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# Evaluation of Phytotoxicity of Effective Microorganism (EM) Treated Distillery Industry Effluent

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**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, distillery effluents decomposition with EM technology into high nutrient content of treated effluent and its impact on plant growth parameters of vignamugo (Green Gram) was studied. The distillery effluents was effectively decomposed by commercial formulation of the EM-1 with complete conversion of fresh distillery effluents into finely very rich in treated effluent with 6.33ppm of total Nitrogen (N), 167.6ppm of total phosphorous (P), 153% of total potassium (K),and 0.72 % of organic matters. Plant growth parameters such as shoot length, leaf surface area, total foliage per count, total leaves and branches emerged in the plants, chlorophyll content of compost treated plant reveals 0.42 mg/g. The study explores the possible augmentation of EM as a major source of conversion of wastes into high nutrition organic manures and thus reducing dumping of industrial effluent into the environment.**

**Key words:** Effective microorganism, Formulations, Decompositions, Compost.

Among the raw material sources for distillery, two very important raw materials are cane sugar molasses and beet sugar molasses. Distillery wastewater (stillage) is the main byproduct originating in distilleries, and its volume is approximately 10 times that of ethanol produced. It is not surprising that the utilization of the stillage raises serious problems, and that many attempts have been made all over the world to solve them. Distillery wastewater is usually comprised of a high volume of greatly acidic matter which presents many disposal and treatment problems. Waste streams generally contain high levels of both dissolved organic and inorganic materials.

Effective Microorganisms, aka EM Technology, is a trademarked term now commonly used to describe a proprietary blend of 3 or more types of predominantly anaerobic organisms.

EM consists of the following five families of micro-organisms: [1] Lactic acid bacteria: these bacteria are differentiated by their powerful sterilising properties. They suppress harmful micro-organisms and encourage quick breakdown of organic substances. In addition, they can suppress the reproduction of Fusarium, a harmful fungus. [2]Yeasts: these manufacture anti-microbial and useful substances for plant growth. Their metabolites are food for other bacteria such as the lactic acid and actinomycete groups. [3]

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Actinomycetes: these suppress harmful fungi and bacteria and can live together with photosynthetic bacteria. [4] Photosynthetic bacteria: these bacteria play the leading role in the activity of EM. They synthesize useful substances from secretions of roots, organic matter and/or harmful gases (e.g. hydrogen sulphide) by using sunlight and the heat of soil as sources of energy. They contribute to a better use of sunlight or, in other words, better photosynthesis. The metabolites developed by these micro-organisms are directly absorbed into plants. In addition, these bacteria increase the number of other bacteria and act as nitrogen binders. [5] Fungi that bring about fermentation these break down the organic substances quickly. This suppresses smell and prevents damage that could be caused by harmful insects.

These effective micro-organisms secrete beneficial substances such as vitamins, organic acids, chelated minerals and antioxidants, when in contact with organic matter they change The soil micro flora and fauna so that disease inducing soil becomes disease-suppressing soil. The anti-oxidation effects of these micro-organisms pass directly to the soil or indirectly to plants maintaining their NPK and CN ratio. This process increases the humus content of the soil and is capable of sustaining high quality food production. By improving soil and plant health, EM assists in germination, flowering, fruiting and ripening in plants thus increasing yields and quality of product [6,7].EM also enhances the efficacy of organic matter as fertilizers and assists the photosynthetic capacity of plants (allowing them to use more of the light spectrum). Other beneficial effects include the suppression of soil borne pathogens and pests, and the ability to suppress weeds thus reducing the reliance on chemicals as control[8,9].

EM cuts fertilizer costs and in time also reduces the volume of weeds[10,11].As EM effective micro-organisms work largely in the anaerobic sector they are especially effective in areas where problems such as putrefaction, sludge and offensive odours occur. EM effective micro-organisms are used in arable farming, vegetable production, orchards and Viniculture. Using EM in fish breeding waters and ponds brings both an improvement in water quality and sludge reduction[12,13].

## MATERIALS AND METHODS

Commercial formulation of effective microorganism (EM) used in this study consist of *Lactobacillus planetarium*, *Candida utilis*, *Streptomyces albus*, *Aspergillus oryzae* and the formulation was maintained, stored and activated as per the manufacture instruction Activation was carried out by instruction by the manufactures.100gms of jaggery 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 10 ml of EM stock. The preparation was kept for 24 hours and used for further study.

**Sample collection**

Distillery effluent collected from mohan distillies limited-Chengalpattu,Kanchipuram District,Tamil Nadu,India in 5 liters plastic can.

**Effluent treatment**

The laboratory experiment was conducted to evaluate the effect of EM on Industrial waste effluents with ten replicates and untreated control. The setup consists of 2l Erlenmeyer flask with 1 litre of Industrial Effluent.100 ml of activated EM culture was added into the sample. The setup was operated continuoustly for 20 days.EM was added each day at the dilution rate of 1:10,000 for five days. The effect of EM was assessed by changes in the pH,oxygen demand, total dissolved solids after the incubation period in the EM treated sample( Table 1).Every 5 days after the EM application the tubs were observed for microbial growth and the process of decomposition. The treated effluent waste was analyzed for pH, organic carbon, humic acid,total nitrogen (N), Total phosphorous (P), and Total potassium (K). Every 5days interval the decomposed sample was analyzed for microbial population of respective EM members and non EM members, extra cellular enzyme activity. The plastic bottle with EM-1 treated distillery effluent waste were observed for effective decomposition.

**Evaluation of heterotropic microbial population during the decomposition**

Total heterotrophic bacterial, actinomycetes yeast and mold population was studied both control and treatment with serial dilution technique. 1 ml of the sample taken at the respective time interval was serially diluted and 1 ml of respective aliquot were added in

the sterile Petri plates, 20 ml of sterile molten nutrient agar, starch casein agar and potato dextrose agar was poured. The seeded plates were incubated at 37°C for 24 hours (bacteria); for 4-7 days (Actinomycetes); at 30°C for 3-4 days (Fungi).After the incubation period.colony count was recorded. After the incubation the seeded plates were observed for microbial colonies, the presence of individual members of EM and non EM members was characterized adapting standard microbial identification methods. The identified respective microbial isolates were maintained on the respective agar slants. (Table 2)

**Determination of nutritional parameters of em-1 treated soil**

The N,P,K parameters were evaluated by Alkali KMnO4, Olsens Method and Flame Photometric method carried out in national agro foundation Limited,Chennai. The content of the organic carbon and humic acid was also studied using the standard soil testing laboratory.

**Effect of em-1 treated effluent soil on seedling emergencleguminous plants**

The Healthy seeds of groundnut, green gram and black gram were immersed in suspension of EM treated effluent soil, and it is incubated overnight at room temperature. After the incubation period, the treated seeds were transferred to plastic pot filled with soil. A weighed quantity of 1ml of finely powdered EM-1 treated effluents is mixed with 5g of sterile loam soil. After the mixing, the soil is transferred to pot. The Diameter of pot is 30 X 10cm (approximately). Fully matured seeds of greengram was sown in each pot. The total number of pots assays were 3. After the sowing the seeds into the pot soil, the pots were transferred to open atmosphere under direct sunlight. Monitoring was done frequently for the evaluation of growth parameters. The pots were evaluated for seedling emergence and plant growth parameters.

**Evaluation of phytotoxic effect of em-1 treated effluents on green gram(vignamugo)**

**Description of the pot assay**

Phytoxicity af EM treated effluent on growth parameters of V.Munga was carried out by pot assay .In this experiments pot of (30 x 10cm) was filled with fertile loam soil and known quantity of raw effluent (Control), consordia EM treated effluent (T1) and commerical formulation of treated EM(T2) was mixed with the soil .The mixture was kept for one hour.The seeds of V.Mungo was sown in respective treatment box. Seeds germination was observed daily.The green gram was sown during second week of project duration at a spacing of 30Í10cm and regularly water was sprayed without conventional agriculture practices, chemical synthetic fertilizers and pesticides were not applied. After 20 days after seeding emergence, the application was initiated with ultra low volume sprayer during evening time at less wind velocity. Control plot was sprayed with raw effluent water only. Three replications were maintained for standard and test EM formulation. After treatment, the shoot length (cm), height of the plant (cm), leaf surface area (cm), total new branches emerged, total foliage density estimation, chlorophyll estimation, soil nitrogen, phosphorous and potassium level was carried out every 5 days after treatment.

## RESULTS AND DISCUSSIONS

Decomposition process was started with immediate application of EM-1 which was visualised clearly after the 5 days of application by the appearance of microbial growth and in subsequent days, decolouration and changes in physical texture and water activity was noted. The same observation was occurring rapidly at successive days. The complete decomposition was noticed on 20th day which is clearly identified by changes in the ph,colur,alkalinity,dissolved oxygen, BOD,COD and total dissolved solids. The treated effluent waste was collected and used for further studies.

**Evaluation of microbial status during decomposition**

All the microbial members in EM formulation was recorded in all tested time period. (Table 2)

**Determination of nutritional parameters of treated distillery effluent waste**

The total N,P,K and organic matter content of the compost reveals 6.33ppm of Total nitrogen, 167.6ppm of total phosphorous, 153ppm of total potassium and 0.72% of organic matter. But in case of control sample, the values were recorded as 8.1ppm of Total nitrogen, 68.8ppm of total phosphorous, 153ppm of total potassium and 0.65% of organic matter .The pie chart

representations of the above values are as below( Table 3- 4).

**Effect of treated effluent waste on seedling emergence of leguminous seeds**

A complete cent percentage of seedling emergence was recorded in all the tested seeds. Seedling emergence was recorded within 48 hours of treated effluent waste with soil application.

**Evaluation of plant growth parameters on greengram**

EM inoculated greengram plants showed distinct differences in all the tested plant growth parameters .These were significant differences (Pd" 0.05) in shoot length, total height of the plant, leaf number per plant, leaf surface area, total new branch emerged, total foliage count per plant and chlorophyll content. (Table 5)In greengram treated with EM-1, shoot length was recorded as 21.0 before treatment and 31.0cm as after the treatment. The control plant reveals 21.0 before the treatment and 27.6 after the treatment. The compost plant reveals 21.0 before the treatment and 34.7 after the treatment. In greengram treated with EM-1, the leaf surface area in test was found to be increased from 6.0 to 6.5 cm, the control plants shows from 4.1 to 7.2 cm and the compost plant shows the variation from 6 to 6.4cm. The total height of the greengram plant was also increased in test than control. The heights of the control plant were observed as from 24.0 to 37.0 cm in test, 24.0 to 27.0 cm in control and from EM-1 the height was observed as from 24.0 to 41.0 cmTotal emergence of new branches in greengram plant was also increased in test. The number of new branches emerged per plant was found to be increased as 1.0 to 2.1cm per plant for EM-1 plant during 4th ,15th and 30th days after

**Table 1.** Change in physic-chemical parameters of effluent treated with EM at different incubation period

| S. No | Parameter | Incubation time (days) | | | |
|---|---|---|---|---|---|
| | | 0 | 5 | 15 | 20 |
| 1. | pH | 9.0 | 8.4 | 7.7 | 7.1 |
| 2. | Alkalinity | 59.0 | 41.0 | 37.0 | 21.0 |
| 3. | Dissolved oxygen (mg/l) | 1.0 | 1.0 | 1.4 | 1.7 |
| 4. | BOD (mg/l) | 2.8 | 2.1 | 1.5 | 0.9 |
| 5. | COD (mg/l) | 164 | 141 | 112 | 109 |
| 6. | Total dissolved solids (mg/l) | 2160 | 1012 | 940 | 902 |

**Table 2.** Evaluation of microbial status during decomposition

| S. No | EM Members | Occurrence (days) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 | 20 |
| 1 | *Lactobacillus bulgaricus* | + | + | + | + | + |
| 2 | Actimomicetes,Streptomyces | + | + | + | + | + |
| 3 | *Aspergillusoryzae* | + | + | + | + | + |

**Table 3.** Determination of nutritional parameters of control effluent soil (N,P,K)

| S. No | Nutrition Parameters | Unit | Results |
|---|---|---|---|
| 1. | Organic matter | % | 0.65 |
| 2. | Nitrate Nitrogen N | PPM | 8.1 |
| 3. | Phosphorous P | PPM | 68.8 |
| 4. | Potasium K | PPM | 153.0 |

**Table 4.** Determination of nutritional parameters of EM treated effluent soil (N,P,K)

| S. No | Nutrition Parameters | Unit | Results |
|---|---|---|---|
| 1 | Organic matter | % | 0.72 |
| 2 | Nitrate Nitrogen N | PPM | 6.3 |
| 3 | Phosphorous P | PPM | 167.6 |
| 4 | Potasium K | PPM | 153.0 |

application in test likewise in control, the new branches emerged per plant was 1.0 and 2.10 cm at respective days and in compost, the new branches emerged per plant was 1.0 and 3.90 cm at respective days.Total foliage count per plant in greengram was also show distinct differences. In test treatment, the total foliage count per plant was from 15 to 40.1 cm in EM-1 and in control from 15.0 to 35.0 cm per plant was recorded and in compost, from 15 to 42.0 cm. There was no distinct differences was observed in chlorophyll content in test and control. 0.32 to 0.39 (mg/g) was recorded in control and in EM-1, from 0.32 to 0.42 (mg/g) and in compost from0.32 to 0.46 (mg/g) ( Table 5).

In this present study, the effect of inoculation of EM on growth, yield, total microbial population from soil and phyllosphere, pest infestation, disease spots, total new branches, total foliage leaves per plant are evaluated. It consists of two treatments. One set of treatment consist of control, while the other treatment consists of EM formulation. After the EM microbial consortia was bored in the pot soil, shoot length and height of the plant, surface area of leaf, total new branches, total foliage leaves, microbial population in soil and chlorophyll content and nitrogen, phosphorous and potassium level, organic carbon and humic acid levels were found to be increased in EM . There was significant difference in leaf chlorophyll content among the control and test. The results from the study demonstrated that growth and yield of ground nut and black gram may be improved by inoculating the plant with effective microorganisms. Increased shoot height stem diameter growth probably reflects allocation of resources into shoots rather than roots. Increase in the number of leaves and leaf area are common occurrences in plants that are provided with proper nutrition and this can increase the photosynthetic activity of the plants. Increase in leaf area and number of leaves should result to higher rates of photosynthesis hence increased plant growth. For

**Table 5.** Effect of EM treated effluent on plant growth parameters of V.Mungo(20 days)

| S. No | Plant Growth parameters | Treatment | Before Treatment (cm) | After Treatment (cm) |
|---|---|---|---|---|
| 1 | Length of shoot | Controlled | 21 | 27.6 |
| | | T1 | 21 | 31 |
| | | T2 | 21 | 34.7 |
| 2 | Leaf surface area | Controlled | 4.1 | 7.2 |
| | | T1 | 6 | 6.5 |
| | | T2 | 6 | 6.4 |
| 3 | Height of Plant | Controlled | 24 | 27 |
| | | T1 | 24 | 37 |
| | | T2 | 24 | 41 |
| 4 | Total New branches emerged in plant | Controlled | 1 | 2.1 |
| | | T1 | 1 | 3 |
| | | T2 | 1 | 3.9 |
| 5 | Total foliage content in plant | Controlled | 15 | 35 |
| | | T1 | 15 | 40.1 |
| | | T2 | 15 | 42 |
| 6 | Chlorophyll content (mg/g) | Controlled | 0.32 | 0.39 |
| | | T1 | 0.32 | 0.42 |
| | | T2 | 0.32 | 0.46 |

plants, a high rate of net carbon assimilation can result in higher biomass accumulation, favouring future growth and reproduction[14].The position and distribution of leaves along the shoot influences the sink strength of the plants. During early stages of leaf growth, synthesis of chlorophyll, proteins and structural compounds is high resulting in high catabolic rates to support energy needs by the plants. Inoculation of effective microorganism can increase the available nutrition for plant roots and improve photosynthesis. In general effective microorganisms seem to have direct impact on growth and yield of green gram and black gram. Further study will helpful to exploit the principles

of EM technology for the treatment of distillery effluent and its possible utilization of agriculture.

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