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Effects of Effective-Microorganism (EM) Treated Fish Pond Effluent on Soil Microbial Activities

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

An experiment was conducted at the Department of Crop/Soil Science, Rivers State University to evaluate effects of Effective Microorganism (EM) treated fish pond effluent on soil microbial activities. The study had two levels of fish pond effluent (Diluted and undiluted) and three levels of treated effluent (0EMAS, EMAS, EMAS+EM5) laid out in a completely randomized design with three replications. Microbial count and Carbon-dioxide evolution were evaluated. Total Carbon-dioxide evolved after 15 weeks was highest in soils treated with EMAS+EM5-N and was least in EMAS+EM5-D. The identified fungal isolates were *Aspergillus* and *Mucor* species, and the identified bacteria were *Bacillus* and *Micrococcus* species but bacteria were more predominant than fungi and recorded highest in soils treated with EMAS+EM5-N. Also Effective Microorganism (EM) increased the biodiversity of microorganism. Data were subjected to analysis of variance.

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1. INTRODUCTION

"In many areas, integrating aquaculture with agriculture has become a channel for increasing the use of limited water resources, decreasing dependence on chemical fertilizers, and providing a greater economic return per unit of water" [1]. "In Nigeria, over a trillion liters of irrigation water per year are used in agriculture that could be used for aquaculture first. In return, fish waste and algae production have the potential to sustain crop growth and yield, while lowering the usage and cost of chemical fertilization. There is a great need for an environmentally safe best management practice that will alleviate the use of these fertilizers, but which will also continue to profit growers" [1].

"An awareness that soil microbial activity might have a role in developing soil structure has existed for many years"" [2]. "Studies have shown that soil bacteria themselves, release binding agents that enhance soil aggregation" [3]. "The bacteria produce extracellular polymeric substances that are released in the form of a peripheral slime and play a role in binding clay particles to form micro aggregates" [4]. "There is a perception that soil fungi play a more dominant role than bacteria in developing soil structure. This arose from a series of incubation studies where organic amendments were added to a poorly structured soil. Soil fungi play several roles in the development of soil structure" [5]. "The hyphal network can entangle soil particles and hold them together" [6]. "Hyphae can release chemical compounds including polysaccharides and glycoprotein mucilage, which act as adhesives to hold soil particles together" [7].

Soil microorganisms are important because they play a vital role in various bio- chemical cycles in the soil ecosystem. Therefore, any changes in the type or quantity of soil microorganisms may disrupt the natural soil ecosystem, which in turn may influence soil fertility. Microorganisms have major roles in pond culture, particularly with respect to productivity and nutrient cycling, the nutrition of the cultured animals, water quality, disease control and environmental impact of the effluent. Management of the activities of microorganisms in food webs and nutrient cycling in ponds is necessary for optimizing production, but the objectives will differ with the type of aquaculture, the species cultivated and the economics.

"Effective microorganisms (EM) on the other hand are mixed cultures of beneficial naturally occurring organisms that can be applied as inoculants to increase the microbial diversity of soil ecosystem. They consist mainly of the photosynthesizing bacteria. Lactic acids bacteria, yeast, actinomycetes and fermenting fungi (These microorganisms are physiologically comparable with one another and can coexist in liquid culture). There is evidence that EM inoculation to the soil can improve the quality of soil, plant growth and yield" [8,9].

"The positive effect of fish pond effluent on organic matter content, nutrients and crop yield has been severally reported" [10-12]. "It is reported that treating a pond with activated EM will keep enough beneficial microbes in the system and improve better fish development. It also reduces algae growth in the pond which compete the fish for nutrients, in addition, EM enhances the activities of beneficial indigenous microorganisms, for example mycorrhizae which fix atmospheric nitrogen thereby supplementing the use of chemical fertilizer and pesticides. There is evidence that EM inoculation to the soil can improve the quality of soil, plant growth and yield" [8].

2. MATERIALS AND METHODS

The research was carried out both in the fishery unit of the Department of Fisheries and Aquatic Environment and the screen house of Crop/Soil Science Department of Rivers State University. The site is located at longitude 4.7923^0N and 6.9825^0E with an elevation of 13m above sea level. The site has an average annual rainfall of about 200.45mm, relative humility is 69.08% and a mean annual temperature of 31.03^0C. Sandy loam soil was obtained from the Teaching and Research farm of Rivers State University, Cat fish (*Clarias gariepinus*) was procured from Momoh Farm Limited, Rivers State and fifty juveniles of cat fish were stocked in three different ponds. The ponds were cleaned using brush and water. The juveniles were fed with 2mm size feed at 30g per pond two times daily, and the feed was changed to 5mm feed size when the fish weighed 300g. The pond water was changed two times a week to enable collection of a good concentration of the effluent. The study had two levels of fish pond effluent (Diluted and undiluted) and three levels of treated effluent (0EMAS, EMAS, EMAS+EM5) laid out in a completely randomized design with three replications. Microbial count and identification were evaluated while Carbon-dioxide evolution was determined with Stotzy method to check the soil microbial activities. The microbial inoculants product (EMAS) which is a derivative of EM that provides nutrient for plants was produced in Rivers State University Crop/Soil Lab. “EM was activated by mixing with molasses and water in a composition of 94% (9400 ml) of water, 3% (300 ml) of molasses and 3% (300 ml) of EM1.The mixture was stirred, dissolved and left for seven days in a plastic container without exposure to direct sunlight. It was ready for use when read a constant pH of 3 giving a sweet and a sour smell. Two liters was then added to the pond twice weekly” [13].

“EM5 which is also a derivative of EM that has a pesticide function was produced in the crop/soil lab of Rivers State University by blending the molasses with warm water to make certain that it has been completely dissolved. Vinegar, distilled spirit, crushed peeled garlic clove, crushed hot peppers, crushed ginger, neem and EM was added to the dissolved molasses. The mixture was poured into a plastic container that was shut tightly to maintain anaerobic condition. The container was stored in a warm place out from direct sunlight and was allowed to ferment for about 2 weeks with a pH of 3.5 and a sweet smell” [13]. Two liters was then added to the pond twice weekly.

3. RESULTS AND DISCUSSION

3.1 Effect of Treated Fish Pond Effluent on Heterotrophic Count

The result of the Total Heterotrophic count of the bacteria present in the various treatments is as shown on Table 1, soils treated with diluted activated effective microorganism (EMAS) as nutrients gave the highest colony forming unit of $1.97x10^7$, generally, the diluted forms of the effluent gave a higher colony unit than the undiluted effluent, however the untreated effluent had the least colony forming unit. In some arid and semiarid regions, the use of fish pond effluent for irrigation is crucial for overall water management Jueschke et. al., 2008 although it may alter soil environment greatly and as a result, affect soil microbes Chen et. al 2008. The increase in the soil microorganisms as observed in the effluent treated soil was as a result of the EM added which is in line with the findings of Singh et al., 2003 that EM has the ability to increase and enhances the activities of beneficial indigenous microorganisms.

Table 1. Total Heterotrophic count in colony forming unit per milliliter

Treatments	CFU (Colony Forming Unit)
0EMAS-N	$1.26X10^6$
0EMAS-D	$1.69\ X10^6$
EMAS-N	$1.29\ X10^6$
EMAS-D	$1.97\ X10^7$
EMAS+EM5-N	$2.12\ X10^6$
EMAS+EM5-D	$2.91\ X10^6$

EMAS= Activated EM (Nutrient), EM5= Activated EM (Pesticides), N=No dilution, D=50% dilution, 0EMAS=Untreated Effluent

3.2 Effect of Treated Fish Pond Effluent on Number of Bacteria and Fungi

The total bacteria and fungi counts are presented in Fig. 1 result shows that bacteria is dominant than fungi. The mean bacteria count ranged between 126-212 with the highest in EMAS+EM5-N while the mean fungi count ranged between 27-54 with the highest in EMAS+EM5-N. Two genera of fungi were isolated from the studied soil which is *Aspergillus* spp. and *Mucor* spp. and two genera were also isolated for bacteria which are Bacillus spp and Micrococcus specie. The variations in microbial population indicate the impact from the root exudates which differed in chemical and in quantity among plants as observed by Bergsma-Vlami et. al., [14] when produced by plant can cause immediate and profound response in the microbial population and may result in a buildup of micro flora specific to a particular plant species and genotype.

3.3 Effect of Treated Fish Pond Effluent on *Aspergillus* and *Mucor* spp.

Fig. 2 reveals 2 genera of fungi isolated from the studied soil which are *Aspergillus* spp. and *Mucor* spp. There was no significant difference among treatment for *Aspergillus* spp.($p>0.05$) but mean difference with the highest count in EMAS-N and the least in 0EMAS-N. For *Mucor* specie there was also no significant difference among treatment but mean difference in the order 0EMAS-D>EMAS+EM5-N>EMAS+EM5-D>EMAS-N>EMAS-D>CONTROL.

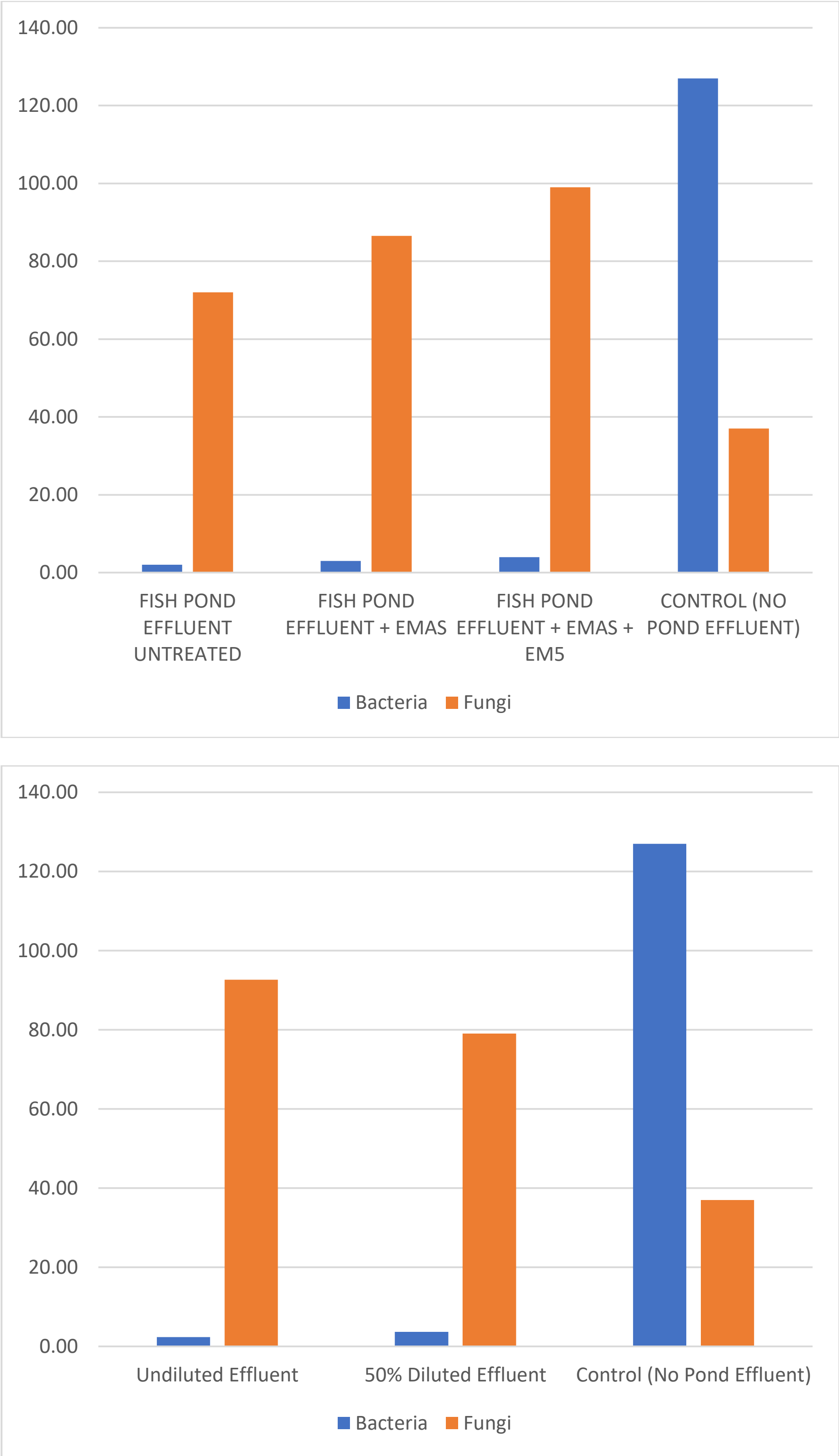

Fig. 1. Effect of treated fish pond effluent on Number of Bacteria and Fungi

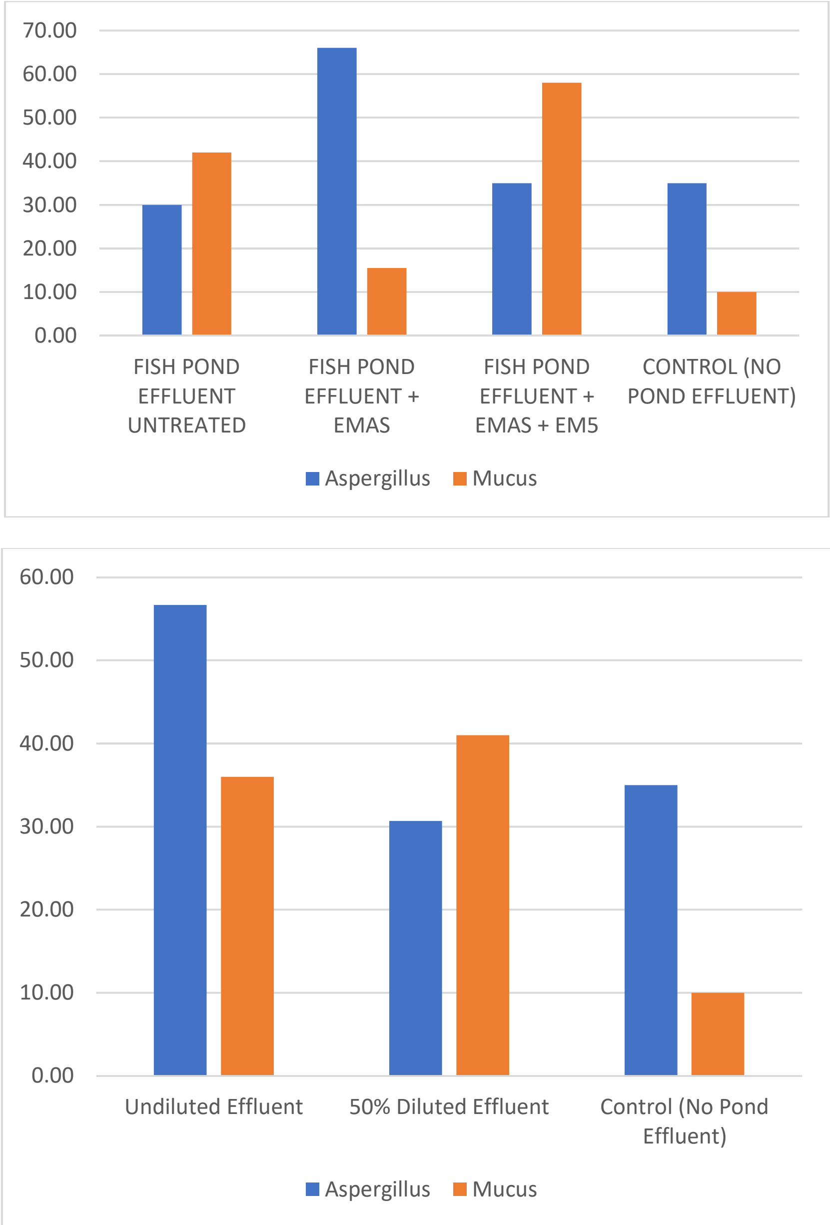

Fig. 2. Effect of treated fish pond effluent on Aspergillus and Mucor spp.

3.4 Effect of Treated Fish Pond Effluent on *Bacillus* and *Micrococcus* spp.

The result of the effect of treated fish pond effluent on bacteria isolate and count is as shown in Fig. 3. *Bacillus* specie was more in abundance when compared to *Micrococcus* specie *Bacillus* specie was present across all treatments while *Micrococcus* specie was seen only in soils treated with 0EMAS-N and absent in other treatments. The *bacillus* spp. count was in the order 0EMAS-N>0EMAS-D=EMAS+EM5-N=EMAS+EM5-D=CONTROL>EMAS-N=EMAS-D with 5, 4 and 3 isolates respectively. This is similar to the observation of Shaikhul Islam et al., [15] who isolated *Bacillus* spp. in a cucumber grown soil.

3.5 Effect of Treated Fish Pond Effluent on Total Carbon Dioxide Evolved

The result of the effect of treated fish pond effluent on total carbon-dioxide evolved after 15 weeks is as shown in Fig. 4. Soils treated with EMAS+EM5-N had the highest carbon-dioxide evolution and was recorded the least in the soils treated with EMAS+EM5-D. However, the undiluted effluent had the highest carbon-dioxide evolution compared to the diluted effluent. As observed by Stoklasa's [16], the amount of CO_2 evolved was dependent on the mechanical condition of the soil, its fertility and crop grown and that intensity of CO_2 produced shows the presence not only of active bacteria but also of easily available organic matter .The determination of CO_2 evolved by a soil under given degrees of moisture and temperature in a certain length of time was also believed by Stoklasa's [16], to furnish a reliable and accurate method of determination of the bacterial activity. EMAS(N) had the highest heterotrophic count which could also be as a result of the high CO_2 evolution and similar to the observation made by Montealegre et al., [17], Carney et al., [18], and Kandler et al. [19] that high carbon-dioxide evolution changes the microbial population of a soil [20-22].

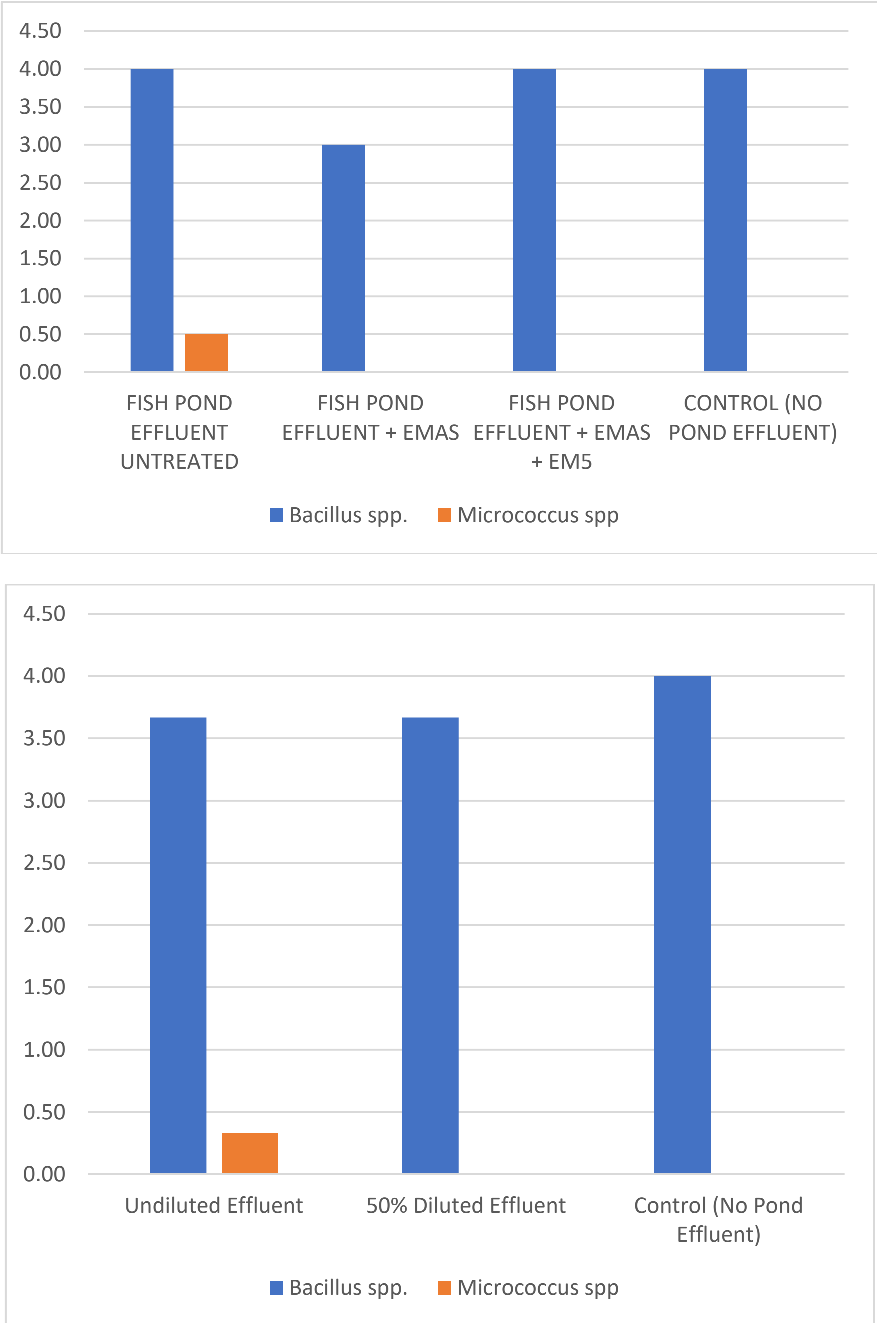

Fig. 3. Effect of treated fish pond effluent on *Bacillus* and *Micrococcus* spp.

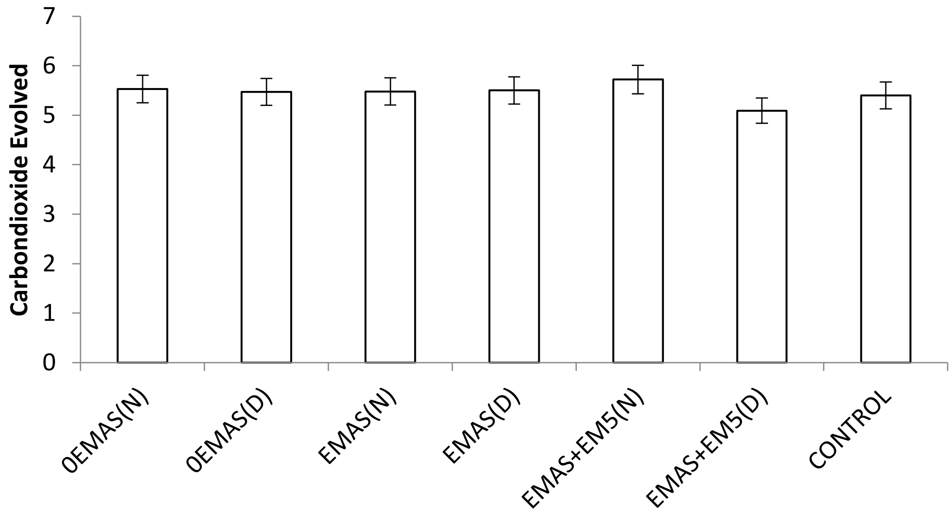

Fig. 4. Effect of treated fish pond effluent on Total Carbondioxide evolved
EMAS= Activated EM (Nutrient), EM5= Activated EM (Pesticides), N=No dilution, D=50% dilution, 0EMAS=Untreated Effluent

4. CONCLUSION

The result of this experiment showed that Effective Microorganism (EM) increased the biodiversity of microorganisms and in turn increases soil quality. Pond effluent encourages organic farming and also encourages the use of water in a sustainable way, As it is generally understood that microbial community benefits crop yields, health, growth and soil productivity, Effective Microorganism (EM) should be applied as a source of nutrient as it increases populations of beneficial microorganisms in the soil helping to control soil diseases through a natural processes by enhancing the competitive and antagonist activities of the microorganisms in the EM inoculants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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