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Use of Vermicompost as Carrier Material for Microbial Inoculants for Enhanced Crop Production

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Present study was conducted whether vermicompost can be used as carrier material for microbial inoculants such as *Rhizobium* sp. and *Bacillus* sp. which were used as nitrogen fixers and phosphate solubilizers in the agriculture. Both the microorganisms were isolated, identified and mass multiplied and used at a population load of 1x 10^4 cells / g of vermicompost [Cow-dung + Leaf litter (1:1)]. Vermicompost was used as a carrier material and this mixture was added to the soil for pot culture studies. Application of vermicompost along with microbial inoculants showed, increase of plant growth and yield parameter when compared to the control.

Key words: *Eudrilus eugeniae*, Vermicompost, *Vigna unguiculata* (cowpea), *Rhizobium* sp., *Bacillus* sp. and *Aspergillus* sp.

Nowadays earthworms are increasingly utilized for decomposing and recycling organic materials worldwide and the process is known as vermicomposting. The major role of earthworms in the soil is decomposition of organic materials, developing soil structure and altering physico-chemical properties. Organic matter decomposition, nutrient cycling, soil structure and plant productivity has been studied by several authors (Lavella 1988; Scheu and Wolters 1991; Zhang and Schrader 1993). The compost thus derived is rich in available nutrients, soil beneficial microbes and plant growth promoting substances (Bhiday, 1994). Vermicompost can be produced from almost all types of organic wastes after suitable processing under controlled processing conditions. Vermicompost is rich in available nutrients required for plant growth (Karmegam and Daniel, 2000-a).Laboratory level as well as field level application of vermicompost showed higher growth rate of plants, increased uptake of nutrients and increased rate of yield in crops like paddy, tomato, green gram and cow pea (Kale and Bano, 1986; Tomati and Galli, 1995; Karmegam *et al*. 1999). An important feature is that, during the processing of the wastes by

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earthworms, many of the nutrients that they contain are changed to forms which are more readily taken up by the plants, such as nitrate, ammonium, nitrogen, exchangeable phosphorus, soluble potassium, calcium and magnesium (Edwards and Bohlen, 1996).

Root exudates selectively influence the growth of bacterial and fungal population by altering the presence of substrates in soil in the vicinity of roots (Jaeger *et al*., 1999; Yang and Crowley, 2000). Leguminous plants have been used as nitrogen source in traditional agricultural practices such as crop rotation and green manuring. Symbiotic nitrogen fixation in the legume- *Rhizobium* symbiosis is of considerable agricultural importance, as it leads to a significant increase in the combined nitrogen content of the soil (Dadarwal and Yadav, 1989). Rhizosphere microorganisms in turn exert strong effects on plant growth and health by nutrient solubilization N_2 fixation or by plant hormones (Hoflich *et al*., 1994; van Loon *et al.,* 1998).

Next to nitrogen it is invariably classified as one of the macronutrients and is an important key element in frequency of use as fertilizer. Several soil bacteria particularly those belong to genera *Bacillus* and *Pseudomonas* posses the ability to bring insoluble phosphate into soluble forms. Studies of rhizosphere microbial communities performed by traditional cultivation techniques indicated that the presence of different plant species influences the composition of the microbial community due to the differential response of bacterial and fungal populations to different root exudation patterns (Miller *et al.,* 1995).

The concept of sustainable agriculture has been recently developed and it involves the successful management of resources (Bhatnagar and Palta, 1996). Thorough knowledge about beneficial microbes has now been obtained with the help of depth research and advancement of technology. We come to know that it also helps for high production of crops. So we can inoculate the microbes along with vermicompost for increasing soil fertility and crop productivity.

The present study reveals that the *Rhizobium* sp. (nitrogen fixing bacteria) and *Bacillus* sp.(phosphate solubilizing bacteria) were isolated from the root nodules and rhizosphere region of *Vigna unguiculata*, and that has been mass multiplied and mixed with casts of the earthworm *Eudrilus eugeniae* and that has been used for the plant growth studies.

MATERIAL AND METHODS

Cassia auriculata Linn. is the common plants widely distributed in the southern part of Tamilnadu. Vermicomposting studies were carried out with the selected organic substrates *i.e*., leaf litters *C.auriculata Linn*. of using the earthworm *Eudrilus eugeniae* following the method adopted by Daniel and Karmegam (1999).The fresh leaves from these plants were collected and shade-dried for a week and then subjected to initial decomposition in rectangular draining cement tanks by sprinkling water, regular mixing and turning the substrates for 15 days.

Earthworm*, E.eugeniae* was collected from the vermiculture unit of the Department of Biology, Gandhigram Rural University. The vermibeds were prepared using pre-decomposed litter waste and cow-dung in 1:1 ratio on dry weight basis in plastic troughs in triplicates. In one set of troughs clitellate earthworm of 6-7 weeks old were introduced. Watering was done regularly to moisten the medium. The vermibeds were mixed well without damaging the earthworms for uniform decomposition. After 90 days the worm-worked (experiment) and the control substrates were taken out air dried, powdered and subjected to physico-chemical and microbial analysis (Daniel *et al*., 1999) and used as carrier material. The vermibed substrates i.e., the control and the worms-unworked substrates were analyzed for various physico-chemical parameters such as p^H, Electrical conductivity(EC),organic carbon, total nitrogen, total phosphorus, total potassium using standard procedures as given in the following table 1.

One gram of the sample was taken in a sterile test tube containing 9 ml of distilled water and shaken in vortex mixer. From this stock various dilutions were prepared from 10^1 to 10^6 with sterile water as described by Kannan (1996). One ml of the dilutions of 10^3,10^4 and 10^6 from each sample was taken and poured in petriplates containing the respective medium such as nutrient agar (bacteria), sabouraud dextrose agar (fungi)

and Kenknight's agar (actinomycetes) for the enumeration of microorganisms (Subba rao, 1995 and Kannan, 1996).

Nitrogen fixing bacteria namely, *Rhizobium* sp. was isolated from the root nodules of *Vigna unguiculata* used the technique of Vincent (1970). The nodules were gently crushed and the exudate was streak plated onto a sterile yeast extract mannitol agar (YEMA) containing 0.025 percent Congo red (Hahn, 1966) and incubated at 28°C +2°C. The white, translucent, glistering and elevated colonies were transferred to YEMA slants.

Phosphate solubilizing bacteria namely, *Bacillus* sp. was isolated from the sites of the rhizosphere of leguminous plants. Serial dilutions of the rhizosphere soil samples were individually plated on Pikovskaya agar medium (PVK) as described by Guar (1990).The colonies exhibiting zones of phosphate solubilization developed within 3-5 days were transferred to agar slant on the PVK medium containing 0.5 percent Tri-calcium phosphate and allowed to grow at room temperature for 3 days. The cultures were streaked repeatedly on nutrient agar medium till pure strains were obtained.

Isolated bacterial cultures were identified up to genus level by cultural, morphological and biochemical characteristics in accordance to the Bergey's manual of determinative bacteriology, Ed No. II, 2001

Identified *Rhizobium* sp. and *Bacillus* sp. was mass multiplied conical flask containing sterile liquid medium such as yeast extract mannitol broth and nutrient broth for 4 - 5 days under controlled conditions. Cell count was determined from fresh broth after suitable dilution in a haemocytometer (Vincent, 1970). After the count, the cell suspension was mixed with vermicompost at the rate of $1x10^4$ cells gram of vermicompost and the mixture was mixed with soil for a pot culture studies.

RESULTS AND DISCUSSION

The physico-chemical characteristics of the control and worm-worked (experiment) composts are showed in Table 2.

The pH of the vermicompost changed from its original value towards neutral. Most of the researchers reported that the vermicasts are more neutral and this may be due to the fact that

Table 1. Methods used for analyzing various physico-chemical parameters of the vermibed substrates after 90 days

Parameter	Method	Reference
p^H	Digital p^H meter	Jackson (1973)
Electrical conductivity (E.C.)	Conductivity bridge	Jackson (1973)
Organic carbon	Potassium-dichromate oxidation method	Walkley and Black (1947)
Total nitrogen	MicroKjeldhal method	Tandon (1993)
Total phosphorous	Spectrophotometric method	Tandon (1993)
Total potassium	Flame photometric method	Tandon (1993)

Table 2. Microbial colony forming units of the control and worm worked compost (90 days)

Vermicompost	Bacteria X 10^7/g	Fungi X 10^3/g	Actinomycetes X 10^4/g
Control	152	110	121
Worm worked	311	182	206

et al., 1999; Mba, 1983).

The microbial colony forming units (CFU) of bacteria, fungi and actinomycetes observed in the experiment and in the control of all three-leaf litters. All the microbial colony-forming units were higher in the worm worked substrates than in the control.

The increase of microorganisms is due to the activity of earthworms and their castings, which encouraged the growth of microorganisms (Tiwari and Mishra, 1993).

The predominant colonies of watery translucent white opaque appearance without absorbing the congo red were identified as Rhizobium , using staining motility tests, bio-chemical test. The predominant colony was identified as *Bacillus* sp. using staining, motility, bio-chemical test. The isolated phosphate solubilizing bacteria was further screened for their ability to solubilize phosphate and insoluble phosphate source quantitatively in liquid medium. The production of clearing zones around the colonies of the organism is an indication of *Bacillus* sp.

The predominant colony was identified as *Rhizobium* sp. and *Bacillus* sp. was mass multiplied through shaker flask technique and their cell count was measured on 5th day was 4.51 and 5.49 × 10^9 /ml.

In the present study, vermicompost along with combination of microbial inoculants were able to initiate rooting and development of roots better than control. Vermicompost application has shown increase in the germination efficiency and yield of plants (Sevugaperumal *et.al*. 1998 and Buckerfield *et al*. 1999). The early appearance of flowers, improved pod parameters were observed largely in plants treated with microbial inoculant enriched vermicompost. Inoculation of *Rhizobium* sp*., Bacillus* sp. and *Aspergillus* sp. significantly increased the nodulation in cowpea. Similar findings have also been reported by Sharma and Namdeo (1999).The phosphate solubilizing bacillus possess the ability to bring soluble 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.

Pot culture studies has shown that the growth and yield parameters of cowpea, *Vigna unguiculata* (L.) walp. were also significantly higher in vermicompost along with microbial inoculants than in vermicompost and control. So the present study indicates that vermicompost can very well be used as carrier material for the microbial inoculants.

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