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EFFECT OF FORMULATION OF EFFECTIVE MICROORGANISM (EM) ON POST TREATMENT PERSISTENCE, MICROBIAL DENSITY AND SOIL MACRONUTRIENTS

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Abstract

Effective Microorganisms (EM), a culture of coexisting beneficial microorganism predominantly consisting of lactic acid bacteria, photosynthetic bacteria, yeast, fermenting fungi and actinomycetes that are claimed to enhance microbial turnover in soil and thus known increase soil macronutrients and increases plant growth and yield. In the present study, Pot trails were conducted to evaluate the effect of various formulations of Effective microorganisms (EM) viz rice washed water, rice bran and oil cake, sugar syrup, rice bran, oil cake and sugar on post treatment persistence of EM and soil nitrogen, phosphorous and potassium level. The experiment was initiated with four groups of five replications. Group one was the negative control with only water added throughout the study. Group two was the second control with respective formulating agents without EM (0.01%). Group three was only EM solution (0.01%) and group four was respective formulations of EM as granulated form (0.01%). Among the formulations rice bran +oil cake +sugar syrup formulation recorded maximum N,P,K level followed by sugar syrup. Moreover the occurrence of respective microbial member was recorded in all the formulations and maximum microbial count was recorded in rice bran, oil cake and sugar syrup formulation. Similarly the pots treated with EM with rice bran+oil cake+ sugar syrup increased the microbial density other than individual members of EM of the soil.

Keywords: Effective microorganism, Formulations, Macronutrients, Microbial density

Introduction

Farmers have adopted the strategy of increasing crop yields by applying large amounts of chemical fertilizers and pesticides. At present, however, the negative effects of heavy applications of chemical imputs, in terms of production, environment and quality deteriorations are becoming apparent [1] The ultimate goal of sustainable agriculture is to develop farming systems that are productive, energy conserving, environmentally sound conserving of natural resources such as soil and water and thus ensure food safety and quality Organic agriculture has much in common with sustainable agriculture. The same stress is placed upon the use of renewable resources, conservation of resources and the maintenance of environmental quality without using chemical imputs [2])Microbial inoculants is one way organic farmers are able to increase yield and quality of crops without a large investment of money and labor [3].

A microbial inoculant containing many kinds of naturally occurring beneficial microbes called 'Effective Microorganisms' has been used widely in nature and organic farming [4].The concept of Effective Microorganisms was developed by Japanese horticulturist Teuro higa from the University of Ryukyus in Japan. He reported in the 1970s that a combination of approximately 80 different microorganisms is capable of positively influencing decomposing organic matter such that it reverts into a life promoting process. The Studies have shown that EM may have a number of applications, including agriculture, livestock, gardening landscaping, omposting, bioremediation, cleaning septic tanks, algal control and household uses [5] The application of EM will improve soil and irrigation water. It can be used in seed treatment. It can be used to make organic sprays for the enhancement of photosynthesis and control of insects, pests and diseases [6] Successful use of EM depends on suitable formulation techniques. The formulation method increased their persistence and dependability on the prevailing environmental condition and offered protection against unfavourable environmental condition. Moreover EM can show better performance if they are mixed with suitable ingredients which may act as nutrients, adhesives or wettable agents [7]. In the present study, the various formulations of Effective Microorganisms (EM) viz rice washed water ,rice bran +oil cake, sugar syrup + rice bran+ oil cake and sugar syrup on post treatment persistence of EM and soil nitrogen, phosphorous and potassium level and influence of microbial density other than respective members of EM under pot trail was studied.

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Materials and Methods

Effective microorganism (EM)

The liquid culture of the EM used in the study was supplied by Environ Biotech and contained a mixture of lactic acid bacteria *Lactobacillus plantarum* (1.0 X 10^4) yeast with 1.0X10^5 CFU/ml *Candida utilis*, actinomycetes *Streptomyces albus* (3.0X10^3 CFU/ml), fermenting fungi *Aspergillus oryzae* (1.1X10^5 CFU/ml). EM solution is a yellowish liquid with a pleasant odour and sweet sour taste with a pH of 3 and stored in cool place without refrigeration

Selection of media for formulation

The following substrates were selected for formulation viz rice washed water, rice bran and oil cake, sugar syrup, rice bran, oil cake and sugar syrup.

Formulation with rice washed water

500gms of rice was soaked in a liter of water overnight and after overnight soaking filtered through cheese muslin cloth. 100 ml of the filtrate was collected in 250 ml of conical flask, steam sterilized for 30 minutes, after cooling, 1ml of EM was inoculated. The preparation was used for soil assay.

Formulation with rice bran and oil cake

100gms of rice bran and 25g of groundnut oil cake were added to 1 liter of distilled water and sterilized by autoclaving. The homogenized substrate was then cooled and mixed with 1ml of EM. The preparation was kept for 24hours and used for soil assay.

Formulation with sugar syrup

100gms of sugar was soaked in 1liter of sterile distilled water for five hours,, syrup was sterilized by filtration through membrane filter and the collected filtrate (150 ml) was mixed with 1 ml of EM . The preparation was kept for 24 hours and used for soil assay.

Formulation with rice bran, oil cake and sugar syrup

40g of rice bran, 30g of oil cake and 30g of sugar were added to 1 liter of distilled water and sterilized by autoclaving. The homogenized substrate was then allowed to cool. 1ml of EM was inoculated into the substrate. This preparation was kept for 24 hours and used for soil assay

Treatment methods

Fertile loam soil was collected from paddy field and it was sieved and about 2 kg of soil was sterilized at 180°C for 24 hours and the sterilized soil was transferred to the twenty five- cm diameter pots. Pots were divided into four groups of five replications. Group one was the negative control with only water added throughout the study. Group two was the second control with respective formulating agents without EM (0.01%) Group three was only EM solution (0.01%) and group four was respective formulations of EM as granulated form (0.01%) scattered over the top of the soil in each pot. All the pots were allowed to sit for 90 days.

Soil total N,P and K assay

Soil samples from respective treatment were analyzed for total nitrogen (alkali $KMnO_4$ method), phosphorous (Olsens method) and potassium (Flame photometric method) contents.

Evaluation of persistence and microbial density

To study the persistence of microbial members of EM, the soil samples were analysed for the occurrence of individual microbial members by soil dilution technique using the method of Yanagida and Shinohara [8]. 1g. of the soil sample from respective treatment taken from depth of 3cm and serially diluted with sterile phosphate buffer and 0.1 ml of the aliquote was spread plated on trypticase soy agar plates (Bacteria), Starch casein agar (actinomycetes) and sabouraud dextrose agar (mold and yeast). After incubation,the number of colony forming units(CFU) per gram was determined to estimate number of viable microbial cells.

Results and Discussion

Effect of Formulations on soil N,P and K

The pots treated with EM formulations (Group IV) showed increased level of N,P and K than Group,I,II and III category and was significantly higher than the other three groups (p=0.0552) (Table 1). The maximum level of NPK was recorded in EM formulated with rice bran+oil cake+ sugar syrup) (176 mg/kg, 249 mg/kg, 99.37 mg/kg), followed by sugar syrup (133 mg/kg, 143 mg/kg, 98.76mg/kg), rice bran and oil cake (94.31 mg/kg, 76.81 mg/kg, 66.2 mg/kg), rice washed water (73.34 mg/kg, 39.89 mg/kg, 58.76 mg/kg).

Table 1.Nitrogen, phosphorous and potassium level of soil treated with various formulations of effective microorganism

S. No	Treatment	Group	Nitrogen (mg/kg)	Phosphorous (mg/kg)	Potassium (mg/kg)
1	Water	I	41.34	27.57	37.34
2	Formulating agents	II			
2a	Rice washed water		63.22	38.23	68.22
2b	Rice bran+oil cake		63.25	39.12	68.07
2c	Sugar syrup		64.24	39.05	68.03
2d	Rice Bran+oil cake +sugar Syrup		67.34	40.12	69.12
3	EM solution without formulating agents	III			
4	EM with respective formulating Agents	IV			
4a	EM+rice washed water		73.34	39.89	58.76
4b	EM +Rice bran+oil cake		94.31	76.81	66.20
4c	EM+sugar syrup		133.00	143.00	98.76
4d	EM+ Rice Bran+oil cake +sugar syrup		176.00	249.00	99.37

In group I category, the available NPK levels was 41.34mg/kg, 27.57 mg/kg, 37.34mg/kg).In group II category, the available NPK levels in rice washed water (63.22mg/kg, 38.23mg/kg, 68.22mg/kg), rice bran and oil cake (63.25mg/kg, 39.12 mg/kg, 68.07 mg/kg), sugar syrup (64.24mg/kg, 39.05mg/kg, 68.03mg/kg), rice bran, oil cake and sugar syrup (67.34 mg/kg, 40.12mg/kg, 69.12mg/kg). Pots treated with EM only showed (Group III) increased level of N,P and K level than Group I and II. But it was not statistically significant than Group IV($p=0.0552$). 111.10,91.37 and 79.12 mg/kg of nitrogen, phosphorous and potassium was recorded in this treatment. Piqueres et al [2] reported that soil EM combined with compost or organic matter increases the activity, persistence of EM and thus increases soil nutrients Impact of EM amended with different organic amendments such as chopped straw and lupine seed meal reveals that total soil organic carbon, total nitrogen mineral N was increased [9] In the present investigation, N,P and K level was found to be higher in EM combined with various formulating agents than EM without any formulations.

Persistence of EM and microbial density other than members of EM

All the microbial members could be recorded in all the formulations treated pots (Table 2). But maximum occurrence of all the microbial members was observed in EM formulated with rice bran + oil cake + sugar syrup. Colony count of respective microbial members reveals. *Lactobacillus plantarum* 21.0 $X10^{7,}$ *Candida utilis* ($11.2X10^{6}$ actinomycetes (*Streptomyces albus* $13.0X10^{7}$ CFU/ml, fermenting fungi *Aspergillus oryzae* ($1.1X10^{6}$ CFU/ml) (Table 2) Followed by rice bran+ oil cake+ sugar syrup EM formulated with sugar syrup recorded maximum populations. Colony count of respective microbial members reveals. *Lactobacillus plantarum* $29.0X10^{6}$ *Candida utilis* $67.1X10^{5}$*, Streptomyces albus* $2.4X10^{5}$ CFU/g. *Aspergillus oryzae* $47.0X10^{5}$ CFU?g. Least populations was recorded in rice washed water formulation. (*L. plantarum* $67.1X10^{5}$ *Candida utilis* $31.4X10^{5}$ *Streptomyces albus,* $29.0X10^{4}$ *Aspergillus oryzae* $11.0X10^{5}$ All the microbial members were recorded in EM without formulating agents treated pots and the count of respective microbial members reveals. *Lactobacillus plantarum* $17.0X10^{5}$, *Candida utilis* $21.5X10^{5}$, *Streptomyces albus* $3.0X10^{4}$ *Aspergillus oryzae* $2.1X10^{5}$ CFU?g. But it was not statistically significant than EM rice bran+ oil cake+sugar syrup. The microbial density other than respective microbial members was found to be increased in the EM rice bran+ oil cake+sugar syrup formulations. Total heterotrophic bacterial, fungal including yeasts and actinomycetes was found to be $12.1X10^{6}$,$21.3X\ 10^{5}$ and $15.1X10^{4}$ CFU/g. in the same formulation treated soils. But the remaining formulations treated soils recorded very least respective microbial populations. Sugar formulation recorded $45.4X10^{5}$ (bacteria) $24.1X10^{4}$ (actinomycetes) $71.2X10^{3}$ (mold and yeast). EM without formulations also stimulate total heterotrophic microbial populations (Table 3). But it was not statistically significant than respective formulation $21.2X10^{3}$ $43.3X10^{2}$ and $11.0X10^{2}$ CFU/g.of bacterial, actinomycetes and mold including yeast populations was recorded. When Effective Microorganisms increase as a community in soils, populations of native effective microorganisms are also increased. Thus, the micro flora becomes rich and the microbial ecosystems in the soil become well-balanced Rice bran contains valuable components such as oil, protein, vitamins and some essential minerals as well as enzymes, microorganisms. It is found to effectively adsorb several organic compounds, such as dichloromethane, chloroform, carbon tetrachloride, trichloroethylene, tetrachloroethylene and benzene.. In recent year, it has been realized that the application of composted materials to the soil is more beneficial than direct application of raw materials, because raw materials might contain phytotoxic organic materials or ammonia, which may cause phytotoxicity to vegetable crops Amendment of soil with oil cake helps in reducing the

soil borne pathogens and it also increases the total microbial population in the rhizosphere. Sugar being present in the soil may stimulate the proliferation of microorganisms[10] EM combined with organic matter or other compost which is an important source of nutrients usable by microorganism for improving soil nutrients and microbial density and thus increase plant growth biomass research studies shows that the chemical, physical and microbiological properties of an organic fertilizer that was inoculated with EM amended with organic matter and compost was found to be drastically increased [11]. In the present study the combination of rice bran+ oil cake + sugar syrup enhances the persistence of EM, increased the microbial density and soil nutrients. Moreover the formulation is cost effective and we recommend this formulation to Farmers for sustainable crop production.

Table 2.Total viable count of individual members of Effective Microorganism (EM) in different formulations of EM treated soil

		CFU/g		
S. No	Formulation	Bacteria	Actinomycetes	Mold and yeast
1	Water	-	-	-
2	Formulating agents	-	-	-
2a	Rice washed water	-	-	-
2b	Rice bran+oil cake	-	-	-
2c	Sugar syrup	-	-	-
2d	Rice Bran+oil cake +sugar Syrup	-	-	-
3	EM solution without formulating agents	$21.2X10^3$	$43.3X10^2$	$11.0X10^2$
4	EM with respective formulating Agents			
4a	EM+rice washed water	$13.1X10^4$	$1.2X10^3$	$47.3X10^2$
4b	EM +Rice bran+oil cake	$37.8X10^5$	$19.1X10^4$	$67.2X10^3$
4c	EM+sugar syrup	$45.4X10^5$	$24.1X10^4$	$71.2X10^3$
4d	EM+ Rice Bran+oil cake +sugar syrup	$12.1X10^6$	$21.3X10^5$	$15.1X10^4$

Table 3.Total viable count of individual members of Effective Microorganism (EM) in different formulations of EM treated soil

		CFU/g			
S. No	Formulation	*Lactobacillus plantarum*	*Candida utilis*	*Streptomyces albus*	*Aspergillus oryzae*
1	Water	-	-	-	-
2	Formulating agents	-	-	-	-
2a	Rice washed water	-	-	-	-
2b	Rice bran+oil cake	-	-	-	-
2c	Sugar syrup	-	-	-	-
2d	Rice Bran+oil cake +sugar Syrup	-	-	-	-
3	EM solution without formulating agents	$17.0X10^5$	$21.5X10^5$	$3.0X10^4$	$2.1X10^5$
4	EM with respective formulating Agents				
4a	EM+rice washed water	$67.1X10^5$	$31.4X10^5$	$29.0X10^4$	$11.0X10^5$
4b	EM +Rice bran+oil cake	$1.0X10^6$	$54.0X10^5$	$70.0X10^4$	$21.0X10^5$
4c	EM+sugar syrup	$29.0X10^6$	$67.1X10^5$	$2.4X10^5$	$47.0X10^5$
4d	EM+ Rice Bran+oil cake +sugar syrup	$21.0X10^7$	$11.2X10^6$	$13.0X10^7$	$1.1X10^6$

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