Effects of powdered chicken eggshells as a soil amendment on the vegetative growth of *Vigna radiata*

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Abstract

The continuous and excessive generation of eggshells as agricultural and industrial waste results in various environmental problems. However, recent studies have shown that eggshells contain essential compounds that promote plant growth and soil condition. Thus, this study aimed to determine the effects of powdered chicken eggshells (PCES) on the vegetative growth of *Vigna radiata*. Chicken eggshells were air-dried, crushed, and powdered. The plants were then grown on PCES-soil compositions of 0%, 10%, and 15% (w/w). The stalk and root length were statistically compared between treatments using one-way ANOVA and post-hoc Tukey-Kramer test. The results indicate that a significant difference existed for both mean plant length scores between plants grown on untreated soil and PCES-treated soil; PCES was found to improve the vegetative growth of *V. radiata*. However, adverse effects were observed at 15% PCES due to excessive calcium uptake. Hence, quantifying the amount threshold of PCES is necessary.

Introduction. - Food industries continuously produce excessive amounts of waste, making it increasingly crucial to formulate solutions to solve this problem. Eggs are one of the products with extensive production quantities under the industry [1]. Hence, considerable amounts of eggshells are being discarded every day, resulting in various environmental issues such as increased waste in landfill sites and pollution [2]. According to the Food and Agriculture Organization of the United Nations (FAO) [3], the world egg production in 2019 was recorded to be about 83.5 million tonnes. In addition, eggshells constitute about 11% of the total egg weight. Thus, the waste generated can be estimated to be about 9.2 million tonnes per year, globally [1].

An eggshell is composed of 94% calcium carbonate, 1% calcium phosphate, 1% magnesium carbonate, and 4% organic substances [4]. Hence, eggshells are known to contain substantial amounts of calcium, and due to this, they can be utilized for the improvement of plant growth [5,6]. A study conducted by Gaonkar and Chakraborty [7] reported that powdered eggshells increase the pH and calcium content of the soil. Furthermore, the study indicated that chicken eggshells contain larger quantities of calcium carbonate than duck eggshells. Additionally, Ok et al. [8] and Soares et al. [9] reported the successful immobilization of heavy metals such as cadmium, lead, and zinc in contaminated soils through treatment with eggshells. Moreover, a study conducted by Wijaya and Teo [5] reported that eggshells significantly improved the height of *Ocimum basilicum* (sweet basil), which is associated with its calcium content.

These findings are mainly associated with the composition of eggshells as previously mentioned. Furthermore, it must be noted that calcium, an essential mineral for plant growth, is the main constituent sought after eggshells in the study [5,11]. Additionally, the mechanism of entry of this mineral cation to plant cells is via passing through Ca^{2+}-permeable ion channels situated in the plasma membrane [10]. This separates the other components of calcium salts such as the carbonate group for the case of calcium carbonate [11].

The aforementioned information and findings highlight the practical application of eggshells in utilizing their properties to strengthen waste management and agricultural production. Hence, its application as a soil amendment to increase the calcium content of the soil will be beneficial to developing countries. Furthermore, this is in line with the promotion of zero hunger, economic growth, and responsible consumption and production, which are some of the Sustainable Development Goals (SDGs) being targeted by the United Nations (UN) [12].

Vigna radiata (mung beans or "monggo") is one of the major crops in the Philippines, commonly used in various local dishes. It is an annual, erect or semi-erect legume that is usually cultivated for its seeds or sprouts across Asia [13]. Additionally, it is one of the cheapest protein sources in the Filipino diet as it is easily cultivated [14]. Furthermore, according to the Philippine Statistics Authority (PSA) [15], a production of about 23.8 thousand tonnes of the legume was observed within the second quarter of 2020. Although it shares a considerable fraction of the Philippine agriculture, the effects of eggshells on the vegetative

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growth of *V. radiata* are yet to be explored. Since it can be easily cultivated, it can serve as a model plant to determine the effects of calcium derived from chicken eggshells towards vegetative growth in general, which is a key factor that determines whether or not a plant proceeds to the reproductive stage [14].

To address this gap, the study investigated the effects of powdered chicken eggshells (PCES) on the vegetative growth of *V. radiata*. Specifically, it aimed to:

(i) measure the stalk length of *V. radiata* grown on PCES-soil compositions of 0%, 10%, and 15% (w/w) at 10, 20, and 30 days after planting;

(ii) measure the root length of *V. radiata* grown on PCES-soil compositions of 0%, 10%, and 15% (w/w) at 30 days after planting; and

(iii) determine if a significant difference exists among the mean values for stalk length and root length of *V. radiata* between treatments.

Methods. - Chicken eggshells were air-dried, crushed, and powdered. Then, the acquired PCES were mixed with 400 g of soil at 0%, 10%, and 15% (w/w). *V. radiata* seeds were planted at the PCES-soil compositions and received 50 mL of distilled water every day. The stalk length was measured at 10, 20, and 30 days after planting, and the root length was measured at 30 days after planting.

Preparation of PCES. Chicken eggshells were collected and air-dried for two weeks. The dried eggshells were then crushed and powdered using an electric grinder (Nima Japan 150 W NM-8300). Subsequently, the PCES was acquired from the resulting solid through a sieve (pore size: 0.16 cm).

Formulation of PCES-soil compositions. The soil compositions were prepared at the following PCES percentages: 0%, 10%, and 15% (w/w). Six pots were allotted for each treatment. For each pot, about 400 g of dry loam soil was homogenized with a mass of PCES corresponding to its assigned treatment.

Growing of V. radiata plants. Five *V. radiata* seeds were planted in each pot and about 50 mL of distilled water was allocated to each pot per day. Moreover, the blocking of the pots was randomized every day.

Measurement of morphological lengths. After 10 and 20 days, the stalk length of the plants was measured using Measure © 2020 Apple Inc. (precision: ±1 cm). After 30 days, both the stalk length and root length of the plants were measured using a vernier caliper (precision: ±0.005 cm).

Data Analysis. One-way analysis of variance (ANOVA) test was conducted to determine significant differences at the morphological lengths between treatments, and a post-hoc Tukey-Kramer test was conducted to determine which treatments significantly differed from each other. The tests were conducted at an alpha level of 0.05 (α=0.05) using the Analysis Toolpak add-in of Microsoft® Excel® for Microsoft 365 MSO (16.0.13901.20436) 64-bit.

Safety Procedure. During the conduct of the study, the use of appropriate personal protective equipment (PPE) was observed. Electricity-powered and hazardous equipment were properly handled according to their safety precautions. Lastly, all organic and inorganic waste were disposed of accordingly.

Results and Discussion. - In this study, a significant difference exists between the total means of the morphological lengths of the plants grown on 0% PCES and PCES-treated soil (10% and 15% PCES) at all intervals. Additionally, there was no significant difference between the stalk length of the plants grown on 10% and 15% PCES at all intervals, but interestingly, a significant difference exists between their root lengths. Simply put, the addition of PCES as a soil amendment significantly improved the vegetative growth of *Vigna radiata* (Figures 1 and 2).

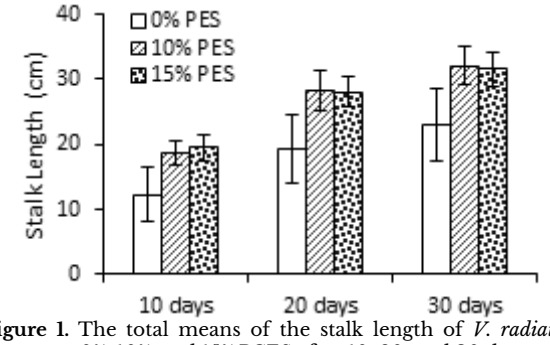

Figure 1. The total means of the stalk length of *V. radiata* grown on 0%, 10%, and 15% PCES after 10, 20, and 30 days.

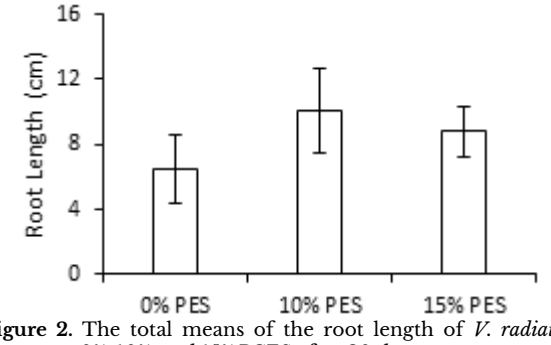

Figure 2. The total means of the root length of *V. radiata* grown on 0%, 10%, and 15% PCES after 30 days.

These findings are mainly associated with the calcium content of eggshells [1,4], where the mineral plays a key role in several physiological processes in plants [5,11]. Furthermore, similar studies conducted by Wijaya and Teo [5] and Gaonkar and Chakraborty [7] reported similar findings as well.

Contrastingly, adverse effects were observed on the plants grown on 15% PCES. As previously mentioned, a significant difference exists between the root lengths of the plants grown on 10% and 15% PCES; it was presented in Figure 2 that this was in favor of the prior treatment. Furthermore, chlorosis was observed on the leaves of the plants grown on 15% PCES. It is known that excessive calcium uptake may lead to disturbances in the ion balance, resulting in an antagonistic effect towards other minerals, such as iron, potassium, and magnesium [11,16]. This results in mineral deficiency, mainly indicated by chlorosis—the decreased green pigmentation in

interveinal areas but an increased pigmentation in the veins of the leaves [11,16,17]. In this study, plants grown on 15% PCES had their third and succeeding trifoliates afflicted with chlorosis. Simply put, the younger leaves were afflicted, which was also observed in previous studies that investigated iron and potassium deficiency [11,17] (Figure 3).

Figure 3. The leaves of a *V. radiata* plant grown on 15% PCES afflicted with chlorosis.

As previously mentioned, the occurrence of chlorosis in the plants may be due to the excessive amount of calcium present in the soil composition. Consequently, this results in the deficiency of essential plant growth minerals, such as iron, potassium, and magnesium, due to the induction of an ion imbalance associated with the antagonistic relationship between calcium and the aforementioned cation minerals [11,16,17]. Moreover, a study conducted by Giel and Bojarczuk [11] reported that other than the induction of mineral deficiencies, the addition of calcium salts such as calcium carbonate increases the total nonstructural carbohydrates (TNC) in the roots and leaves of plants. This limits the utilization of photosynthetic products and subsequently causes growth inhibition. Furthermore, this may be attributed to the observed significant difference between the root length of the plants grown on 10% and 15% PCES.

In summary, PCES significantly improved the vegetative growth of *V. radiata* in terms of the morphological lengths measured. However, excessive calcium uptake may have occurred at the plants grown on 15% PCES, which resulted in chlorosis. Furthermore, it is critical to note that calcium uptake was not quantified in this study. The occurrence of chlorosis has been associated with excessive calcium uptake since it is the major component of the PCES—the variable that was varied among treatments. In addition, there is existing literature that correlates mineral deficiencies to interveinal chlorosis, and only the plants grown on 15% PCES were afflicted with chlorosis despite the randomization of the blocking of the pots on a daily basis and the homogenization of the soil compositions.

Limitations. Only two parameters were evaluated to observe vegetative growth, namely, stalk and root length, since the plants were observed for only 30 days due to time constraints. Furthermore, no tests or analyses were done regarding soil parameters, calcium uptake, mineral and TNC content, and chlorosis due to the unavailability of equipment since the study was conducted during a pandemic.

Conclusion. - Powdered chicken eggshells significantly improved the vegetative growth of the *V. radiata* plants. However, excessive amounts induced adverse effects on the plants.

Recommendations. - It is recommended to observe the plants until the reproductive stage to quantify other morphological structures. Furthermore, the analysis of variables correlated with excessive calcium uptake such as mineral and TNC content is advised to quantify the influence of excess calcium. This is to determine which mineral deficiency occurred due to their identical visual indicator—chlorosis. Regarding this, reflectance spectroscopy is suggested to quantify the occurrence of chlorosis. Lastly, it is advised to determine the amount threshold of PCES with regards to the adverse effects and the ideal PCES supplementation for the best mean scores.

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