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Impact of effective and indigenous microorganisms manures on *Colocassia esculenta* and enzymes activities

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This study deals with the evaluation of the effect of effective microorganisms (EM) and indigenous microorganisms (IMO) manure on *Colocasia esculenta* (Taro) in Bambili-Cameroon. A randomized complete block design (RCBD) with three treatments (EM manure, IMO manure and control) and six replications were conducted. Investigations were performed taking into account morphological and agronomical parameters as well as disease incidence, total phenol contents, peroxidase (Pox) and polyphenoloxidase (PPO) activities. There was a significant difference ($p < 0.05$) in the height of plants and number of leaves throughout the period of research in plants treated with EM manure. Plants treated with EM manure gave the heaviest corms and cormels (15.549 ± 2.17 tons/ha) followed by plants treated with IMO manure (12.335 ± 1.69 tons/ha) and then the control plants (10.539 ± 2.24 tons/ha). Both EM and IMO manures were ineffective in controlling taro leaf blight disease that emerged in the field. Total phenolic content as well as Pox and PPO activities increased significantly during the first 5 months of development with EM manures producing the highest quantities followed by that of IMO manure. This is due to the microbial diversity of the manures which in turn improves soil quality and enhances the growth and yield of *C. esculenta*. These results suggest that EM and IMO manures can be used to ameliorated taro productivity but cannot be used to combat disease.

Key words: Taro, yields, phenolic content, peroxidase, polyphenoloxidase.

INTRODUCTION

Taro (*Colocasia esculenta* L.) is an herbaceous perennial plant widely cultivated in West and Central Africa. It is the third most important staple root/tuber crop after yam and cassava in Nigeria and second after cassava in Cameroon and first in Ghana (Echebiri, 2004). The main nutrient supplied by taro is carbohydrate (Jirarat et al., 2006), and it also contains proteins, vitamins and minerals (Duru and Uma, 2002). The prevalence of taro leaf blight caused by *Phytophthora infestans* (Brunt et al., 2001) and the declining soil fertility have had a negative impact on the yield of taro. Changes in dietary habits and preferences for exotic foods, and introduction of other crop species with better comparative advantages such as sweet potato and cocoyam have hindered taro production

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(Joughin and Kalit, 1986). During growth processes, many enzymes play physiological roles in plants. Phenolic compounds are essential for the growth and productivity of plants; they act as metal chelators by directly scavenging molecular species of active oxygen or by inhibiting lipid peroxidation by trapping the lipid alkaxyl radical (Michalak, 2006). Peroxidases (Pox) are a family of isoenzymes found in all plants and they are involved in the scavenging of reactive oxygen species (ROS), produced throughout plant development in response to biotic and abiotic stresses. Polyphenoloxidase (PPO) is important in the oxidation of phenolic compounds to quinines and the reinforcement of physical barriers of cells through the process of lignification (Campos-Vargas and Saltveit, 2002).

The increased use of chemical fertilizers and some organic fertilizers in agriculture helped the country in achieving self sufficiency in food production. However, it has also polluted the environment and caused slow deterioration of soil health. The chemical residues in the food product are also causing injury to human beings and animal production. To combat these problems and in the light of sustainable agriculture, green technology is now used by most farmers (Piqueres et al., 2005).

It has led to increased research efforts on the biological components of dynamic soil microorganisms that are beneficial for plant growth, resulting in rapid mineralization of organic matter, suppression of soil-borne pathogens and increased crop yield and quality (Higa, 1996). Such products include indigenous microorganisms (IMO) and effective microorganisms (EM) (Helen et al., 2006). IMO are beneficial members of the soil including filamentous fungi, yeast and bacteria collected from non-cultivated soils near the area where they are applied. IMO is collected from the environment surrounding the farm and its use is aimed at protecting life and integrity of the natural world. However, a high degree of farm management needs to be put in place if maximum benefits from IMO need to be obtained (Helen et al., 2006). EM consists of mixed culture of beneficial and naturally occurring microorganisms that can be applied as inoculants to increase the microbial density of soils and plants (Zimmermann and Kamukuendjie, 2008). Research has shown that the inoculation of EM cultures to the soil/plant ecosystem can improve soil quality, soil health and the growth, yield and quality of crops (Daly and Steward, 1999).

However, the use of mixed cultures of beneficial microorganisms as soil inoculants to enhance growth, health and quality of crops is still questionable by researchers since the claim lacks conclusive scientific proof (Szymanski and Patterson, 2003). In fact, data to justify the use of EM and IMO in cultivation and production of crops in Cameroon are lacking. Therefore, the aim of this study was to evaluate the effect of two organic manures (EM and IMO) on field cultivation of *C. esculenta* through morphological, agronomic and biochemical markers.

MATERIALS AND METHODS

Location

The experiment was carried out at the research farm of Higher Teacher Training College (HTTC) Bambili, University of Bamenda (Cameroon). It is located at Latitude 5°99'0'' north and longitude 10°15'00'' east. Bamenda is in the North West Cameroon highlands, having a mean temperature of about 24°C, a humid tropical climate with an average rainfall of 2000 mm and an altitude of 900 m above sea level (Gwaabe, 2000).

Land preparation

The land for planting was cleared and raked, and then ridges (70 to 100 cm) were formed. The experimental design was the randomized complete block design (RCBD) with three treatments and six replications: EM manure, IMO manure and control.

Manure preparation and application

The EM manure was prepared according to the method of Higa (1991), whereas IMO manure was prepared according to the method of Helen et al. (2006), using local farming field material. The treatments were applied 1 week before planting and 3 months after planting at a concentration of 20 g per hole. Cormels of *C. esculenta* (100 to 125 g) with 3 to 4 buds were planted at 1 m interval. Weeds were controlled manually and by mulching done 3 months after planting.

Measurement of morphological parameters

Plant height was measured with a tape at 2 weeks intervals across 5 months. Plant height was taken from the plant base to the tip of the top most leaf. The mean number of leaves for each ridge and treatment was also recorded.

Harvest

The taro cormels were harvested 9 months after planting when all the leaves had turned yellow. The number and weight of cormels for each treatment were recorded.

Disease incidence

Disease incidence was evaluated by counting number of leaves in each treatment showing yellowish-brown-soaked lesions. This was done at monthly intervals for 6 months.

Phenol analysis

Fresh leaves (0.5 g) were ground at 4°C with 80% methanol, and then centrifuged at 6 000 g for 15 min after 30 min incubation. The total phenolic compounds were determined using Folin-Ciocalteu reagent according to the method described by Macheix et al. (1990). Results were expressed in µg equivalent of chlorogenic acid by reference to the standard.

Extraction of proteins and analysis of peroxidase and polyphenoloxidase

Fresh leaves (200 mg) were extracted with 3 ml Tris-maleate buffer

Table 1. Variation of the height of *C. esculenta* plants under different treatments with time.

Duration (weeks)	Control	EM manure	IMO manure
2	3.500 ± 0.985[a]	3.778 ± 0.732[a]	3.278 ± 0.958[a]
4	6.278 ± 1.127[a]	6.611 ± 1.037[a]	6.389 ± 1.037[a]
6	9.389 ± 0.698[a]	9.944 ± 1.552[a]	9.667 ± 0.686[a]
8	11.389 ± 0.979[a]	11.944 ± 1.162[a]	11.611 ± 0.979[a]
10	14.556 ± 1.042[a]	15.222 ± 1.801[a]	14.556 ± 1.042[a]
12	18.111 ± 1.451[a]	19.944 ± 2.014[b]	18.667 ± 0.907[b]
14	20.000 ± 1.237[a]	21.667 ± 0.970[b]	21.000 ± 0.840[a]
16	21.222 ± 1.166[a]	22.500 ± 1.200[b]	21.667 ± 0.907[a]
18	20.389 ± 0.850[a]	22.667 ± 1.138[b]	20.722 ± 1.127[a]
20	20.500 ± 1.618[a]	22.278 ± 1.776[b]	20.389 ± 1.145[a]

Means with same letter in the same line are not significantly different at $P < 0.05$ (Student Newman and Keuls test).

(0.1 M, pH 6.5) containing Triton X-100 (0.1 $g.L^{-1}$) and centrifuged for 15 min at 6 000 g after 1 h incubation at 4°C. The supernatant obtained was used as the crude protein extract. Pox activity was assayed spectrophotometrically at 470 nm using guaiacol as a substrate. Twenty-five microliters of enzyme extracts was added to 2.5 ml of reaction mixture containing a solution of 0.1M Tris-maleate buffer (pH 6.5) and 25 mM guaiacol. Reactions were initiated with 20 µl of H_2O_2 (10%) and ascorbic acid (0.25 mM) and stopped after 2 min. PPO activity was determined by measuring the oxidation of 0.2 M catechol at 420 nm. Enzyme activity was expressed as enzymatic unit.mg^{-1} fresh weight (EU.mg^{-1} FW)

Data analysis

The data obtained were expressed as means ± SD and were statistically analyzed using the SPSS statistical software Version 17.0 (SPSS Inc., Chicago). The significant difference between mean values was determined using analysis of variance (ANOVA). Student Newman-Keuls test was used to compare means at 0.05 level of significance.

RESULTS

Variation of the height of *C. esculenta* plants

Results showed a gradual increase in height of plants for all treatments up to week 16 with control having the longest plants. From week 18 till the end of the experiment, EM manure produced the longest plants. No significant difference was detected for plant treated with IMO manure and that of control, while a significant difference ($P < 0.05$) was noted for plants treated with EM manure (Table 1).

Variation of the number of leaves of *C. esculenta* plants

The number of leaves per plant increased in all treatments till the 12th week of the experimental period. Plants treated with EM manure and IMO manure showed a significant difference in number of leaves during the early stages of experiment (up to week 8) when compared with control plants. Between weeks 16 and 18, there was a significant difference ($P < 0.05$) in number of leaves in plants treated with both EM and those treated with IMO manure compared with control plants. During the last week, there was a significant difference in the number of leaves for plants treated with EM manure only (3.222 ± 0.732) (Table 2).

Evaluation of productivity

Significant differences at $P < 0.05$ were observed for mean weight of cormels produced per treatment. Plants treated with EM manure recorded cormels that weighed the most (15.549 ± 2.17 tons/ha) followed by plants treated with IMO manure (12.335 ± 1.69 tons/ha) and then control plants (10.539 ± 2.24 tons/ha) (Figure 1).

Evaluation of the disease incidence

The disease incidence increased for all treatments up to the 4th month and started decreasing. After 1 month, control plants had the highest disease incidence (2.5 ± 1.25) followed by plants treated with IMO manure (2.16 ± 1.72) and then plants treated with EM manure (1.83 ± 0.87). At the 4th month, this disease increased in all plants, with plants treated with IMO manure having the highest value (8.66 ± 3.74) over plants treated with EM manure (7.83 ± 1.47) and control plants (7.66 ± 3.47). These values decreased at 6 months with EM manure plants recording the highest and significant value at $p < 0.05$ (6.00±1.26) followed by control plants (4.02 ± 1.78) and IMO manure plants (4.83 ± 2.71) (Figure 2).

Evaluation of the total phenol content

After 2 months of growth, EM manure treated plants

Table 2. Variation of the number of leaves of *C. esculenta* plants under different treatments with time.

Duration	Control	EM manure	IMO manure
2	3.111 ± 0.963[a]	2.778 ± 0.943[a]	3.333 ± 0.907[a]
4	3.944 ± 0.873[a]	4.556 ± 1.199[b]	4.611 ± 0.778[b]
6	4.444 ± 0.922[a]	4.756 ± 1.097[a]	4.944 ± 0.873[a]
8	4.944 ± 1.162[a]	5.644 ± 0.984[b]	5.889 ± 0.758[b]
10	5.778 ± 1.114[a]	5.722 ± 0.826[a]	5.833 ± 0.707[a]
12	6.056 ± 1.110[a]	6.156 ± 0.938[a]	6.111 ± 1.132[a]
14	5.389 ± 0.850[a]	5.278 ± 1.127[a]	5.222 ± 1.309[a]
16	4.222 ± 0.943[a]	4.611 ± 1.145[b]	4.611 ± 1.092[b]
18	3.244 ± 0.938[a]	3.922 ± 0.575[b]	3.833 ± 0.857[b]
20	2.667 ± 0.594[a]	3.222 ± 0.732[b]	2.778 ± 0.808[a]

Means with same letter in the same line are not significantly different at P < 0.05 (Student Newman and Keuls test).

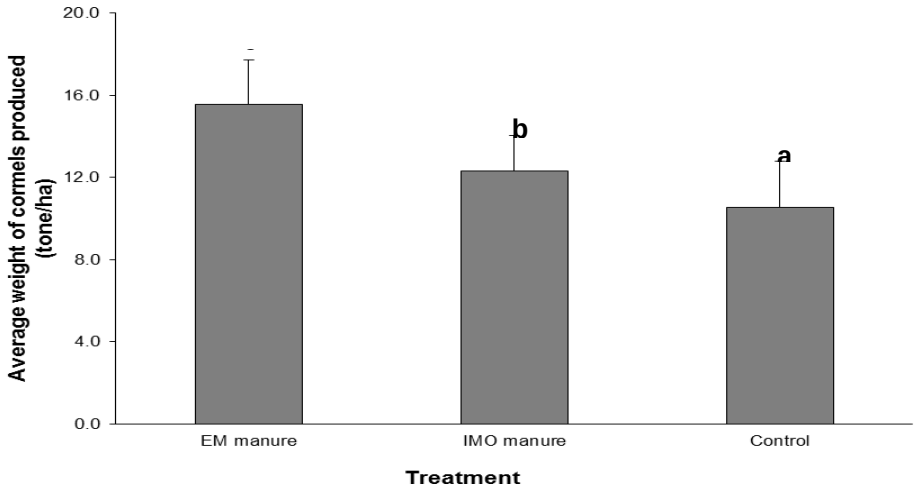

Figure 1. Average weight of cormels of *C. esculenta* produced (tons.ha^{-1}) per treatment. Histograms with same letters are not significantly different at P<0.05 (Student Newman and Keuls test).

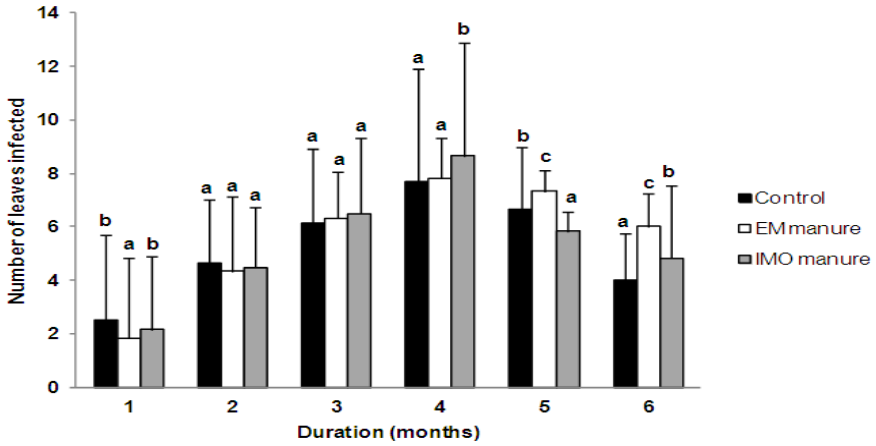

Figure 2. Evaluation of the disease incidence in *C. esculenta* plants under different treatments with time. Histograms with same letters are not significantly different at P<0.05 (Student Newman and Keuls test).

Table 3. Variation of the total phenol (µg of CA.mg^{-1} FW) content in the leaves of *C. esculenta* plants under different treatments with time.

Duration	Control	EM manure	IMO manure
2	3.929 ± 1.396^{a}	6.346 ± 0.779^{b}	4.596 ± 0.618^{a}
3	13.717 ± 0.926^{a}	15.408 ± 1.280^{b}	13.996 ± 0.591^{a}
4	18.772 ± 0.704^{a}	23.116 ± 0.484^{b}	25.670 ± 1.306^{b}
5	16.743 ± 3.957^{a}	19.732 ± 0.453^{ab}	23.791 ± 2.294^{b}

Means with same letter in the same line are not significantly different at P < 0.05 (Student Newman and Keuls test).

Table 4. Variation of POX activity (EU.mg^{-1} FW) in leaves of *C. esculenta* under different treatments with time.

Duration	Control	EM manure	IMO manure
2	4.680 ± 0.195^{ab}	5.617 ± 0.168^{b}	4.172 ± 0.231^{a}
3	4.595 ± 0.168^{a}	6.468 ± 0.132^{b}	5.01 ± 0.384^{a}
4	7.411 ± 0.502^{a}	7.517 ± 0.162^{a}	7.303 ± 0.768^{a}
5	9.184 ± 0.524^{ab}	9.34 ± 0.539^{b}	8.581 ± 0.475^{a}

Means with same letter in the same line are not significantly different at P < 0.05 (Student Newman and Keuls test).

Table 5. Variation of PPO activity (EU.mg^{-1} FW) in leaves of *C. esculenta* under different treatments with time.

Duration	Control	EM manure	IMO manure
2	0.936 ± 0.12^{a}	1.163 ± 0.189^{b}	0.814 ± 0.165^{a}
3	1.212 ± 0.32^{a}	1.356 ± 0.22^{b}	1.109 ± 0.097^{a}
4	1.39 ± 0.245^{a}	1.691 ± 0.479^{ab}	1.814 ± 0.506^{b}
5	1.498 ± 0.174^{a}	1.976 ± 0.169^{b}	1.773 ± 0.186^{ab}

Means with same letter in the same line are not significantly different at P < 0.05 (Student Newman and Keuls test).

produced the highest phenolic content (6.346 ± 0.779 µg of CA. mg^{-1} of FW) followed by plants treated with IMO manure (4.596 ± 0.618 µg of CA. mg^{-1} of FW) and that of control plants (3.929 ± 1.396 µg of CA. mg^{-1} of FW). This content increased with time and peak was achieved after 4 months after which it decreased in all treatments. At this time, the total phenolic content noticed was highest in plants treated with IMO manure (25.670 ± 1.306 µg of CA. mg^{-1} of FW) followed by that of EM manure (23.116 ± 0.484 µg of CA. mg^{-1} of FW) and lowest in control (18.772 ± 0.704 µg of CA. mg^{-1} of FW). At 5 months, the total phenolic content decreased in all treatments, but at this time, IMO manure plants produced the highest quantity of phenolic content followed by that of EM manure, then control plants (Table 3).

Evaluation of Pox activity

The evaluation of Pox activity showed that after 2 months of development, this activity was significantly higher ($P < 0.05$) in plants treated under EM manure (5.617 ± 0.168 EU.mg^{-1} FW) than that of IMO manure-treated plants (4.172 ± 0.231 EU.mg^{-1} FW) and control plants (4.680 ± 0.195 EU.mg^{-1} FW). This activity increased significantly during growth independent of treatment. After 4 months of growth, there were no significant differences of the activities of plants treated with EM manure and control plants (Table 4).

Evaluation of PPO activity

The PPO activity increased significantly during the development of plants independent of the treatment. This activity was highest in plants treated with EM manure along the process followed by plants treated with IMO manure and with control plants. After 5 months, this activity was 1.976 ± 0.169 EU.mg^{-1} FW; 1.773 ± 0.186 EU.mg^{-1} FW and 1.498 ± 0.174 EU.mg^{-1} FW for EM manure plants, IMO manure plants and control plants, respectively (Table 5).

DISCUSSION

Results obtained in this study showed that plant height increased gradually up to week 16 for control and plants treated with IMO and then week 18 for plants treated with EM manure. This result of differences in plant heights may be attributed to the gradual release of essential nutrient elements as required by *C. esculenta* plants. EM and IMO manure continuously supply nutrients to plants which enhance growth. The microorganisms associated with these amendments enhance the production of plant growth regulators (Arshad and Frankenberger, 1992). The longest plants obtained with EM manure could be attributed to the effect of EM stimulating mineralization of organic matter, with subsequent release of more nutrients into the soil-plant system (Daly and Stewart, 1999).

The production of more leaves by plants treated with manures than control plants was because EM and IMO manure had a steady supply of nutrients resulting from the gradual breakdown of the manure components. Similar results were obtained by Xu et al. (2000), where application of EM increased fruit yield and plant growth of a tomato crop. Apparently, the application of EM manure into the soil is usually associated with an increase in soil microbial biomass, which increases the rate of symbiotic biological nitrogen fixation through increases in *Azotobacter* bacteria (Hussain et al., 1994). The EM contains phytohormones and other biologically active substances delay senescence in plants (Yamada and Xu, 2000). Depletion of nutrients during the cultivation period explains why control plants had the least number of leaves.

The application of EM and IMO manure was ineffective in controlling taro leaf blight that emerged in the field. EM and IMO manures in the soil served as a good substrate for microorganisms, some of them pathogenic, like those causing taro leaf blight. The results are similar to those obtained by Ncube et al. (2010), where application of EM had no effect on the control of tomato leaf blight. It is possible that in other instances where EM has been found to have positive effects on leaf blights, the weather was not favorable for blight attack compared with our environment where the weather is at times very conducive for the development of blight and the results clearly indicate that EM may not be effective in controlling it. The IMO manure was also ineffective in controlling taro leaf blight, showing very high disease incidence especially in the 3rd and 4th months. The overall population dynamics of indigenous microbial communities during composting is influenced by a number of factors such as available nutrients, moisture, temperature, oxygen and pH (Epstein, 1997). Consequently, factors such as different bulking materials, composting and storage conditions are expected to influence the population dynamics of IMO and ultimately pathogen growth potential. Therefore the increased occurrence of disease was attributed to the inability of IMO to compete with harmful microorganisms in the presence of limited nutrients and unfavorable conditions, inability to produce inhibitory compounds or secondary metabolites and the absence of highly active IMO to lyse harmful microorganisms.

Total phenol contents are widespread in plant and tissue and could more or less accumulate depending on the physiological condition of plants (Mbouobda et al., 2010). General tendency indicates relevant activation of phenol contents, which increases with time and dependent on the manure used. Phenol content as well as Pox and PPO activities were high in plants treated with EM and IMO manures during growth development (5 months). These biochemical activities played an important role in the growth process of plants. Poxs are related to manure detoxification presumably by catalyzing the phenol oxidation at the expense of hydrogen peroxide (Wang and Balligton, 2007). They are also involved in lignin biosynthesis as a physical barrier against several stresses (Belaqziz et al., 2008). The PPOs are highly reactive intermediates whose secondary reactions are believed to be responsible for the oxidative browning which accompanies plant senescence, wounding, and responses to pathogens (Friedman and Baker, 2007). Bioactive substances such as hormones and enzymes produced by yeasts promote active cell and root division. Their secretions are useful substrates for EM and IMO such as lactic acid bacteria and actinomycetes which are intermediate to that of bacteria and fungi producing antimicrobial substances from amino acids secreted by photosynthetic bacteria and organic matter.

Conclusion

Application of EM and IMO manures increased growth parameters, yields as well as biochemical parameters of *C. esculenta*. However, they did not show any potential for controlling taro blight disease that affected the *C. esculenta* crops during growth season.

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