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VERMIWASH: BIOCHEMICAL AND MICROBIOLOGICAL APPROACH AS ECOFRIENDLY SOIL CONDITIONER

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ABSTRACT

Vermiwash was found to contain enzyme cocktail of proteases, amylases, urease and phosphatase. Microbiological study of vermiwash revealed that it contains nitrogen-fixing bacteria like *Azotobactre*r sp., *Agrobacterium* sp. and *Rhizobium* sp. and some phosphate solublizing bacteria. Laboratory scale trial showed effectiveness of vermiwash on Cowpea plant growth.

Keywords: Vermiwash, soil conditioner, enzymes, nitrogen, bacteria, cowpea.

INTRODUCTION

Vermiculture is a mixed culture containing soil bacteria mixed and an effective strain of earth worms (NIIR Board, 2008). Earthworm has efficiency to consume all types of organic rich waste material including vegetable waste, industrial and other organic waste. Vermicroposting refers to the production of plant nutrient rich excreta of worms.

Earthworms play a vital role in plant growth. It is a quite possible to effect quick change over for sustainable agriculture by harnessing brand new vermicompost technology to the soil. In recent times, the commercial vermin culturists have started promoting a product called vermiwash. This vermiwash would have enzymes, secretions of earthworms which would stimulate the growth and yield of crops and even develop resistance in crops receiving this spray. Such a preparation would certainly have the soluble plant nutrients apart from some organic acids and mucus of earthworms and microbes (Shivsubramanian and Ganeshkumar, 2004). But so far there are no experimental evidences to quantify the effect of such spray.

Microbes in the environment significantly influence the biogeochemical cycle of phosphorus. The organic phosphorous compounds are decomposed and mineralized by enzymatic complexes like posphatases produced by microbes. In the ecosystem, a mixed population of microbes is essential to promote enzymatic degradation of naturally occurring phosphorous compounds (Trivedi and Bhatt, 2006). Shweta and Singh (2007) reported that presence of plant growth promoting substance in vermicompost and in an article published in The Hindu Newspaper by Subasashri (2004), vermiwash was reported for an effective biopesticide.

The present study was carried out to evaluate composition of vermiwash by considering biochemical and microbiological approaches for sustainable development of plant growth promoting factor or as a soil conditioning agent on cowpea plant.

MATERIALS AND METHODS

Earthworms

Earthworms were collected from vermicompost soil.

Preparation of extract

The earthworms (10g) were immersed in 25ml warm water and kept for 30 minutes at room temperature. Secreted enzyme extracts was centrifuged to remove the insoluble materials at 3000 rpm for 10 minutes. The filtrate was made cell free using 0.2µ membrane filtration.

Preparation of soil extract agar

Soil (10%) was taken in water and filtered through simple filter paper. The filtrate with 2.5% agar was used for preparation of soil extract agar medium and sterilized. To the sterilized extract added 5% (v/v) of filter-sterilized vermiwash.

Extracellular enzymes

Exracellular enzymes secretion from earthworms was screened by qualitative and quantitative methods. Qualitatively, protease, amylase and lipase secretions were tested on gelatin, starch and oil emulsion agar plate medium respectively. The zone of clearance was measured. Quantitatively, Protease (caseinase and gelatinase) assay was performed as per Zambare *et al.* (2007) with 1% casein and gelatin substrates. Amylase assay was performed as per Raghuramula *et al.* (1988) with 1% starch substrate. In addition to these enzymes, urease, phosphatases and nitrate reductases were also studied. Protein content of vermiwash was estimated by method of Lowery *et al*. (1951).

Microbiological studies

Microflora of Vermiwash for *Azotobactor, Agrobacterium, Rhizobium* and Phosphate sollublizing microbes was isolated on different media like Johnson's medium, Rhizobium medium and soil extract agar medium, respectively.

RESULTS AND DISCUSSIONS

Vermicomposting contains mainly earthworm excreta and decomposed matter. As the main substrates presented in the waste is of rich source of macromolecules. Resultant complex materials can easily broken by secretary enzymes of earthworms. Soil with simpler substances is the best suitable media to growth of

nitrogen fixing and phosphate solubilizing microbes. Vermiwash is a collection of excretory products and excess secretions of earthworms along with micronutrients from soil organic molecules. It was prepared in boiling water and its estimated enzyme profile on agar medium showed amylase and protease secretion while absence of lipase (Table-1).

Table-1. Qualitative method of estimation of enzyme secretion from earthworms.

Enzymes	**Zone of clearance** (mm)
Gelatinase	17.3 ± 2.7
Amylase	1.85 ± 0.5
Lipase	0
Urease	Positive

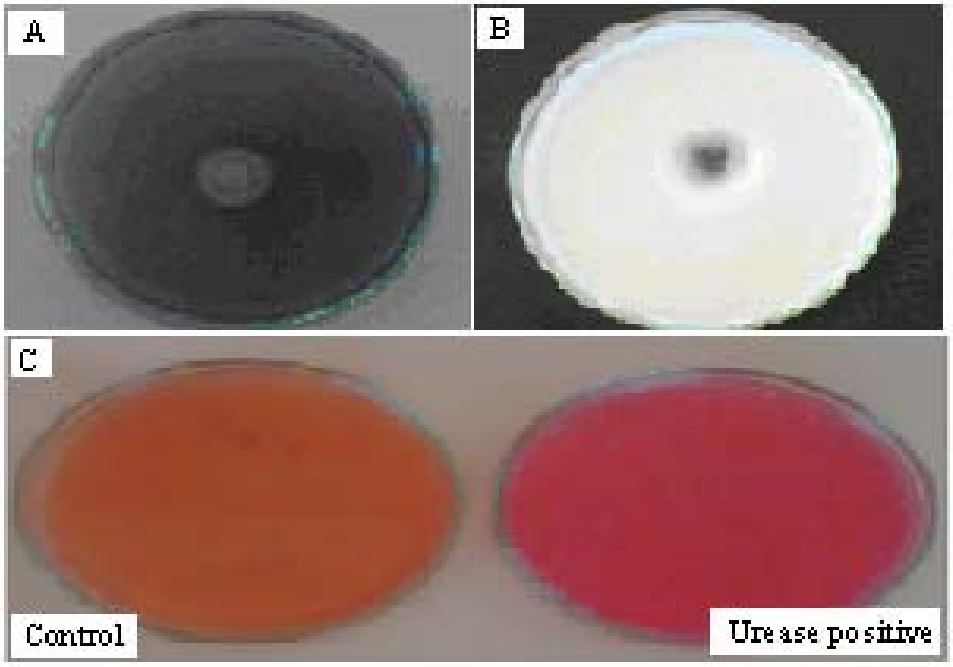
All experiments are conducted in duplicate.

Figure-1A shows zone of clearance produced by amylase and Figure-1B shows presence of protease. Quantitatively, highest enzyme activity was observed for gelatinase enzyme followed by amylase and caseinase with specific activity in same trend as shown in Table-2. Presence of proteases in soil helps in seed germination while amylases help for availability of simple carbon source for enhancement of plant growth and productivity as well.

Table-2. Quantitative method of estimation of enzyme secretion from earthworms.

Enzymes	**Enzyme activity** (U $ml^{-1}min^{-1}$)	**Specific activity** (U mg^{-1} Protein)
Gelatinase	115.60 ± 17.23	16.50
Amylase	75 ± 25	10.71
Caseinase	48.16 ± 19.27	6.88

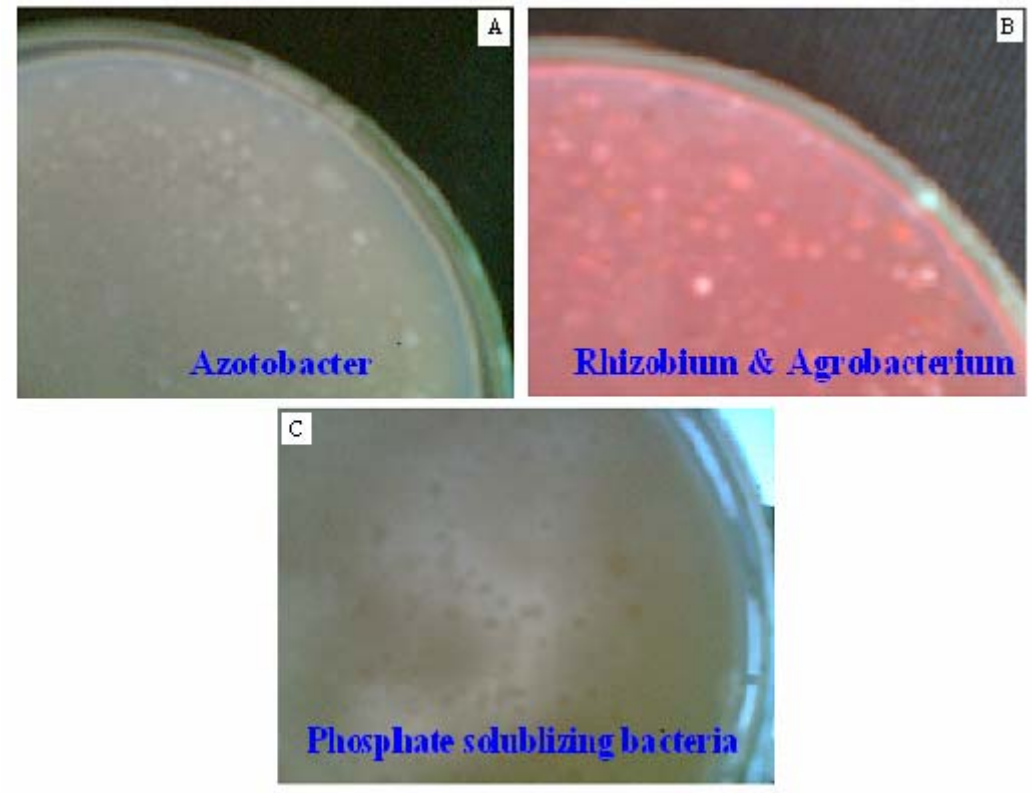
All experiments are conducted in duplicate; Protein content 7 mg ml^{-1}.



(A) amylase test; (B) protease test and (C) Urease test.

Figure-1. Enzyme profile of vermiwash- qualitative method.

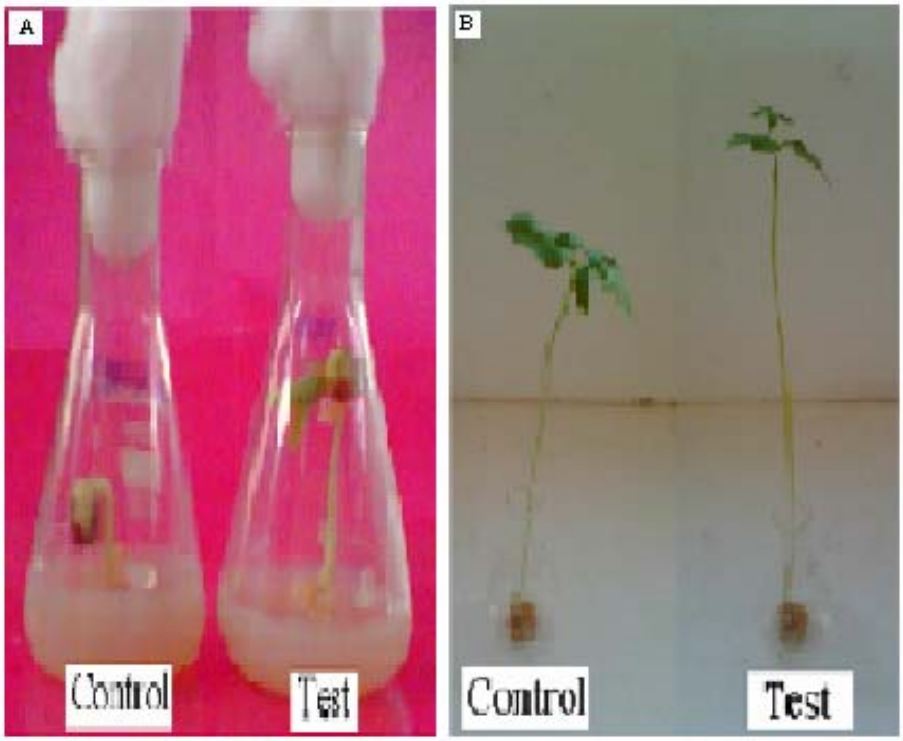
Soil born microflora is essential for growth of plants because organic nitrogenous compounds and phosphorous are decomposed and mineralized by different enzymes produced by nitrogen fixing and phosphate solublizing bacteria (Chaudhary, 2005). In vermiwash, urease producing microbes were found which convert red colored urea agar into pink color as shown in Figure-1C. Vermiwash microflora contains *Azotobactor, Agrobacterium, Rhizobium* and phosphate sollublizing microbes. *Azotobactor* are shown with translucent appearance (Figure-2A), *Agrobacterium* with red color colonies (Figure-2B), *Rhizobium* with white color colonies (Figure-2B) and phosphate sollublizing microbes with halo zone and turbid background (Figure-2C). Presence of these microbes makes available inorganic nitrogen, amino acids, and inorganic phosphates to plants through aminofication and nitrification processes. Likewise Prabhu (2006) reported presence of large number of beneficial microorganisms that help in plant growth and protects it from a number of infestations. It was also reported that vermiwash improves the germination percentage of the seeds and seedling vigour of seeds such as cowpea and paddy crops.



(A) Translucent appearance of *Arthrobacter* species; (B) White color *Rhizobium* species and red color *Agrobacterium* species and (C) Phosphate soliblizer with hollow zones and turbid background.

Figure-2. Microbiological analysis of vermiwash.

The effect of vermiwash was tested on growth of Cowpea plant on soil extract agar medium at laboratory scale experiments. The growth pattern was observed up to 15 day only. Figure-3A shows growth at 3rd day and Figure-3B shows growth at 15th day. Vermiwash supplemented medium showed high Cowpea plant growth as compared to without supplementation. From Figure-4, it is seen that in vermiwash supplemented medium from 2 to 7 days the growth is linear and latter with less linear. Likewise, effect of vermiwash was seen on the growth and productivity of Marigold (Shivsubramanian and Ganeshkumar, 2004). Also, George *et al.* (2007) reported the effect of vermiwash spray on significantly maximum dry chilli yield. The positive effect of vermiwash on crop growth and yield in the present study is in conformity with the studies of Bucker field *et al.* (1999) who reported that weekly applications of vermiwash increased radish yield by 7.3% and Thangavel (2003) who observed that both growth and yield of paddy increased with the application of vermiwash and vermicast extracts.



(A) 3rd day; (B) 15th day.

Figure-3. Effect of vermiwash in Cowpea plant growth.

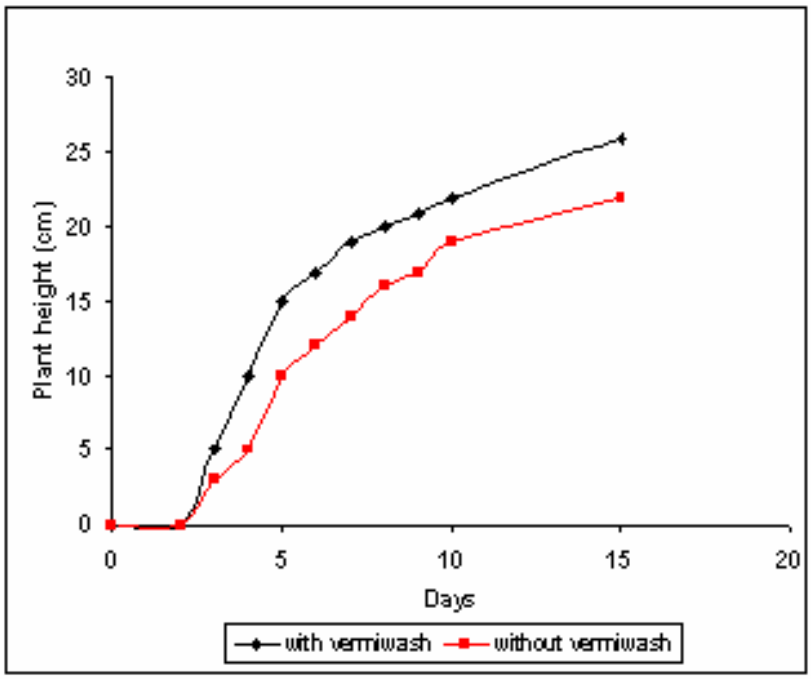


Figure-4. Effect of vermiwash on Cowpea plant growth with respect to time.

CONCLUSIONS

Vermiwash revealed potential application in sustainable development in agriculture biotechnology with respect to its origin, cost effectiveness, easily availability, time saving, reproducibility, reliability and eco-friendliness.

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