanidermatum* did not show any disease incidence throughout the experimental period. There were no significant differences among the treatments with respect to yield (Table 6). However, the highest yield was recorded in T_9 (PGPR Mix- II) followed by T_6 (*Trichoderma* sp + *Bacillus* sp.) with 49 g plant^{-1}. The lowest yield was recorded in control with 39.71 g plant^{-1}. In general, the biofilm based inoculant comprising of TCH (*Trichoderma* sp.) + BCH (*Bacillus* sp.) (T_2) was the most promising treatment for the management of both collar-rot and root-rot along with growth enhancement in cowpea. Microorganisms associated with plants generally protect the hosts against the pathogen. However, there is a need to use the combination of consortia of microorganisms having different functional attributes. In the present studies, biofilm based consortia inoculant of *Trichoderma* sp. (TCH) and *Bacillus* sp. (BCH) was the most promising treatment. These results are in agreement with earlier reports where Anabaena- *Bacillus subtilis* biofilm treatment recorded significantly higher plant and soil nutrient parameters in cotton due to useful traits beneficial for effective multiple nutrient and pest management. It has also been reported that combination of strains of bacteria/fungal antagonists are more efficient in biocontrol than monocultures which is in agreement with present study.

Table 6. Effect of different treatments on the yield of cowpea after challenge inoculation with *Pythium aphanidermatum* (30 DAS) under pot culture experiment

Treatments	**Yield at harvest (g plant^{-1})**	**Projected yield (t ha^{-1})**
T1: Biofilm based *Trichoderma* sp. (TPZ) + *Bacillus* sp.	47.00^{bc}	3.48
T2: Biofilm based *Trichoderma* sp.(TCH) + *Bacillus* sp.	47.42^{bc}	3.51
T3: Biofilm based *Trichoderma* sp. (TMT) + *Bacillus* sp.	45.28^{c}	3.35
T4: *Bacillus* sp. (KAU ref.culture)	44.28^{c}	3.27
T5: *Trichoderma* sp. (KAU ref.culture)	45.85^{bc}	3.39
T6: *Bacillus* sp. + *Trichoderma* sp.	49.00^{ab}	3.69
T7: Carbendazim + Mancozeb (2 g / l as soil drenching)	44.85^{c}	3.32
T8: Package of practices (KAU, 2011)	44.14^{c}	3.26
T9: PGPR Mix – II @ 2.5 kg / ha	51.71^{a}	3.83
T10: Absolute control	39.71^{d}	2.94
CD (5 %)	3.63	-

T1 –T7: Organic Package (KAU, 2009); T8 : Package of practices (KAU,2011); T9 : Organic Package (KAU, 2009) + PGPR Mix – II @ 2.5 kg / ha

Population of Trichoderma and Bacillus at the time of flowering and final harvest

The maximum population of *Trichoderma* sp. at the time of flowering was recorded in T1 (*Trichoderma* sp. (TPZ) + *Bacillus* sp (BPN) (46.2 × 10^4 cfu g^{-1}) followed by T_2 *Trichoderma* sp (TCH) + *Bacillus* sp. (BCH) (39.5 × 10^4 cfu g^{-1}). The lowest *Trichoderma* sp. population was recorded in T_6 (*Bacillus* sp. + *Trichoderma* sp.) (25.2 × 10^4 cfu g^{-1}). The maximum population of *Bacillus* sp. at the time of flowering was recorded in T3 (TMT *Trichoderma* sp. + BML *Bacillus* sp.) (38.1 × 10^4 cfu g^{-1}) followed by T_1 (TPZ+BPN) (35.2 × 10^4 cfu g^{-1}). The lowest *Trichoderma* sp. population was recorded in T_6 (*Trichoderma* sp + *Bacillus* sp.) (22.7 × 10^4 cfu g^{1}).At harvest, *Trichoderma* sp. population was highest in T_1 (TPZ+BPN) (3.6 × 10^2 cfu g^{-1}) followed by T_6 (*Bacillus* sp. + *Trichoderma* sp.) (3.1 × 10^2 cfu g^{-1}). The lowest *Trichoderma* sp. population was recorded in T_3 (TMT+BML) (2.1 × 10^2 cfu g^{-1}). At harvest, *Bacillus* sp. population was maximum in T_3 (4.9 × 10^2 cfu g^{-1}) followed by T_1 (4.1 × 10^2 cfu g^{-1}). The lowest *Bacillus* sp. population was recorded in T_6 (*Bacillus* sp. + *Trichoderma* sp.) (2 × 10^2 cfu g^{-1}). In the present study, the inoculated biofilm based inoculants in the potting mixture showed decline in the population of *Trichoderma* sp. from107 to 10^2 cfu g^{-1} of potting mixture at the time of harvest. However, the highest *Trichoderma* sp. was recorded in the case of biofilm based inoculant with TPZ (*Trichoderma* sp.) + BPN (*Bacillus* sp.) (T_1) followed by *Trichoderma* sp. (T_5) (3.1 x 10^2 cfu g^{-1}). Among all the treatments, biofilm based inoculants performed better than the other treatments. In the case of *Bacillus* sp., population before and after the experiment revealed that the population declined from 10^8 to 10^2 cfu g^{-1}. However, the population of biofilm based inoculants was higher than the other treatments. In general, population of inoculated microorganisms declines more/less rapidly due to introduction into a natural soil which is microbiologically undisturbed soil. The decline in the population might be due to abiotic stress factors such as soil texture, pH, temperature, moisture content and substrate availability which largely determines the survival and activity of introduced microorganisms (Gray, 1975). In the present studies, the biofilm based inoculants performed better with respect to the population of *Trichoderma* sp. It has been reported that biofilm formulations provide protection against environment stresses, antimicrobial compounds and acquisition of new genetic traits which is in agreement with the present results (Stewart, 2002; Rafique *et al*., 2015). Microbial species associated with surface and enclosed in extra cellular polymeric matrix provides enhanced survival ability to the species under adverse environmental conditions which is in agreement with the present study where the population of *Trichoderma* sp. was better in biofilm based inoculants.

Among all treatments, biofilm based *Trichoderma* sp. (TCH) + *Bacillus* sp. (BCH) was the most promising inoculant for the management of diseases and growth promotion in cowpea. The studies indicated that *Trichoderma* based *Bacillus* sp. biofilm could be a promising inoculant for the growth promotion and biocontrol of plant pathogen due to the dual attributes of the inoculants. The combination of traits for plant growth promotion and antagonistic activities are more effective than single inoculant.

However, further studies are needed to confirm the results under field conditions.

ACKNOWLEDGEMENT

The facilities and support provided by Kerala Agricultural University, Thrissur is greatly acknowledged.

REFERENCES

Cavaglieri, L., Orlando, J., Rodriguez, M. I, Chulze, S. and Etcheverry, M. 2005 Biocontrol of *Bacillus subtilis* against *Fusarium verticillioides in vitro* and at the maize root level. *Research Journal Microbiology*. **156**: 748-754.

Chen, X., Zhang, Y., FU, X., Li, Y. and Wang, Q.2016 Isolation and characterization of *Bacillus amyloliquefaciens* PG12 for the biological control of apple rot. *Post-harvest Bioligical Technology*. **115**. 113-121

Cherif, H., Silini, A., Yahiaoui, B., Ouzari, I. and Boudabous, A.2016. Phylogenetic and plant-growth-promoting characteristics of *Bacillus* isolated from the wheat rhizosphere. *Ann. Microbiol.* [DOI: 10.1007/s13213-016-1194-6].

Deka, N. 2014. Comparison of tissue culture plate method, tube method and Congo red agar method for the detection of biofilm formation by coagulase negative *Staphylococcus* isolated from non-clinical isolates. *International Journal of Current Microbiology and Applied Science*. **3**(10):8 10-8153.

Elsas, V. J. D., Kijkstra, A. F., Govaert, J. M. and Veen, V. J. A. 1986. Survival of *Pseudomonas fluorescens* and *Bacillus subtilis* introduced into two soils of different texture in field microplots. *FEMS Microbiology Ecology*. **38** .151-160.

Flemming, H. C. and Wingender, J. 2001. Relevance of microbial extracellular polymeric substances (EPSs) – Part I: Structural and ecological aspects. *Water Science Techno*logy. **43**: 1-8

Jayasinghearachchi, H. S. and Seneviratne, G. A. 2004. Bradyrhizobial *Penicillium* spp. biofilm with nitrogenase activity improves N2 fixing symbiosis of soybean. *Biology and Fertility of Soils*. **40**. 432-434.

Khare, A., Singh, B. K, and Upadhyay, R. S. 2010. Biological control of *Pythium aphanidermatum* causing damping-off of mustard by mutants of *Trichoderma viride* 1433. *Journal of Agricultural Technology*. **6**(2): 231-243.

Mathur, T., Singhal, S., Khan, S., Upadhyay, D. J, Fatma, T. and Rattan, A. 2006. Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. *Indian Journal Medical Microbiology*. **24**(1):25-29.

Mota, F. F., Gomes, E. A. and Seldin, L. 2008. Auxin production and detection of the gene encoding for the auxin efflux carrier (AEC) protein in *Paenibacillus polymyxa. Journal of Microbiology*. **56**: 275-264.

Pan, S., Mukherji, R. and Bhagat, S. 2013. Evaluation of *Trichoderma* spp. against soil borne plant pathogens. *Annuals. Plant Protection Science*. **21**(1): 176- 223.

Satish, L., Uday, B., Lodha, S. and Burman, U. 2000. Efficacy of composts on nitrogen fixation, dry root rot (*Macrophomina phaseolina* intensity and yield of Legumes. *Indian Journal of Agricultural Science*. **70**:846-849.

Timmusk, S., Grantchavora, N., Gerhart, E. and Wanger, H. 2005.*Paenibacillus polymyxa* invades plant roots and forms biofilms. *Applied and Environmental Microbiology*. **71**: 343-347.

Triveni, S., Prasanna, R., Shukla, L. and Saxena, A.K. 2012. Evaluating the biochemical traits of novel *Trichoderma*-based biofilms for use as plant growth promoting inoculants. *Annuals of Microbiology*.10.

Warmink, J., A, Nazir, R., Corten, B. and Van Elsan, E. D.2011. Hitchhikers on the fungal highway: the helper effect for bacterial migration via fungal hyphae. *Soil Biology and Biochemistry*. **43**: 760-765.

Webb, J. S., Thompson, L. S., James, S., Charlton, T., Tolker-Neilsen, Koch B., Givskov, M. and Kjelleberg, S. 2003.Cell death in *Pseudomonas aeruginosa* biofilm development. *Journal of Bacteriology*. **185**: 4585-4592

Vinaykumar, B. and Surendra Gopal*, K.
Department of Agricultural Microbiology, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India.

***Corresponding author**
ks.gopal@kau.in