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RESEARCH ARTICLE

EFFECTS OF GUANO MANURE ON HEIGHT GROWTH AND FOLIAR NUTRIENT CONCENTRATIONS OF SELASIH (*OCIMUM TENUIFLORUM* L.)

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ABSTRACT

The herbal industry in Malaysia is facing problem of scarcity of the natural herbs due to excessive exploitation of natural forest. It is therefore necessary to cultivate these herbs and research is urgently needed on its agronomic aspects. A pot trial study was therefore carried with the following objectives: (i) to determine the effects of different organic fertilizer rates on height growth, and (ii) to measure selected foliar and soil nutrient concentrations. Treatments evaluated were: (i) 10% of guano manure mixed with 90% of soil (T1), (ii) 20% of guano manure mixed with 80% of soil (T2), (iii) 30% of guano manure mixed with 70% of soil (T3), (iv) 40% of guano manure mixed with 60% of soil (T4), (v) 50% of guano manure mixed with 50% of soil (T5), (vi) 60% of guano manure mixed with 40% of soil (T6), (vii) 70% of guano manure mixed with 30% of soil (T7), (viii) 80% of guano manure mixed with 20% of soil (T8), (ix) 90% of guano manure mixed with 10% of soil (T9), and (x) Soil without guano manure (control) (T10). The experiment was conducted using a Completely Randomize Design (CRD) with five replications. Plant height for each treatment was recorded on monthly basis for four months duration. Selected nutrient concentrations in the plant leaves after harvest and in the soil before planting and after harvest along with the soil pH were analysed. Soil total N was determined using Kjeldahl method while total P was determined using Aqua regia method. The exchangeable K, Ca, Mg, Zn, Cu, and Na were extracted using double acid method and their concentrations determined using atomic absorption spectrophotometry (AAS). Dry ashing method was used for the determination of P, K, Ca, Mg, Zn, Cu, and Na concentrations in plant leaves while total N concentrations was determined using Kjeldahl method. Guano manure showed significant effects on plant height, and concentrations of total N, total P, Ca, Mg and Zn in the leaves. The T3 recorded significantly higher plant height at 76.50 cm while the lowest was in T9 at 50.60 cm. The concentrations of K, Cu and Na on the other hand recorded no significant effects. Nutrient concentrations in the soil after harvest decreased compared to before planting while soil pH increased as level of manure increased in the soil before planting and after harvest. It is advisable to conduct field trial experiment for further study.

KEYWORDS

foliar nutrient, guano manure, height growth, *Ocimum tenuiflorum*

1. INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. It is sometimes referred to as Herbalism or Botanical Medicine. It is the use of herbs for their therapeutic or medicinal value. Herb is a plant or plant part valued for its medicinal, aromatic or savoury qualities. Herbal plants produce and contain a variety of chemical substances that act upon human body. The World Health Organization (WHO) estimates that 80% of the world population used herbs for their primary health needs (WHO, 2019). Therefore, major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. This may lead to over exploitation of this natural resources of medicinal plants in forest leading to extinction. Hence, to avoid the valuable herbs from extinction, it necessary to cultivate them.

One example of the medicinal plant is Selasih (*Ocimum tenuiflorum* L.). This plant is native to India and grown in the tropical and subtropical regions of the world. *O. tenuiflorum* prefers partial to full sunlight and thrives on well-drained soil rich in organic matter. It can be found growing along roadsides and in wastelands, as it can adapt well to its environment (Samy et al., 2005). *O. tenuiflorum* is an erect, hairy, branched, fragrant and an annual herb. It may attain a height of about 75 to 90 cm when mature. Its leaves are nearly round and up to 5 cm long with the margin being entire or toothed. They are aromatic because of the presence of a kind of scented oil in them. In Ayurveda (Indian traditional remedy), the leaves, flowers and occasionally the whole plant of *O. tenuiflorum* are used medicinally in the treatment of heart and blood diseases, asthma, bronchitis, and purulent discharges of the ear (John, 2001). The infusion of the leaves is given in malaria and as a stomachic in gastric diseases of children. Juice of the leaves could be taken internally and is very effective as a cure of skin diseases such as itches, ringworm, and in impurities of the blood.

On another aspect, plants need water, air, light, suitable temperature, and nutrients to grow. Plants get carbon, hydrogen and oxygen from the air and water while other nutrients are from the soil. Although some soils are naturally fertile, most need the addition of some form of fertilizer. A

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fertilizer is any material, organic or inorganic, natural or synthetic, that supplies plants with the necessary nutrients for plant growth for optimum yield (Salleh et al., 2015). There are two major kinds of fertilizers mainly the organic and inorganic or chemical fertilizers. Organic fertilizers are natural materials of either plant or animal origin, including livestock manure, green manures, crop residues, household waste, compost, and woodland litter. On the other hand, inorganic or mineral fertilizers are fertilizers mined from mineral deposits with little processing such as lime, potash, or phosphate rock, or industrially manufactured through chemical processes such as urea. A study on the nutrient uptake of plants could be done by conducting soil testing and leaf diagnosis. Soil testing has the advantage of being able to measure the level of nutrients available in the soil, and the extent to which these will be available to the crop during the growing period. The leaf diagnosis on the other hand indicated nutrient status of the plant at particular time of sampling.

The international market for herbal medicines has an immense impact on the populations of medicinal plant species and their habitats. As demand for herbs, phytotherapies, and naturally derived pharmaceuticals products increases, it can often lead to over-harvesting of desired medicinal plant species. Therefore, preventive steps must be taken to ensure the availability and existence of particular herbs and other medicinal plants. Thereby, the present study was conducted as an attempt to get basic knowledge about the suitable rate of organic fertilizer which must be applied to enhance growth and foliar nutrient concentrations of the medicinal plant. Specifically, the present study aimed to determine the effects of different rates of guano manure organic fertilizer on height growth and selected foliar nutrients of Selasih (*Ocimum tenuiflorum* L.) and soil nutrient concentrations before planting and at harvest.

2. Materials and Methods

2.1 Experimental Design and Treatments

The pot-trial experiment was conducted under net house conditions using a Completely Randomize Design (CRD). The herb (Selasih) *O. tenuiflorum* was grown in the polybag using mixed soil. Plant cuttings of about 3 months old were used with 1 plant cutting grown per polybag. The polybags were arranged randomly. A total number of 50 experimental units consisted of 10 levels of treatments with 5 replications were involved. The organic fertilizer used was guano manure. Treatments applied were a mixture of guano manure with mixed soil by volume basis (Table 1). Treatments arrangements were as shown in Table 2.

Table 1: Treatments Composition

Treatment	Composition
T1	10% of guano manure mixed with 90% of soil
T2	20% of guano manure mixed with 80% of soil
T3	30% of guano manure mixed with 70% of soil
T4	40% of guano manure mixed with 60% of soil
T5	50% of guano manure mixed with 50% of soil
T6	60% of guano manure mixed with 40% of soil
T7	70% of guano manure mixed with 30% of soil
T8	80% of guano manure mixed with 20% of soil
T9	90% of guano manure mixed with 10% of soil
T10 (Control)	Soil without guano manure

Table 2: Arrangement of Treatments

ROW 1	ROW 2	ROW 3	ROW 4	ROW 5
T6 a	T8 d	T2 a	T3 b	T9 b
T8 b	T5 e	T9 e	T7 d	T5 a
T4 c	T2 b	T6 e	T9 c	T1 d
T3 e	T9 a	T10 b	T4 e	T7 e
T5 a	T1 a	T5 b	T6 c	T4 b
T1 c	T4 c	T1 e	T10 d	T8 a
T9 d	T10 e	T7 b	T1 b	T10 c
T2 e	T7 c	T3 d	T5 d	T3 c
T7 a	T3 a	T8 c	T2 d	T6 d
T10 a	T6 b	T4 a	T8 e	T2 c

2.2 Data Collection and Statistical Analysis

Plant height of each treatment was recorded at the end of the experiment (four months after planting). Foliar nutrient concentrations after harvest and nutrient concentrations in the soil before planting and after harvest and soil pH were also analysed and recorded. Soil total N was determined using Kjeldahl method while total P was determined using Aqua regia method. The exchangeable K, Ca, Mg, Zn, Cu, and Na were extracted using double acid method and their concentrations determined using atomic absorption spectrophotometry (AAS). Dry ashing method was used for the determination of P, K, Ca, Mg, Zn, Cu, and Na concentrations in plant leaves. All data were analysed using the analysis of variance (ANOVA) at $p \leq 0.05$ followed by the mean comparison analysis of Duncan multiple range test (DMRT) using the Statistical Analysis System (SAS) software.

3. Results and Discussion

3.1 Effects of Guano Manure on Plant Height

Plant height is the actual measurement of plant from the soil surface to the tip of the tallest panicle or shoot. It could be one of the visual indicators of plant growth rate. As shown in Table 3, there were significant differences at $p \leq 0.05$ among treatments with T3 recorded the highest height at 76.50 cm while T9 recorded the lowest height at 50.60 cm. The T3 thus appeared to be the optimum guano level for plant growth. A group researchers stated that organic manure could result in significant effect on plant growth including plant height (Maheshbabu et al., 2007). According to some researcher's other factors such as nutrients availability and soil pH also influenced plant growth (Salleh et al., 2015). In contrast, T9 showed the lowest plant height probably due to the toxic effect of over fertilization since T9 consist of a mixture of 90% guano manure with only 10% of soil. As stated by plants need all nutrients in sufficient amounts for optimum growth but too much of some nutrients can cause negative effects on plant growth (White and Brown, 2010).

Table 3: Effects of Guano Manure Treatments on Plant Height

Treatment	Plant Height (cm)
T1	65.30 ^{ab}
T2	70.90 ^{ab}
T3	76.50 ^{a}
T4	68.86 ^{ab}
T5	65.30 ^{ab}
T6	68.90 ^{ab}
T7	67.40 ^{ab}
T8	69.70 ^{ab}
T9	50.60 ^{c}
T10	62.60 ^{b}

Note: Means within the same column with different letters are significantly different at $p \leq 0.05$

3.2 The Soil Nutrient Concentrations Before Planting and at Harvest

Nutrient concentrations of total N, total P, exchangeable K, Ca, Mg, Zn and Ca were significantly different at $p \leq 0.05$ between treatments except for exchangeable Cu (Table 4). In the case of total N in the soil before planting, T8 showed the highest concentration (0.15%) while T3 recorded the lowest value at 0.08%. On the other hand, T9 showed the highest value of exchangeable Ca at 0.77% while the lowest was T10 at 0.17%. For exchangeable Mg, T6 recorded the highest value at 490.40 ppm while T8 recorded the lowest value at 160.80 ppm. The highest value of exchangeable Zn in the soil before planting was recorded in T2 (113.07 ppm) while the lowest was in T9 (5.60 ppm). There was no significant difference in the concentrations of exchangeable Cu in the soil before planting. The exchangeable Na showed significant difference among treatments with the highest value was T1 at 232.53 ppm while the lowest was T9 at 124.80 ppm.

In the case of total N in the soil after harvest (Table 4), T8 recorded the highest concentration at 0.06% while other treatments (T3, T5, T6, T7, T9, and T10) recorded the lowest value at only 0.03%. The highest total P concentration was recorded in T9 at 3.70% while the lowest was in T10 at 0.19%. The highest value of exchangeable K was in T7 at 73.20 ppm while the lowest was in T8 at 20.10 ppm. T9 showed the highest value of exchangeable Ca at 0.53% while the lowest was T10 at 0.17%. For exchangeable Mg, T7 recorded the highest value at 352.50 ppm while T10 was the lowest at 61.40 ppm. Highest value of exchangeable Zn was in T3 (27.14 ppm). Meanwhile, T1 showed the highest value of exchangeable Cu at 2.66 ppm while T9 the lowest at 0.35 ppm. The highest value of exchangeable Na was recorded in T2 (75.40 ppm) while the lowest was in T10 (37.70 ppm).

Table 4: Soil Nutrient Concentrations Before Planting and at Harvest

	Total N	Total P	Ca	K	Mg	Zn	Cu	Na
			Exchangeable					
Treatments	%		ppm					
Soil Nutrient Concentrations Before Planting								
T1	0.09bc	0.03f	0.55e	348.67a	232.93c	97.47a	11.60a	232.53a
T2	0.08c	0.18e	0.63d	258.13b	210.93cd	113.07a	11.07a	220.67ab
T3	0.08c	0.41d	0.64d	215.60bc	171.73cd	90.67ab	9.60a	214.53ab
T4	0.11bc	0.64bc	0.69bc	162.80c	182.67cd	55.87bc	8.67a	202.53ab
T5	0.10bc	0.75b	0.66c	169.47c	189.07cd	47.73cd	8.00a	193.73ab
T6	0.09bc	0.91a	0.72b	326.93a	490.40a	12.80de	8.80a	135.60cd
T7	0.11bc	0.72b	0.66c	260.27b	408.13b	38.27cde	17.73a	198.53ab
T8	0.15a	0.54c	0.72b	97.73d	160.80d	41.33cde	8.13a	179.20bc
T9	0.12b	0.63bc	0.77a	80.27d	382.93b	5.60e	8.00a	124.80d
T10	0.10bc	0.05f	0.17f	362.40a	188.67cd	13.20de	9.07a	125.20d
Soil Nutrient Concentrations at Harvest								
T1	0.04b	0.43f	0.32cd	40.90bc	168.80bc	22.40a	2.66a	64.50bc
T2	0.04b	0.96e	0.34c	32.50bc	146.40bcd	26.81a	2.37a	75.40a
T3	0.03b	1.12e	0.28de	36.20bc	112.50cd	27.14a	2.56a	73.30ab
T4	0.04b	1.11e	0.26ef	32.40bc	109.00cd	27.06a	2.58a	72.70ab
T5	0.03b	2.05d	0.25ef	32.60bc	84.30cd	26.06a	1.61b	72.70ab
T6	0.03b	2.45cd	0.24ef	48.50b	138.60bcd	11.68bc	0.56c	53.20d
T7	0.03b	2.60c	0.21f	73.20a	352.50a	15.07b	0.59c	56.80cd
T8	0.06a	3.26b	0.47b	20.10c	102.20cd	21.83a	1.46b	66.80ab
T9	0.03b	3.70a	0.53a	26.20bc	219.70b	5.59d	0.35c	47.10d
T10	0.03b	0.19f	0.17g	37.80bc	61.40d	7.58cd	1.22b	37.70e

Notes: Means within the same column with different letters are significantly different at $p \leq 0.05$

The total N concentrations in the soil before planting and after harvest were significantly different between treatments. T8 showed the highest value of total N in the soil before planting at 0.15% and also in the soil after harvest at 0.06%. Decreasing amounts of total N concentration in the soil before planting as compared to after harvest might be caused by factors such as nutrient uptake by plant, leached from soil or being used up by soil organisms. Dennis stated that the soil is the main source of nutrients for plants thus nutrient availability in the soil will decrease with time (Dennis, 2005). Other nutrients which showed the same trend before planting and after harvest are exchangeable K, Ca, Mg, Zn, Cu and Na. A group researchers stated that nutrient deficiency for plant uptake can occur as a consequence of inadequate supply of the nutrients and leaching losses (Roy et al., 2007). Other than that, the root is a plant organ that also responsible for nutrient uptake. The longer the root, the better the plant's ability to absorb nutrients due to wider range of coverage (Putra et al., 2020). However, total P concentration showed an opposite trend whereby P concentration in the soil after harvest was much higher compared to before planting. T9 showed the highest total P concentration in the soil after harvest at 3.70% while in the soil before planting, the concentration was only 0.63%. According to microbial activities could influence nutrient amount in the soil thus breakdown of organic residues with high amounts of phosphate will increase the amount of phosphorus in the soil (Dennis, 2005). However, microbial activity depends much on temperature and is the highest in the range of 30 to 45^{0}C (Mengel et al., 2001).

3.3 Comparison of pH Values in Soil Before Planting and at Harvest

There were significant differences in soil pH before planting and at harvest. As shown in Table 5, soil pH increased with increasing levels of manure. Among treatments, T10 recorded the lowest pH value at 4.82 (KCl) and 6.47 (water) in the soil before planting, and 5.89 (KCl) and 6.77 (water) in the soil at harvest. This indicated that the control treatment (soil without manure) have higher acidic value. In contrast, T9 recorded the highest pH value at 7.55 (KCl) and 7.61 (water) in the soil before planting and 7.56 (KCl) and 7.61 (water) in the soil at harvest. In general, there was an increasing trend in soil pH as the level of guano manure increases. According to pH will increase with increasing rate of organic manure application (Salleh, 2015). Therefore, organic manure can be used to remediate problems of acidic soil as it will increase soil pH when being applied to the soil. However, soil pH from 6.50 to 7.00 provides the most suitable condition for optimum plant growth (Mengel et al., 2001). Hence, in the present study, T3 with soil pH at 7.00 recorded the highest plant height compared to the other treatments. Dennis stated that soil pH ranges near neutral pH is important to ensure proper plant growth because most essential elements reach near maximal availability and most plant toxic elements become unavailable in this pH range (Dennis, 2005).

Table 5: Soil pH Before Planting and at Harvest

Treatments	Before Planting		At Harvest	
	pH			
	KCl	Water	KCl	Water
T1	6.99^{g}	6.89^{e}	6.57^{f}	7.14^{e}
T2	6.99^{g}	6.93^{e}	6.70^{e}	7.22^{de}
T3	7.00^{g}	7.18^{d}	7.00^{d}	7.31^{cd}
T4	7.30^{de}	7.42^{b}	7.19^{c}	7.40^{bc}
T5	7.27^{e}	7.43^{b}	7.29^{bc}	7.35^{c}
T6	7.40^{c}	7.27^{c}	7.34^{b}	7.48^{b}
T7	7.32^{d}	7.27^{c}	7.23^{bc}	7.50^{b}
T8	7.21^{f}	7.43^{b}	7.30^{bc}	7.49^{b}
T9	7.54^{b}	7.56^{a}	7.56^{a}	7.61^{a}
T10	4.82^{h}	6.47^{e}	5.89^{g}	6.77^{f}

Note: Means within the same column with different letters are significantly different ($p \leq 0.05$)

3.4 Foliage Nutrient Concentrations at Harvest

As shown in Table 6, T4 recorded the highest N concentration at 0.70% while T9 recorded the lowest value (0.35%). The highest total P concentration was in T9 at 0.50% whereas the lowest was in T4 with only 0.28%. The concentration of exchangeable K however showed no significant difference among treatments. The highest value of exchangeable Ca was recorded in T9 at 3.89% while the lowest was in T4 with only 3.21%. For exchangeable Mg, T9 recorded the highest value at 0.43% while the lowest was in T5 at 0.13%. The highest value of exchangeable Zn was in T8 (96.25 ppm) while the lowest value was in T4 (50.00 ppm). Meanwhile, T3 recorded the highest value of exchangeable Cu (987.50 ppm) while T5 was the lowest with only 200.00 ppm. However, all of the treatments are not significantly different at $p \leq 0.05$. In the case of exchangeable Na, it was also found that there was no significant difference among all treatments. The highest value of exchangeable Na was in T9 at 0.81% while the lowest was in T8 at 0.34%.

Table 6: Foliage Nutrient Concentrations at Harvest								
	Total N	**Total P**	**K**	**Ca**	**Mg**	**Na**	**Zn**	**Cu**
				Exchangeable				
Treatments	%				ppm			
T1	0.53ab	0.41ab	2.60a	3.47ab	0.30b	0.45a	73.63abc	631.30a
T2	0.49ab	0.42a	2.16a	3.58ab	0.27bc	0.65a	81.50abc	650.00a
T3	0.56ab	0.40abc	2.45a	3.21b	0.20cd	0.46a	83.38abc	987.50a
T4	0.70a	0.28c	2.42a	3.21b	0.16d	0.43a	50.00c	793.80a
T5	0.60ab	0.39abc	2.49a	3.55ab	0.13d	0.37a	73.13abc	200.00a
T6	0.70a	0.45a	2.43a	3.83a	0.28bc	0.42a	81.75abc	412.50a
T7	0.49ab	0.40abc	2.28a	3.67ab	0.31b	0.37a	84.75ab	768.80a
T8	0.56ab	0.46a	2.37a	3.53ab	0.32b	0.34a	96.25a	550.00a
T9	0.35b	0.50a	2.61a	3.89a	0.43a	0.81a	79.88abc	600.00a
T10	0.49ab	0.29bc	2.51a	3.82a	0.32b	0.35a	56.63bc	293.80a

Note: Means within the same column with different letters are significantly different ($p \leq 0.05$)

In general, the concentrations of total N, total P, exchangeable Ca, Mg and Zn showed significant differences among treatments while exchangeable K, Cu and Na showed opposite trend with no significant difference. For total N concentration, T9 recorded the lowest value of foliar nitrogen at harvest. This might cause by the change during growing stages since as plants mature, the concentration of nitrogen in the leaves, stem and roots will decrease as a result of conversion to protein stored in the plants (Barker and Pilbeam, 2007). In contrast, concentration of total P in T9 showed the highest value as compared to the other treatments. This condition may be due to physiological changes as the plants mature and produced flowers and fruits. As stated by phosphate is of particular importance for fruit setting, fruit quality and resistance to diseases hence insufficient phosphate nutrition may delay fruit maturation (Mengel et al., 2001). Exchangeable K concentrations in the leaves of *O. tenuiflorum* do not show any significant difference among treatments. The lowest value was shown by T2 while the highest was showed by T9. It shows that potassium supplied by the mixture of guano manure with soil was sufficient for plant growth since potassium is the second most abundant mineral nutrient in the plants after N (Roy et al., 2007).

Concentrations of exchangeable Ca, Mg and Na showed the highest amount in T9 since it has the highest organic manure. A group researchers stated that Ca ranks with Mg in the group of least abundant nutrients in plants (Roy et al., 2007). Ca is immobile in the phloem and is involved in cell division, growth, root lengthening and activation or inhibition of enzymes. The exchangeable Ca in plant leaves was higher compared to exchangeable Mg. High calcium concentration in the soils sometimes limit magnesium accumulation and may cause magnesium deficiency symptoms (Barker and Pilbeam, 2007). However, exchangeable Na showed no significant difference among treatments. Barker and Pilbeam, stated that sodium and potassium have similar chemical properties but these two elements have very different roles and are treated very differently by mechanisms involved in short- and long-range transport (Barker and Pilbeam, 2007). For the exchangeable Zn, T8 showed the highest amount but with only little amount compared to other nutrients. According to plant requires only trace amount of zinc which functions to promote RNA synthesis which in turn is needed for protein synthesis (Roy et al., 2007).

4. Conclusion

Application of guano manure as organic fertilizer in this study showed significant effect on plant height. The optimum rate of fertilizer which produced the tallest plant was the T3 (30% of guano manure mixed with 70% of soil). In contrast, T9 (90% of guano manure mixed with 10% of soil) appeared to retard height growth may be due to over fertilization. In the case of nutrient concentrations in the leaves of *O. tenuiflorum*, total N, total P, exchangeable Ca, Mg and Zn showed significant differences among treatments while exchangeable K, Cu and Na showed no significant difference at $p \leq 0.05$. The soil nutrients before planting recorded higher concentrations compared to after harvest probably due to several factors such as nutrient uptake by plant, leaching from soil or being used up by soil organisms. Soil pH increased as the level of fertilizer increased for both soil before and after harvest. For the control treatment, the soil without fertilizer was found to be highly acidic while guano manure was found to be more alkaline. Organic manure seems to act as a soil conditioner and soil amendment to improve soil characteristics including soil acidity. Findings of the present study might be further confirmed by conducting multi-location field trial experiments.

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