

BIOFERTILIZERS FROM ANIMAL INGESTA: EFFECT ON THE SOIL, TISSUES AND AGRONOMIC TRAITS OF ADLAY (*Coix lacryma-jobi* L.)

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Abstract: Adlay (*Coix lacryma-jobi* L.) is highly responsive to organic fertilizers. The study was conducted to determine the effect of formulated biofertilizers derived from ingesta of cow and pigs which are rich in essential elements and K-solubilizers. Soil grown to adlay had slightly increased pH values and P and K contents. Tissue analysis showed that adlay seedlings have sizeable amounts of N and K. Results further revealed that the liquid biofertilizer treatments significantly influenced the agronomic characteristics of adlay seedlings, except on plant height at 30 DAS and percent dry matter at 60 DAS. The combination of cow-pig ingesta liquid biofertilizer (CPILBF) is best for adlay in pot culture at the rate of 18-28 L/ha. Nonetheless, validation trials using the same formulations are recommended for adlay and other crops to compare the results.

Keywords: adlay, cow ingesta, pig ingesta, cow-pig ingesta, potassium solubilizers

INTRODUCTION

The Sustainable Development Goals (SGD) of the United Nations aim to achieve a better and sustainable future for all humans and two of these goals are zero hunger, and good health and well-being (UNDP, *n.d.*). Food insufficiency and malnutrition are among the stumbling blocks in building a healthy human resource of the nation like the Republic of the Philippines. Among Filipinos, there is deficiency in energy and protein sources and one of the potential alternatives that could address these problems is Adlay or Job's tears (*Coix lacryma-jobi* L.). Adlay is an additional staple crop in the Philippines (Aradilla, 2018). The chemical analysis released by the Food and Nutrition Research Institute (FRNI) mentioned that adlay is superior in terms of its food energy (356kcal), carbohydrate content (79.3 g), protein (12.8g), fat (1.0g), and total dietary fiber (0.3g) (DA-BAR, 2016). It is a promising cereal crop that grows in marginal soils with good ratooning ability (Aradilla, 2016). It is said to be highly responsive to organic fertilizers and formulated liquid biofertilizers with low input requirement (Monteroyo and Aradilla, 2014).

It is a fact that in modern agriculture, the use of synthetic chemicals and fertilizers is highly encouraged to increase crop yields. However, food crops produced in this type of agricultural systems are not nutrient-rich and contribute to air, soil and water pollution (Youssef *et al.*, 2014). Hence, there is a paradigm shift in search for alternatives to synthetic fertilizers and pesticides to produce 'nutrient-rich, chemical free and high quality food' to ensure bio-safety of consumers (Bhardwaj *et al.*, 2014). In sustainable farming, biofertilizers could be derived from manures and plant extracts (Bhattacharjee *et al.*, 2014) to increase soil fertility and improve yield of crops. The microorganisms present in biofertilizers help convert lock-up nutritionally essential nutrients to available forms through biological processes (Verma *et al.*, 2011; Singh *et al.*, 2013). These biofertilizers could be used as pre-treatment of seeds, and either applied on plant surfaces, or soil promoting growth of host plants (Vessey, 2003). Biofertilizers need a carrier for microbial inoculants that is high in organic matter content and water-holding capacity with favorable H⁺ concentration (Gandhi and Gaur, 1990). These should be free from contaminations to optimize the growth of microorganisms (Phua *et al.*, 2012). It was observed that liquid biofertilizers (LBF) are promising and an updated technology of the conventional carrier-based production technology (Sharma *et al.*, 2010). LBF caters to long survival of the organism by providing suitable medium sufficient for the entire crop cycle. Liquid inoculant formulations use various broth cultures amended with agents that promote cell survival in the package, and after application to seed or soil (Girisha *et al.*, 2006; Kumaresan *et al.*, 2011). The application of liquid broth formulation increases the shelf life of *Enterobacter hormaechei*, a potassium solubilizer comparing to the solid formulation (Prajapati *et al.*, 2014). Biofertilizers enhance soil fertility and supply or mobilize plant nutrients for crop nutrition with four categories: N fixers; P-solubilizing microorganisms; P mobilizers and organic matter decomposers. These may include cyanobacteria, symbiotic and free living bacteria and *Arbuscular Mycorrhizal* fungi (Khare *et al.*, 2012). The

plant growth-promoting *rhizobacteria* (PGPR) present in biofertilizers are active in ecosystem processes like biological control of plant pathogens, nutrient cycling and seedling establishment. PGPR may colonize the rhizosphere, the root surface, and intercellular spaces of plants. Nitrogen fixers are not only significant for legumes, but also non-legumes whereinsome strains have multiple functions for plant growth. Phosphate (P)-and potassium (K)-solubilizing bacteria enhance mineral uptake by plants through solubilizing insoluble P and releasing K from silicate in soil (Verma *et al*, 2011; Chandra *et al*, 2005).

Liquid fertilizer from the manure of cows, guinea pigs and pigs had been studied by Criollo *et al* (2011) while Leksono *et al* (2014) used animal manure and urine. Nevertheless, there areno reports on the use of animal ingesta liquid biofertilizer as nutrient source for adlay. Hence, this study sought to determine the effect of three formulated biofertilizers on the soil, tissues and agronomic characteristics of adlay in a pot experiment.

MATERIALS AND METHODS

Materials. The materials used in the study were the formulated liquid biofertilizers (LBFs), adlay seeds (Gulian variety), inorganic fertilizers, 30 pcs of 10" x 12" plastic pots, 0.5 kg tie wire, bamboo poles, nylon net, 300 kg top soil, shovel, hand trowel, bolo, camera, record notebook, pen, ruler, meter stick, portable sprayer (atomizer), and weighing scale.

Collection and Preparation of Soil Samples for Laboratory Analysis. A three kilogram soil sample was collected randomly from the field in a zigzag manner at about six inches deep. This was mixed thoroughly, dried, pulverized and sieved through a 2-mm opening wire mesh. One half kilogram of the composite soil sample was submitted to the Soil and Plant Analysis Laboratory (SPAL)-CMU for analysis of chemical properties of the soil such as soil pH, organic matter (OM) content, total nitrogen (TN), extractable phosphorous (EP), exchangeable potassium (EK), and fertilizer recommendations. After the pot experiment, one-half kilogram of soil sample collected from Treatment 3 (applied with cow ingesta liquid biofertilizer), Treatment 4 (pig ingesta liquid biofertilizer), and Treatment 5 (50% cow and 50% pig ingesta liquid biofertilizer) were submitted to the same Laboratory for analysis.

Preparation of Soil Media and Potting. The soil for the pot experiment was taken from the 1,443.75 m² area. This was prepared one week before sowing by thoroughly pulverizing the soil using a shovel and mixed thoroughly. Ten kilograms of well pulverized soil were placed in each pot and labeled based on treatment assignments with two pots per treatment combination. The soil-filled pots were placed in a partially shaded makeshift green house to protect the seedlings from the intense heat of the sun.

Experimental Design and Treatments. The three LBFs were tested for their efficacy on adlay plants. The experiment was laid out in a Randomized Complete Block Design (RCBD) with five treatments, replicated three times with a total of fifteen experimental units. The different treatments were the following:

T₁ – Control (unfertilized)

T₂ – 20 – 120 – 70 N-P₂O₅-K₂O kg/ha

T₃ – 5 ml of Cow Ingesta Liquid Biofertilizer (CILBF)/500 ml water

T₄ – 5 ml of Pig Ingesta Liquid Biofertilizer (PILBF)/500 ml water

T₅ – 5 ml of Cow-Pig Ingesta Liquid Biofertilizer (CPILBF)/500 ml water

Seed Preparation, Planting, and Thinning. Adlay seeds used in the experiment were procured from the CMU-Department of Agriculture-Bureau of Agricultural Research (CMU-DA-BAR) Adlay R and D Project in Musuan, Bukidnon. Prior to sowing, adlay seeds were pre-germinated by soaking and incubating for eight and four hours, respectively. Five pre-germinated seeds were sown in every pot and covered with a fine soil layer at approximately three centimeters. Thinning was done at 15 days after sowing (DAS) leaving only two healthy seedlings per pot.

Application of Fertilizers

1. Inorganic Fertilizers. The recommended rate of inorganic fertilizer was applied in one application after thinning at 15 DAS using 3 grams of 16-20-0 and 0.585 grams of 0-0-60 per pot.
2. Liquid Biofertilizers. The three liquid biofertilizer treatments were applied to adlay with equal volume of spray solution. This was done by spraying 500 ml per treatment (5 ml LBF/500 ml water) solution at one week interval starting at 15 DAS until 50 DAS.

Water Management. Adlay throughout its growing season needs moisture. Rainfall is its main source of moisture but in scarcity of rainwater, sprinkler irrigation was provided as needed.

Cultivation and Weeding. After thinning, fertilizer application was done, followed by hilling up using a hand trowel. Subsequent weed growths were removed by hand weeding.

Termination of the Experiment. This was done at 60 DAS and four (4) plants per treatment were uprooted and washed to remove dirt. Data on shoot length, root length, number of vegetative tillers and fresh weight of seedlings were taken. The seedlings were sun-dried until the weight per sample became stable. The dried seedlings were pulverized using a Laboratory Willy Mill and sieved in a 0.2 mm wire mesh and a 25 grams sample were placed in a small polyethylene bag for chemical analysis at the SPAL, College of Agriculture, CMU.

Data Gathered

A. **Plant Tissue Analysis.** The dried adlay plant samples were analyzed for Total Nitrogen (%), Total Phosphorous (%) and Total Potassium (%). The methods in taking the data is presented in Table 1.

B.

Table 1. Methods used in plant tissue analysis

PARAMETER	METHODS OF ANALYSIS	REFERENCE
Total Nitrogen, %	Micro-Kjeldahl method	PCARRD (1980)
Total Phosphorus, %	Dry Ashing/Vanado-molybdate method	PCARRD (1980)
Total Potassium, %	Flame Photometer	PCARRD (1980)

Source: Soil and Plant Analysis Laboratory, CMU, Musuan, Bukidnon

B. Agronomic Characteristics of Adlay Seedlings

These data were taken from four (4) sample plants per treatment.

1. Plant Height (cm) – This was obtained by measuring the height of adlay seedlings per treatment from the soil level to the tip of the longest leaf using a meter stick taken at 20, 40, and 60 DAS.
2. Number of Vegetative Tillers per Plant – This was obtained by counting all the tillers that developed from the sample plants at termination time.
3. Shoot Length (cm) – This was obtained at termination time by measuring the shoot starting from the first node above the node bearing the roots to the tip of the longest leaf of the plants sample using a meter stick.
4. Root Length (cm) – This was obtained at termination time by measuring the roots starting from the node that bear the roots to the tip of the longest roots of the sample plants and measured using a meter stick.
5. Fresh Weight (g) - This was determined by weighing all sample plants per treatment at termination time with the use of a digital weighing scale.

6. Dry Weight of the Plants (g) – This was obtained by sun drying the plant samples and the weight were taken in three weighing periods until the weight became stable and measured using a digital weighing scale.

7. % Dry Matter (DM) – This was determined using the formula:

$$\% \text{ Dry Matter (DM)} = \frac{\text{Dry Weight (g) of Seedlings}}{\text{Fresh Weight (g) of Seedlings}} \times 100$$

Statistical Analysis. The data gathered were tabulated and analyzed statistically using the Analysis of Variance (ANOVA) in Randomized Complete Block Design (RCBD). Honestly Significant Difference (HSD) test was used to compare significant differences among treatment means.

RESULTS AND DISCUSSION

Chemical Properties of the Soil Media Before and After Application of LBFs. The initial soil pH was moderately acidic (pH 4.43). However, with the application of LBFs in each treatment, the pH values increased to 4.70, 4.74, and 4.76 for CPILBF, CILBF and PILBF, respectively. This implies that the liquid biofertilizer formulations have the ability to reduce soil acidity by increasing the pH. The initial soil organic matter content was 5.47%; it decreased slightly to 5.38 %, 4.97 % and 4.00 % for CPILBF, CILBF and PILBF, respectively (Table 1). There was a slight decrease inorganic matter content in the soil attributed to faster rate of decomposition due to higher temperature during the conduct of the experiment.

Table 1. Chemical properties of the soil before and after the pot experiment

SOIL PROPERTIES	BEFORE APPLICATION	AFTER APPLICATION		
		Cow	Pig	Cow-Pig
		Ingesta	Ingesta	Ingesta
pH	4.43	4.74	4.76	4.70
Organic Matter (OM) %	5.47	4.97	4.00	5.38
Total Nitrogen, %	0.29	0.11	0.08	0.05
Extr. Phosphorus (ppm)	4.40	9.74	20.19	9.10
Exch. Potassium (ppm)	51.00	189.00	204.00	180.00

Source: Soil and Plant Analysis Laboratory, Central Mindanao University, Musuan, Bukidnon

The removal of all plants from the soil at termination wherein no plant biomass was left is also one of the reasons for the decrease of organic matter content in the soil after the application of LBFs. In this experiment, the application of LBFs which had *Enterobacter sp.* also contributed to faster decomposition of organic matter resulting to, the decline of organic matter content in the soil.

A decreasing trend in Total Nitrogen in the soil was also observed in all soils applied with LBFs attributed to the higher intake of nitrogen by the plants. However, the extractable phosphorus available in the soil approximately doubles in CPILB and CPILBF applied plants. Extractable P has quadrupled in PILBF and a very noticeable increase in exchangeable potassium ranging from 180 ppm to 240 ppm for CPILB, CILBF and PILBF, respectively. Results of the study showed decreasing trends in organic matter and subsequently the nitrogen content of soils treated with LBFs. This finding contradicts to that of Dahmardeh (2013) who reported that biofertilizers increased the organic matter content and nitrogen availability in canola and improve the soil physiological structure. On the other hand, Suprpta, et al (2014) reported that the intake of macro nutrients by rice plants is higher on plants treated with LBFs.

Plant Tissue Analysis of Adlay Seedlings. Tissue analysis results show that adlay plants treated with any of the three liquid biofertilizers have higher total nitrogen ranging from 2.17% to 2.37% for CILBF, PILBF and CPILBF, respectively compared to plants applied with inorganic fertilizer with N content of 1.98%, and 1.12% in the control plants.

There was a slight increase in total P for LBF-treated plants with 0.20% (CILBF), 0.25 (PILBF) and 0.27 (CPILBF) compared to 0.14% from the control treatment. Total K noticeably increased in all LBF-treated plants than those treated with inorganic fertilizers and the untreated plants. The result suggests that the intake of macro nutrients by adlay plants is higher on plants treated with LBFs (Table 2). This finding conforms to that of Suprapta, *et al* (2014) who stated that rice plant treated with *Enterobacter cloacae* increased the macro nutrient content in the leaves. Hellal, *et al* (2011) also reported that partial substitution of mineral nitrogen fertilizer with biofertilizer on Dill (*Anethum graveolens*) or applying biofertilizer alone or in combination with chemical N fertilizer increased the growth, yield and chemical constituents of dill compared to the untreated plants.

Table 2. Tissue analysis of adlay at vegetative stage as affected by application of liquid biofertilizers (LBFs)

TREATMENT	TOTAL NITROGEN (%)	TOTAL PHOSPHOROUS (%)	TOTAL POTASSIUM (%)
T ₁ – unfertilized	1.12	0.14	1.05
T ₂ - 20-120-70 N-P ₂ O ₅ -K ₂ O kg/ha	1.98	0.24	2.08
T ₃ - 5 ml of CILBF/500 ml water	2.17	0.20	2.98
T ₄ - 5 ml of PILBF/500 ml water	2.37	0.25	3.85
T ₅ - 5 ml of CPILBF/500 ml water	2.24	0.27	3.10

Source: Soil and Plant Analysis Laboratory, Central Mindanao University, Musuan, Bukidnon

Plant Height at 20, 40, and 60 DAS. The average plant height of adlay at 20, 40 and 60 DAS is presented in Table 3. At 20 DAS, Gulian seedlings were of comparable heights regardless of fertilizer treatments although inorganic fertilizer-treated plants were taller (58.80 cm) compared to LBF-supplied adlay (41.38 cm to 43.63 cm) and unfertilized plants were the shortest (41.07 cm). At 60 DAS, synthetic fertilizer-treated plants were the tallest (103.83 cm), and with comparable height to CILBF-treated plants (91.50 cm). Among the LBFs used, the combination of cow and pig ingesta produced taller plants attributed by higher NPK contents. Suprapta *et al* (2014) found out that 30 days old transplanted rice treated with biofertilizer (*Enterobacter cloacae*) increased height by 26.71% to 30.62%. In contrast, Oladele *et al* (2014) mentioned that lowland rice inoculated with biofertilizers were not significantly ($P < 0.05$) taller than those without biofertilizer.

Table 3. Plant height (cm) of adlay at 20, 40, and 60 DAS as influenced by application of liquid biofertilizers (LBFs)

TREATMENT	20 DAS	40 DAS	60 DAS
T ₁ - unfertilized	22.23	41.07 ^b	71.37 ^c
T ₂ - 20-120-70 N-P ₂ O ₅ -K ₂ O kg/ha	26.97	58.80 ^a	103.83 ^a

T ₃ - 5 ml of CILBF/500 ml water	25.27	42.67 ^b	82.00 ^{bc}
T ₄ - 5 ml of PILBF/500 ml water	25.30	43.63 ^b	87.17 ^b
T ₅ - 5 ml of CPILBF/500 ml water	23.82	41.38 ^b	91.50 ^{ab}
F-test	ns	*	**
CV (%)	12.59	12.08	8.96

Means followed by the same letter(s) are not significantly different at 5% level of significance based on HSD.

ns - not significant

* - significant

** - highly significant

Number of Vegetative Tillers. At 60 DAS, CPILBF-applied plants had the most number of tillers (4.67 pcs), followed by those with CILBF (4.33 pcs), and at par with those with inorganic fertilizer (4.0 pcs). PILBF-treated plants had only 3.33 tillers while unfertilized plants had the least tillers (2.33 pcs). Results show that LBFs have comparable effects to that of synthetic or chemical fertilizer (Table 4). Suprapta *et al* (2014) recorded significant ($p < 0.05$) increase in the number of tillers, chlorophyll content in the leaf, dry weight of shoots and roots of rice applied with biofertilizers.

Table 4. Number of vegetative tillers, shoot length (cm), and root length (cm) of adlay as affected by the application of liquid biofertilizers (LBFs)

TREATMENT	NUMBER VEGETATIVE TILLERS	SHOOT OF length (cm)	ROOT length (cm)
T ₁ – unfertilized	2.33 ^c	74.24 ^c	68.08 ^b
T ₂ - 20-120-70 N-P ₂ O ₅ -K ₂ O kg/ha	4.00 ^{ab}	105.33 ^a	95.25 ^a
T ₃ - 5 ml of CILBF/500 ml water	4.33 ^{ab}	86.78 ^{bc}	81.08 ^{ab}
T ₄ - 5 ml of PILBF/500 ml water	3.33 ^{bc}	88.38 ^{bc}	86.06 ^a
T ₅ - 5 ml of CPILBF/500 ml water	4.67 ^a	92.38 ^{ab}	88.71 ^a
F-test	*	*	*
CV (%)	16.22	8.56	10.17

Means followed by the same letter(s) are not significantly different at 5% level of significance based on HSD

ns - not significant

* - significant

** - highly significant

Shoot Length. The shoot length (cm) of adlay seedlings was significantly affected by the application of liquid LBFs (Table 4). Among the LBF-treated plants, those with CPILBF had the longest shoot (92.38 cm) comparable to those with inorganic fertilizers (105.33 cm), followed by plants with PILBF and CILBF with means of 88.38 and 86.78 cm, respectively. The shortest shoot were the unfertilized plants with a mean of 74.24 cm. The result conforms to that Agamy, *et al* (2012) on the effect of soil amendment using yeast as biofertilizers on the growth and productivity of sugar beet. Yeasts (*P. transvaalensis*, *K. waltii* and *S. cataeensis*) as biofertilizer significantly increased the yield and enhanced the growth of sugar beet. The two doses (50 and 100 ml/pot) significantly increased plant height, number of leaves, root length, root diameter, fresh and dry weight of shoots and roots, particularly at 100 ml/pot.

Root Length. There was a similar trend in roots lengths of adlay, and CPILBF-treated had the longest roots (88.71 cm) comparable to plants with inorganic fertilizers (95.25 cm) followed by PILBF and CILBF-applied with lengths of 86.06 and 81.08 cm, respectively (Table 4). Elefan (2015) concluded that the use of biofertilizers significantly influenced some growth parameters in horticultural crops. Suprapta *et al* (2014) also noted that application of biofertilizer (*Enterobacter cloacae*) increased the root length of rice by 47.95% to 52.39% and that the treatment promotes both the growth of shoots and roots of the rice plant as well as improved the root system of the plant.

Fresh Weight and Dry Weight of Adlay Seedlings. The fresh weight and dry weight (g) of adlay seedlings were significantly affected by the application of LBFs (Table 5). CPILBF-treated plants had the heaviest fresh weight of 440.00 g. at par with plants applied with inorganic fertilizers (613.33 g) followed by PILBF and CILBF-treated plants with a mean of 370.00 g and 360.00 g, respectively. As to the dry matter content, adlay seedlings with CPILBF and CILBF had the same weight of 53.33 g comparable to synthetic fertilizer-treated plants (66.67 g). The PILBF-applied adlay weighed only 43.33 g, yet still heavier than the unfertilized plants (20 g). This indicates that LBFs influenced the fresh and dry weight of adlay seedlings. Biofertilizers influence shoot and root growth as well as length, fresh weight and number of roots of lowland rice (Oladele, 2014). The dry weight of shoots and roots of rice applied with biofertilizer (*Enterobacter cloacae*) was significantly ($p < 0.05$) higher compared to control plants (Suprapta, *et al*, 2014).

Table 5. Fresh weight (g), dry weight (g) and % dry matter of adlay at 60 DAS as affected by application of liquid biofertilizers (LBFs)

TREATMENT	FRESH weight (g)	DRY weight (g)	% DRY MATTER
T ₁ – unfertilized	140.00 ^c	20.00 ^c	13.83
T ₂ - 20-120-70 N-P ₂ O ₅ -K ₂ O kg/ha	613.33 ^a	66.67 ^a	10.94
T ₃ - 5 ml of CILBF/500 ml water	360.00 ^b	53.33 ^{ab}	15.90
T ₄ - 5 ml of PILBF/500 ml water	370.00 ^b	43.33 ^b	11.08
T ₅ - 5 ml of CPILBF/500 ml water	440.00 ^{ab}	53.33 ^{ab}	14.46
F-test	**	*	Ns
CV (%)	28.36	24.24	36.48

Means followed by the same letter(s) are not significantly different at 5% level of significance based on HSD

ns - not significant

* - significant

** - highly significant

Percent Dry Matter. Treatment plants did not vary in this parameter implying that their dry matter yields were more or less comparable which ranged from 10.94% to 15.90% (Table 5). However, CILBF-treated plants had the highest percent dry matter (15.90%) followed by CPILBF-applied (14.46%) but plants supplied with inorganic fertilizer had lesser percent dry matter (10.94%) attributed to higher moisture content of fresh adlay seedlings. LBF-treated plants have more dry matter compared to inorganic-fertilized although unfertilized plants had higher percentage of dry matter compared to plants with CILBF and PILBF.

CONCLUSION AND RECOMMENDATION

The three LBF formulations are potential source of natural nutrients in adlay production. The application of biofertilizers slightly increased the pH, extractable P and exchangeable K in soils grown to adlay. Tissue analysis of the test crop showed that it contains sizable amounts of nitrogen (2.17% - 2.37%) and potassium (2.98% - 3.85%). All agronomic parameters of the LBF-applied adlay plants significantly differed, except for plant height at 30 DAS and percent dry matter at 60 DAS. Cow-pig ingesta liquid biofertilizer (CPILBF) is best for Adlay in pot culture at the rate of 18-27.5 L/ha. However, validation trials using the three LBFs (CILBF, PILBF and CPILBF) are recommended for adlay and other crops to compare the results.

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