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Phytochemical Screening of *Lactuca sativa* L. As Influenced by Various Nutrient Sources Under Soil and Ecological Conditions of Marawi City, Philippines

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Flavonoids, Alkaloids, Tannins, Phytosterols, Phenolics, R. Sugar.

ABSTRACT

The study was conducted to determine the phytochemical components of Lettuce applied with different types and levels of organic and inorganic fertilizers. Objective of the study is to determine the phytochemical properties of Lettuce using different fertilizer sources and compute its Return on Investment (ROI). Randomized Complete Block Design (RCBD) was used with three replications conducted at Mindanao State University, Marawi City. Results of the study revealed that all three replications of T3 showed double positive (++) in tannins indicating that T2 is having harmful levels of the phytochemicals. The results showed that all other treatments are safe from harmful phytochemical components. Result of the study, considering that T3 (Rec. Rate (RR) Inorganic Fertilizer) treated plants are high in tannins, it is therefore safe to recommend the use all other treatments. In urban areas, this study showed that the use of pots can be a practical alternative to increase production and provide food in urban settings.

INTRODUCTION

Phytochemical component has emerged as a major concern in agriculture food production worldwide. It has been a challenge to produce foods free from toxic elements which might be detrimental to human health. Types of fertilizer used to crop therefore are very important in crop production. It plays an important role in the cropping systems. In addition, relying on inorganic fertilizers is a major constraint due to its prohibitive cost though identified an important factor in meeting the food requirements of a growing population.

Eleazu et al. (2012) defined phytochemicals as chemical compounds formed during the plants normal metabolic processes. These chemicals are referred to as secondary metabolites which comprises of several classes and these includes; alkaloids, flavonoids, phenols, tannins, (Ndukwe et al., 2013; Nduche et al., 2015), coumarins, glycosides, gums, polysaccharides, terpenes, terpenoids, (Oludare and Bamidele, 2015; Singh, 2012). Plants may contain other substances apart from the aforementioned chemicals.

Guo et al. (1994) suggested that vegetable consumption may be inversely linked and salt preserved foods positively related to risk of brain cancer. A case control of 129 subjects in northeast China paralleled these findings (Hu et al., 1999). In particular, a strong inverse relationship to cancer risk was found with high consumption of onions. Both Guo et al., (1994) and Hu et al., (1999) postulated that N-nitroso compounds (NOCs) from salted foods may be the reason for increased risk of brain cancer. It has been shown that both organosulfur compounds (Shenoy and Choughley, 1992) and flavonoids (Law et al., 1999; Shutenko et al., 1999) found in onions have a protective effect against NOCs.

The same Netherlands Cohort Study used to evaluate stomach carcinoma risk (Dorant et al., 1996) was also used for analysis of risk of lung cancer. Although high onion intake was associated with lower lung cancer risk in stratified analysis, upon correction for dietary and non-dietary determinants of lung cancer, the correlation was not statistically significant (Dorant et al., 1994). It was postulated that

dietary absorption of and/or quantity of flavonoids and organosulfur compounds in the onions consumed were inadequate to produce effective results. Khanduja et al. (1999) performed an in vivo study of quercetin effects on mice with N-nitrosodiethylamine-induced lung tumorigenesis. The flavonoid was found to decrease tumor incidence by 32% in the initiation phase, but had no effect on already present carcinomas. The mechanism of benefit was thought to be a consequence of antioxidant activity and suppression of lipid peroxidation. The researchers used a dosage of 9mg/mL H20 but failed to quantify how much each mouse ingested daily per kg body weight. Therefore, it can be concluded that there is a possible inhibitory effect on lung carcinoma risk from ingestion of onions, but it is unknown if the levels needed are feasible to consume.

In addition, Tobacco smoking has been cited as the major cause of urinary bladder cancer in humans (Malaveille et al., 1996). Human urine was shown to contain dietary phenols that had an antimutagenic effect on a known tobacco-smoke related carcinogen (Malaveille et al., 1996; 1998). Extracts from onions and wine were shown to have corresponding effects as the phenols extracted from urine, suggesting both absorption of flavonoids after ingestion and a possible role in protection against tobacco carcinogens from dietary intake of vegetables. Mechanisms of these effects were thought to alter absorption rates, modify enzymes that activate heterocyclic amines, and react with or tightly bind toxic substances or metabolites.

Significant research has been done on the effect of onion consumption on diabetic conditions. The organosulfur compounds S-methylcysteine sulfoxide (SMCS) and S-allylcysteine sulfoxide (SACS) were linked to significant amelioration of weight loss, hyperglycemia, low liver protein and glycogen, and other characteristics of diabetes mellitus in rats (Sheela et al., 1995). They found that the use of SMCS and SACS (200mg/kg/day) gave results comparable to treatment with insulin or glibenclamide but without the negative side effect of cholesterol synthesis stimulation. Similarly, Baba Suresh and Srinivasan (1997) found that a 3% onion powder diet also reduced hyperglycemia, circulating lipid peroxides, and blood cholesterol (LDL-VLDL exclusively). In vivo analysis of the effects of quercetin on human diabetic lymphocytes showed a significant increase in the protection against DNA damage from hydrogen peroxide at the tissue level (Lean et al., 1999). Antioxidant activity was shown, but non-diabetic controls were not used and symptom relief was not mentioned. Further human studies should assess the ability of a high flavonoid diet to attenuate diabetic conditions.

Bone fractures due to osteoporosis are a health care burden. Dairy and soy have both been proposed as dietary sources of compounds (calcium, phytoestrogens) with potential for improving bone health, but neither has been confirmed as helpful in clinical trials with humans. Mulbauer and Li (1999) demonstrated that onion intake by rats was responsible for increasing bone mass, bone thickness, and bone mineral density. Onions inhibited bone resorption by 20% when consumed at a rate of 1g per day per kg of body weight. This was slightly higher than the rate of bone resorption obtained from the calcitonin that is typically used to treat postmenopausal osteoporosis. These findings suggest that onion intake may be a useful dietary approach to improving bone health.

Reduction of heart disease via dietary intake of phytochemicals has been examined (Fitzpatrick et al., 1993; Hertog et al., 1995; Augusti, 1996). Researchers who studied 12,763 men from seven countries found an inverse relationship between flavonoid intake and coronary heart disease (Hertog et al., 1995). Inhibition of LDL oxidation and platelet aggregation were proposed as mechanisms of benefit against cardiovascular disease (Janssen et al., 1998). Quercetin exerts its beneficial effects on cardiovascular health by antioxidant and anti-inflammatory activities (Kuhlmann et al., 1998). Adenosine and paraffinic polysulfides (PPS) are compounds isolated from onions with purported antiplatelet effects (Makheja and Bailey, 1990; Augusti, 1996; Yin and Cheng, 1998).

However, phytochemical properties can also be harmful to human body. Limited studies on crops had been made to evaluate the phytochemical components which are deemed toxic and harmful to human health if the amount exceeds safe levels. Hence, this study focused on the effects of inorganic fertilizers and organic fertilizer to the phytochemical constituents of lettuce.

METHODS AND MATERIALS

The experiment was laid out using Randomized Complete Block Design (RCBD) with seven treatments replicated three times. Distance between blocks is one meter. Shown below are the treatments and their respective amount and types of fertilizers used. There were four (4) treatment combinations used in the study. Each treatment was replicated three (3) times and consists of fifteen (15) pots per replicate. Randomization was done through drawing of lots. The treatment combinations are the following:

T_1	= Control
T_2	= Recommended Rate (RR) Inorganic Fertilizer (60-40-60 kg N, P_2O_5, K_2O/ha)
T_3	= RR Organic Fertilizer (30t/ha; 30g/pot)
T_4	= RR Foliar Fertilizer (60ml/ 16 li H_2O)

**Treatment (T)*

The following materials were used in this study: plastic pots, fertilizer materials (complete fertilizer, muriate of potash, ammonium sulfate, foliar fertilizer (crop giant) and vermicompost), garden soil, spade, standard ruler, and weighing scale.

A total of one hundred eighty (180) pots were used corresponding to the number of treatments and the number of replications per treatment. Each pot was 8" in diameter and 10" in depth. The soil was homogenized or mixed thoroughly to ensure that each pot for each treatment and replicate is filled with the same soil quality.

Three seeds were sown per pot. Thinning was done to keep one plant per pot as the seedlings have developed their first true leaves. Watering was done uniformly and as necessary for all treatments throughout the study period. The amount of water applied per pot was 200ml per watering. Insect pests were controlled by a combination of handpicking and spraying with crushed /pounded chili solution. The pots were kept weed-free by manual hand pulling where needed and prior to treatment application.

Table 1. Treatment, rate, method, and time of application of fertilizer

Treatment	Description	Rate of Application per Plant	Method of Application	Time of Application
T1	Control	-	-	-
T2	Rec. Rate (RR) Inorganic Fertilizer (60-40-60 kg N, P_2O_5, K_2O/ha)	-2.86 g Complete (C) -3.33 g Muriate of Potash (MOP) - 9.52 g Ammonium Sulfate (AS)	Side dress	-7 days after planting (DAP) for C and MOP -14 days DAP for AS
T3	RR Organic Fertilizer (30t/ha; 30g/pot)	-30 g	Basal	-Before planting
T4	RR Foliar Fertilizer (60ml/ 16 li H_2O)	33.33 ml at a rate (3.75 ml per liter of water)	-Foliar spray	-7 DAP -14 DAP -21 DAP

**The weight of soil in 1 ha is 2,000,000kg*
** The weight of soil per pot is 2kg*

Phytochemical Properties

Screening of phytochemical constituents were carried out following the standard methods described in the laboratory manual for the UNESCO (1990). The various phytochemical constituents tested were the following: alkaloids, flavonoids, phytosterols, tannins phenolics and reducing sugar.

Test for tannins. An aliquot of 0.5 mL extract of the sample plants as added to 10 mL of distilled water in a test tube and was filtered. Two mL of 5% Ferric Chloride (FeCl) was added to the filtered sample. Brownish green or black coloration observed indicates the presence of tannins.

Test for alkaloids. Five ml of the extract was prepared in a beaker and 200 mL of 10% CH_3COOH in ethanol (C_2H_5OH) was added. The mixture was filtered and the extract was allowed to become concentrated in a water bath until it reached one fourth of the original volume. Concentrated NH_4OH was added. Formation of the white precipitate or turbidity indicated the presence of alkaloids (Trease and Evans, 1983)

Test for flavonoids. Few drops of 1% Ammonia (NH_3) solution was added to 5 mL extract of plant sample in a test tube. Yellow coloration indicated the presence of flavonoids.

Test for phytosterols. About 0.05g of the crude ethanolic extract was treated with 2mL chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Test for phenolics. About 0.03g of crude ethanolic extract was weighed and 1mL of 1% ferric chloride solution was added. Appearance of blue or green colour indicates the presence of phenols.

Test for reducing sugar. About 0.03 g of crude etahnolic extract was treated with 1mL of Fehling's A and B solutions. The resulting solution was heated. Formation of red precipitate indicates the presence of reducing sugars.

In addition, profitability using Return of Investment (ROI) was computed using the formula below. This included all the inputs used for the study which begin from the seedling stage up to the harvesting using the formula:

$$\text{Return on Investment (ROI)} = \frac{\text{Net Income}}{\text{Cost of Production}} \times 100$$

RESULTS AND DISCUSSION

Phytochemical Analysis of Lettuce

Table 2 shows a positive result of alkaloids, flavonoids, phytosterols, tannins and phenolics. However, T3 (Rec. Rate (RR) Inorganic Fertilizer) shows a double positive (++) in tannins. All treatments of the study show negative (-) in R. Sugar. The result of the study agrees with Mueller-Harvey (2006) study on "The Conundrum of Tannins in Animal Nutrition and Health". Results showed that the harmful effects of tannins in ruminant and monogastric animals can range from producing chronic or systemic disorder. Many monogastric animal species appear to be more sensitive to tannins than ruminants.

Table 2. Summary of Results on Phytochemical Analysis of *Pechay* leaves with three Replications in RCBD

Treatments		Phytochemical Components					
		Alkaloids	Flavonoids	Phytosterols	Tannins	Phenolics	R. Sugar
T_1	Control	+	+	+	+	+	-
T_2	RR Organic Fertilizer	+	+	+	+	+	-
T_3	Rec. Rate (RR) Inorganic Fertilizer	+	+	+	++	+	-
T_4	RR Foliar Fertilizer	+	+	+	+	+	-

Legends: Absent (-)
Present (+)
Concentrated (++)

Return on Investment (ROI)

The cost of inputs used for this study which began from the procurement of materials up to the harvesting and the output was computed to determine the profitability of the production. Return on investment measures the overall effectiveness of management generating profits with its available assets.

Table 3. Price per Crop Based on Treatment

	Treatments	Unit Price per kg (in Peso)
T_1	Control (garden soil)	-
T_2	RR Organic Fertilizer (30t/ha; 30g/pot)	120.00
T_3	Rec. Rate (RR) Inorganic Fertilizer (60-40-60 kg N, P2O5, K2O/ha)	100.00
T_4	RR Foliar Fertilizer (60ml/ 16 li H2O)	110.00

Computation of the Return on Investment (ROI) showed an ROI of 100.37% which shows that the production is profitable. T2 (RR Organic Fertilizer (30t/ha; 30g/pot)) turned out the highest sales followed by T4 (RR Foliar Fertilizer (60ml/ 16 li H2O)), and T3 (RR Inorganic Fertilizer (60-40-60 kg N, P2O5, K2O/ha)). This means that even if T3 showed to have the highest marketable yield, T2 which used vermicompost (organic fertilizer) has the greatest potential for more profit due to low fertilizer cost.

Table 4. Sales per Treatment

Treatment		Replication				
		R_1	R_2	R_3	Treatment Total	Total Sales Per Treatment (kg)
T_1	Control (garden soil)	-	-	-		
T_2	RR Organic Fertilizer (30t/ha; 30g/pot)	108.22	108.99	108.75	325.96	39,115.20
T_3	Rec. Rate (RR) Inorganic Fertilizer (60-40-60 kg N, P2O5, K2O/ha)	77.82	77.90	77.93	233. 65	23,365.00
T_4	RR Foliar Fertilizer (60ml/ 16 li H2O)	79.25	79.69	79.44	238.38	26,221.8
FINAL TOTAL						88,702.00

Table 5. Production Expenses

MATERIAL COST	*Lettuce*
Seeds	75.00
Organic Compost (Vermi) and	100.00
Pots	200.00
Inorganic Fertilizer 1. Complete Fertilizer 2. Ammonium Sulfate 3. Muriate of Potash 4. Crop Giant	500.00
Subtotal	875.00
TOTAL COST	875.00
NET INCOME	87,827.00

$$\text{ROI} = \frac{\text{Net Income}}{\text{Cost of Production}} \times 100$$

$$= \frac{\mathbf{87{,}827.00}}{\mathbf{875.00}} \times 100$$

$$\text{ROI} = \mathbf{100}.37\%$$

Conclusion AND RECOMMENDATIONS

Based on the result of the study, T_2 (RR Inorganic Fertilizer) revealed a double positive (++) in tannins which means that *Lettuce* contained chemical components that is may be harmful to human health and T_2 topped the sales due to much lower production cost, the use of all organic fertilizer is a very practical alternative to higher and more profitable *Lettuce* production which is safe for human consumption. Result of the study showed that the use of organic fertilizers is highly recommended. In areas where land area is a problem, this study revealed that the use of pots can be a viable alternative to increase vegetable production and provide food in urban areas.

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