Effect of vermicompost tea on seed germination of Green Romaine (*Lactuca sativa* L. var. Jericho) and Green Batavia (*Lactuca sativa* L.var Concept) Lettuce

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ABSTRACT: Vermiculture is a technology which uses earthworm and microorganism to convert solid waste to become organic fertilizer. It can be collected vermicompost tea during decomposition of vermicompost. Vermicompost tea is liquid biofertilizer which can use to boot up plant nutrients and plant productions. Vermicompost tea was normally diluted being apply to plant and avoid plant damage. The objective of this study was to investigate and to find a suitable concentration of vermicompost tea for growing seed of Green Romaine (*Lactuca sativa* L. var. Jericho**)** and Green Batavia (*Lactuca sativa* L.var Concept) Lettuce. The experiment was arranged in a Complete Randomized Design with 3 replications in the petri dish. Vermicompost tea concentration was diluted to 0, 25, 50, 75, and 100% and applies to lettuce seeds both varieties. 13 days of the experiment, seed germination percentage, shoot length, root length, seed vigor index, fresh weight, and dry weight were collected. The results showed that 25 and 50 % of vermicompost tea were not significantly increase seed germination of Green Romaine and Batavia compared to control ($p>0.05$) but (75 and 100%) were significantly decrease the seed germination than the control. Nevertheless, 50 % vermicompost tea was significant increase in shoot length, root length, seed vigor index, fresh weight and dry weight of both lettuce varieties compared to other treatments ($p>0.05$). Therefore, 50% vermicompost tea could be used to improve seed growth of both lettuce varieties.

Keywords: Vermicompost tea, Green Romaine, Green Batavia, and seed germination

Introduction

Due to the growing population, agriculture sector is under pressure in producing more food in order to support the demand. During food processing, there is a huge amount of organic waste such as fruits and vegetable. In 2014, the total production of fruit and vegetable were around 35 million tons and 289.8 million tons; moreover, Asia produces fruits (73.3%) and vegetable (86.3%) for supply the world production (Liakou et al., 2018). The waste products of fruits and vegetable were around 512, 000 tons and 11millions tons (Liakou et al., 2018). It has been observed that this organic waste can cause environmental pollution such as water pollution, air pollution, and disease. Nevertheless, the organic and inorganic waste

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might contain high nutrients which can convert and recycle waste in an agricultural field in order to fulfill the requirement of the crop.

Vermicomposting technology is the technique which uses earthworms to stabilize the organic waste material and to produce microorganism-rich in the composting process (Quaik et al., 2012). Vermicompost is a sustainable source of macro and micronutrient, plant growth hormone, suppressive microbes and enzymes which not only enhance microbial population but also hold nutrients for a long time (Garg and Gupta, 2011). During the vermicomposting, it can be collected vermicompost tea which is designed to allow the percolation water through these passages and collected the nutrient and microorganism along with it. It was already acknowledged that vermicompost tea can be able to improve the plant health, crop yield and nutritive quality. Additionally, it was already reported that vermicompost tea contains soluble mineral nutrients, organic acids and water-soluble plant growth which have positive effect to on initial root development and plant development (Esteban et al., 2017). Besides, it also contains growth hormones like cytokines, gibberellins and indole-3-acetic acid (Esteban et al., 2017). Using vermicompost tea as organic fertilizer can also assist the farmers reduce of using chemical fertilizer which has negative effect on the environment as well as consumer's health.

Lettuce is one of the essential vegetable in agricultural market. Romaine and Batavia lettuce are contained high level of nutrient such antioxidants, other crucial vitamins and minerals, manganese, and more compared to other varieties. Nevertheless, lettuce seed is expensive in the market.

Seed germination is a critical stage which ensures the plant reproduction and controls dynamic of plant population, thus it is the critical test for crop productivity. Therefore, it can be used vermicompost tea to improve the seed germination. Vermicompost tea has been studying for its effects on seed germination with different kind of seed. Carlo et al. 2008 reported using 50% vermicompost tea as liquid fertilizer can improve maize (*Zea mays* L) seeding. It was also reported that 10% vermicompost tea was the most effective to enhance saluyot (*Corchorus olitorius*) germination (Esteban et al., 2017). The experiment on vermicompost and vermicompost tea use as the supplement to improve the seeding plant growth and yield in flaxseed (*Linum usitassimum* L). For organic agriculture indicated that 50% of vermicompost tea can improve the performance seed, root and stem (Makkar et al., 2017). Even though vermicompost tea had been used with some crop seed; the information about vermicompost tea on lettuce seed is still limited especially the suitable concentration for lettuce germination. The objectives of this research were to (i) determine the effects of vermicompost tea on seed germination of Green Romaine (*Lactuca sativa* L. var. Jericho) and Green Batavia (*Lactuca sativa* L.var Concept) lettuce and (ii) to find the suitable range of vermicompost tea for growing seed of Green Romaine (*Lactuca sativa* L. var. Jericho) and Green Batavia (*Lactuca sativa* L.var Concept) lettuce.

Methodology

Preparation of vermicompost and vermicompost tea

Vermicompost was prepared as described by Iwai et al. (2011). Briefly, Roi et series, cow manure, vegetable waste, rice husk ash were used as the components for vermicompost with ratio 4:4:3:1 (W/W). The manure was exposed 5 days to remove the harmful organism and noxious gases. Vermibed was conducted

with pot size 15 cm width and 30 cm length with the 19 cm height. Pre-compost was 15 days to avoid thermophilic stage (increased hot temperature in vermicompost cause earthworm death in vermicomposting process). Earthworms (*Eisenia foetida)* epidemic were collected from The Research Developing and Learning Centre on Earthworm for Agriculture and Environment located Khon Kaen University. There are 18 vermibeds with 150 earthworms per pot (12kg/pot) and the bed was covered tored and useless pokets. Each bed was maintained 80% moisture by using periodic sprinkling of adequate quantity of tape water and the bed contained drainage which can collected vermicompost tea during watering. The tea was collected until vermicomposting was manually turned up to 8 weeks.

Physical and chemical parameters analysis of vermicompost tea

The pH and EC of vermicompost tea were measured by using Cyberscan PC 300 pH and EC meter (EUTECH instrument, Singapore). Total N was measured by Kjeldahl method using the concentration of H_2SO_4, K_2SO and HgO to digest the sample (Carlos et al., 2008). The concentration of NH_4^+ and NO_3^- were determined by distillation with magnesium oxide (MgO) (Carlos et al., 2008). The concentration of P_2O_5 was determined by using HNO_3 and $HClO_4$ with spectrophotometry following the method of Horwitz and Latimer. (2005). The concentration of K_2O was also analyzed by using HNO_3 and $HClO_4$ with Flame photometer (Horwitz, 2000). The other nutrients Ca, Mg, Fe, Mn, Zn and Cu were determined by HNO_3 and H_2O_2 by using Atomic Absorption Spectrophotometry (Horwitz, 2000).

Vermicompost tea text on Roman (*Lactuca vativa* L.var. Jericho) and Batavia

(*Lactuca sativa* L.var. Concept) seed germination.

The germination test on Roman (*Lactuca sativa*l l. var. Jericho) and Batavia (*Lactuca sativa* l.var. Concept) lettuce was conducted for 13 days. The experiment design was Completely Random Design (CRD) with three replications. Lettuce seed was washing with deionized water for 15 minutes to remove microbe from the seed. Fifty seeds from each varieties were put on Whatman Grade 181 No. 1 filter papers in 9cm petri dishes and treated with different concentration of vermicompost tea (0, 25, 50, 75 and 100%) and deionized water was used in 0% instead of vermicompost tea then incubated at 25 ^{o}c in 12 darkness and 12 hours light for 13 days in an incubator (Lazcano et al., 2010). After completed of seed germination, seed from each treatment was measured for germination percentage, root length, shoot length, seed vigor index, fresh weight, and dry weight

Seed germination percentage

The total numbers of seed germination percentages in each treatment were counted on 13 days and the percentages were calculated following (Manisha and Angoorbala, 2015).

$$\text{Germination (\%)} = \frac{\text{Total number of seed germinated in particular treatment}}{\text{Total number of seed treated in particular treatment}} \text{ X } 100$$

Root and shoot length estimation

The 25 seeds from each treatment were selected random from all replications to measure root and shoot length after 13 days of germination test. The shoot length was measured from the based of the primary leaf and root length was measured from the tip of the primary root.

Seedings fresh and dry weight estimation

Fresh and dry weight was measured by using an Ohaus PA 2102 electronic balance.

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After fresh weight measurement, seeding weight was placed in Binder hot air oven (Model C 170, Germany) at 80°c for 24 hours and the dry weight was measured again with the electronic balance.

Seed vigor index

Seed vigor index is an important parameter which determined the level of the activities of the seed during seed germination. Seed germination, root length, and shoot length were used to calculate seed vigor index, which followed the formula (Abdul Baki and Anderson, 1973)

Vigor index= (Mean root length +Mean shoot length) x Percentage germination

Statistical analysis

All the data were used to analysis of variance (One Way ANOVA) and treatment

means were compared using Least Significance Difference (LSD) at P< 0.05 by using the Statistic 10 software (version 10, USA).

Results and Discussion

Characteristics of vermicompost tea

The chemical properties of the vermicompost tea used in this study are presented in **Table 1**. On the average, the pH of 25, 50, 75 and 100% vermicompost tea were 8.46±0.02, 8.43±0, 8.42±0.02, and 8.35±0.01, respectively. The EC were 1.84±0.02, 3.43±0.04, 5.10±0.11 and 6.69±0.10 dS/m, respectively. The vermicompost tea contained macronutrients such as total nitrogen (N), ammonium (NH_4^+), nitrate (NO_3^-), phosphorus (P_2O_5), potassium (K_2O), calcium (Ca), and magnesium (Mg) which are good fertilizer properties for agriculture. Moreover, it also contained micronutrients such as iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu)

Table 1 Chemical characteristic of vermicompost tea

Parameters	Units	Concentration of vermicompost tea			
		25%	50%	75%	100%
pH	-	8.46±0.02	8.43±0.00	8.42±0.02	8.35±0.01
EC	dS/m	1.84±0.02	3.43±0.04	5.10±0.11	6.69±0.10
total N	mg /l	382.5±21.6	765±22.6	1147±22.6	1530 ±23.6
NH_4^+	mg /l	1.5±0.49	3 ±0.24	4.5 ±0.39	6.02 ±0.4
NO_3^-	mg /l	87.15±0	174.3 ±1.2	261.4±3.2	348.6 ±2.3
P_2O_5	mg /l	7.275±1.4	14.55 ±1.5	21.8 ±1.7	29.1 ±1.85
K_20	mg /l	1231±143	2462 ±153	3693 ±123	4924 ±113
Ca	mg /l	74.75±22	149.5 ±21	224.2 ±19	299 ±17.2
Mg	mg /l	44 ±2.4	88 ±2.59	132 ±2.39	176 ±2.79
Fe	mg /l	81±23.1	162 ±23.2	243 ±22.8	324 ±23.8
Mn	mg /l	0.61 ±0.02	1.25 ±0.04	1.87 ±0.05	2.5 ±0.07
Zn	mg /l	0.37 ±0.01	0.75 ±0.02	1.12 ±0.05	1.5 ±0.09
Cu	mg /l	0.12 ±0.00	0.25 ±0.01	0.37 ±0.02	0.5 ±0.05

Table 2 Effect of vermicompost tea at different concentration on seed germination and seed growth of green Romaine

Treatments	seed germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	seed vigor index
0%	86±2.00 a	0.56±0.01 c	2.66±0.05 b	0.73±0.01 c	0.02±0.00 c	276.91±5.70 c
25%	88±0.00 a	1.33±0.15 b	3.14±0.40 b	1.29±0.04 b	0.05±0.00 b	394.24±49.00 b
50%	91±2.30 a	2.62±0.62 a	4.57±0.06 a	2.30±0.04 a	0.1±0.01 a	656.91±49.00 a
75%	36±1.40 b	0.83±0.18 bc	2.51±0.80 b	0.41±0.19 d	0.01±0.01 cd	115.25±40.00 d
100%	0±0.00 c	0±0.00 d	0±0.00 d	0±0.00 e	0±0.00 d	0±0.00 e
F test	**	**	**	**	**	**
CV (%)	10.63	27.92	16.92	9.53	19.05	12.07

** significant differences at the 0.01 level
Values in same letters in the columns are not significantly different (LSD test, P≤ 0.05)

Table 3 Effect of vermicompost tea at different concentration on seed germination and seed growth of green Batavia

Treatments	seed germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	seed vigor index
0%	88 ±0.00 bc	1.34±0.21 c	3.38±0.23 bc	0.86±0.19 c	0.03±0.00 c	412.79±6.90 c
25%	91± 1.15 bc	2.29±0.83 b	3.65±0.21 b	1.49±0.20 b	0.06±0.00 b	543.29±63.0 b
50%	93±1.15 a	3.48±0.22 a	6.12±0.01 a	2.59±0.09 a	0.16±0.02 a	896.63±24.0 a
75%	76±9.17 c	1.70±0.08 bc	2.98±0.48 cd	0.81±0.16 c	0.01±0.00 d	359.07±73.0 c
100%	63±16.70 d	1.08±0.14 c	2.78±0.33 d	0.92±0.06 c	0.01±0.00 d	242.79±58.0 d
F test	**	**	**	**	**	**
CV (%)	10.41 10.4	20.47	7.83	11.46	13.94	10.57

** significant differences at the 0.01 level
Values in same letters in the columns are not significantly different (LSD test, P≤ 0.05)

which are essential elements for improving plant growth, soil fertility and plant productivity (**Table 1**). On the other hand, EC were high with 75 and 100% vermicompost tea.

Seed germination

Different concentration of vermicompost tea has different effect to the seed germination percentage of green Romaine and green Batavia lettuce (**Table 2 and 3**). It was showed that 0, 25, and 50% of the vermicompost tea did not show significant increase in seed germination of green Romaine (86±2.00, 88±0.00 and 91± 2.30%, respectively) and Batavia (88±2.00, 91±1.15 % and 93±1.15, respectively) due to the LSD test (P≤ 0.05) which showed in **(Table 2 and 3)**; however, 75

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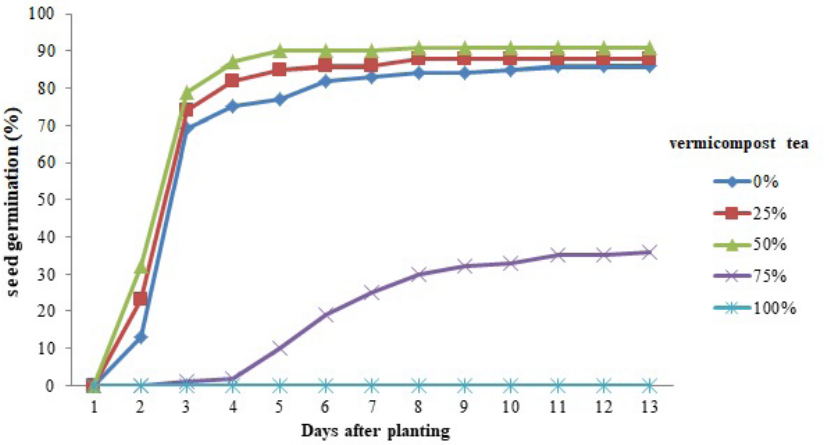

Figure 1 Percentage of green Romaine seed germination in different concentration of vermicompost tea with different days.

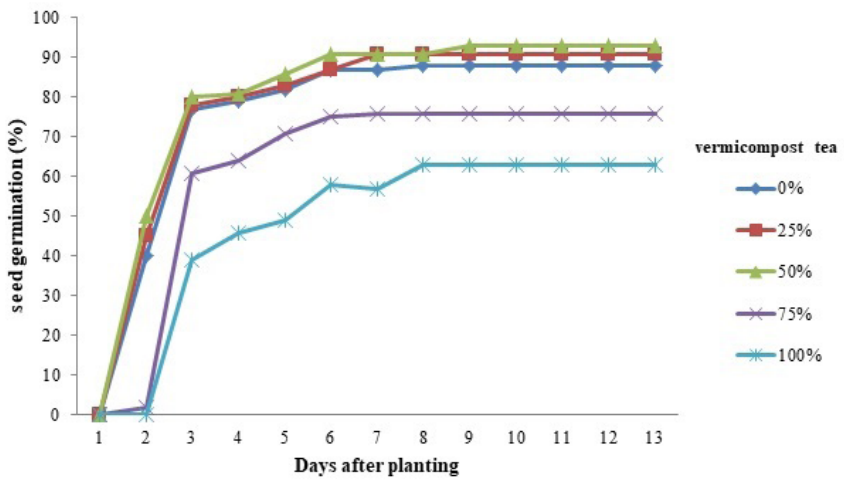

Figure 2 Percentage of green Batavia seed germination in different concentration of vermicompost tea with different days.

and 100% vermicompost tea decreased seed germination of green Romaine (36±1.40 and 0±0 %, respectively) and Batavia (76±9.17 and 63±16.7%, respectively). Arancon et al.(2008) reported that petunia seed showed lower germinated with 75 to 100 % vermicompost tea and it might be the substitution of vermicompost, particularly animal manure which attributed to the higher salt contain (i.e. electrical conductivity) or excessive nutrient levels in more concentration. From (**Table 1**), 75 and 100% vermicompost tea have high electrical conductivity (5.10±0.11 and 6.69±0.10 dS/cm, respectively). Huy and Iwai (2018) reported that seed cannot germinate when the solution contains more EC more than

3.08 dS/m. The reduction of seed germination might be the toxicity of high osmotic pressure which can decrease the water uptake to the seed during germination or it might be high toxicity of high concentration of chloride ions (Bazai and Achakzai, 2006).

Shoot and root length

Vermicompost tea showed significant effect on the shoot and root length of green Romaine and Batavia ($P<0.01$). The longest shoot and root of both varieties were found in 50% vermicompost tea (2.62±0.62 and 4.57±0.06 cm) and (3.48±0.22 and 6.12±0.01 cm) (**Table 2 and 3**). It was reported that the lower concentration of vermicompost tea can promote the growth and ecomorphological characters such as plant height and shoot length (Esteban et al., 2017). Vermicompost tea can be able to supply the balance nutrients to the plant root and growth; increasing the organic matter and humic substance which affect to nutrient accumulation and promote root growth (Esteban et al., 2017). Ingham (2003) reported that vermicompost tea increase plant growth and yield by improving plant nutrition and by a growth- stimulating effect independent of improving nutrient status. Tomati et al. (1988) also reported that the interaction between earthworms and microorganisms in organic matter degradation has been shown to produce significant amounts of plant growth regulation including auxins (IAA), cytokinins and gibberellins. It was also report that auxin (20.16 µg/100ml), cytokine (0.68 µg/100ml) and abscisic acid (0.009 µg/100ml) can improve the shoot and root of plant (Iwai et al., 2011). Nevertheless, it was observed that 100 % vermicompost tea inhibited the root and shoot length of lettuce in both vanities **(Table 2 and 3)**. Similar results with the report of Arancon et al. (2008) who found that shoot and root of petunia were poor at higher concentration of vermicompost tea. Arancon et al. (2006) also reported that the high vermicompost tea concentration reduced the plant height cause of high concentration of plant growth hormones such as auxins and humic acid produced by microorganism during vermicomposting. When apply auxins at higher concentration, it can reduce the rate of growth and development of plant; however, it can increase the growth with the lower concentration (Hopkins and Huner, 2004).

Fresh weight

The obtained results (**Table 2 and 3**) indicated that vermicompost tea showed significant effect to fresh weight of green Romaine and Batavia ($P<0.01$) and the fresh weight of both varieties were higher with 50% vermicompost tea than other treatments (2.30±0.04 and 2.59±0.09g, respectively) ($P<0.05$). Generally, vermicompost tea contains high amount of the plant nutrient which can improve yield of plant. Pant et al. (2011) were reported that vermicompost tea has soluble nutrient and microbial products. These properties can enhance nutrient uptake from the soil to the plant and it had a great potential to promote the plant growth, root development and biomass. However, the 100 % vermicompost tea contains high electrical conductivity (6.69±0.10 dS/m) which was highly soluble salt level. It was also reported that the development of seed could be decreased because high level of salinity. High salinity concentration decreased the water and nutrient uptake to the seed (Huy and Iwai, 2018).

Dry weight

From the experiment was indicted that the concentration of vermicompost tea was significantly affected to dry weight ($P<0.01$) of the lettuce seed. The highest dry weight of green Romaine and Batavia seeding was found in 50% vermicompost tea treatment (0.11± 0.01 and 0.16±0.02 g, respectively)

(**Table 2 and 3**). Vermicompost tea showed a much greater at lower concentration by increasing shoot and root dry weight more than the higher concentration (Arancon et al., 2008). The increasing of dry weight of the seed length at lower concentration of vermicompost tea might influent the fresh weight of green Romaine and Batavia also. Tomati et al. (1988) also indicated the application of vermicompost and lower concentration of vermicompost tea contains synthetic hormones which promotes root and shoot biomass.

Seed vigor index

The results showed that vermicompost tea was significantly affected to seed vigor index ($P<0.01$). The 50% of vermicompost tea promoted the highest seed vigor index of Romaine and Batavia (656.9±49 and 896.6±24, respectively) **(Table 2 and 3)**. Seed vigor index showed the activity and performance of the seed during germination. If shoot and root showed good result, the vigor index will be high (Huy and Iwai, 2018).

Duration of seed germination

(**Figure 1 and 2**) showed that seed germination of green Romaine and Batavia grew faster (88±2.0 and 91±2.30 %) and (91± 1.15 and 93±1.15%) with 25 and 50% vermicompost tea than other treatments. Nevertheless, seed germination of both varieties was discourage towards with 75 and 100% of vermicompost tea (36±1.40 and 0±0) and (76±9.17 and 63 ±16.7%).The delayed of the seed germination of both varieties cause of high soluble salt or osmotic pressure containing in higher concentration of vermicompost tea. Singh et al. (2012) reported that the germination of the morning-glory was decreased under osmotic potential which caused water stress condition to seed during germination. Dash (2012) also reported that osmotic pressure of the effluent increases at higher concentration contained high total salt which is more difficult to restart the germination.

Conclusions

The finding of this study indicated that higher concentration (75 and 100%) were inhibited seed germination and seed growth of green Romaine and Batavia. However, 50% of vermicompost tea was significantly increased shoot and root length, seed vigor index, fresh weight and dry weight of green Romaine and Batavia compared to the other treatments because the lower concentration of vermicompost tea contained hight plant nutrients and lower electrical conductivity. These conditions were suitable for seed germination. Thus, dilution vermicompost tea properly can be used as liquid fertilizer since it can promote seed performance which means that seeding is health and vigor when establish in the file.

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