DETERMINATION OF THE EFFECT OF FERMENTATION ON THE RATE OF PLANT NUTRIENT RELEASE FROM PLANT TISSUES

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ABSTRACT

The use of fermented plant extracts (FPE) as a top dress in crop production is a natural process that can provide plant nutrients and suppress diseases. However, there is scanty information on the use of plant extracts and the bulky of it is not documented. This study aimed at determining the effectiveness of fermentation in extracting plant nutrients from plant tissues for use as top dress in crop production. Materials from Lantana (Lantana camara, L), stinging nettle (Urtica dioica L) and Tithonia (Tithonia diversifolia, G), common plants that are locally available in most agricultural areas were investigated. A complete randomized design (CRD) was used to set up the experiments in the laboratory. Tender shoots and leaves of these plants were fermented in water and the levels of nitrogen, phosphorous and potassium (NPK) contained were measured at intervals of 1, 5, 10, 15, 20 and 25 days. The concentration of lactic acid bacteria (LAB) yeasts and the pH were also determined at each interval. Standard foliar feeds (Agro-Feed and Power Booster) commonly used by farmers were used as control. The results demonstrated that Lantana and stinging nettle were not significantly different in the percentage of N released. Phosphorous was highest in stinging nettle at 5.26%, Tithonia had 5.12% while Lantana had the lowest at 4.48%. However, potassium was highest in Tithonia at 4.38%, stinging nettle had 3.64%, while Lantana had the lowest at 3.63%. There were also high significant differences in concentration of lactic acid bacteria (LAB), Yeast and pH at $p<0.05$. The findings of the study showed that nutrients can be extracted from plant materials by fermentation although in less quantities as compared to commercial foliar feeds.

Keywords: Plant Nutrients, Fermentation, Plant Tissues.

1. INTRODUCTION

Food safety and environmental concerns in many countries has led to the belief that the use of inorganic fertilizers and pesticides poisons the soil and results in a low quality product (Polyxeni *et al.,* 2016). This has led to development and adoption of environmental standards in crop production. Many farmers especially those producing horticultural crops are increasingly converting to these standards in order to continue selling their produce to the lucrative international markets. It is expensive and requires a lot of skills for the majority of the small holder farmers to meet the requirements for good agricultural practices while using the synthetic agro-chemicals (Rattiya & Ulrike, 2017), some of which has stringent application requirement.

The awareness of these problems and the possibility of higher cost in the future has created need to produce food in a manner that enhances food safety, does not affect the environment and is affordable to the majority small holder farmers. This has brought about two systems of alternative crop production: organic agriculture and integrated production (IP). In organic

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agriculture, the use of synthetic mineral fertilizers is not allowed. In integrated production the use of synthetic mineral fertilizers is allowed provided that the nutrient balance on the farm is in equilibrium (Abdur *et al*., 2020). The use of natural fertilizers is therefore an attractive alternative way to avoid the use of synthetic chemical fertilizers. In recent years, fermented plant extracts (FPEs) or fermented plant juices (FPJ) have been extensively used as natural liquid fertilizer. They are easily produced from agricultural crops or agricultural waste. These FPEs promote plant growth and act as bio-control agents depending on the type of plants being used as well as soil conditioners (Katarzyna *et al*., 2021). There is a Korean natural farming philosophy that strongly recommends that farmers produce their agro-inputs rather than purchasing them from the market (Hans and Atsushi, 2000). This ensures production of healthy food without poisoning the soil and other environmental processes. Since FPEs are useful in eliminating problems associated with the use of chemical fertilizers and pesticides, they are now being widely applied in natural farming, organic agriculture and Integrated production farming. FPEs are produced by lactic acid fermentation (Shinsuke *et al*., 2012).

Lactic acid bacteria (LAB) and yeasts are normally found in fermented plant products because their habitats and some of their physiological properties are similar (Luc De Vuyst & Frédéric Leroy, 2020). The fermentation processes have been widely utilized for the production of alcohols including wines, the production of soy sauces and soybean pastes, the production of fermented milk products such as cheeses, and the production of pharmaceuticals. In general, fermentation is a phenomenon that organic matter is decomposed by an action of the bacteria, to produce a useful substance (Luc De Vuyst & Frédéric Leroy, 2020). Generally, plants are composed of juice, chlorophyll and fiber and there are about 100, 000 to 150, 000 microorganisms per one cm^3 of leaf extract (Ashraf *et al*., 2018). Most of the micro-organisms in the plant extracts are lactic acid bacteria and yeast. Through fermentation, plant juice and chlorophyll are produced as a rich enzyme solution full of these bacteria which are useful for invigorating plants. Since chlorophyll doesn't dissolve in water or oil but in weak alcohol; microorganisms in the process of fermentation produce small quantities of alcohol that extract the chlorophyll (Hugo Scheer, 2012)

2. MATERIALS AND METHODS

Extracts were prepared according to the method described by Merckens in 1973 (Bozsik, 1996). Fresh and tender leaves and shoots from growing Lantana, stinging nettle and Tithonia plants were obtained from farmers' fields.

Plate a: Tithonia diversifolia   *Plate b: Lantana camara*   *Plate c: Urticadioica masaica,*

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Each of the materials was chopped into small pieces less than one inch long. The materials were weighed using Ashton Meyers scale, Model No 776. An equal weight (500 grams) of each plant species chopped material was taken and put in a plastic container and then mixed with two liters double distilled water. The containers were tightly closed. The experiment was set up in the science laboratory of the Coast Institute of Science and Technology (CIST) in Voi town, Kenya, where it ran for 25 days with stirring after every 2 days. Samples of 100ml plant teas were collected initially (immediately after preparation) and after every 5 days intervals, that is, 0, 5, 10, 15, 20 and 25 days, placed in sterilized screw capped bottles and taken to the Kenya Bureau of Standards (Kebs) laboratories in Mombasa for analysis of NPK content. The pH values, concentration of LAB and yeast in the mixtures were also determined during the test intervals.

Nitrogen content in the plant teas from the three plant species was determined using Kjedhal method. Potassium content was determined by flame photometry method while Phosphorus was determined using ultra violet (UV) visible spectrophotometer a method described by Cary and Beckman in the 1940 (Robert *et al.,* 2003) and has since then been improved to modern UV-visible spectrophotometer. The process begun with the decomposition of the sample material using the Kjeldahl method and the determination of the concentration of lacto acid bacteria (LAB) was done using the viable cell count standard method (colony count, or CFU/ml) according to the method of Harrigan (1998).

Yeast cell concentration was determined indirectly by measuring the optical density (absorbance) of a culture sample (Barnett *et al*. 2000; Middelhoven, 2002). Five portions of 5mls, 10mls, 15mls, 20mls and 25.0 mls of the test solution were put into separate 100 cm^3 volumetric flask and topped up to 50 cm^3 by adding distilled water. 50 cm^3 of distilled water was put into another 100 cm^3 flask as a blank solution. The Spectrophotometer was set to 600 nm and the absorbance of the solutions measured in order of increasing concentration followed by that of the blank solutions. The results were recorded in a Table with the absorbance versus the corresponding concentration. The yeast content was then calculated. The pH values of the plant teas were determined when taking the samples periodically by inserting the Ph meter into the plant teas.

3. RESULTS

NPK extracted from *Lantana camara*, *Urtica dioica* and *Tithonia diversifolia* plant tissues

Figure 1 shows that *Lantana camara* and *Urtica dioica* released 3.48% nitrogen and Tithonia had the lowest amount of 2.45%. Phosphorous was highest in *Urtica dioica* at 5.26% while Tithonia had 5.12% and Lantana had the lowest phosphorous at 4.48%. Highest percentage of potassium was released from Tithonia at 4.38% while Urtica dioica had 3.64% and Lantana had the lowest at 3.63%.

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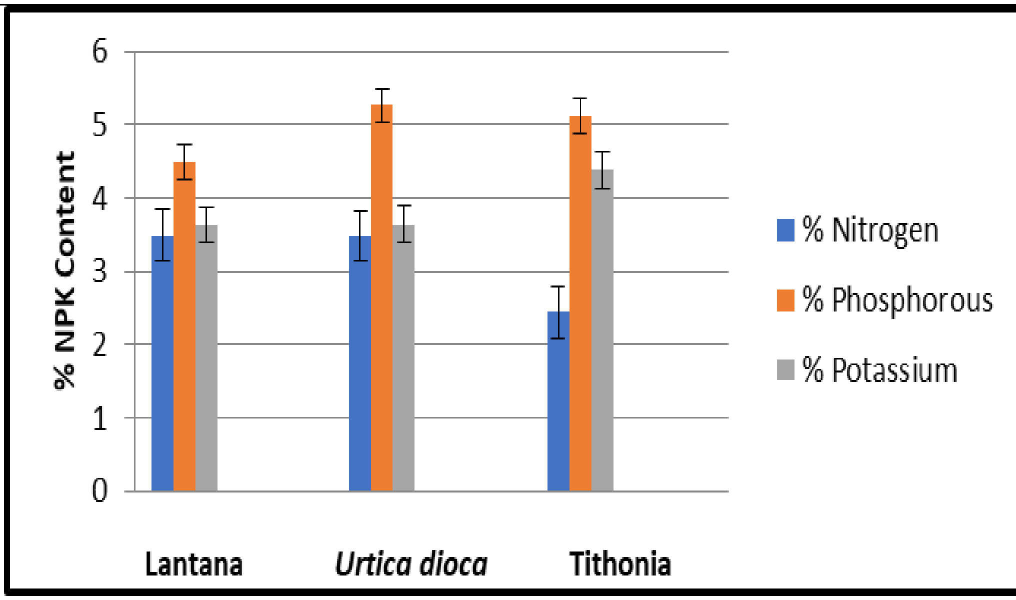

Fig. 1: Percentage of NPK extracted from Lantana, stinging nettle and Tithonia plant tissues

Period Taken to Release Plant Nutrients from Plant Tissues

The comparison across time was done to determine the period taken for the highest amount of nutrients extracted from the three plant species (Table 1). The extraction of nitrogen was highest at 10 days after fermentation (DAF) across all the plant species with *Urtica dio*ica registering the highest quantity (6.6%) while Tithonia had the lowest quantity of 4.0%. The highest phosphorus was recorded at 1DAF from Lantana (10.0%) and *Urtica dioica* (9.7%) while Tithonia recorded its highest P on 5 DAF at 9.7%. Highest K (7.3%) was released by Tithonia at 15 DAF while Lantana and *Urtica dioica* released their highest amount of K at 5 DAF with 6.0% and 5.6% respectively. In general, Lantana and *Urtica dioica* recorded similar mean percentage of N (3.48%) which was higher than the mean percentage from Tithonia (2.45%). However, Tithonia recorded the highest mean percentage of K at 4.38%. Table 1 also shows a general trend of increase of the percentage of nitrogen released from the plant tissues up to 5 DAF and then dropping to lowest percentage at 25 DAF. The percentage of phosphorus was highest at 1DAF from Lantana and *Urtica dioica* and dropped as fermentation period increased. Across the three plant species, lowest percentages of NPK were released at 25 DAF.

Table 1: Effect of time on nutrient release from tissues of Lantana, stinging nettle and Tithonia

DAF	*Lantana camara*			*Urtica dioica*			*Tithonia diversifolia*		
	% N	**% P**	**% K**	**% N**	**% P**	**% K**	**% N**	**% P**	**% K**
1	3.1a	10.0a	4.8a	3.0a	9.7a	4.5a	3.0a	7.2a	4.9a
5	3.3a	6.3b	6.0b	3.3a	6.5b	5.6b	3.1a	6.7a	5.3a
10	6.5b	5.5b	4.7a	6.6b	5.6b	4.3a	4.0b	9.7b	5.5a
15	3.5a	3.1c	3.5c	3.5a	4.7bc	4.1a	3.3a	3.5c	7.3b
20	3.3a	1.6d	1.7d	3.3a	3.8d	2.8c	0.8c	2.1d	2.8c
25	1.2c	0.4 d	1.1d	1.2c	1.3d	0.6d	0.5c	1.5e	0.5d
Mean	**3.48**	**4.48**	**3.63**	**3.48**	**5.26**	**3.64**	**2.45**	**5.12**	**4.38**
LSD (p<0.05)	**0.51**	**0.86**	**0.62**	**0.51**	**0.97**	**0.62**	**0.38**	**0.95**	**0.84**

Key: DAF = Days after fermentation. In a column, values having common letter(s) do not differ significantly at $p \leq 0.05$ as per LSD

Comparison of NPK content of Fermented Plant Extracts with Common Foliar Feeds

Comparing the FPEs with controls indicated that Agro Feed and Power Booster have higher nitrogen content of 12.0 % and 14.0% respectively, while *Lantana camara* and *Urtica dioica* have 3.48% and *Tithonia diversifolia* has 2.44%. The two commercial foliar feeds also have more than double the amount of phosphorous and potassium when compared to the FPEs as shown in Figure 2. This shows that the plant extracts have low mineral contents than the commercial foliar feeds

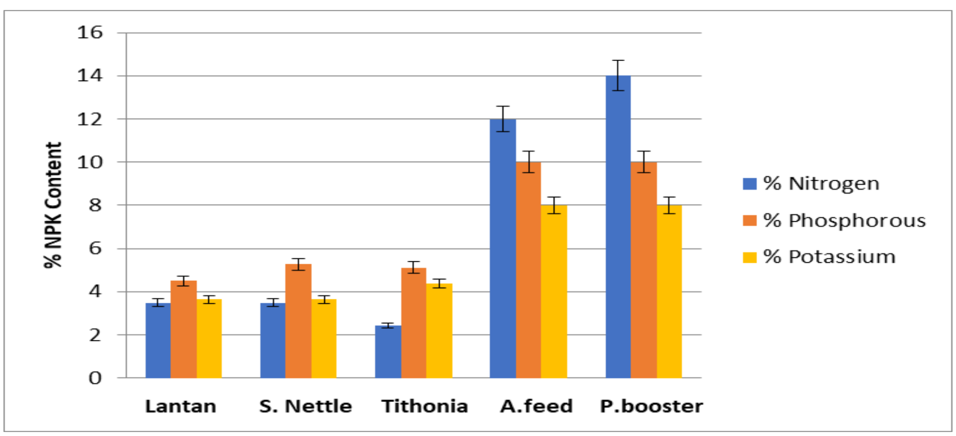

Fig 2: Comparison of nutrient content of fermented plant extracts with common foliar feeds

Variation of Lactic Acid Bacteria, Yeast and Ph during fermentation of *Lantana camara*, *Urtica dioica* and *Tithonia diversifolia*

According to Table 2, there was an exponential increase in concentration of lacto acid bacteria (LAB) from day one up to day 10, after which a downward trend was observed up to day 25. The increase in LAB coincided with the period of higher Ph. Lacto acid bacteria decreased gradually with decrease in Ph. The trend in yeast was contrary to that of LAB. Yeast was less when Ph was higher, up to 10 days, and increased with decrease in Ph.

Table 2: Variation of Lactic Acid Bacteria, Yeast and Ph during fermentation of *Lantana camara*, *Urtica dioica* and *Tithonia diversifolia*

Day	Lantana camara			*Urtica dioica*			*Tithonia diversifolia*		
	LAB (Cfu/Ml)	YEAST (Cells/ml)	PH	LAB (Cfu/Ml)	YEAST (Cells/ml)	PH	LAB (Cfu/Ml)	YEAST (Cells/ml)	PH
1(24 hrs)	0.3a	0.2a	6.9a	0.2a	0.2a	6.9a	0.2a	0.3a	6.9a
5	471.2b	3.5a	6.8b	469.4b	2.5a	6.8b	475.0b	3.0a	6.8b
10	1593.8c	91.0b	6.6c	1605.3c	89.7b	6.6c	1596.0c	90.4b	6.6c
15	1192.5d	2989.5c	5.3d	1191.5d	2986.0c	5.3d	1190.2d	2990.4c	5.2d
20	875.3e	3841.7d	4.9e	868.6e	3839.2d	4.9e	879.8e	3850.7d	4.8e
25	29.3f	921.5e	3.8f	32.1f	919.2e	3.9f	31.5f	897.0e	3.9f
Mean	**693.57**	**1307.9**	**5.71**	**694.52**	**1306.13**	**5.73**	**695.45**	**1305.3**	**5.70**
LSD (*p<0.05*)	**27.16**	**43.05**	**0.05**	**27.16**	**43.05**	**0.05**	**27.17**	**43.04**	**0.05**

Same letters on same column mean no significant differences

4. DISCUSSION

Although the NPK released by the plant tissues of the three plants was lower than that of commercial foliar feeds, the fermented plant extracts (FPEs) are prepared from plant materials that are available locally and therefore affordable by many farmers. Few farmers use the commercial foliar feeds because they are expensive. The commercial foliar feeds could also be environmentally hazardous. Different researchers have found varying NPK contents in FPEs and this could be attributed to the growth stage of the plant tissues. For instance, Gitahi and Mureithi (2002) found that Lantana had Nitrogen, Phosphorous and Potassium contents of 2.5%, 0.26% and 1.93% and for tithonia it was 3.97 %, 0.30 % and 4.6%. Ranasinge *et al.*, (2019) in an analysis of Sri-Lankan weeds reported that the highest nitrogen content was recorded in *Mikania scandens* (3.44%) followed by *Tithonia diversifolia* (3.39 %) and the least N content was observed in *Panicum maximum* (1.20%). However, the highest phosphorous was recorded in *Tithonia diversifolia* at 0.37%. In this study the NPK content from the three plant species are relatively higher and this could be attributed to method of extraction and growth stage of plant materials used.

Gachengo and Niang, (1996) demonstrated that maize yields were higher with incorporation of tithonia biomass than with commercial mineral fertilizer at equivalent rates of N, P and K. This was attributed to the increased soil microbial activity, in addition to providing soil nutrients. Bunyasi (1998) observed that tithonia and stinging nettle plants are good at gathering minerals from the soil that include iron, calcium, sulphur, sodium, potassium, phosphorus, silica and other essential trace elements. The counts for lacto acid bacteria (LAB) and yeast increased initially and then decreased with the fermentation period. Lactic acid bacteria and yeasts are normally found in fermented plant products because their habitats and some of their physiological properties are similar (Gosh *et al.*, 2004).

In a similar experiment with wild forest noni (*Morinda coreia*) fermentation, Kantachote and Kowpong, (2009) observed that LAB increased rapidly during the first 7days and were the dominant population until after day 21. The yeasts began to dominate when LAB decreased in population. According to Kantachote and Charernjiratrakul, (2008) and Olstorpe *et al.* (2008), LAB became predominant at day 7 presumably because the anaerobic conditions and high sugar content were ideal for the proliferation. Although these conditions are also probably suitable for yeast, their natural specific growth rates are much lower than those of the LAB (Olstorpe *et al.* 2008) so the LAB population out competed the yeast population. This was also found to be the case in this study where up to day 10 LAB predominated over yeast but gradually decreased up to day 25. From day 10, yeast predominated up to day 25.

In this study, the Ph of the solution reduced drastically after the tenth day. Oboh, (2006) had observed similar changes and concluded that since chlorophyll does not dissolve in water or oil but in weak alcohol, microorganisms in the process of fermentation produce small quantities of alcohol that dissolve chlorophyll to release the minerals. The concentration of LAB and Yeast was high between day 10 and day 20. This could imply that the rate of nutrient release was highest in that period hence fermentation period should not go beyond day 20. During the period between day 10 and day 20, LAB and Yeast are available in high quantities and these provide additional benefits such as conditioning the soil, feeding other micro-organisms and moderating the soil pH (Kengo and Hui-Lian, 2000).

5. CONCLUSION

The study indicated that the three plants showed high means of extractable Nitrogen, Phosphorous and Potassium. This shows that high amounts of nutrients can be extracted from the three plant materials and made available for crop production. To obtain high amounts of nutrients, the FPES from the three plant species can be combined. From the findings of this study, it can be concluded that small holder farmers could use extracts from these plant materials to increase the productivity of their soils without necessarily having to incur large costs of buying inorganic fertilizers. From the study it was observed that, fermentation can be used to extract nitrogen, phosphorous and Potassium from plant tissues. The methodology, equipment and skills required are simple and within the reach of the small holder farmers. The lacto acid bacteria and yeast that are used in fermentation are naturally occurring. The byproducts of this process are not harmful to the environment and can be reused to provide further benefits to the soil

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