

## ***In vitro* Solubilization of Rock Phosphate and Biocontrol of Tea Pathogens by Microorganisms Isolated from Vermicompost**

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(Received: 15 August 2012; accepted: 20 September 2012)

Fungi isolated from vermicompost were studied for their phosphate solubilizing potential and antagonistic activity. These microorganisms were grown *in vitro* for 30 days in Synthetic liquid medium amended with rock phosphate and their phosphate solubilizing activity was determined. Rock phosphate solubilization of these isolates ranged from 27.60-67.97%. Among the test fungi, *Trichoderma viride* has shown the highest potential in solubilizing rock phosphate in the synthetic medium and solubilization of rock phosphate has been observed to be ranging from 40.50-67.97%. For antagonism study (2mm), the test organisms were grown in potato dextrose agar media at 2cm apart from the tea pathogen, *Fomes lamaroensis* and their antagonistic colony interaction were determined. *Aspergillus flavus*, *Aspergillus niger* and *Trichoderma harzianum* were found to inhibit the growth and overgrew the brown root rot of tea causing organism i.e. *Fomes lamaroensis*. *Trichoderma atroviride* and *Trichoderma* sp. showed mutual intermingling of growth while *Trichoderma viride* showed intermingling growth in which the pathogen ceased to grow and was overgrown by the antagonist. Therefore, it can be suggested that the organisms showing antagonism to the said pathogen and phosphate solubilization potential may be tried under the field condition in Barak valley where above mentioned pathogen is a serious problem to the tea plantations of the area and faster rock phosphate solubilization is a required of the tea industry at large.

**Key words:** Antagonistic activity, biocontrol *in vitro*, phosphate solubilization, Rock phosphate, *Trichoderma viride* and vermicompost.

Vermicomposts are finely divided peat-like materials with high porosity, aeration, drainage, and water holding capacity. Vermicompost is often considered as a supplement to fertilizers as it releases the major and minor nutrients slowly with significant reduction in C/N ratio, synchronizing with the requirement of plants (Pattnaik and Reddy, 2010). Vermicompost is rich in microbial diversity, population, and activity and vermicast contains enzymes such as proteases, amylases, lipases, cellulase and chitinase which continue to

disintegrate organic matter even after they have been removed (Sharma, *et. al.*, 2005). In the course of the present work, vermicompost was prepared to observe its potential as organic amendment in the soil of tea agroecosystem of Barak Valley (South Assam) in general. While exploring the potential, it was also observed whether the mycoflora isolated from the finished vermicompost have the potential for phosphate solubilization and antagonistic potential to control the difficult root pathogens of tea i.e. *Fomes lamaroensis*, so that the role of mycoflora of vermicompost in phosphate solubilization and biocontrol in the soil can be established.

Phosphate solubilizing microorganisms play a very significant role in making phosphorus available to plants by dephosphorilating

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phosphorus bearing organic compounds and also by bringing about favourable changes in soil reaction in the soil micro-environment leading to solubilization of inorganic phosphate sources. Many microorganisms can solubilize inorganic phosphates which are largely unavailable to plants (Chabot *et al.*, 1993). Addition of rock phosphate coupled with inoculation of phosphate solubilizing microorganisms has given good response in many crops. These microorganisms solubilize not only insoluble forms of soil phosphates and phosphatic fertilizers such as rock phosphate, bone meal, etc but also increases the efficiency of soluble forms of phosphatic fertilizers applied to soil

Antagonists are biological agents with the potential to interfere in the life process of plant pathogens. The fungal antagonists are known to be the most effective in the biological seed treatments (i.e. *Chaetomium sp.*, *Penicillium sp.* and *Trichoderma sp.* *Trichoderma* species, those have gained maximum popularity due to their highly antagonistic properties against several soil borne plant pathogens. The method of application of antagonists may be either direct inoculation to the soil or by stimulating the antagonists with the help of organic/inorganic soil amendments (Dutta and Issac, 1979 ; Deb and Dutta, 1991 ; Deb and Dutta, 1988). Biocontrol potential of *Trichoderma* spp. against cotton root rot has been indicated in number of reports ( Jakhar *et al.*, 1997; Suriachandraselvan and Aiyathan, 1988). Keeping the above in view some observations were made in the the present study on the vermicomposting, phosphate solubilizing and biocontrol potential of some fungal organisms isolated from vermicompost against a tea root pathogen *in vitro* i.e. *Fomes lamaoensis*.

## MATERIALS AND METHODS

### Vermicomposting

The earthworm seeds of *Eisenia foetida* were brought from Midpu laboratory which is situated near the Rajiv Gandhi university, Arunachal Pradesh. A thin layer of sand was spread in the lowest layer of the vermicomposting tank. Mother culture ( i.e earthworm seeds along with vermicompost) was inoculated to the vermicomposting tank. Then, chopped Guatemala grass (3cm) was spread over the layer of mother

culture. Chopped banana (2 cm) was spread over Guatemala grass layer followed by spreading of tea waste, rock phosphate and micromajic (a commercial product) respectively. Cowdung was spread over the layer of rock phosphate and micromajic. This made one complete layer of vermicomposting process. One complete layer was made in 10 days interval. Layering of one complete layer was done nine times. Mixing of materials was also done after every two weeks for quick decomposition. Water was sprinkled regularly for maintaining adequate moisture and body temperature of the earthworms. Vermicompost was harvested and spread for drying under shade.

### Isolation of microorganisms from the vermicompost

For fungal isolation, samples were collected from the vermicomposting tank. The samples were brought to the laboratory in sealed container and fungi were isolated within 24 hour of sampling. A modified soil dilution plate method (Timonin, 1940) was adopted for the isolation of soil fungi using Rose Bengal agar media (Tsao, 1964). The inoculated plates were incubated for 5-7 days at  $25 \pm 1^\circ\text{C}$  for fungi (Devi *et al.*, 2012).

### Identification of the Fungal species

Fungal species were identified with the help of standard literature (i.e Manual of the genus *Aspergillus* by K.B. Raper and D.I. Fennel, 1973, the *Penicillia* by K.B. Raper and C. Thom, Soil fungi by Gilman, 1956, Illustrated Genera of Imperfect fungi by H.L. Barnett and B.B Hunter, 1972 and Monographic contribution on *Trichoderma* by A. Nagamani, C. Manoharachary and D.K. Agarwal and P.N. Chowdhry, 2002).

### Solubilization of rock phosphate

Solubilization of Rock phosphate by fungi was studied in a synthetic liquid medium as per the method suggested by Sen and Paul (1957).

### Method for analysis of soluble phosphate

50 ml of the synthetic liquid medium was taken in each 100 ml conical flasks and sterilized for half an hour. The flasks were then inoculated with the 8 days old fungal culture grown in slants. A conical flask with the medium without any inoculum was also incubated simultaneously as a control for blank adjustment in Spectrophotometric estimation. All the flasks including control were incubated at  $25 \pm 2^\circ\text{C}$  for varying time intervals (i.e

15 days, 20 days, 25 days and 30 days respectively).

After the expiry of the incubation period, contents of the flasks were shaken vigorously and filtered through Whatman filter paper No. 44. The residue on the filter paper was saved carefully for the determination of the growth of fungi during the incubation period. The oven dry weight of the fungi was recorded after drying. 10ml of the filtrate was diluted to 100 ml with water for the analysis of soluble phosphate.

5 ml of the diluted liquid medium was taken in 50ml volumetric flask and phosphorus was determined as per the modified method described by Bray and Kurtz (1945). 5ml of the filtrate was added with 25 ml Bray I extractant solution (0.03 N  $\text{NH}_4\text{F}$  and 0.1 N HCl) and was shaken for 5 minutes in a shaker.

1ml of the above solution was added with 10ml Ammonium molybdate and distilled water was added to make it near to 50 ml mark in 50 ml volumetric flask. The contents of the flasks were shaken well. 1ml of dilute Stannous chloride was then added to the above solution. Blue colour developed within 2-3 minutes. The intensity of the blue colour was recorded in 680 nm Spectrophotometer.

Amount of soluble phosphorus was calculated as per the following formula :

$$\text{Phosphorus(\%)} = \frac{X \times \text{Volume of the solution (ml)}}{10 \times 1 \times \text{Oven dry weight of the sample}} \times 100$$

Where X=optical density of the Spectrophotometer

Volume of the solution=solution taken for analysis i.e 5ml.

#### Antagonism study

To ascertain whether antagonism existed between the organisms isolated from vermicompost and brown root rot disease of tea causing organism (*Fomes lamoensis*), a 4mm disc of the antagonistic fungi were placed separately in the petridishes containing potato dextrose agar medium at 2 cm apart from the pathogen, i.e., *Fomes lamoensis*. Respective controls were also made with the test organisms (*Aspergillus flavus*, *Aspergillus niger*, *Trichoderma atroviride*, *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma sp.*, *Fusarium sp.*) and *Fomes lamoensis*. All the plates were separately incubated at  $25 \pm 1^\circ\text{C}$  for 4-5 days and antagonistic colony interaction were examined according to the classification of Skidmore and

Dickinson (1976). The inhibition of colony diameter was recorded. It is the most common method of determining antagonistic activity. The organisms are allowed to grow against each other and one of the following reactions is normally observed.

- Mutual intermingling of the two organisms.
- Inhibition of one organism on contact ; the other organism continues to grow unchanged or at a reduced rate through the colony of the inhibited organism.
- Mutual inhibition on contact ; the space between the two colonies is small, but clearly marked.
- Inhibition of one organism at a distance ; the antagonist continues to grow through the resulting clear zone at an unchanged or reduced rate.
- Mutual inhibition at a distance.
- overgrowth by the antagonists

## RESULTS

Through dilution plate, the following fungal organisms i.e. *Aspergillus flavus*, *Aspergillus niger*, *Fusarium sp.*, *Trichoderma atroviride*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma lignorum*, *Trichoderma citrinoviride* and *Trichoderma sp.* were isolated from the vermicompost and identified. These organisms were studied for their phosphate

**Table 1.** Colony interaction between fungal species isolated from the vermicompost and *Fomes lamoensis*

| Fungal species                | Colony interaction* |
|-------------------------------|---------------------|
| <i>Aspergillus flavus</i>     | Bi                  |
| <i>Aspergillus niger</i>      | Bi                  |
| <i>Fusarium sp.</i>           | D                   |
| <i>Trichoderma atroviride</i> | A                   |
| <i>Trichoderma harzianum</i>  | Bi                  |
| <i>Trichoderma viride</i>     | Bii                 |
| <i>Trichoderma sp.</i>        | A                   |

\*A : Mutual intermingling growth

Bi : overgrowth by the antagonists

Bii : intermingling growth in which the test fungus, pathogen has ceased growth and is overgrown by the antagonist

D : Not detected

(Classified according to the Skidmore and Dickinson 1976)

**Table 2.** Growth of antagonistic fungi in monoculture and in dual culture with *Fomes lamaroensis* on Potato dextrose agar medium on the 5th day of observation

| Fungi                         | Colony diameter on the 5th day of observation |              |                          |
|-------------------------------|---|--------------|--------------------------|
|                               | Monoculture                                   | Dual culture |                          |
|                               |   | Antagonists  | <i>Fomes lamaroensis</i> |
| <i>Aspergillus flavus</i>     | 82  | 80           | 28                       |
| <i>Aspergillus niger</i>      | 80  | 72           | 29                       |
| <i>Fusarium</i> sp.           | 55  | 32           | 30                       |
| <i>Trichoderma atroviride</i> | 74  | 68           | 36                       |
| <i>Trichoderma harzianum</i>  | 79  | 76           | 37                       |
| <i>Trichoderma viride</i>     | 80  | 74           | 31                       |
| <i>Trichoderma</i> sp.        | 72  | 62           | 39                       |
| CD (5 %)                      | 8.70  | 15.04        | 4.23                     |
| CD (1%)                       | 12.80   | 22.13        | 6.                       |

solubilizing and biocontrol potential against the tea root pathogen (*Fomes lamaroensis*) *in vitro*.

The solubilization of rock phosphate in liquid medium by different species of fungi (isolated from vermicompost) over a period of time was determined and recorded ( Fig. 1-6). Six test organisms i.e *Aspergillus flavus*, *Aspergillus niger*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma lignorum* and *Trichoderma citrinoviride* were grown in 100ml of synthetic liquid medium in 250ml conical flask. From the experiment it was observed that *Trichoderma viride* had the highest potential in solubilizing rock phosphate followed by *Aspergillus niger* and *Trichoderma lignorum*. However, it was also observed that *Trichoderma harzianum*, *T. citrinoviride* and *A. flavus* were also capable of solubilizing rock phosphate but their contribution was low as compared to *T. viride*, *T. lignorum* and *A. niger*. (Fig. 1-6). Further, it was observed that with the increase in time intervals, solubilization increased.

*Trichoderma viride* was found to cause highest solubilization of rock phosphate among the fungal isolates from the vermicompost. As the number of days of incubation increased, solubilization of rock phosphate by *Trichoderma viride* was also found to have increased. During the same period of time, solubilization of rock phosphate by *Trichoderma viride* was found to be 67.97% (Fig. 3). In general, solubilization of rock phosphate by the test fungal species (isolated from vermicompost). Solubilization of rock phosphate by *Aspergillus niger* was found to be 64.4% (Fig.

1). While by *Aspergillus flavus* it was found to be 50.1% (Fig. 2) and in *Trichoderma harzianum* it was found to be 52.75% after 30 days of incubation (Fig. 4). While *Trichoderma lignorum* solubilized 59.4% of rock phosphate (Fig. 5) and *Trichoderma citrinoviride* has solubilized 49.24% of rock phosphate (Fig. 6) respectively.

The colony interaction between the fungi isolated from vermicompost and the tea pathogen, *Fomes lamaroensis* was observed *in vitro*. *Aspergillus flavus*, *Aspergillus niger* and *Trichoderma harzianum* were observed to inhibit the growth and to overgrow over the brown root rot causing organism ('Bi' type according to the Skidmore and Dickinson, 1976 ). *Trichoderma harzianum* also restricted the growth of the pathogen. *Trichoderma atroviride* and *Trichoderma* sp. showed mutual intermingling of growth ('A' type). While *Trichoderma viride* showed intermingling growth in which the pathogen ceased to grow and was overgrown by the antagonist ('Bii' type). *Fusarium* sp. didn't show any inhibition ('D' type) (Table 1).

Studies on antagonism revealed that *Aspergillus flavus*, *Aspergillus niger*, *Trichoderma atroviride*, *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma* sp occupied more area on PDA medium as compared to the pathogen, *Fomes lamaroensis* by the 5<sup>th</sup> day of observation in the dual culture ( Table 2). This was due to the faster growth rate and parasitism potential of the antagonists. In monoculture, *Aspergillus flavus*, *Aspergillus niger* and *Trichoderma viride* was observed to occupy more area on the PDA medium

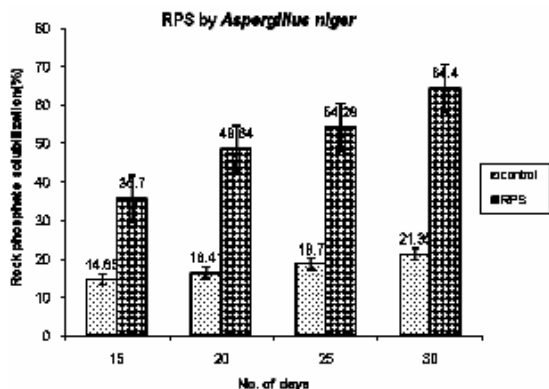


Fig. 1. Rock phosphate solubilization by *Aspergillus niger* in liquid medium at different time intervals as compared to control

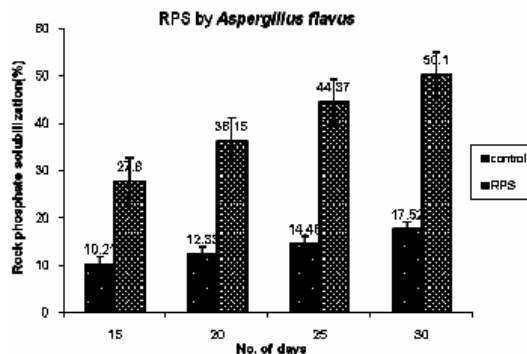


Fig. 2. Rock phosphate solubilization by *Aspergillus flavus* in liquid medium at different time intervals as compared to control

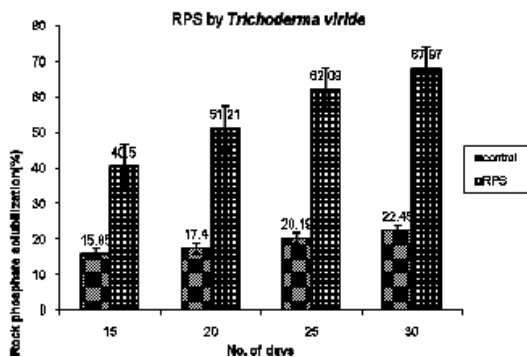


Fig. 3. Rock phosphate solubilization by *Trichoderma viride* in liquid medium at different time intervals as compared to control

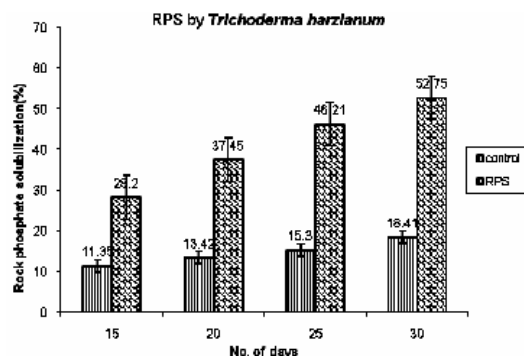


Fig. 4. Rock phosphate solubilization by *Trichoderma harzianum* in liquid medium at different time intervals as compared to control

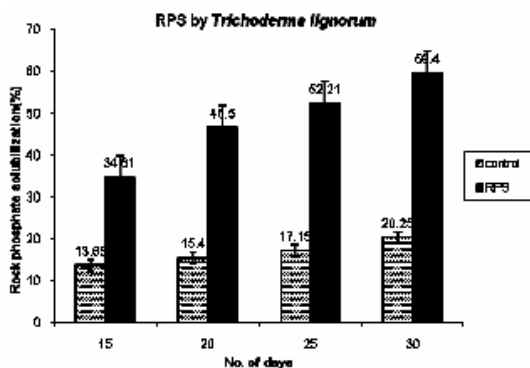


Fig. 5. Rock phosphate solubilization by *Trichoderma lignorum* in liquid medium at different time intervals as compared to control

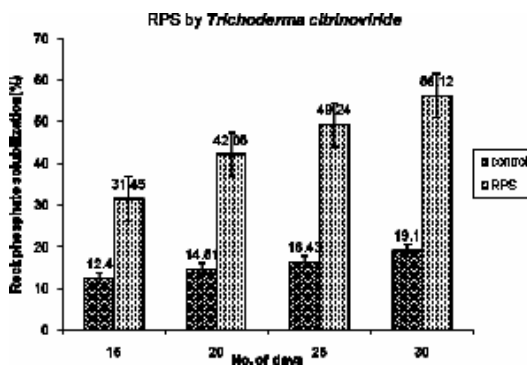


Fig. 6. Rock phosphate solubilization by *Trichoderma citrinoviride* in liquid medium at different time intervals as compared to control



compared to other fungi isolated from the vermicompost (Table 2).

### DISCUSSION

With the increase in time intervals, solubilization of rock phosphate by the fungal species isolated from vermicompost increased in the synthetic liquid medium. *Trichoderma viride* showed highest potential of solubilization of rock phosphate after 30 days of incubation. This was followed by *Aspergillus niger*, *T. harzianum*, *T. lignorum*, *T. citrinoviride* and *A. flavus* respectively. They also showed rock phosphate solubilizing potential in synthetic liquid medium but their contribution was low as compared to *T. viride* and *A. niger*. Phosphate solubilizing microorganisms (PSMs) play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphatic fertilizers. Application of PSMs in the field has been reported to increase crop yield. Fungi have been reported to possess greater ability to solubilize insoluble phosphate than bacteria (Nahas, 1996).

A fall in pH during the growth of phosphate dissolving microorganisms has been reported in liquid medium (Bardiya and Gaur, 1974, Gaur, 1972). While a rise in pH above neutral has been observed in certain cases (Goswami and Sen, 1962). The optimum pH for maximum solubilization of inorganic phosphate has been found to be in neutral or slightly acidic liquid medium with pH 4.0-5.0 for fungi such as *Penicillium* sp. and *Aspergillus* sp. Phosphorus is an essential nutrient limiting plant growth. Despite its wide distribution, it is deficient in most of the soils, owing to high P-fixation capacities of the soil. Rock phosphate is theoretically the cheapest fertilizer and most abundant but its direct application in the soils is not always agronomically effective due to its low solubility. An environmentally acceptable and economically sound alternative to traditional processing of these deposits is the use of phosphate solubilizing microorganisms. Therefore, the focus of the present study has been to use rock phosphate and phosphate solubilizing microorganisms in consortium as a source of P-nutrient for tea as a crop. Preliminary experiments were performed in order to characterize the phosphate solubilizing activity using rock

phosphates as the sole source of P-nutrient to *A. niger*, *A. flavus*, *T. viride*, *T. harzianum*, *T. lignorum* and *T. citrinoviride* under laboratory conditions.

The fungi isolated from the vermicompost were found to grow very fast in dual culture. On the other hand, the slow growth rate of *Fomes lamaoensis* suggests a more rapid utilization of nutrients by the antagonists when grown together. Nutrient depletion, space and production of toxic substances (antibiotic or antibiotic like substances) by the fungi are known to play a dominant role in antagonism and these factors are usually governed by the physicochemical nature of the environment (Burgess and Griffin, 1967). Therefore, it can be suggested that fungi isolated from the vermicompost or amendment of vermicompost itself may be tried to control this tea root pathogen (i.e. *Fomes lamaoensis*) under field condition. The present *in vitro* study suggested that positive antagonistic effect of *Aspergillus flavus*, *Aspergillus niger* and *Trichoderma harzianum* which inhibited the growth and overgrew on the brown root rot causing organism of tea (*Fomes lamaoensis*). *Trichoderma harzianum* also restricted the growth of the pathogen. *Trichoderma atroviride* and *Trichoderma* sp. showed mutual intermingling of growth. *Trichoderma viride* showed intermingling of growth in which the pathogen ceased growth and was overgrown by the antagonists. However, *Fusarium* sp. didn't show any inhibition zone. The results also suggest that application of vermicompost not only add organic matter to the soil but may also be able to give biocontrol of tea root pathogen i.e. *Fomes lamaoensis*. Biological control offers a powerful means to increase yield by suppression or destruction of pathogen inoculums, protect plants against infection, or increase the ability of plants to resist pathogens. The result obtained in the present work suggests that some of the fungi isolated from vermicompost can be used as biocontrol agent against brown root rot pathogen of tea i.e. *Fomes lamaoensis*. Most of the soil fungi isolated from the tea agroecosystem/vermicompost are known to survive saprophytically in nature. Release of inhibitory substances/ metabolites produced by *Trichoderma viride* into the host organism is known to result in direct inhibition of the growth of the pathogen by disintegrating the

hyphal wall resulting penetration, absorption and lysis of the mycelium.

The present results on antagonism and phosphate solubilization suggests that fungi isolated from the vermicompost have the potential to be used in biological control of tea pathogen (i.e *Fomes lamaoensis* ) causing brown root rot disease of tea, phosphate solubilization and application of vermicompost in tea agroecosystem will not only add organic matter but also add the potential antagonists which can act as biocontrol agent against tea root pathogens i.e *Fomes lamaoensis* and addition of phosphate solubilizing microbes which will make the unavailable phosphate into available form and should contribute to the growth and root proliferation, in turn higher absorption of phosphate reflecting on the productivity of tea at large.

#### REFERENCES

1. Pattnaik, S. and Reddy, M. V. Nutrient status of vermicompost of urban green waste processed by three earthworm species- *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus*. *Applied and Environmental Soil Science*, 2010; Article ID 967526: 1-13.
2. Sharma, S., Pradhan, K., Satya, S. and Vasudevan, P. Potentiality of earthworms for waste management and in other uses. *The Journal of American Science*, 2005; **1**(1) : 4-16.
3. Chabot, R., Antoun, H. and Cescas, M. P. Stimulation of growth of maize and lettuce by inorganic phosphorus solubilizing microorganisms. *Canadian J. Microbiol.*, 1993; **39**: 941-947.
4. Dutta, B. K. and Isaac, I. Effect of inorganic amendment (NPK) to soil on rhizosphere mycoflora of Antirrhinum plants infected with *Verticillium dahliae* Kleb. *Plant and soil*, 1979; **52**: 56-59.
5. Deb, P. R. and Dutta, B. K. Studies on biological control of foot rot disease of soyabean caused by *Sclerotium rolfsii* Sacc. *J. of Plant Diseases and Protection*, 1991; **98**(5) : 539-546.
6. Deb, P. R. and Dutta, B. K. (*Trichoderma* as a biocontrolling agent against *Sclerotium rolfsii*. The Faculty Press 88, Great Britain. *Microbial Letters* , 1988; **37**: 107-113
7. Jakhar, S. S., Chauhan, M. S. and Duhan, J. C. Management of root rot of cotton (*Gossypium* species) caused by *Rhizoctonia* species. *Plant Dis. Res.*, 1997; (13): 115-118.
8. Suriachandraselvan and Aiyathan Integrated management of dry root rot of cotton incited by *Macrophomina phaseolina* (Tassi.) Goid. *J. cotton Res. Dev.*, 1988; **12** (2) : 226-232.
9. Timonin, M. I. The interaction of higher plant and soil microorganisms I. Microbial population of seedlings of certain cultivated plants. *Can J. Resistant* ,1940; (18) : 307-317.
10. Tsao, P. H. Effect of Certain Fungal Isolation Agar Media on *Thielaviopsis basicola* and on its Recovery in Soil Dilution Plates, *Phytopathology* , 1964; **54**: 548-555.
11. Devi, O. G., Dutta, B. K. and Das, A. K. Effect of organic and inorganic amendments to soil on the microflora of tea [*Camellia sinensis* L. (O) Kuntze] agroecosystem. *Journal of mycopathological research*, 2012; **50**(1): 35-41.
12. Raper, K. B. and Fennel, D.I. *The Genus Aspergillus*, Robert E. Krieger Publishing Company, Huntington, New York, 1973.
13. Gilman , J. C. *A Manual of Soil Fungi*. Oxford and IBH Publishing Co, revised second edition. New Delhi, 1956.
14. Barnet, H. L. and Hunter, B. B. *Illustrated Genera of Imperfect Fungi* ( III<sup>rd</sup> Edition). Burgess Publishing Company, USA, 1972.
15. Nagamani, A., Manoharachary, C., Agarwal, D. K., and Chowdhry, P. N *Monographic Contribution on Trichoderma* Pers. ex Fr. Associated Publishing Company, New Delhi, 2002.
16. Sen, A. and Paul, N. B. Solubilization of phosphates by some common soil bacteria. *Curr. Sci.*, 1957; **26**: 222-223.
17. Skidmore, A. M. and Dickinson, C. H. Colony interactions and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Trans British Mycological Society*, 1976;(66) : 57-64.
18. Nahas, E Factors determining rock phosphate solubilization by microorganisms isolated from soil. *World J. Microbiol. Biotechnol.*, 1996; **12**: 567-572.
19. Bardiya, M. C. and Gaur, A. C. Isolation and screening of microorganisms dissolving low-grade rock phosphate. *Folia Microbial* , 1974; **19** : 386-389.
20. Gaur, A. C. Role of phosphate solubilizing microorganisms and organic matter in soil productivity, *Symp. Soil productivity*, 1972.
21. Goswami , K. P. and Sen, A . Solubilization of calcium phosphate by three strains of a phosphobacterin. *Indian Journ. Agric. Sci.*, 1962; **32**(2) : 96-101.
22. Burgess, L. W. and Griffin, D. M. Competitive saprophytic colonization of Wheat straw. *Annals of Applied Biology* , 1967; **60**: 137-142.