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ORIGINAL ARTICLE

Effect of *Aspergillus* sp. as a Bioinoculant In Vermicompost With Special Reference To Phosphate Enrichment

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Natarajan Manivannan, Nooruddin Thajuddin, Thilagavathy Daniel and Muthukumaran Gunasekaran: Effect of *Aspergillus* sp. as a Bioinoculant In Vermicompost With Special Reference To Phosphate Enrichment

ABSTRACT

Aspergillus sp. is well known fungi than can potentially mobilize phosphate from inorganic sources. The present study aimed at utilizing the *Aspergillus* sp. to enrich the total phosphate content in vermicompost. Phosphate solubilizing *Aspergillus* sp. was isolated from the vermicompost. The isolate was grown in mass and the spore count was done periodically. Pre-prepared vermicompost was mixed with a spore suspension of *Aspergillus* sp. at $1x10^8$ spores/g. Treated vermicompost was mixed with soil, for pot culture studies. The composition of phosphate solubilizing fungi with vermicompost showed higher productivity of *Vigna unguiculata* (L.) Walp. (cowpea).

Key words: *Aspergillus* sp., Phosphate solubilization, Vermicompost, *Vigna unguiculata* (L.) Walp. (cowpea).

Introduction

The current environmental conditions the soil loses its natural fertility due to the chemical fertilizers and this ultimately results in declining crop yields. Nowadays organic compost has been identified as an alternative to chemical fertilizer to increasing soil fertility and crop production in sustainable agriculture. Hence the use of vermicompost and biofertilizers as organic manure will have to be the technique to be adopted in the nutrient requirements of crops and ensure the production of more food materials. Vermicomposting is a biotechnological process of composting, in which certain species of earthworms are used to enhance the process of waste conversion and produce a better end product. Vermicomposting has been recognized as an eco-friendly technology for converting organic wastes into high value organic manure (Kale *et al*., 1982; Senapathi, 1993). The soil is an abiotic factor, which inhabits varieties of microflora and macrofauna. The microorganisms and macroorganisms are interdependent and the nutrient cycling in the soil is highly influenced by the activity of these organisms; to exploit the organic resources available in the litter and soil, the microorganisms generally and mutually associate with macroorganisms like earthworms and *vice versa* (Moore, 1988; Clarholm, 1985 and Lavelle *et al*.,1995). Phosphorous has been called the 'key of life' because it is directly involved in most of life processes. Next to nitrogen, it is invariably classified as one of the macronutrients and is an important key element in frequency of use as fertilizer. Microbes which solubilize the bound phosphates and rock phosphates are called phosphate solubilizing microorganisms. The present study has been carried out with *Aspergillus* sp. (phosphate solubilizing fungus) isolated from the vermicompost and that has been mass multiplied and mixed with the casts of the earthworm, *Eudrilus eugeniae* Kinberg and analyzed for their efficiency in plant growth promotion.

Materials and Methods

Isolation of Phosphate Solubilizing Fungus:

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The casts of the earthworm, *Eudrilus eugeniae* Kinberg was collected. One gram of casts was serially diluted up to10^6. The dilution 10^4, 10^5 and 10^6 were plated on sabouraud dextrose agar plate incorporated with rock phosphate. The petri plates were incubated at room temperature for 4 - 5 days. The microorganism isolated on sabouraud dextrose agar plates was identified on the basis of transparent zones of clearing around the microbial colonies (Paul and Sundararao, 1971). The fungal isolates were identified based on their cultural characteristics and the structure of their conidiophores in microscopy using Lactophenol cotton blue staining technique.

Mass cultivation:

The identified colonies were mass multiplied on double sterilized (25gram) barley. Spore suspension of the mother culture was inoculated into the barley and was kept under room temperature for 12 days and shaked on 7^{th}, 9^{th} and 11^{th} day. Spore count was done by normal plate count method to determine the viable count in 8th, 10th and 12th day at 25 ± 2^oC incubation. Similarly the spore count was determined from fresh broth after suitable dilution in a haemocytometer (Vincent, 1970). Then the spore suspension was mixed with vermicompost at the rate of $1x10^8$ spores per ml for one gram of vermicompost and the mixture was mixed with soil for a pot culture studies.

Pot culture studies:

Vigna unguiculata (L.) Walp. (cowpea) seeds used in this study were surface sterilized with 70 % sodium hypochlorite for 90 sec at room temperature**.** Vermicompost used in this study was prepared indigenously and the parameters of which were recorded (data not shown). *Aspergillus* sp. culture suspension was mixed with vermicompost at the rate of 1 x 10^8 spores/g of vermicompost. The experimental design adopted in the present investigation is as described in table 1.

Table 1: Experimental design for pot culture studies with *Vigna unguiculata* (L.) Walp. (cow pea).

S. no.	Treatments	Notations
1	Seeds + plain soil (control)	T0
2	Seeds + soil mixed with vermicompost	T1
3	Seeds + soil mixed with vermicompost + *Aspergillus* sp.	T2

Results and Discussion

The predominant fungal colony was isolated from the vermicast of *Eudrilus eugeniae* and it was identified as *Aspergillus* sp. using Lacto phenol cotton blue stain. The isolated phosphate solubilizing fungus was further screened for their ability to solubilize phosphate and insoluble phosphate was measured quantitatively in liquid medium. The production of clearing zones around the colonies of the organism is an indication of *Aspergillus* sp. (Fig. 1) were the dominant organism found almost in vermicompost.

The phosphate solubilizing fungus, *Aspergillus* sp. was mass multiplied in sterilized barley and kept for 8 - 12 days at room temperature (Fig. 2). The spore count increased from 4.16 to 7.24 x 10^9 and viability was also analyzed, as given in the Fig. 3.

Phosphorus is the second limiting nutrient next to nitrogen in majority of soils for crop production. The major mechanism of mineral phosphate solubilization is the production of organic acids, and acid phosphatases are playing a major role in the mineralization of organic phosphorus in soil. The principal mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms. Synthesis of organic acids results in acidification of the microbial cell and its surroundings. The production of Gluconic acid seems to be the most frequent agent of mineral phosphate solubilization. Also, 2-ketogluconic acid is another organic acid identified in strains with phosphate solubilizing ability.

The mechanisms of rock phosphate solubilization have been discussed by different authors (Goldstein, 1995; Kim *et al*., 1997a). Some of them considered that the organic acid produced by microorganisms have an important role in solubilization of inorganic phosphates **(**Venkaterswarlu *et al*., 1984; Vazques *et al*., 2000**).** According to Goldstein (2000) the bioprocess of rock phosphate ore involves much more processes the simple acid dissolution. Obviously the bioconversion was performed in a complex heterogeneous system. Significantly higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere. In addition, the fungal genera such as *Penicillium* and *Aspergillus* have this capacity (Suh *et al*., 1995; Whitelaw *et al*., 1999).

Vermicastings along in combination with microbial inoculants were able to initiate rooting and development of roots better than control. In the present study the result showed the increased growth rate of root and shoot initiation, development and weight. Vermicompost application has shown increase in the germination efficiency, root growth and yield of plants (Sevugaperumal et.al. 1998 and Buckerfield *et al*. 1999; Kulkarni *et*

al., 1996). Pod parameters are an important character in the life cycle of plant growth. In the present study, the appearance of pods were observed more in plant treated with vermicompost along with *Aspergillus* sp.

Growth and yield performance of cowpea (*Vigna unguiculata*) is given in Fig.4. Cowpea performed well in *Aspergillus* sp. enriched vermicompost formulation. Soil fungus belonging to the genera *Aspergillus* sp. possess the ability to bring insoluble phosphate into soluble forms by secreting organic acids such as formic, acetic, propionic, etc. These acids lower the pH and bring about the dissolution of bound forms of phosphate. Some of the hydroxyl acids may chealate with calcium and iron resulting in effective solubilization and utilization of phosphates (Gerretsen, 1948; Sperber *et al*., 1958). Application of phosphate solubilizing microorganisms in the agricultural fields has already been reported to increase crop yield. (Young, 1994; Young *et al*., 1998; Goldstein *et al*., 1999)

Fig. 1: Colony morphology of *Aspergillus* sp. on sabouraud dextrose agar showing a profound diffuse zone of clearing around the colony

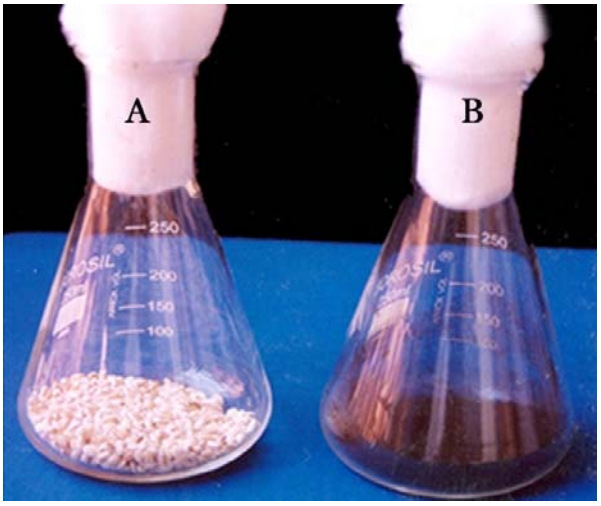

Fig. 2: Culturing *Aspergillus* sp. on double sterilized barley. A. Double sterilized barley B. *Aspergillus* sp. grown on barley.

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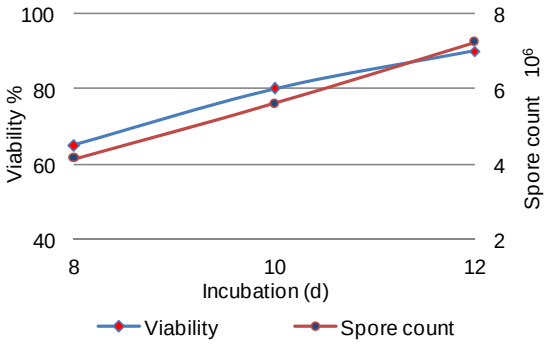

Fig. 3: Viability and spore count of Aspergillus sp., as evaluated with barley as a growth substrate

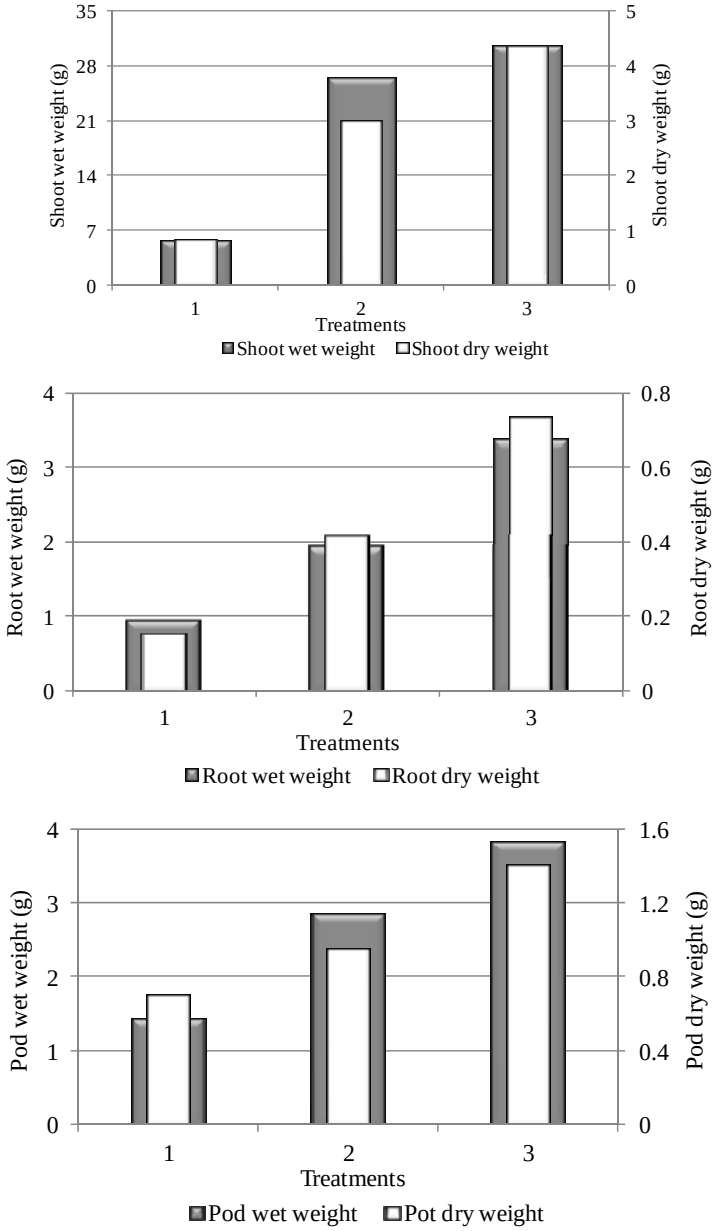

Fig. 4: Morphometric and yield parameters of cowpea (*Vigna unguiculata* (L.) Walp.) under different organic formulations. 1 – Control, 2 – Vermicompost, 3 - Vermicompost with *Aspergillus* sp.

Pot culture studies has shown that the wet and dry weight of the shoot, root and pod were also higher in vermicompost enriched with microbial inoculants than in vermicompost alone and in control. The maximum growth performance and yield of a cow pea was observed in the *Aspergillus* sp. enriched vermicompost. This is probably due to more phosphorus content in the soil formulation.

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Reference

Buckerfield, C.J., C. Tamara, F. Kenneth, E. Lee and A.K. Webster, 1999. Vermicompost in solid and liquid as a plant growth promoter. Pediobiologica, 43: 753-759.

Clarholm, M., 1985. Interactions of bacteria, protozoa and plant leading to mineralization of soil and peat soil Biol. Biochem., 12: 49-57.

Gerretsen, F.C., 1948. Influence of microorganisms on the phosphorus uptake by the plant. Pl. Soil., 1: 51-81.

Goldstein, A.H., 1995. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilizationby Gram negative bacteria. Biol. Agric. Hort., 12: 185-193.

Goldstein, A.H., 2000. Proc. IFA Technical Conference, 2000, New Orleans, pp: 1-21.

Goldstein, A.H., K. Braverman, N. Osorio, 1999. Evidence for mutualism between a plant growing in a phosphate-limited desert environment and a mineral phosphate solubilizing (MPS) bacterium. FEMS Microbiol. Ecol., 3: 295-300.

Kale, R.D., K. Bano and R.V. Krishnamoorthy, 1982. Potential of Perionyx excavatus for utilization of organic wastes. Pedobiologia, 23: 419-425.

Kim, K.Y., D. Jordan, H.B. Krishnan, 1997a. Rahnella aqualitis, a bacterim isolated from soybean rhizosphere, can solubilize hydroxyapatite.FEMS Microbiol. Lett., 153: 273-277.

Kulkarni, B.G., V.G. Nalawadi and R.S. Giraddi, 1996. Effect of vermicompost and vermiculture on growth and yield of china aster (Callistephus Chinensis Ness). CV. Ostrich plume mixed. South Indian horticulture., 44(1 and 2) : 33-35.

Lavelle, P., C. Lattaud, D. Trigo and I. Barois, 1995. Mutualism and biodiversity in soils. In: The significance and regulation of soil biodiversity (Ed: Collings, H.P. Robertson, G.P. and Klug, M.J.). pp: 23-33.

Moore, J.C., 1988. The influence of microarthropods on symbiotic and non-symbiotic mutualism in detrital based ground food webs. Agri. Ecos Environ., 24: 147-159.

Paul, N.B. and W.V.B. Sundararao, 1971. Phosphate dissolving bacteria in rhizosphere of some cultivated legume. Pl.soil., 25(1): 127-32.

Senapathi, B.K., Vermitechnology in India, N.S. Subba Rao, C. Balagopalan, S.V. Ramkrishna, 1993. Editors , New Trends in Biotechnology, Oxford and IBH, New Delhi pp: 347-358.

Sevugaperumal, R., K. Jaisankar and K. Jeyaraj, 1998. Comparative analysis of the effect of vermicompost and *Azospirillum* on Sorghum, *Sorghum valgare* Linn. ANJAC Journal., 15: 18-21.

Sperber, J.I., 1958. A solubilization of apatite by soil bacteria. Nature., 180: 994-95.

Suh, J.S., S.K. Lee, K.S. Kim, and K.Y. Seong, 1995. Solubilization of Insoluble Phosphate by *Pseudomonas putida, Penicillium* sp. and *Aspergillus niger* Isolated from Korean Soils.JKSSF, 28(3): 278-286.

Vazques, P., G. Holguin, M.E. Puente, A. Lopez-Cortes, Y. Bashan, 2000. Biol. Fertil. Soils, 30,

Venkaterswarlu B., Rao A.V., Raina P., 1984 J. Indian Soc. Soil Sci., 32: 273-277.

Vincent, J.M., 1970. A manual for the practical study of root nodule Bacteria. I.B.P. Hand book, No. 15, Blackwell Scientific Publisher, Oxford, pp: 164.

Whitelaw, M.A., R.J. Harden, K.R. Helyar, 1999. Phosphate solubilisation in culture by the soil fungus *Penicillium radicum.* 31: 655-665.

Young, C.C., 1994. Selection and application of biofertilizers in Taiwan. Food and Fertilizer Technology Center. Tech. Bull., 141: 1-9.

Young, C.C., C.H. Chang, L.F. Chen, C.C. Chao, 1998. Characterization of the nitrogen fixing and ferric phosphate solubilizingbacteria isolated from Taiwan soil. J. Chin. Agricult. Chem. Soc. 36, 201–210 (in Chinese). 460-468.