Role of Microorganisms, Soluble N and C Compounds in Fermented Bio-Extract on Microbial Biomass C, N and Cowpea Growth

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Abstract

Previous field and pot experiments showed that the sole application of the bio-extract did not increase plant dry weight and yield. However, application of bio-extract (BE) with organic fertilizers increased plant dry weight (leaf, stem and yield). It has been hypothesized that the bio-extract may act as a direct source of beneficial microorganisms or/and substrates to stimulate soil microbial activities in decomposing organic fertilizer and to release nutrients to plants.The objectives of this pot experiments were to investigate the roles of live microorganisms and/or chemical constituents of bio-extract combined with or without compost on microbial biomass and crop growth.

Autoclave treatment on the bio-extract showed that the living microorganisms contained in the bio-extract were not significant source of microorganisms responsible for decomposing organic fertilizer in the soil. There were no significant differences in microbial C and N in the soils treated with either original or autoclaved bio-extracts in the short term (8 days). Bio-extract after autoclaving could still enhance crop growth (top dry weight) when being combined with municipal compost. Moreover, cowpea treated with original, autoclaved or artificial (only soluble C and N) bio-extracts did not show significant difference in top dry weight.

Our studies suggest that some substrates, such as soluble organic acids, amino acids, in the bio-extract might play some important roles to stimulate soil microbial activities and enhance crop growth.

Keywords: Bio-extract, Biofertilizer, Cowpea, Microbial biomass, Soluble C and N

Introduction

Many small farmers in Northeast Thailand have been using the bio-extracts (BE) produced from plant and animal residues to reduce or replace chemical fertilizer and pesticide use. They use bio-extract either singly or in combination with other organic amendments in crop production. However, the real benefit(s) of bio-extracts in crop production remain unclear.

Previous field and pot experiments tested the effect of bio-extract on growth and yield of cowpea. Sole application of the bio-extract did not increase plant dry weight and yield. However, combined bio-extract with organic fertilizers increased plant dry weight (leaf, stem and yield) (Kamla et al., 2007). Khaliq et al. (2006) reported that combining the supply of effective microorganisms (EM) with organic and inorganic nutrient sources significantly increased cotton seed yield

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in a field experiment when compared to addition of organic material or EM alone. Similarly, Daly and Stewart (1999) reported that combining EM with molasses applied to onion, pea and sweet corn increased yield up to 29, 31 and 23 %, respectively. The beneficial effects of EM suggest that the bio-extract might supply a mixture of microorganisms that enhances crop growth when combined with organic fertilizers.

Suggesting that bio-extract may contain a significant source of microorganisms and/or substrates to stimulate soil microbial activities to decompose of organic fertilizer and release nutrients for the plant. It could be hypothesized that either microorganisms and /or chemical constituents of the bio-extract might be the key active ingredients. Bio-extract microorganisms might originate more soil microorganisms to decompose organic fertilizer.

An alternative hypothesis is that soluble organic nitrogen and carbon, main chemical constituents of bio-extracts may stimulate soil microorganisms to enhance nutrient availability to plants. McGill et al. (1981) proposed that soluble organic carbon in the soil is an immediate source of carbon for soil microorganisms.

The first objective of the experiments was to confirm the role of microorganisms and chemical constituents in fermented bio-extract combined with municipal compost on soil microbial activities by measuring microbial biomass. The second objective was to investigate the role of bio-extract microorganisms and chemical constituents, soluble C and N, on early growth of cowpea.

Materials and methods

Experiment 1

Soil preparation and experimental design

There were three treatments and four replications in this study. The treatment combinations were:

1. Municipal compost
2. Municipal compost + BE (original)
3. Municipal compost + BE (autoclaved)

The experiment was laid out in randomised complete block design and conducted in a glasshouse at Murdoch University, Australia. The soil was sieved through a 4 -mm mesh and visible organic materials removed by hand Each pot with the diameter of 30 cm and height of 10 cm was filled with 1 kg of sieved loamy sand soil . In bio-extract treatment, the bio-extract was applied to the soil at 1.1 ml mixed with 250 ml of water before application. Meanwhile the municipal compost treatment was applied at 6.1g/pot with the same volume of water used in the bio-extract treatment. Its chemical characteristics were as follows; organic matter by wet oxidation method was 5 g kg-1 (Walkley and Black, 1934), pH (1: 1 H_2O) was 6.5, total N by the Kjeldahl method was 0.3 g kg-1 (Bremner, 1960), extractable P by the Bray II method was less than 0.05 g kg-1 (Bray and Kurtz, 1945), exchangeable K and Ca were 63 and 620 ppm, respectively (Schollerger and Simmon, 1945).

Microbial biomass N and C

Microbial biomass C and N were measured on the same day on fresh soil samples immediately after sampling. Microbial biomass N is determined by the chloroform fumigation-extraction technique (Amato and Ladd, 1988). After 36-hour fumigation the soil samples (10 g) was extracted with 50 mL of 1 M KCl. An unfumigated sample was extracted immediately after sampling. Microbial biomass N was determined by the ninhydrin-reactive N method and calculated as the difference between the fumigated minus the unfumigated sample. A kN factor of 3.1 was applied in the calculation according to Amato and Ladd (1988).

Microbial biomass C was determined by the choroform fumigation-extraction method (Vance et al., 1987). Pot soil sample with equivalent of 10 g oven-dry soil (fumigate and non-non-fumigated) was extracted with 50 ml 0.5 M K_2SO_4. Total organic-C in the soil extracts was determined by wet digestion method as describe by Jenkinson and Powson (1976). Microbial biomass C (BC) was estimated from BC = EC (Vance et al., 1987), where EC =[(organic C extracted from fumigated soil) - (organic C extracted from unfumigated soil)]. Sample soils for microbial biomass were measured at the time 0, 2, 4, and 8 day after application of bio-extract. Total microbial biomass C was calculated using a kc factor of 0.45 (Jenkinson, 1988).

Experiment 2

Soil preparation and experimental design

The experiment was laid out in randomised complete block design and conducted in a glasshouse at Murdoch University, Western Australia. The soil was sieved through a 4 -mm mesh and visible organic materials removed by hand. Pots were filled with 4.0 kg of the soil and laid out in complete block design. The chemical properties of soil are as described in experiment 1. There were six treatments and five replications in this study. The treatment combinations were;

1. Control
2. Municipal compost
3. Municipal compost + BE (original)
4. Municipal compost + BE (autoclaved)
5. Municipal compost + soluble N (NH4Cl) and soluble C (sucrose)
6. Municipal compost + soluble N (amino acids) soluble C (organic acids)

Table 1 shows chemical compositions of municipal compost, original bio-extract, artificial bio-extract and soluble C + N in treatment 2-6.

Table 1 Chemical composition of soil amendments (g per kg for manure; g per litre for soluble compounds).

Type of soil amendment	Total C	Total N	Total P	Total K	Total Ca	C/N ratio
Municipal compost	310	20	5.0	11.0	22.0	15.50
BE(original)	99	42	5.2	9.3	2.4	2.36
BE (autoclaved)	99	42	5.2	9.3	2.4	2.36
NH4Cl+sucrose	99	42	-	-	-	2.36
BE(artificial)	99	42	-	-	-	2.36

Treatment descriptions and management

Municipal compost for application was collected from the municipality of Murdoch. The organic fertilizer was applied at 24.44 g/pot. The compost was mixed homogeneously throughout the soil. Bio-extract was made from fish fermented with molasses at approximately 3:1.2 (fish: molasses) by w/w. The fish-based extract was prepared by storing in a plastic container and addition with molasses at that ratio. The BE was not addition of the inoculum into a plastic container and allowed for fermentation about 3 months before application to the crop. During incubation period, the BE was kept in the warehouse of laboratory at Murdoch University. Otherwise, there was not control the temperature and pH during fermentation. The pots used in the experiment have the height of 16 cm and diameter of 20 cm. At each time of application, the liquid solution was applied at 0.6 ml per pot by mixing with the water at ratio 1: 250 (BE: water) to form dilute bio-extract.

For chemical treatments, soluble C and N contents were kept at the same concentration and rate similar to the bio-extract treatment.The artificial bio-extract of treatment 6 was prepared from 6 organic acids and 11 amino acids frequently found in the bio-extract. The proportion of organic acid consisted of butyric (20%), acetic (20%), propionic (20%), lactic (20%) succinic (10%) and formic (10%). Meanwhile mixed amino acids solutions were comprised of glutamic (25%), lysine (12%), alanine (10%), leucine (10%), isoleucine (8%), glycine (8%), valine (6%), aspartic (6%), threonine (5%), phenylalanine (5%) and methionine (5%). For treatment 5, all C and N were supplied in the form of sucrose and NH4Cl. Application frequency of the bio-extract and chemical solution was at once a week in all treatments. All pots were added with water to field capacity before planting. The soil moisture was checked by weighing periodically at every alternate day and kept the soil moisture close to field capacity.

Plant management and sampling

In this experiment, each pot was filled with 4.0 kg of sieved loamy sand soil. Four to five seeds of cowpea Vita 3 cultivar were planted and covered with the soil. The plant was thinned at 4 days after emergence. The soil was incubated one day before planting. The cowpea was sampled when the age of the plant was 21 days after emergence. The plant growth parameters were plant height, leaf width and leaf length of the first fully expanded tri-foliate leaf. Plant samples were divided into leaves and stems and oven-dried for dry weight measurement.

Statistical analysis

Data were arranged in a randomised complete block design and subjected to one way analysis of variance. The DMRT was used to compare the treatments means when the F-test was significant.

Results

Experiment 1

All treatments show similar pattern of changes in both C and N microbial biomass and there were no significant different on size of microbial biomass C and N between autoclaved and non-autoclaved bio-extract (Table 2). The microbial biomass C was peaked at day 2 and decreased afterward. After removal of live microorganisms from the bio-extract, microbial C biomass was still higher than the control having municipal compost alone and was not significantly different from that of the original bio-extract treatment when combined with municipal compost from day 2 to day 8 (Table 2). Meanwhile both original and autoclaved bio-extract treatments significantly contained higher microbial N biomass than the addition of compost alone even at day 4 and 8 (Table 2).

Our studies may imply that the microorganisms in fermented bio-extracts were not directly responsible for increased carbon soil microbial biomass, as there were no significant differences in soil microbial C and N between autoclaved and original bio-extract treatments (Table 2). It can be postulated that addition of bio-extract could stimulate soil microbial activity by other means. One possibility may be the bio-extract supplying some chemical substrates, e.g. organic acid and amino acid, to stimulate growth of soil microorganisms as measured in C and N forms.

Table 2 Response of microbial biomass C, N (MBC, MBN) to autoclaved/non-autoclaved bio-extract combined with municipal compost over 8 days.

Treatments	MBC (mg C kg^{-1})			
	Day 0	Day 2	Day4	Day 8
1. Municipal compost (MC)	43 a	155 b	76 b	65 b
2. MC + BE (original)	47 a	225 a	164 a	121 a
3. MC + BE (autoclaved)	46 a	228 a	143 a	113 a
	MBN (mg N kg^{-1})			
1. Municipal compost (MC)	9.7 a	47.6 b	54.5 a	23.5 b
2. MC + BE (original)	10.1 a	72.1 a	65.7 a	57.3 a
3. MC + BE (autoclaved)	9.3 a	67.8 a	58.6 a	64.1 a

Means in a column followed by the same letter were not significantly different ($P<0.05$).

Experiment 2

At 21 days, cowpea from the treatments having municipal compost combined with all types of bio-extracts and even with soluble C and N in form of sucrose and NH_4Cl showed significantly better growth (dry weight and related parameters) than the treatment having municipal compost alone (Table 3 and 4). Autoclaved, non-autoclaved and artificial bio-extract treatments increased top biomass by 27.2 and 28.8 % and 27.8 % respectively, as compared to application of municipal compost alone. Addition of NH_4Cl with sucrose significantly increased top dry weight of cowpea by 7.4 % as compared to application compost alone. And addition of NH_4Cl with sucrose enhanced crop growth quite small as compared to addition of artificial bio-extract treatment

This may imply that the mixture of amino acids and organic acids in fermented fish bio-extract could play an important role to enhance crop growth via soil microorganism activities provided that there are some organic fertilizers available.

Table 3 Effect of soil amendments on growth of cowpea at 21days after planting.

Treatments	Growth parameters(cm)		
	Leaf dry weight	Stem dry weihgt	Top dry weihgt
1.Control	11.7 d	3.9 d	7.6 d
2. Municipal compost (MC)	13.8 c	4.3 c	8.6 c
3. MC + BE (original)	18.9 a	4.8 a	9.9 a
4. MC + BE (autoclaved)	19.0 a	4.9 a	9.7 a
5. MC + soluble C and N (sucrose and NH4Cl)	16.3 b	4.4 b	8.1 b
6. MC + soluble C and N (artificial BE)	17.5 a	4.6 b	9.5 a

Means in a column followed by the same letter were not significantly different ($P<0.05$).

Table 4 Effect of soil amendments on top dry weight of plant parts at 21days after planting.

Treatments	Dry weight (mg $plant^{-1}$)		
	Leaf dry weight	Stem dry weihgt	Top dry weihgt
1.Control	272 d	126 c	398 d
2. Municipal compost (MC)	339 c	161 b	500 c
3. MC + BE (original)	454 a	182 a	636 a
4. MC + BE (autoclaved)	460 a	184 a	644 a
5. MC + soluble C and N (sucrose and NH4Cl)	388 b	149 b	537 b
6. MC + soluble C and N (artificial BE)	431 a	208 a	639 a

Means in a column followed by the same letter were not significantly different ($P<0.05$).

Discussion

The present results suggested that the microorganisms contained in the bio-extract were not directly responsible for crop growth, as there were no significant differences in the effects between autoclaved and original bio-extract treatments. That is, the bio-extract after removal of live microorganisms by autoclaving and later applied with municipal compost still increased both microbial biomass and plant dry weight. Moreover, there were no significant differences in the dry weight of plants treated with original bio-extract, autoclaved and artificial bio-extract treatments. This result was supported by El- Tarabily et al. (2003) who concluded that the resident microorganisms in commercial fish emulsion were not capable of enhancing plant growth on their own, as there was not significant difference between autoclaved and non-autoclaved treatments. In contrast, several authors still suggested that the microorganisms in the ferment EM products play an important role to increase crop yield (Hussain et al., 1999; Khaliq et al., 2006).

In this pot experiment, it has been hypothesized that all nutrient supply to the plant has been originated from decomposition of compost by microorganism. During 8 days observation, sustainable increases in microbial biomass in both soils treated with either autoclaved or original bio-extract over that of the addition compost alone might suggest that the active decomposing microorganism could derive from the soil not the from bio-extract. The bio-extract alone may contain small populations of microorganism when added to the soil as compared to microorganisms in bulk soil and hence have limited direct effect on soil biological activity. In 88 samples of farmer's bio-extract; the range of densities of microorganism population decrease was from 102-105 cfu ml-(Nop-amornbodee et al., 2004). By contrast, it has been estimated the population of soil bacteria was ranging from 106- 109 cfu per gram soil (Barns and Nierzwicki-Bauer, 1997). Supposing that the bio-extract was added 1 time a week to the pot with 500 g soil and the concentration of the microorganism in the bio-extract and the soil were 105 cfu ml and 106 cfu per gram soil, the microbial population in the pot would consist of 100,000 and 500,000,000 from the bio-extract and the original soil respectively. The bio-extract may become the main microorganism supplier to the soil only when the soil has been sterile.

Application of artificial bio-extract in form of mixture organic C and amino acid could also significantly increase growth and dry weight of cowpea similarly to that of bio-extract treatment. This would imply that soluble organic and amino acid from either artificial or original bio-extract might play an important role to enhance crop growth.

By contrast, the supplying of soluble N and C in form of sucrose and NH4Cl had much weaker positive effect on crop growth. Suggesting that the form of soluble C and N supplying in the soil play as an important role to enhance crop growth.

Dissolved organic nitrogen has been recognized as a major component in soil ecosystem. However, its role is remaining poorly understood (Christou et al., 2005). The fish based-extract was reported to contain inorganic elements and soluble N and C compounds, e.g. amino acids such as histidine, methionine, lysine, serine, typtophan (Miller et al., 1989; Lopetcharat et al., 2001) and organic acid such as lactic acid, acetic acid, formic acid etc. (Park et al., 2001). This implies that addition of bio-extract could increase soluble N in the soil and the amino acids it contains are capable of uptake by plant roots (Persson and N?sholm, 2001; Jones et al., 2005) and fast utilization by microorganisms (Jones and Shannon, 1999).

Several studies have classified the carbon compounds into three major classes (extractives, cellulose and lignin). Extractive fractions are rapidly consumed or converted during initial stages of decomposition (McClaugherty, 1983). However, there is little knowledge about the nature of the compounds in theses different of DOM pools, but it is generally implied that the labile DOM comprises mainly of simple carbohydrate molecules (i.e., glucose, fructose, sucrose), low molecular of organic acids (i.e. acetic, oxalic, succinic acid), amino acid, amino sugars, and low-molecular-weight proteins (Guggenberger et al., 1994; Kusel and Drake, 1999). Theses compounds can directly be utilized by large number of different microorganisms, therefore do not require a special set of enzymes to catalyze their breakdowns (Lynch, 1982).

Based on the usual fermentation products of protein and carbohydrate materials, the main constituents of bio-extract, comprise predominantly short-chain and branch-chain organic acids, and soluble N and sulfur-containing compounds (Ramsay and Pullammanappallil, 2001; Park et al., 2001; Liu, 2003). Hence it can be postulated that combining bio-extract with compost may accelerate the degradation of organic materials and release nutrients to supply crop growth due to a continuous supply soluble C and N to the soil microorganisms. An application of the bio-extracts both in our research and in farmers' fields was scheduled every 7 days providing a continuous supply of soluble C and N compounds for soil microorganisms. This may be the reason for increased crop growth in the low fertile sandy soil when bio-extract was applied together with municipal compost compared to the application of compost alone.

Conclusion

It can be concluded that in the early crop growth microorganisms contained in the bio-extract were not responsible for enhanced crop growth and for increased microbial biomass. Moreover, there were no significant difference of cowpea dry weight between original bio-extract and artificial bio-extract treatments. Either combined original bio-extract or artificial bio-extract increased cowpea growth. Suggesting that soluble organic C and amino acids in the bio-extract play as an important role to enhance crop growth. It may be concluded that the bio-extract contains a significant source of substrate for microbial to increase biomass. That could accelerate decomposition rate of organic fertilizers and release nutrients for the crops. However, this study has not ruled out the involvement of precursors for microbial biosynthesis of plant growth regulators in the bio-extract on crop growth.

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