

Original Research Article

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Evaluation of Bio-controlling Agents against Potato Foliar Pathogens

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ABSTRACT

Bio-controlling of potato foliar pathogens could be an alternative and eco-friendly management. Therefore, in this trial, the antagonistic potential of some bio-controlling agents (BCAs) of *Trichoderma* spp. (*Trichoderma asperillum*, *Trichoderma longibrachiatum* and *Trichoderma harzianum*) and *Bacillus* spp. (*Bacillus cereus*, *Bacillus siamensis*, *Bacillus amyloliquefaciens*, *Bacillus safensis*, *Bacillus subtilis*, *Bacillus flexus* and *Bacillus megaterium*) were assessed against six foliar pathogens of potato origin (*Alternaria solani*, *Alternaria alternata*, *Phomaexigua*, *Curvularia lunata*, *Bipolaris sorokiniana* and *corynospora cassicola*) through *in vitro* and *in vivo* trials. *Trichoderma harzianum* and *Bacillus subtilis* were better than other BCAs against the pathogens. The overall inhibition was 39.2-79.2% with *Trichoderma harzianum* and 26.7-70.7% with *Bacillus subtilis*. Under glasshouse condition, the infection was best controlled with *Trichoderma harzianum* soil treatment @ 10 g/kg + *Bacillus subtilis* seed treatment @ 10 g/kg following foliar application of pathogen @45 days of planting. From this, it was concluded that BCAs could effectively be used for controlling the infection of potato foliar diseases. Hence it is recommended for sustaining potato farming. Further study on validation of above findings through location specific field trials is recommended.

Keywords

Potato disease,
Foliar fungi, Bio-
control,
Trichoderma sp,
Bacillus sp.

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Introduction

Potato (*Solanum tuberosum* L.) is a staple food crop for millions of people to fight against malnutrition and hunger. But its worldwide annual production is reducing due to diseases of bacterial, fungal, viral and physiological kind (Kehr *et al.*, 1964). Foliar pathogens are of greatest concern among these diseases. Foliar pathogens affect the

yield through hampering the photosynthetic ability of the leaves (Rotem, 1994). Among the foliar diseases, early blight is most serious and devastating; it lends both quantitative and qualitative loss (CIP, 1996). Besides potato, it affects but also tomato, chilli, eggplant and many other cultivated and wild plants. Early blight occurs due to *Alternaria solani* and *A. alternata* which are air-borne microbes with wide host range (Pandey and Vishwakarma,

1998). Early blight appears as dark brown to black concentric rings on leaves, which later produces a target board effect. They are difficult to control and presently few cultivars possess resistance against these pathogens. Early blight occurs at all potato growing areas, but its significance could notice only in warm and wet weather when the pathogens multiply faster and spread rapidly (Hausladen and Leiminger, 2011).

The other important foliar diseases occur due to *Phomaexigua*, *Curvularia lunata*, *Bipolaris sorokiniana* etc. *Phoma* lends 20% loss particularly during *Kharif* season (Gupta, 2007) and *C.lunata* causes 16% loss through foliar necrosis. *B. sorokiniana* affects many other crops besides potato. Therefore, it is of utmost importance to control these pathogens to sustain the potato production.

The use of chemical fungicides reduced the infection level (Djéballi and Belhassen, 2010), but chronic treatment with these fungicides lead to the emergence of resistant strains. In addition, the use of these chemical fungicides is costly for farmers, human health and environment (Vurro and Gressel, 2006). As a consequence, it is discouraged. Recently, the trend is diverted towards biological measures (Mishra and Singh, 2012).

In biological measures, new or resident living organisms are purposefully used to suppress the activity of pathogens by direct/indirect manipulation of reproduction of microorganisms (Pal and Gardener, 2006). A number of bio-controlling agents (BCAs) are available. But *Trichoderma* sp. and *Bacillus* sp. are the most promising because of its wide host range and environmental conditions (Chen *et al.*, 1983). Therefore, the present study has been undertaken on the efficacy of BCAs of *Trichoderma* sp. and *Bacillus* sp. against pathogenic foliar fungi of potato origin.

Materials and Methods

The study was conducted in the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, West Bengal. For routine phytopathological and analytical works, standard literatures were followed.

The test pathogens namely *A. solani*, *A. alternata*, *P. exigua*, *C. lunata*, *B. sorokiniana* and *C. cassicola* were isolated from potato leaves having the disease symptoms through tissue segment method (Rangaswami, 1958). The morphological identities of the isolated fungi were confirmed using the text of Booth and Sutton (1984) and Chowdhry *et al.*, (2000). Reproducibility of disease reaction/virulence by the isolates was confirmed following the detached leaflet technique (Foolad *et al.*, 2000) on potato cultivar var. *Kufri Chandramukhi*.

BCAs used were *Trichoderma asperillum*, *Trichoderma longibrachiatum*, *Trichoderma harzianum*, *Bacillus cereus*, *Bacillus siamensis*, *Bacillus amyloliquefaciens*, *Bacillus safensis*, *Bacillus subtilis*, *Bacillus flexus* and *Bacillus megaterium* among the spp. They were procured from Indian Institute of Oil Seed Research (IIOR), Telangana. *Trichodermas* spp. were sub-cultured in PDA and preserved at 5⁰C. *Bacillu* spp. were sub-cultured in NAS following the aseptic technique. The cultures were renewed at 10 days interval to maintain the purity and potency.

The antagonistic potential of *Trichoderma* against the test pathogens was assessed through the dual culture technique (Morton and Straube, 1955). Both pathogen and *Trichoderma* were belonging to same age while testing. 6 mm diameter blocks of the pathogen and *Trichoderma* were inoculated at the same time on the opposite sides of the

PDA in petriplates (9 cm dia.). Then, the plates were incubated at $28\pm 1^{\circ}$ C for 8 days. In each test, a control plate was maintained to compare the result. The antagonistic ability of *Trichoderma* was assessed on the modified Bell's scale (Bell *et al.*, 1982). The hyphal interactions were assessed by growing them on the cellophane membrane placed over the solidified PDA (Dennis and Webster, 1971). Both the fungi when came into contact to each other, the contact zone was cut using sterile scalpel and taken out along with the cellophane. Then, it was gently washed with sterile distilled water, mounted under 0.1% lactophenol cotton blue over a clean glass slide and observed under a microscope. The hyphal interaction was photographed.

For *in-vitro* assessment of *Bacillus* spp., sterile PDA was poured into the sterilized petri-plates. After solidification of the medium, a loop of 24-48 hrs, old culture was taken from slants and streaked on one side of the plate. Fungal plugs were carefully placed on the opposite side of the bacterial streak. Both the bacteria and fungi of same age were used. Incubation was done in a BOD incubator at $30\pm 2^{\circ}$ C for 3-4 days. The length of fungal and bacterial growth and zone of inhibition was measured using a scale (mm). In each test, one control plate was maintained for comparison.

After *in vitro* assessment, the BCAs were evaluated under glasshouse condition in polythene bags (30 x15 cm) against *Alternaria* sp. following Thilagavathi *et al.*, (2007) and Abeysinghe (2009). Briefly, a talc-based formulation was first prepared. For seed treatment, the tubers were mixed with the formulation (@10 g/kg of seed) and shed-dried (Nandakumar *et al.*, 2001). For soil treatment, the talc-based formulation was mixed with soil (@10 g/kg). And then seed tubers hand dipped into each polythene bag. The plants were watered daily @ 50 ml/ bag.

The design of experiment followed was completely randomized block design (CRBD) with two replicates for each combination. The percent disease index (PDI) was calculated following Mayee and Datar (1986).

Results and Discussion

Antagonistic potential of bio-control agents

All *Trichoderma* spp. showed antagonistic effect on potato foliar fungi, that is- *A. alternata*, *A. solani*, *C. cassicola*, *C. lunata*, *B. sorokiana* and *P. exigua* (Plate-1). The inhibition was varied from 54-72% in *A. alternata* (Figure 1a). Maximum inhibition (72%) has shown by *T. harzianum*, followed by *T. asperillum* (*viridae*) and *T. longibrachiatum*. The inhibition was 49.2-76% in *A. solani* (Figure 1b). Maximum inhibition (76%) was shown by *T. harzianum*, followed by *T. longibrachiatum* (56.0 %) and *T. asperillum* (49.2 %). The inhibition was 39.2-71.2% in *C. lunata* (Figure 1c). Maximum inhibition was shown by *T. harzianum* (71.2%) followed by *T. longibrachiatum* and *T. asperillum*. The inhibition was 74.0-79.2% in *C. cassicola* (Figure 1d). Maximum inhibition was shown by *T. harzianum* (79.2%) followed by *T. asperillum* (*viridae*) and minimum (74.0%) by *T. longibrachiatum*.

The inhibition was 41.2-59.2% in *B. sorokiniana* (Figure 1 e). Maximum inhibition was shown by *T. harzianum* (54.9%) followed by *T. longibrachiatum* and *T. asperillum*. The inhibition rate was 67.2-79.2% in *P. exigua* (Figure 1f). Maximum inhibition was shown by *T. harzianum* (79.2%) followed by *T. longibrachiatum* and *T. asperillum*. The direct mycoparasitic activity of *Trichoderma* is one of the major mechanisms involved in this inhibition effect (Bruce *et al.*, 1995; Haran *et al.*, Pandey (2010).

Similarly, all *Bacillus* BCAs such as *B. cereus*, *B. siamensis*, *B. amyloliquefaciens*, *B. safensis*, *B. subtilis*, *B. flexus* and *B. megaterium* showed antagonistic effect on the test pathogens, i.e., *A. alternate* (Plate-2.a), *A. solani* (Plate-2.b), *C. lunata* (Plate-2.c), *C. cassicola* (Plate-2.d), *B. sorokiniana* (Plate-2.e) and *P. exigua* (Plate-2.f) during *in vitro* assessment. The inhibition was 27.45-52.72% in *A. alternate* (Figure 2a), 26.75-56.60%, in *A. solani* (Figure 2b), 34.83-66.02% in *C. lunata* (Figure 2c), 48.30-68.51% in *C. cassicola* (Figure 2d), 29.46-45.53% in *B. sorokiniana* (Figure 2e) and 56.55-70.75%, in *P. exigua* (Figure 2f). This corroborated the findings of Souja *et al.*, (2014) and Abdallah *et al.*, (2015). This inhibitory effect could be attributed to secretion of hydrolytic enzymes (Fujimoto and Kupper, 2016.), peptide antibiotics (Mannanov and Sattarova, 2001), mycosubtilin, and zwittermicin (Pal and Gardener, 2006), volatile extracellular metabolites (Podile *et al.*, 1987), mycosubtilin, and zwittermicin (Pal and Gardener, 2006). Maximum inhibition shown by *B. subtilis* was due to secretion of Fengycin and bacillomycin (Cao *et al.*, 2011) and by *B. amyloliquefaciens* was due to g-polyglutamic acid synthesis (Liu *et al.*, 2010).

During the *in vitro* assessment, *T. harzianum* and *B. subtilis* were better than other BCAs against the pathogens in terms of inhibition of mycelial growth. Thus they were assessed under glass condition in various combinations against *Alternaria* sp. following the foliar application of pathogen at 45 days after planting (DAP). The magnitudes of PDI and crop yield were varied from treatment to treatment (Table 1, Plate 3). PDI was 8.5% with *T. harzianum* soil treatment @ 10 g/kg + seed treatment with *B. subtilis* @ 10 g/kg, 11.1% with seed treatment with *T. harzianum* @ 10 g/kg + soil treatment with *B. subtilis* @ 10 g/kg, 12.0% with soil treatment with *T. harzianum* @ 10 g/kg, 12.2% in healthy plant

with no treatment, 13.4% with seed treatment with *B. subtilis* @ 10 g/kg, 15.5% with seed treatment with *T. harzianum* @ 10g/kg and 16.4% with *B. subtilis* soil treatment @ 10 g/kg + foliar application of pathogen when compared with 20.4% in healthy plant with disease inoculation. The yield was 125.1 g/pot with *T. harzianum* soil treatment @ 10 g/kg+ seed treatment with *B. subtilis* @ 10 g/ kg, 95.15 g/pot with seed treatment with *T. harzianum* @ 10 g/kg + soil treatment with *B. subtilis* @ 10 g/kg, 92.80 g/pot with soil treatment with *T. harzianum* @ 10 g/kg, 88.65 g/pot with seed treatment with *B. subtilis* 10 g/kg, 71.30 g/pot with soil treatment with *T. harzianum* @ 10 g/kg, 71.30 g/pot with seed treatment with *T. harzianum* @ 10g/kg and 82.60 g/pot in a plant with in treatment (negative control) when compared with the yield of 62.60g/pot in plant with disease inoculation (positive control). This indicated that the *T. harzianum* soil treatment + *B. subtilis* seed treatment is most effective against the infection of *Alternaria* sp. This corroborated the findings of Suleiman *et al.*, (2016) and Rani *et al.*, (2017).

Seed treatment with *B. subtilis* has reduced the disease outbreak through microbial competition, antibiosis, hyper parasitism and systemic acquired resistance in the host plants (Hoitink *et al.*, 2001). BCAs have remarkable multiplication capability, thus, when the tubers treated with them, it multiplied in the exponential ratio and formed thick walled spores around the tubers to overcome with the stress from the pathogens (Bharath *et al.*, 2005). Further it promoted crop growth and yield through increased uptake of nutrients and stimulation of growth of the promoting factors such as IAA and GA₃ and reduction of levels of enzymes owing to colonization of roots (Idris *et al.*, 2007; Abeysinghe, 2009).

In the light of above results, the study could be concluded that the foliar pathogens could

be controlled using the BCAs of *Trichoderma* and *Bacillus*. *T. harzianum* and *B. subtilis* are the best BCAs against the potato foliar pathogens. During *in vitro* condition, following foliar application of pathogen @ 45 DAP, *T. harzianum* soil treatment @ 10 gm/kg + *B. subtilis* seed treatment @ 10

gm/kg + is best against the emergence of *Alternaria* sp. From this, it is suggested for wide use against the infection of potato foliar pathogens for sustainable potato production. Further study is recommended for validation of above findings through more location specific field trials.

Table.1 Effect of BCAs on *Alternaria* sp infection during glass house condition

Treatments	Combinations	PDI (%)	Decrease in PDI over disease control (%)	Yield (g/pot)	Increase in yield over disease control (%)
T1	Healthy plant + No treatment (Negative control)	12.2	40.77	82.60	31.9
T2	Healthy plant + Disease inoculation (positive control)	20.6	-	62.60	-
T3	Seed treatment with <i>T. harzianum</i> @10g/kg + foliar application of pathogen at 45 DAP	15.5	24.74	71.30	13.89
T4	Soil treatment with <i>T. harzianum</i> @10 g/kg + foliar application of pathogen at 45 DAP	12.0	41.74	92.80	48.24
T5	Seed treatment with <i>B. subtilis</i> 10 g/kg + foliar application of pathogen at 45 DAP	13.4	34.95	88.65	41.61
T6	<i>B. subtilis</i> soil treatment @ 10 g/kg + foliar application of pathogen at 45 DAP	16.4	20.38	70.55	12.69
T7	Seed treatment with <i>T. harzianum</i> @ 10 gm/kg + soil treatment with <i>B. subtilis</i> @ 10 gm/kg + foliar application of pathogen at 45 DAP	11.1	46.11	95.15	51.99
T8	<i>T. harzianum</i> soil treatment @ 10 g/kg + Seed treatment with <i>B. subtilis</i> @ 10 g/kg + foliar application of pathogen at 45 DAP	8.5	58.73	125.1	99.68
	SEm ±	1.32	4.59	6.92	10.42
	CD (p=0.05)	4.41	15.36	23.13	34.85

Plate.1a The antagonistic potential of *T. harzianum* (A), *T. asperillum* (B) and *T. longibrachiatum* (C) against *A. alternata*(1) and *A. solani*(2)

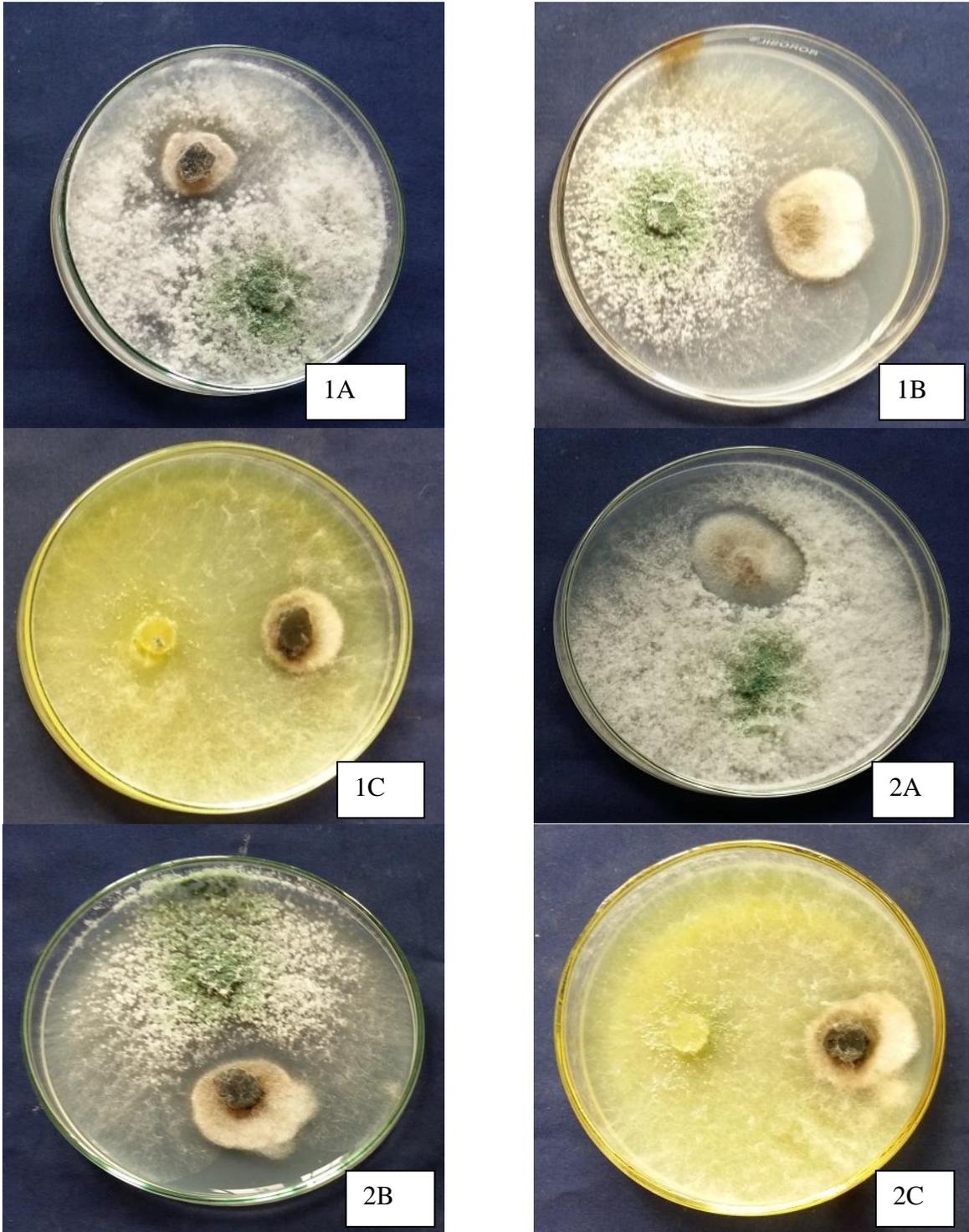


Plate.1b The antagonistic potential of *T. harzianum* (A), *T. asperillum* (B) and *T. longibrachiatum* (C) against *C. cassicola* (3) and *C. lunata* (4)

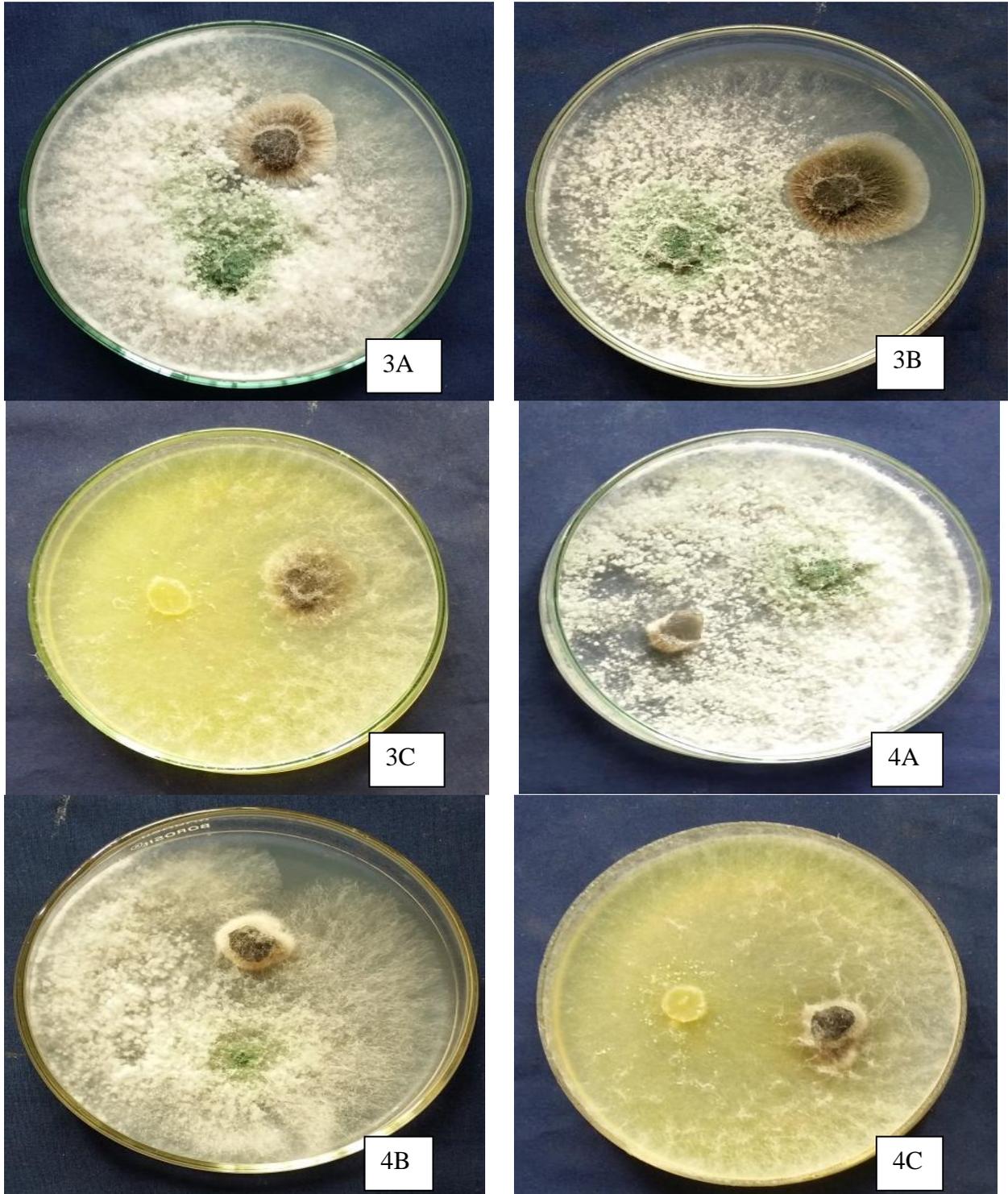


Plate.1c The antagonistic potential of *T. harzianum* (A), *T. asperillum* (B) and *T. longibrachiatum* (C) against *B. sorokiniana* (5) and *P. exigua* (6)

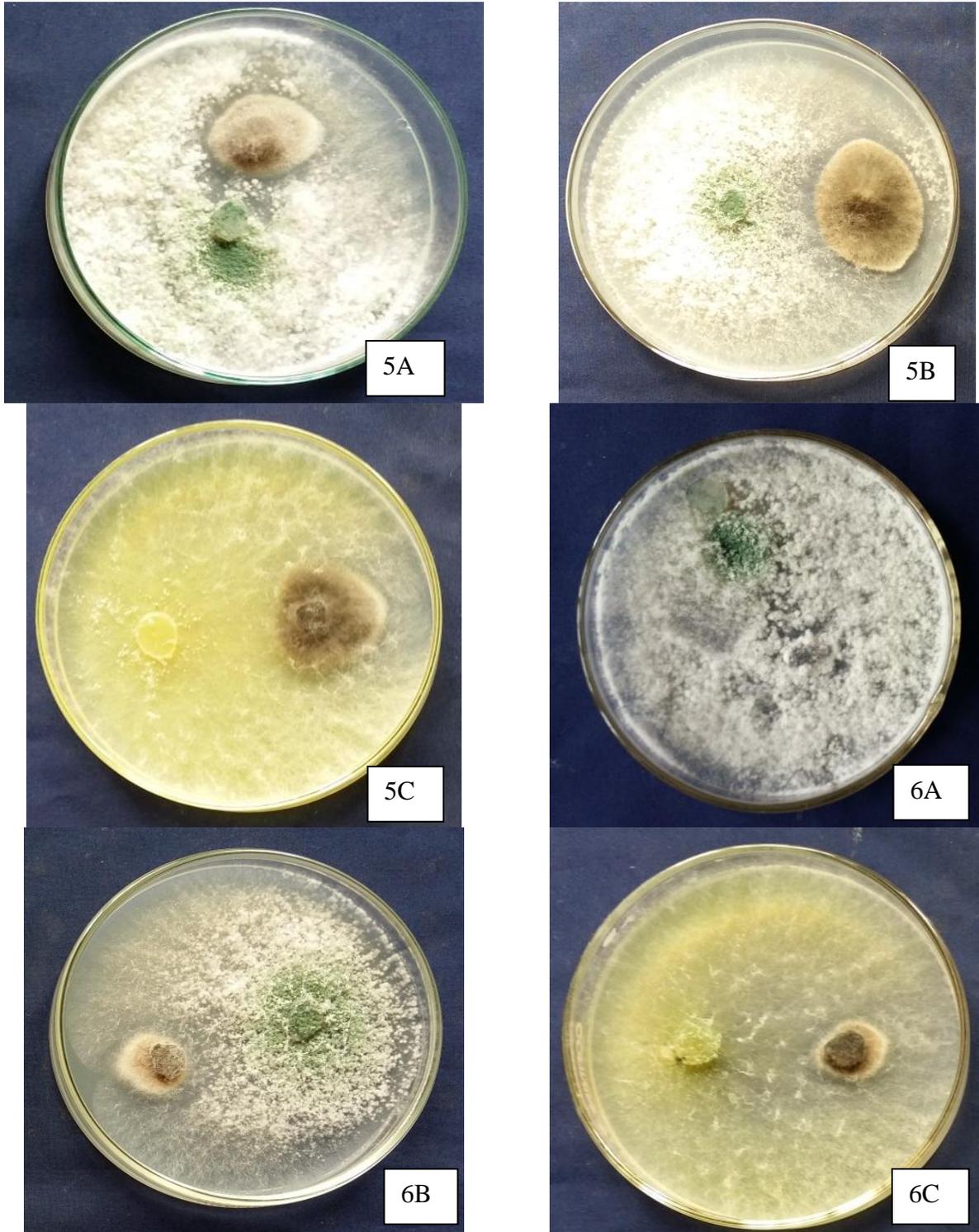


Plate.2a (A-G) Antagonistic effect of *B. flexus* (A), *B. cereus* (B), *B. amyloliquefaciens* (C), *B. megaterium* (D), *B. subtilis* (E), *B. safensis* (F), *B. siamensis* (G) against *A. alternata*

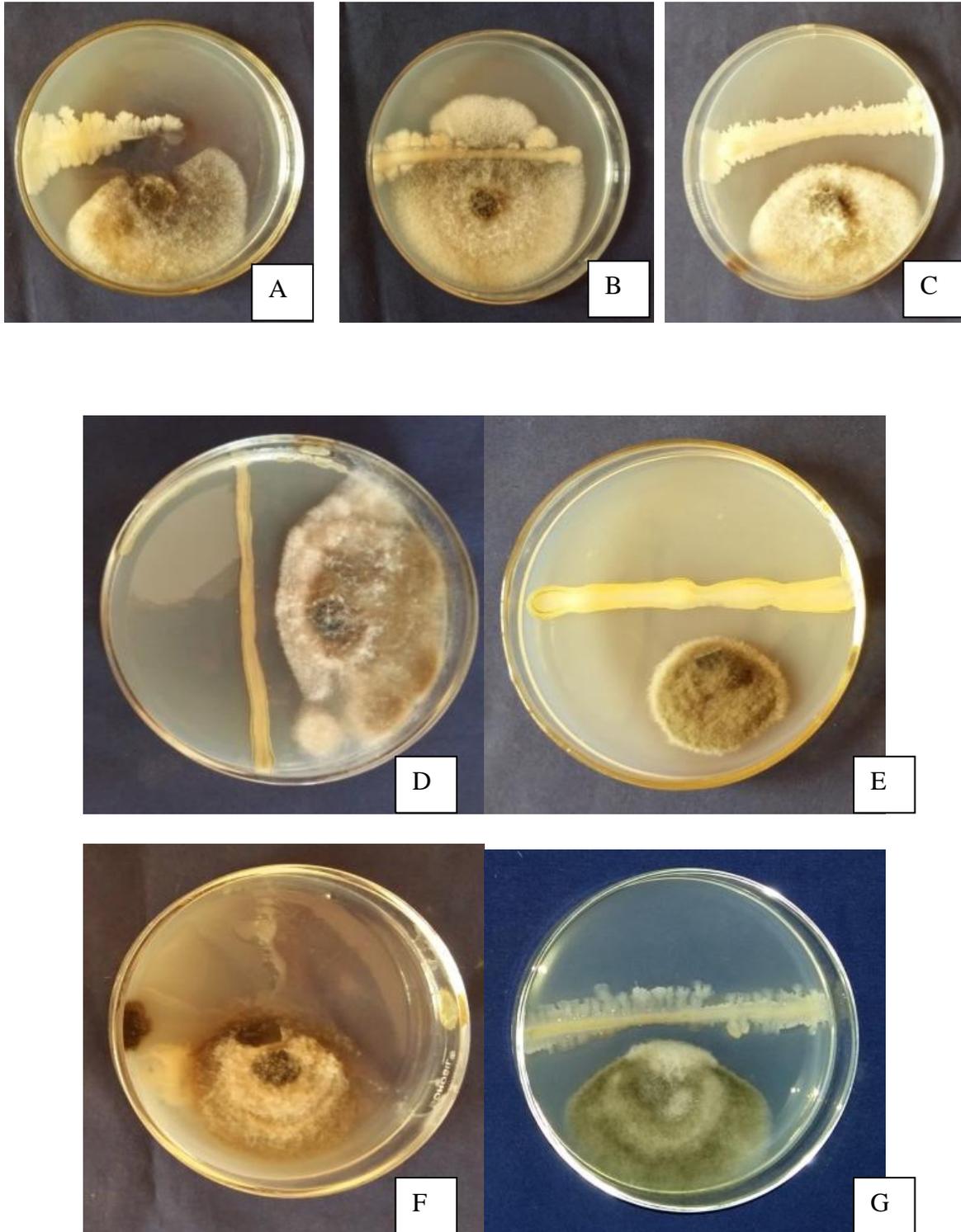


Plate 2b Antagonistic effect of *B. flexus* (A), *B. cereus* (B), *B. amyloliquefaciens* (C), *B. megaterium* (D), *B. subtilis* (E), *B. safensis* (F), *B. siamensis* (G) against *A. solani*

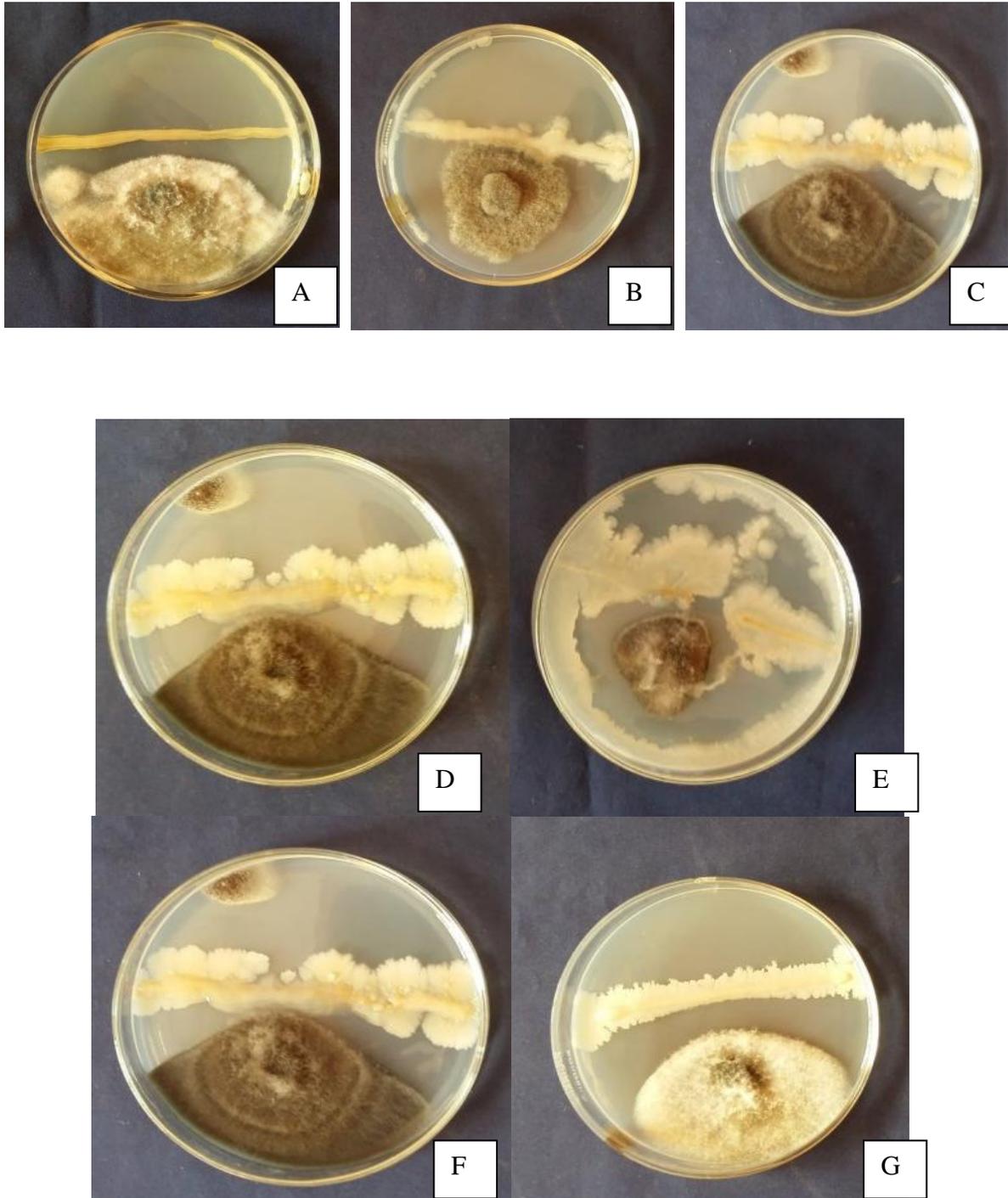


Plate 2c Antagonistic effect of *B. flexus* (A), *B. cereus* (B), *B. amyloliquefaciens* (C), *B. megaterium* (D), *B. subtilis* (E), *B. safensis* (F), *B. siamensis* (G) against *C. lunata*

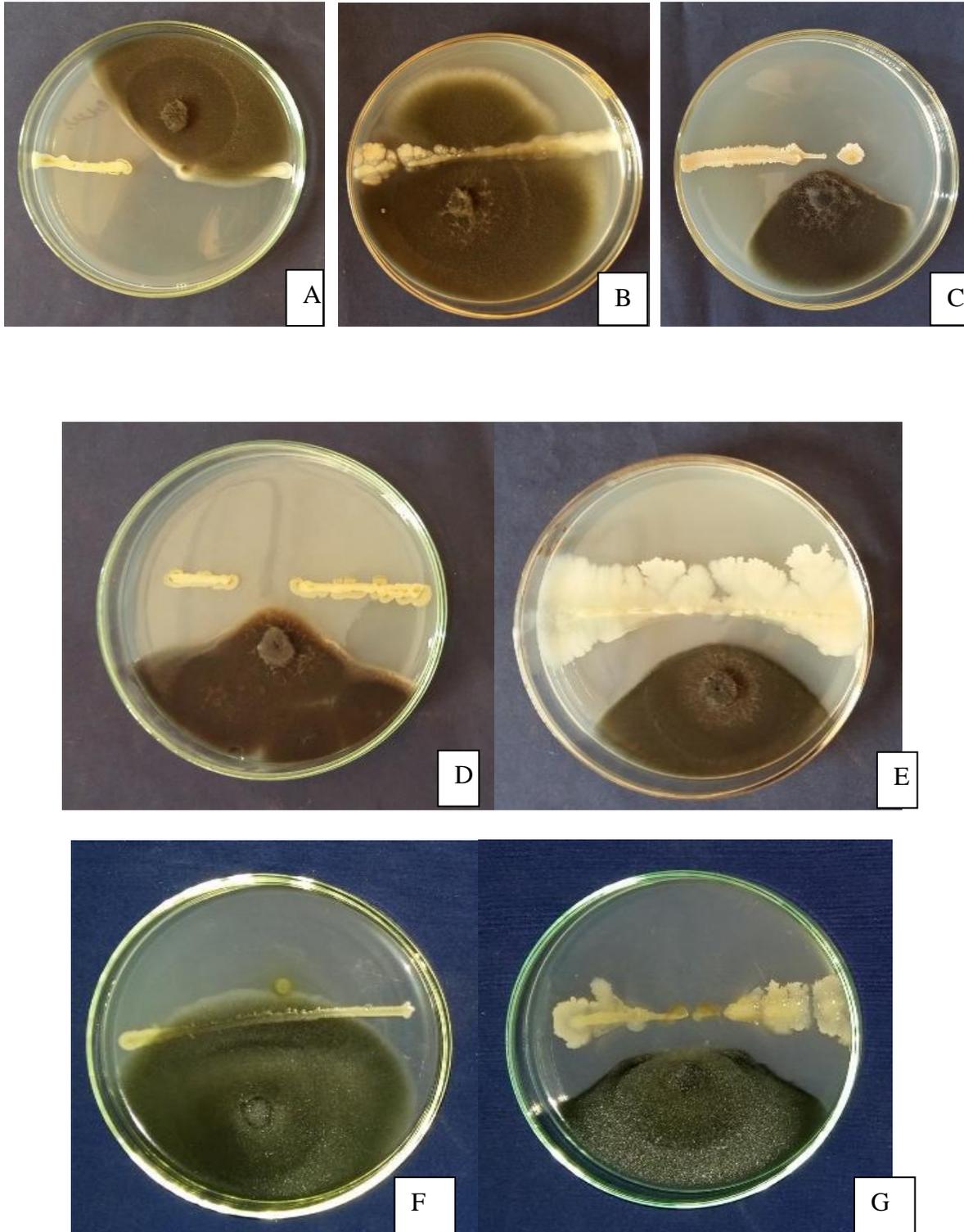


Plate 2d Antagonistic effect of *B. flexus* (A), *B. cereus* (B), *B. amyloliquefaciens* (C), *B. megaterium* (D), *B. subtilis* (E), *B. safensis* (F), *B. siamensis* (G) against *C. cassicola*

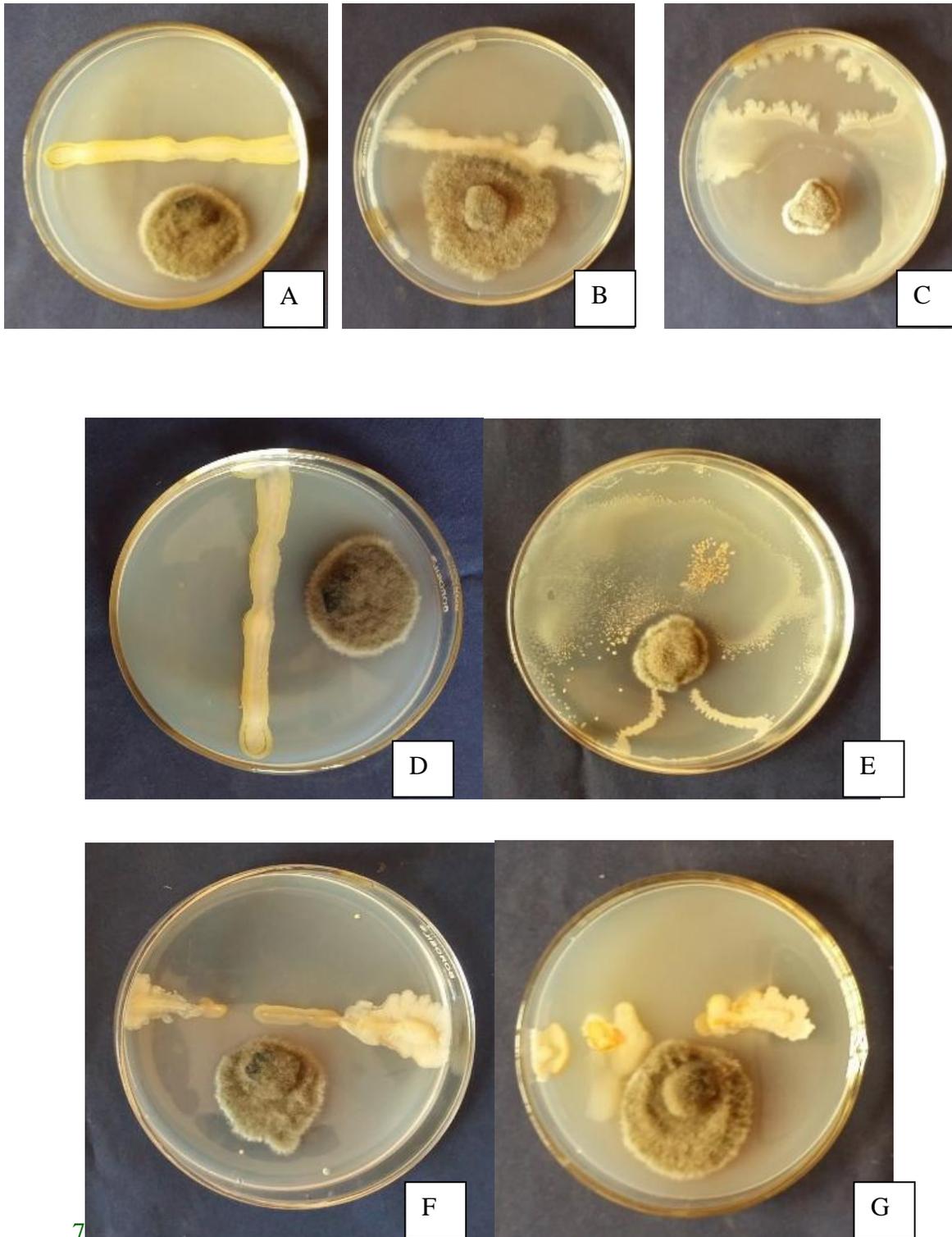


Plate 2e Antagonistic effect of different *B. flexus* (A), *B. cereus* (B), *B. amyloliquefaciens* (C), *B. megaterium* (D), *B. subtilis* (E), *B. safensis* (F), *B. siamensis* (G) against *B. sorokiniana*

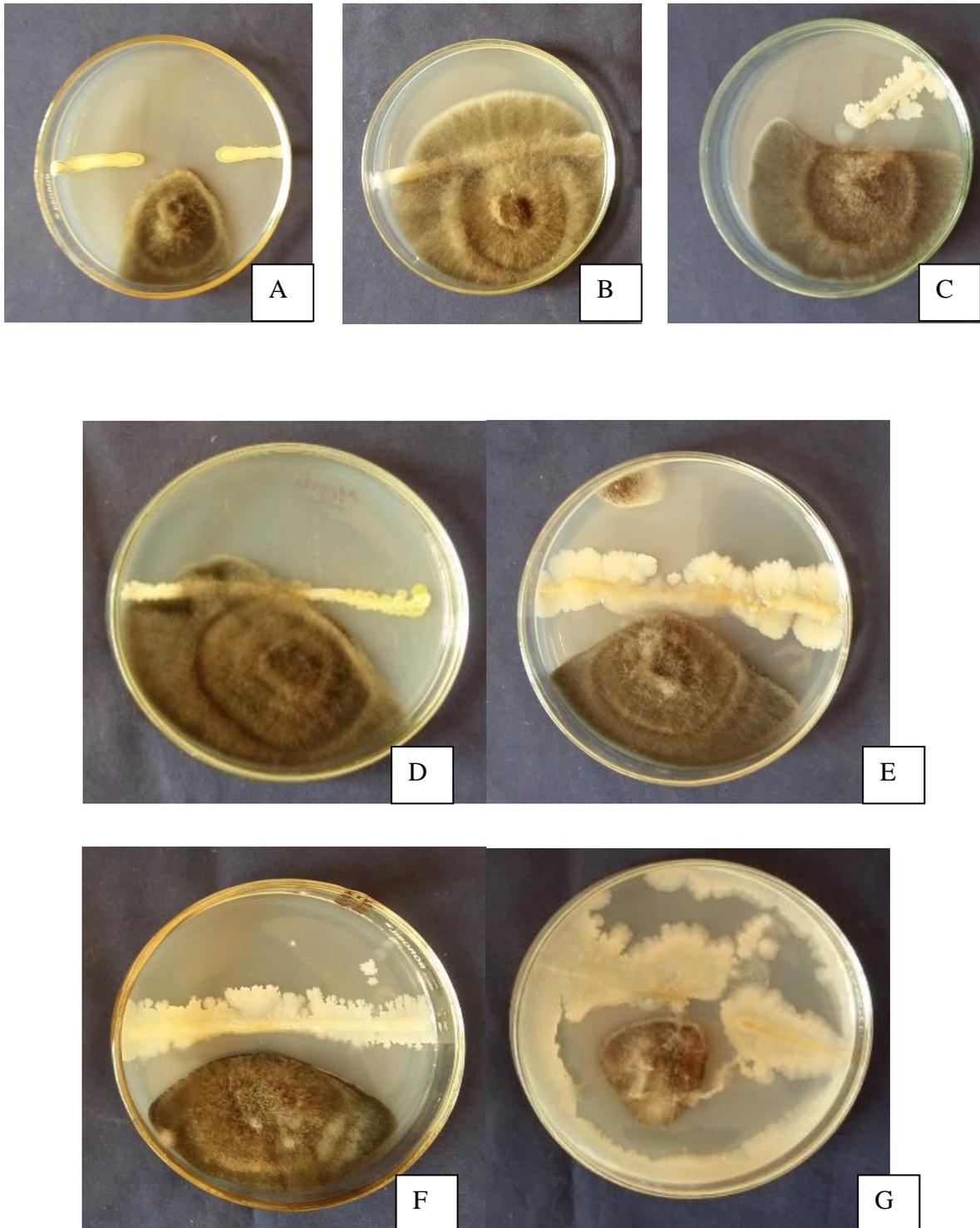


Plate 2f Antagonistic effect of *B. flexus* (A), *B. cereus* (B), *B. amyloliquefaciens* (C), *B. megaterium* (D), *B. subtilis* (E), *B. safensis* (F), *B. siamensis* (G) against *P. exigua*

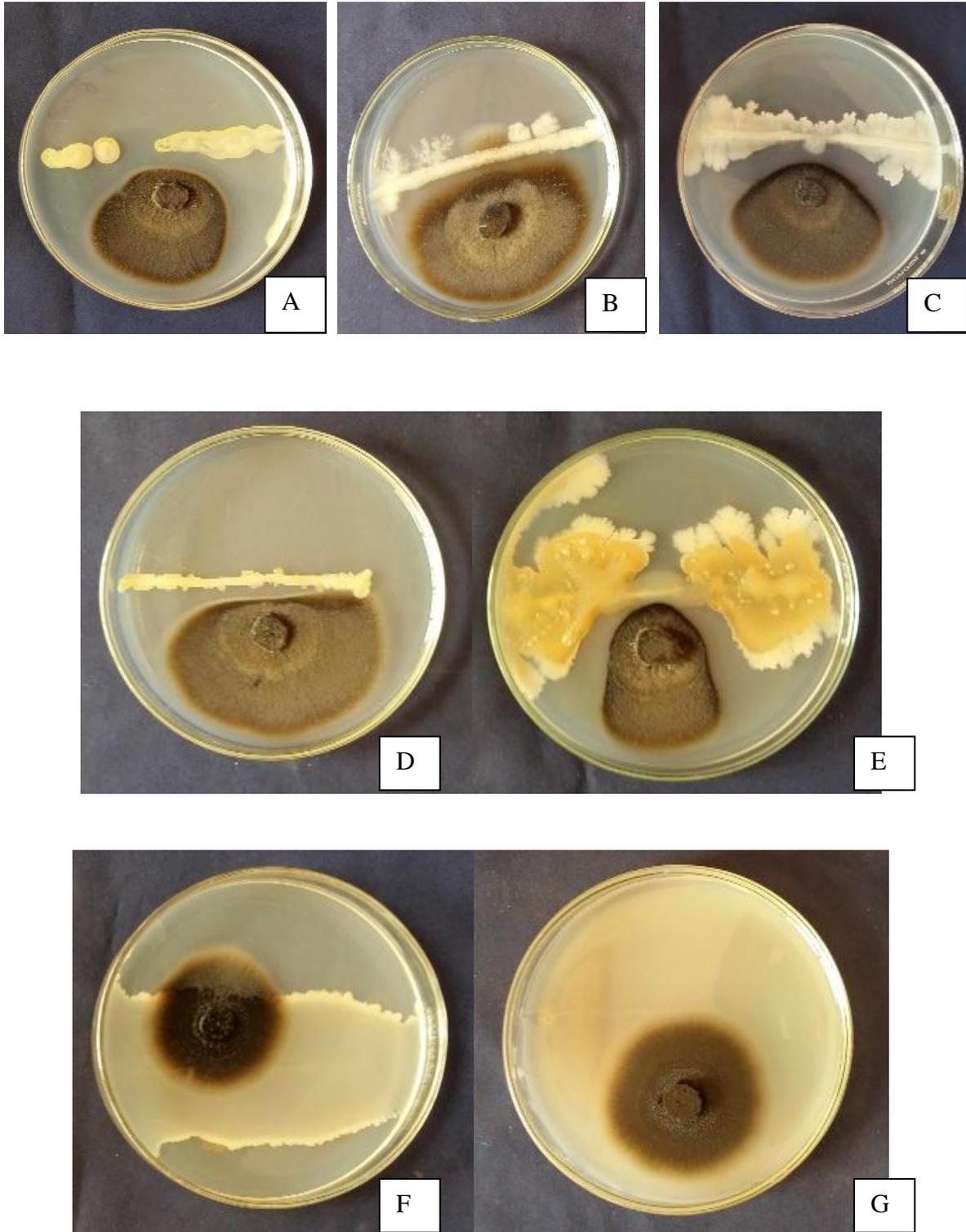


Plate.3 Glass house experiment





T



T6

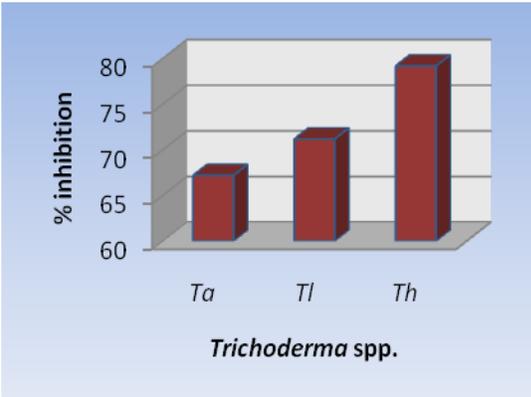


T7

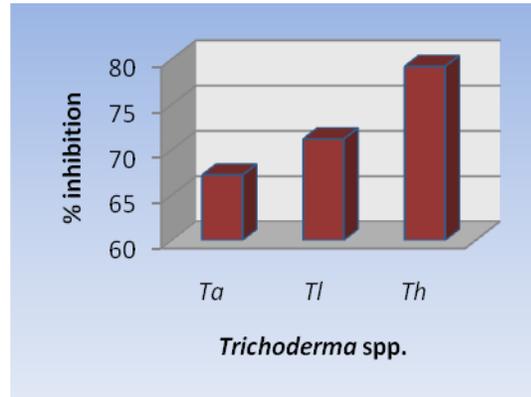


T8

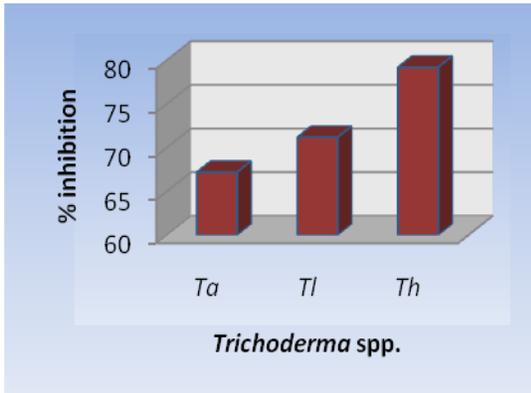
Fig.1 (a-f) Figures showing percentage inhibition in different potato foliar pathogens using *Trichoderma* spp.



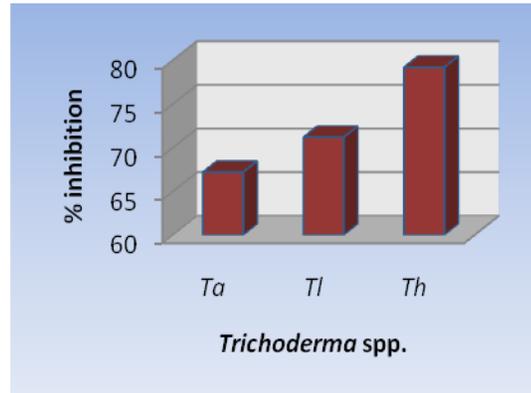
a. Percentage inhibition in *A.alternata* using *Trichoderma* spp



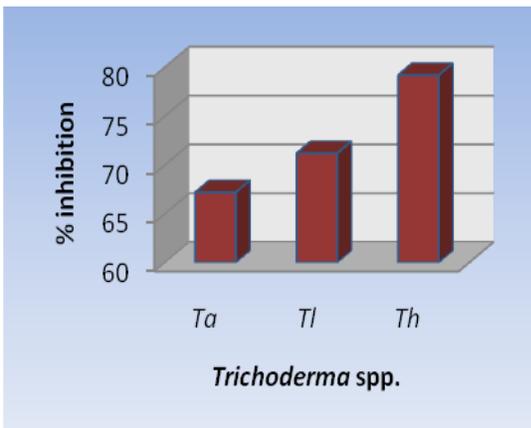
b. Percentage inhibition in *A.solani* using *Trichoderma* spp.



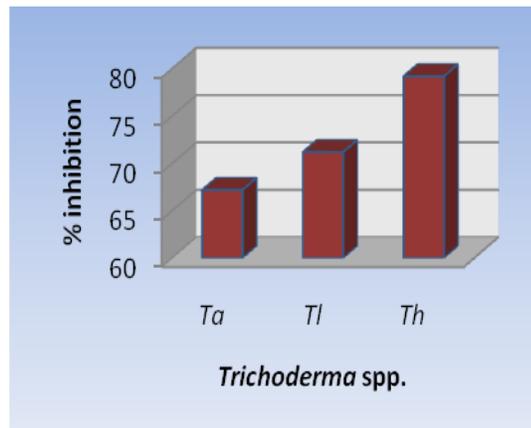
c. Percentage inhibition in *C.cassicola* using *Trichoderma* spp.



d. Percentage inhibition in *C.lunata* using *Trichoderma* spp.

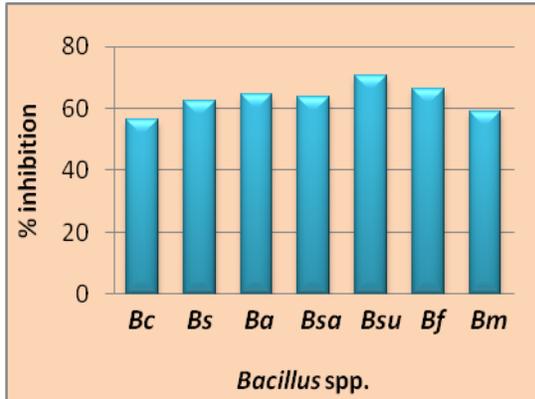


e. Percentage inhibition in *B.sorokiniana* using *Trichoderma* spp.

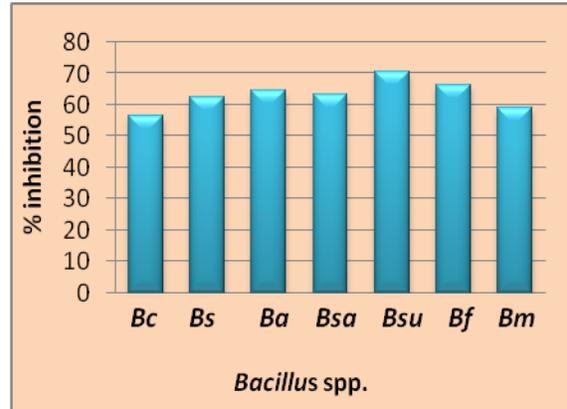


f. Percentage inhibition in *P.exigua* using *Trichoderma* spp.

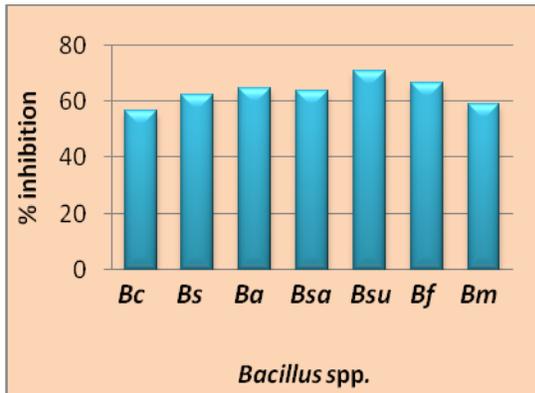
Fig.2 (a-f) Figures showing percentage inhibition in different potato foliar pathogens using *Bacillus* spp.



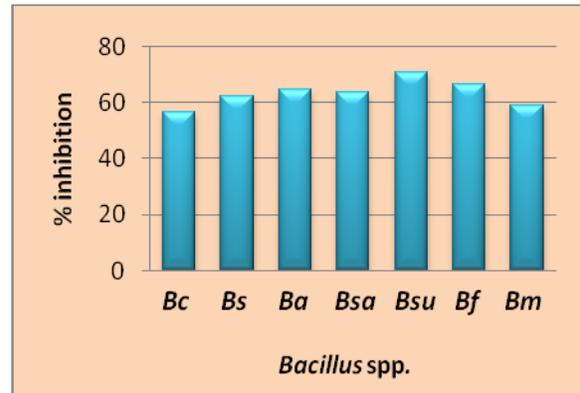
a. Percentage inhibition in *A.alternata* using *Bacillus* spp.



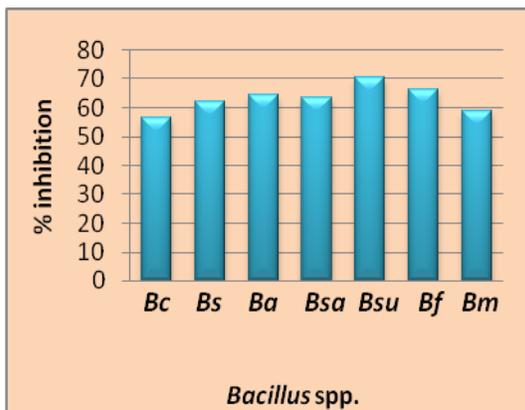
b. Percentage inhibition in *A.solani* using *Bacillus* spp.



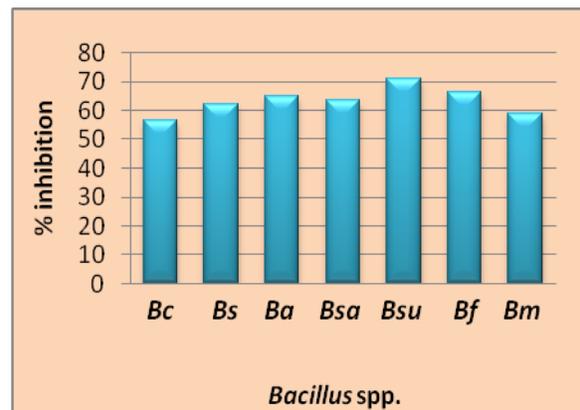
c. Percentage inhibition in *C.lunata* using *Bacillus* spp.



d. Percentage inhibition in *C.cassicola* using *Bacillus* spp.



e. Percentage inhibition in *B.sorokiniana* using *Bacillus* spp.



f. Percentage inhibition in *P.exigua* using *Bacillus* spp.

In conclusion, out of three *Trichoderma* spp. (*T. asperillum*, *T. longibrachiatum* and *T. Harzianum*), *T. harzianum* was the best biocontrolling agent against the foliar pathogens. The inhibition rate was 54-72% in *A. alternata*, 49.2-76% in *A. solani*, 39.2-71.2% in *C. lunata*, 74.0-79.2% in *C. cassicola*, 41.2-59.2% in *B. sorokiniana* and 67.2-79.2% in *P. exigua*. Out of seven *Bacillus* spp. (*B. cereus*, *B. siamensis*, *B. amyloliquefaciens*, *B. safensis*, *B. subtilis*, *B. flexus* and *B. Megaterium*, *B. subtilis* was best biocontrolling agent against the foliar pathogens except for *C. cassicola* and *B. sorokiniana* where *B. amyloliquefaciens* has given best result. The inhibition rate was 27.45-52.72% in *A. alternata*, 26.75-56.60%, in *A. solani*, 34.83-66.02% in *C. lunata*, 48.30-68.51% in *C. cassicola*, 29.46-45.53% in *B. sorokiniana*, 56.55-70.75% in *P. exigua*.

During *in-vivo* assessment of bio-control agents, the PDI was found 8.5% with *T. harzianum* soil treatment @ 10 gm/kg + Seed treatment with *B. subtilis* @ 10 gm/kg + foliar application of pathogen at 45 days after planting (DAP), 11.1% with Seed treatment with *T. harzianum* @ 10 gm/kg + soil treatment with *B. subtilis* @ 10 gm/kg + foliar application of pathogen at 45 DAP, 12.0% with soil treatment with *T. harzianum* @ 10 gm/kg + foliar application of pathogen at 45 DAP, 12.2% in healthy plant + no treatment (negative control), 13.4% with seed treatment with *B. subtilis* 10 gm/kg + foliar application of pathogen at 45 DAP, 15.5% with seed treatment with *T. harzianum* @ 10g/kg + foliar application of pathogen at 45 DAP, 16.4% with *B. subtilis* soil treatment @ 10 gm/kg + foliar application of pathogen at 45 DAP in comparison with 20.4% in healthy plant + disease inoculation (positive control). Thus, from this study, it is clear that combination of *T. harzianum* soil treatment @ 10 gm/kg + seed treatment with *B. subtilis* @ 10 gm/kg + foliar application of pathogen at 45 DAP is best against the potato foliar disease.

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