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Growth Response of Corn to Nitrogen-Fixing Bacteria Enriched Compost

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ABSTRACT

Efforts are made to introduce strains of selected N-fixing bacteria; *Bacillus subtilis* and *Bacillus licheniformis*, as free living 'asymbiotic N fixers' into compost that made from Empty Fruit Bunches (EFB) and Palm Oil Mill Effluent (POME). A total of 7 soil treatments were established to evaluate the influence of N_2-fixing bacteria-enriched compost on vegetative growth and performance of corn; 4 treatments of selected N_2-fixing bacteria strains (namely BT28, BT29, BT30 and BT31) into compost meanwhile 1 treatment with nil inoculum and 2 treatments of control plots of 100% of inorganic fertilizer applications. All treatments were replicated 4 times and arranged in a randomized complete randomized design. The results showed that application of *Bacillus* sp. inoculated EFB compost significantly ($p<0.05$) increased total dry weight as compared to those plots applied with inorganic fertilizers. Growth of corn was significantly affected by the application of selected N_2 fixing strains of *Bacillus subtilis* and *Bacillus licheniformis* particularly on the vegetative growth and performance of corn. Soil pH, nutrient concentration especially K and Mg were significantly increased due to presence of *Bacillus* sp. enriched EFB compost.

Key words: Oil palm, EFB, POME, N_2-fixing bacteria, corn

INTRODUCTION

Palm oil mill usually generate Empty Fruit Bunches (EFB) and the volume of Palm Oil Mill Effluent (POME) as 'solid' and 'liquid' waste products, respectively. Composting of these EFB and POME is an effective way of waste management in oil palm industry and is one of the most promising renewable energy resources (Leng, 2003). Introduction of free-living N_2-fixing bacteria or Plant Growth Promoting Rhizobacteria (PGPR) to this compost would enrich the nutrients especially Nitrogen content. Free-living and associate diazatrophs either depends on organic carbon or simple low molecular weight carbon compound from compost, soil or root exudates as their energy sources (Michiels *et al.*, 1989). Numerous papers are found on the effect of PGPR on the performance of rice, cotton, corn and fruits (Schonwitz and Ziegler, 1984; Sid *et al.*, 2003; Radziah *et al.*, 2003; Naher *et al.*, 2009; Panhwar *et al.*, 2011; Radziah *et al.*, 2011).

Microbial population in the rhizosphere often responds to the changes in exudation patterns. Schonwitz and Ziegler (1984) reported that the amount of exudation which contains sugars and vitamins is high enough to influence and stimulate rhizosphere microbes. Interactions between roots of corn and microbes at rhizosphere seem to be important as most of the nutrient that cornacquires actually move through small zone of 2 mm thickness surrounding the roots (Krishna, 2012). The soil microbes collectively called 'asymbiotic N_2 fixers' actively harbor on rhizoplane, in the rhizosphere and in bulk soil. In this thesis study four isolates, two strains of each *Bacillus subtilis* and *Bacillus licheniformis*, selected from EFB compost are tested for its PGPR properties as well as N fixation (Krishna, 2012). There were several evidences on the ability of *Bacillus* sp. to fix N_2 from atmosphere. Mollica *et al.* (1985) quoted a study by Hino and Wilson (1958) on the occurrence of N_2 fixing ability in the genus *Bacillus*. By keeping the above matters in mind, it was decided to evaluate the effect of *Bacillus subtilis* and *Bacillus licheniformis* on the vegetative growth and performance of corn (*Zea mays*). The main objectives of this glasshouse study were (1) To determine effect of N_2-fixing bacteria-enriched EFB compost on vegetative growth and performance of corn and (2) To determine the effect of N_2-fixing bacteria-enriched EFB compost on soil chemical properties.

MATERIALS AND METHODS

The soil used in this trial was Bungor series taken from Tanah Merah Estate, Sepang, Selangor, Malaysia. This soil series is a fine, kaolinitic, isohyperthermic red-yellow and Typic Paleudult (Paramananthan, 2000). The soil polybags were filled up with 10 kg of soil and arranged at 0.6 m apart, on the cemented platform. Three seeds were sown in each bag but only one seed in normal growth is allowed to grow. All plants were watered with 100 mL of sterile distilled water daily throughout the 45 days of growth. Physical and chemical properties of the soils selected for this experiment were shown in Table 1.

Ten week old of EFB compost was collected and sent to Malaysian Nuclear Agency (MNA), Bangi, Selangor for sterilization through gamma radiation at 40 KGray scale. The bagged-sterilized EFB compost were then inoculated with respective isolates i.e., BT28, BT29, BT30 and BT31.

The four strains of *Bacillus* sp. (BT28, BT29, BT30 and BT31) were prepared in N-free Jensen Broth. The content in the flask was placed on a shaker shaken at 120 rpm for three days at room temperature.

Table 1: Selected properties of soil used in the experiment

Soil properties	Values
Cation exchange capacity (CEC cmol(+)kg^{-1})	7.2
pH (Water 1:25)	3.7
Organic carbon content (%)	1.71
Soil texture (%)	
Clay	25
Silt	15
Fine sand	53
Coarse sand	3
N (%)	0.072
P (ppm)	
Total	328
Available	6.0
Exchangeable K (cmol+kg^{-1})	0.06
Exchangeable Mg (cmol+kg^{-1})	0.07
Exchangeable Ca (cmol+kg^{-1})	0.12

Twenty milliliter of each isolate (approximately at 10^7 cfu mL^{-1}) was injected by sterilized syringe into respective sterilized EFB compost. All inoculated sterilized EFB compost were kept at room temperature in a dry room for one week. Throughout the week, all pre-bagged, inoculated EFB compost were gently mixed for better bacterial growth.

The trial was established in Glasshouse in Nursery, West Estate, Carey Island (2°53'59.55"N, 101°21'43.80"E). A total of 7 soil treatments were established, namely (A) Sterilized EFB compost with N_2-fixing bacteria BT28, (B) Sterilized EFB compost with N_2-fixing bacteria BT29, (C) Sterilized EFB compost with N_2-fixing bacteria BT30, (D) Sterilized EFB compost with N_2-fixing bacteria BT31, (E) Sterilized EFB compost without N_2-fixing bacteria, (F) Inorganic fertilizer applied with 100% of straight fertilizers (Control I) and (G) Inorganic fertilizer applied except for Nitrogen fertilizer (Control II). For treatments A to D, inoculated sterilized EFB compost (200 g) prepared earlier were used. Meanwhile for treatment E, Jensen nutrient broth without inoculums was applied to sterilized EFB compost. The treatments were replicated 4 times. All the 84 polybags are laid out in a Randomized Complete Randomized Design (RCRD). For treatments F and G, straight fertilizers of Ammonium Sulphate (AS), Christmas Island Rock Phosphate (CIRP) and Muriate of Potash (MOP) were used as source of nitrogen, phosphate and potassium respectively. For treatment F, 4 g AS, 2.75 g CIRP and 0.75 g MOP were applied on the 15th day after sowing and 20 g AS, 3.5 g CIRP and 1.0 g MOP were applied on the 25th day after sowing. Similarly, treatment G was also treated with same type of fertilizers except for AS. For treatments applied with *Bacillus* sp.-inoculated EFB compost (treatments A-E) only 75% dosage of treatment F was given. Over all, fertilizer rates were based on recommendation from Malaysian Agricultural Research and Development Institute (MARDI), i.e., 149 N: 61 P_2O_5: 67 K_2O kg ha^{-1}. The corn seeds used were 313 sweet corn varieties. The corn seeds were soaked in a 500 mL beaker containing respective *Bacillus* sp. culture for ten minutes before planting. Corn seeds were planted about 1 cm depth in the polybags.

The leaf chlorophyll content was determined at 45 days after planting, using portable chlorophyll meter (MINOLTA™ SPAD-502). The SPAD reading was taken from the leaf seven to leaf nine. Each value is the mean of 3 readings taken along each leaf in each corn plant. The reading was taken at an approximately 2 cm from them edge of leaf and 3-4 cm from the leaf base. Shoot height was the measured stem height by using the measuring tape. For each treatment, three plants were sampled at 45th day of growth.

Soil was analyzed for total nitrogen following Kjeldahl method (Bremner, 1996), Soil Organic Carbon (OC) according to Walkley and Black (1934) method, soil available P determined by Molybdenum Blue method (Bray and Kurtz, 1945) and exchangeable K, Ca, Mg and Na was determined by leaching method. Soil total populations of bacteria, fungi and actinomycetes were determined using dilution plate technique (Parkinson *et al.*, 1971).

Dry weight was the weight of the plant above (shoot) and below ground (root). Dry weight was determined by drying the whole plant (separately in shoot and root portions) in an oven at 70°C for 16 h. For each treatment, three plants were sampled during destructive analysis stage i.e., at 45th day of growth.

The concentrations and uptake of N, P, K and Mg were determined in finely grounded-dried leaves and tissue. Sample of 0.25 g in weight was digested in 5 mL sulfuric acid (H_2SO_4) on hot plate at 450°C in a fume chamber for 7 min. The solution was then cooled and 10 mL of hydrogen peroxide (H_2O_2) was added into the mixtures and heating continued for another 4 min. The solution was made up to 100 mL with distilled water and filtered with filter paper. Contents of N and P were measured using an auto-analyzer while K and Mg was measured using an Atomic Absorption Spectrophotometer.

Analysis of variance (ANOVA) was performed on all measurements, wet and dry weight properties for all corns planted in polybags i.e., 7 (inoculation treatments)×4 (replications) in a Randomized Completely Randomized Design (RCRD). If the treatments are significantly different, Multiple Comparison Tukey Test (MCTT) was used to detect differences among the treatments.

RESULTS

Effect of N_2-fixing bacteria enriched EFB compost on corn growth: There was a significant ($p<0.05$) effects of *Bacillus* sp. inoculated EFB compost on corn shoot biomass at 45 days of growth (Table 2). Corn grown under *Bacillus* sp. inoculated EFB compost plots in treatment A, B and C had recorded significantly dry shoot weight ranging from 6.15-6.76 g per plant against those in control plots (I and II) where an average dry shoot weight ranging from 2.46-3.16 g per plant recorded. In terms of dry root weight, there was a significant difference among all treatments. Treatments A, B, C, D and Control II had recorded significantly higher dry root weight compared treatment E and Control I.

Total dry weight was found significantly ($p<0.05$) higher for plots applied with BT28, BT29 and BT30 treated EFB compost compared to plots with application of inorganic fertilizer (Control I and II).

The root to shoot ratio was found to be significantly affected by *Bacillus* sp.-inoculated EFB compost as observed at harvesting. With regards to the fresh root production, *Bacillus* sp.-inoculated EFB compost seemed to be effective than those treated with inorganic fertilizers (Fig. 1). Similarly to total dry weight, there was a significant difference recorded in terms of vegetative measurements and SPAD values (Table 3).

Table 2: Effect of N_2-fixing bacteria enriched compost on shoot and root (dry weight g plant^{-1})

Treatments	Shoot	Root	Total	Root-shoot ratio
BT28	6.76^{a}	0.58^{a}	7.34^{a}	0.086^{b}
BT29	6.15^{a}	0.52^{a}	6.67^{a}	0.085^{b}
BT30	6.49^{a}	0.55^{a}	7.04^{a}	0.085^{b}
BT31	4.85^{ab}	0.36^{a}	5.21^{ab}	0.074^{b}
No bacteria	3.81^{bc}	0.26^{b}	4.07^{bc}	0.068^{c}
Control I	2.46^{c}	0.29^{b}	2.78^{c}	0.118^{a}
Control II	3.16^{bc}	0.43^{a}	3.59^{bc}	0.136^{a}

Means in each column with same letter's were not significantly different based on MCTT at 5% confidence level ($p>0.05$)

Table 3: Effect of N_2-fixing bacteria enriched compost on vegetative measurement and SPAD value of corn

Treatments	No. of leaves	Length of leaf (cm)	Shoot length (cm)	SPAD reading
BT28	10.4^{ab}	76.6^{ab}	56.5^{a}	33.5^{ab}
BT29	10.2^{ab}	76.3^{ab}	51.9^{ab}	34.0^{a}
BT30	10.7^{a}	78.0^{a}	54.0^{ab}	33.9^{a}
BT31	9.8^{abc}	72.4^{abc}	46.8^{abc}	31.7^{ac}
No bacteria	9.0^{bcd}	66.8^{bc}	44.2^{bcd}	32.5^{abc}
Control I	7.8^{d}	52.7^{d}	34.3^{d}	31.9^{bc}
Control II	8.4^{cd}	64.3^{c}	37.8^{cd}	31.1^{c}

Means in each column with same letter's were not significantly different based on MCTT at 5% confidence level ($p>0.05$)

Fig. 1: Effect of treatments on the roots of corn at harvesting

Fig. 2: Effect of *Bacillus*-inoculated EFB compost on the growth of corn

In terms of number of leaves, all *Bacillus* sp.-inoculated EFB compost except treatment D (BT31) recorded significantly higher number of leaves and longer leaf length than those of control plots I (Table 3). As such, *Bacillus* sp.-inoculated EFB compost significantly recorded better vegetative growth as compared to those not treated with *Bacillus* sp.-inoculated EFB compost (Fig. 2).

The EFB compost inoculated with isolates BT28, BT29 and BT30 significantly ($p<0.05$) recorded higher SPAD values as compared to those in Control II (i.e., inorganic fertilizer except for nitrogen input-Table 3).

Figure 2 shows the effect of *Bacillus* sp.-inoculated EFB compost on the growth of corn at harvest (45th day). It was evident that corn applied with *Bacillus* sp.-inoculated EFB compost performed better than those of control plots I and II.

Effect of N_2-fixing bacteria enriched EFB compost on corn nutrient concentration: Corn seedling applied with BT28 and BT29-inoculated EFB compost significantly ($p<0.05$) increased nitrogen concentration compared to treatment E and Control II (Table 4). Except for B concentration, BT28 and BT29-inoculated EFB compost significantly ($p<0.05$) influenced Mg and Ca concentrations as compared to those in Control II . Control plot I recorded significantly ($p<0.05$) lower K and higher Ca as compared to *Bacillus* sp.-inoculated EFB compost. Meanwhile, control II has recorded significantly ($p<0.05$) lower P and higher Mg and Ca as compared to *Bacillus* sp.-inoculated EFB compost. However, both *Bacillus* sp.-inoculated EFB compost and inorganic fertilizer application showed no significant ($p>0.05$) difference in B uptake by corn.

Effect of N_2-fixing bacteria enriched EFB compost on soil chemical properties: Results of soil analysis indicated that there was no or less variation among the treatments at the time of sowing the corn seeds particularly in terms of total N and CEC (Table 5).

The soil pH was significantly ($p<0.05$) higher in treatment A and B as compared to Control I (Table 6). At the harvesting (45th day after sowing), all treatments showed higher soil pH except for Control I. Soil organic carbon was found to remain almost constant in all treatments.

There was insignificant ($p>0.05$) effect of *Bacillus* sp.-inoculated EFB compost on soil total N at the time of harvesting (Table 6). Except for treatments A and B, there was generally an increase in soil N from the day of sowing (Table 5) till day of harvesting (Table 6). It seems that there was a greater usage of N from soil reserves as corn in treatments A and B recorded better performance (Table 4). As expected there was basically no difference in soil total N for treatment G which received nil application of N fertilizer.

Table 4: Effect of N_2-fixing bacteria enriched compost corn nutrient concentration

	Dry matter (%)					
	N	P	K	Mg	Ca	B
Treatments	(mg kg^{-1})					
A-BT28	3.80^{a}	0.306^{abc}	1.29^{a}	0.16^{b}	0.190^{cd}	20.75^{a}
B-BT29	3.61^{ab}	0.334^{ab}	1.35^{a}	0.16^{b}	0.207^{cd}	10.00^{a}
C-BT30	3.21^{bc}	0.296^{ab}	1.24^{ab}	0.15^{b}	0.226^{c}	12.25^{a}
D-BT31	3.22^{abc}	0.338^{a}	1.32^{a}	0.17^{b}	0.217^{cd}	10.00^{a}
E-No bacteria	2.75^{c}	0.221^{cd}	1.16^{ab}	0.14^{b}	0.165^{d}	9.50^{a}
F-Control I	3.57^{ab}	0.226^{bcd}	0.92^{b}	0.18^{b}	0.300^{b}	10.25^{a}
G-Control II	2.98^{c}	0.158^{d}	1.02^{ab}	0.26^{a}	0.400^{a}	8.50^{a}

Means in each column with same letter's were not significantly different based on MCTT at 5% confidence level ($p>0.05$)

Table 5: Effect of N_2-fixing bacteria enriched compost on soil nutrient content at sowing day

Treatments	Soil pH	Org C (%)	Total N (%)	Exch-K (cmol(+)kg^{-1})	Exch Mg (cmol(+)kg^{-1})	Total P (ppm)	Av. P (ppm)	CEC(cmol(+)kg^{-1})
A-BT28	4.1^{a}	2.4^{b}	0.148^{ab}	0.858^{ab}	0.50^{a}	370.1^{a}	4.4^{a}	8.11^{a}
B-BT29	4.2^{a}	3.4^{a}	0.141^{ab}	1.037^{ab}	0.50^{a}	362.6^{a}	5.5^{a}	8.51^{a}
C-BT30	4.0^{a}	2.3^{b}	0.141^{b}	0.782^{ab}	0.50^{a}	354.6^{a}	6.1^{a}	9.38^{a}
D-BT31	4.4^{a}	2.4^{b}	0.144^{ab}	1.449^{a}	0.80^{a}	370.7^{a}	13.6^{a}	8.58^{a}
E-No bacteria	4.0^{a}	2.4^{b}	0.145^{ab}	0.785^{ab}	0.52^{a}	315.0^{a}	6.3^{a}	8.37^{a}
F-Control I	4.1^{a}	2.3^{b}	0.166^{a}	0.294^{b}	0.68^{a}	363.8^{a}	20.8^{a}	7.14^{a}
G-Control II	4.3^{a}	2.3^{b}	0.144^{ab}	0.238^{b}	0.68^{a}	391.5^{a}	23.5^{a}	7.38^{a}

Means in each column with same letter's were not significantly different based on MCTT at 5% confidence level ($p>0.05$)

Table 6: Effect of N_2-fixing bacteria enriched compost on soil nutrient content at harvest

Treatments	Soil pH	Org C (%)	Total N (%)	Exch-K (cmol(+)kg^{-1})	Exch Mg (cmol(+)kg^{-1})	Total P (ppm)	Av. P (ppm)	CEC (cmol(+)kg^{-1})
A-BT28	5.1^{a}	2.5^{a}	0.122^{a}	3.503^{a}	2.16^{a}	567.0^{a}	58.0^{a}	8.93^{a}
B-BT29	5.0^{a}	2.4^{a}	0.109^{a}	2.628^{ab}	1.92^{ab}	530.2^{ab}	50.4^{a}	8.78^{a}
C-BT30	4.7^{ab}	2.5^{a}	0.171^{a}	3.206^{ab}	1.88^{ab}	520.2^{ab}	61.3^{a}	9.63^{a}
D-BT31	4.7^{ab}	2.4^{a}	0.163^{a}	2.431^{ab}	1.53^{abc}	456.8^{abc}	42.6^{a}	8.87^{a}
E-No bacteria	4.8^{ab}	2.5^{a}	0.164^{a}	1.850^{bc}	1.11^{bc}	387.8^{c}	38.8^{a}	7.55^{a}
F-Control I	4.1^{b}	2.3^{a}	0.172^{a}	0.518^{c}	0.61^{c}	446.1^{bc}	54.6^{a}	8.17^{a}
G-Control II	4.5^{ab}	2.4^{a}	0.143^{a}	0.406^{c}	0.60^{c}	447.1^{abc}	72.8^{a}	8.26^{a}

Means in each column with same letter's were not significantly different based on MCTT at 5% confidence level ($p>0.05$)

Soil exchangeable K and Mg levels were significantly recorded high in all EFB compost applied plots as compared to inorganic fertilizer plots i.e., control I and II (Table 6). Soil exchangeable K and Mg levels were improved at harvesting as compared to those levels recorded at the sowing of corn seeds (Table 5).

Soil available P level was in treatment E recorded significantly ($p>0.05$) lower than those under influence of *Bacillus* sp.-inoculated EFB compost in treatment A, B and C at the time of harvesting (Table 6).

No significant ($p>0.05$) effect of *Bacillus* sp.-inoculated EFB compost was observed on CEC at the time of harvesting but there was a slight increment in CEC values at the harvesting (Table 5, 6).

DISCUSSION

Application of *Bacillus* sp., inoculated EFB compost significantly ($p<0.05$) increased total dry weight as compared to those plots applied with inorganic fertilizers. Total dry weight was found significantly ($p<0.05$) higher for plants applied with BT28, BT29 and BT30 treated EFB compost. At harvesting, all *Bacillus* sp.-inoculated EFB compost except treatment D (BT31) recorded significantly higher number of leaves than those of control plots I and II. Corn growth in control plots was solely dependent on nutrients supplied through inorganic fertilizer.

The soil pH was significantly ($p<0.05$) higher in treatment A and B as compared to Control I. At the harvest (45th day after sowing), all treatments showed higher soil pH except for Control I. *Bacillus* sp. inoculated EFB compost acted as a soil amendment as soil pH was improved from the time of sowing to harvesting. Increment in soil pH also resulted in a conducive environment for the growth of microbes.

Except for treatments A and B, there was generally an increase in soil N from the day of sowing till day of harvesting. It seems that there was a greater usage of N from soil reserves as corn in treatments A and B recorded better performance. As expected there was basically no difference in soil total N for treatment G which received nil application of N fertilizer.

The EFB compost inoculated with isolates BT28, BT29 and BT30 significantly ($p<0.05$) recorded higher chlorophyll as measured by SPAD values as compared to those in Control II (i.e., inorganic fertilizer except for nitrogen input). This shows that the soil medium used was not able to supply the required N as compared to other treatments. Control II which received nil nitrogen fertilizer input has recorded significantly lower number of leaves and leaf length as compared to treatments with *Bacillus* sp. inoculated EFB compost. There were several evidences on the ability of *Bacillus* sp. to fix N_2 from atmosphere. As quoted by Mollica *et al.* (1985) on a work by Hino and Wilson (1958) on the ability of N_2 fixing in the genus *Bacillus*.

Growth of corn was significantly affected by the application of selected N_2 fixing strains of *Bacillus subtilis* and *Bacillus licheniformis* particularly on the vegetative growth and performance of corn. Soil pH, nutrient concentration especially K and Mg were significantly increased due to presence of *Bacillus* sp. enriched EFB compost.

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