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Research Article

Seed priming using moringa leaf extract and application of *Tithonia* compost on shallot growth

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ABSTRACT

Early establishment of shallot growth is important in order to ensure optimum yield. This study aimed to evaluate the effect of seed priming with moringa leaf extract (MLE) and the application of Tithonia compost on the growth of shallots. The research was conducted at the experimental farm belonging to the Faculty of Agriculture, Hasanuddin University, Makassar, from September to December 2022. The study was arranged in a factorial design with three replications. The first factor was priming, consisting of five levels: unprimed, hydropriming, 12.5% MLE, 25% MLE, and 37.5% MLE. The second factor was the level of Tithonia compost, consisting of four levels: 0, 5, 10, and 15 tons ha^{-1}. The results revealed that shallots treated with a combination of priming 37.5% MLE followed by application of 15 tons ha^{-1} of Tithonia compost in the field had the highest chlorophyll index (25.97). Priming with 37.5% solely resulted in the highest average shallots height (38.23 cm), the number of plant leaves (10.55), leaf weight (15.67 g), and bulb weight (29.92 g). Application of Tithonia compost solely of 15 tons ha^{-1} produced shallot with the highest average plant height (37.72 cm), the number of plant leaves (9.74), leaf weight (13.68 g), and bulb weight (27.01 g). Seed priming using MLE and application of Tithonia compost are prospective to enhance shallots growth, however, it is important to further evaluate the effect of priming on germination traits and economic evaluation on using Tithonia compost for practical applications in the field.

Keywords: botanical seed; organic matter; leaf extract

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INTRODUCTION

Shallot (*Allium ascalonicum* L.) is an important commodity in Indonesia for domestic consumption as well as an export product. In 2022, the export volume of fresh and processed shallots reached 47,955 tons (BPS, 2022a). Domestic consumption of shallots in Indonesia reaches 56 g per capita per week (BPS, 2022b). However, domestic shallot production fluctuates resulting in the scarcity of shallot bulbs in particular periods. Such scarcity causes price spikes in the market that stimulates price inflation. As a result, Kustiari (2017) noted that shallot is classified as a sensitive agricultural product because it directly affects national inflation. Therefore, stabilizing shallots production is important in Indonesia.

Recently, true shallot seeds (TSS) are getting popular as planting material in Indonesia. There are various advantages of shallot cultivation using TSS, including the need for less planting material, free from soil-borne diseases, and higher productivity results in increasing farmers' profit (Basuki, 2009; Sembiring et al., 2014 Palupi et al., 2017). However, propagation using TSS required a longer time than that of bulb, therefore, early establishment of shallots growth such as through seed priming and compost application is important in order to ensure optimum yield using TSS.

Seed priming has many benefits, such as accelerating the germination process, better seed uniformity, and helping plants to adapt to environmental stress (Rhaman et al., 2020). The effect of seed priming is not only on seedling establishment, but the effect continues into the crop production in the field as concluded by many studies on soybean, long bean, rice, and onion (Agawane & Pahre, 2015; Thejeshwini et al., 2019; Karim et al., 2020; El-Sanatawy et al., 2021). One of priming agent that can be utilized is moringa leaf extract. Moringa leaves contain active compounds of cytokinin, antioxidant compounds, amino acids, flavonoids, carotenoids, and vitamins that are able to stimulate plant growth and production (Sheikha et al., 2022).

In the cultivation of shallots, farmers usually apply a large number of inorganic fertilizers. In the long run, applying excess inorganic fertilizers causes negative effects on soil fertility and environmental pollution (Putri et al., 2012). Therefore, the application of organic fertilizers such as compost to the soil can be a solution to enhance soil fertility with a low negative impact on the environment. Although many sources of compost are already known, selected compost ideally should consider the availability and easiness of materials (Nugroho et al., 2019; Ayilara et al., 2020).

Tithonia or Mexican sunflower (*Tithonia diversifolia* (Hemsl.) A. Gray) is invasive weed species (Ajao & Moteetee, 2017) and abundant in Makassar, South Sulawesi. Biomass has been widely used to improve soil fertility because of its ability to decompose quickly and release nitrogen, phosphorus, and potassium into the soil (Ajao & Moteetee, 2017). Turmundi et al. (2019) reported that applying *Tithonia* compost on peanuts at a level of 20 tons ha^{-1} increased plant height, plant dry weight, and production by 71%. Furthermore, research conducted by Hafifah et al. (2016) also found a positive effect on applying *Tithonia* compost on cauliflower because the compost contains organic C, total N, C/N ratio, total P, and total K of 31.76%, 4.46%, 7.12, 0.61%, and 3.75%, respectively; the nutrient content is higher than cow manure. The objective of the research was to evaluate the effect of seed priming with MLE and supplementing shallots growing with *Tithonia* compost on their growth.

MATERIALS AND METHODS

Location and experimental design

The research was conducted in the experimental field belonging to the Faculty of Agriculture, Hasanuddin University, Makassar. The research was conducted from September to December 2022. The average daily temperature was 29.51 ± 0.55 °C.

The study was arranged in a factorial design of two factors with three replications. The first factor was priming, consisting of five levels: unprimed, hydropriming, 12.5% MLE, 25% MLE, and 37.5% MLE. The second factor was the level of *Tithonia* compost consisting of four levels: without compost, 5 tons ha^{-1}, 10 tons ha^{-1}, and 15 tons ha^{-1}. True shallot seeds of Sanren F1 variety were used. The seed was obtained from a commercial market.

Preparing Tithonia compost

Tithonia plant biomass (*Tithonia diversifolia*) was obtained from the Experimental Farm, Faculty of Agriculture, Hasanuddin University. Harvesting the plants was done by cutting the leaves and the young stems, then chopping them into smaller sizes (Figure 1a). The biomass was then dried under sunshine for three days.

Fungi of *Gliocladium virens* Miller were added to the dry biomass. The fungi suspension was dissolved with water as much as 100 g in 5 L, and then evenly sprinkled on the plant biomass for about 50 kg. To make 1,000 kg of *Tithonia* compost it was required at least 2,500 kg of fresh biomass. After being sprinkled and mixed, the biomass was incubated in a plastic bag for 30 days (Figure 1b).

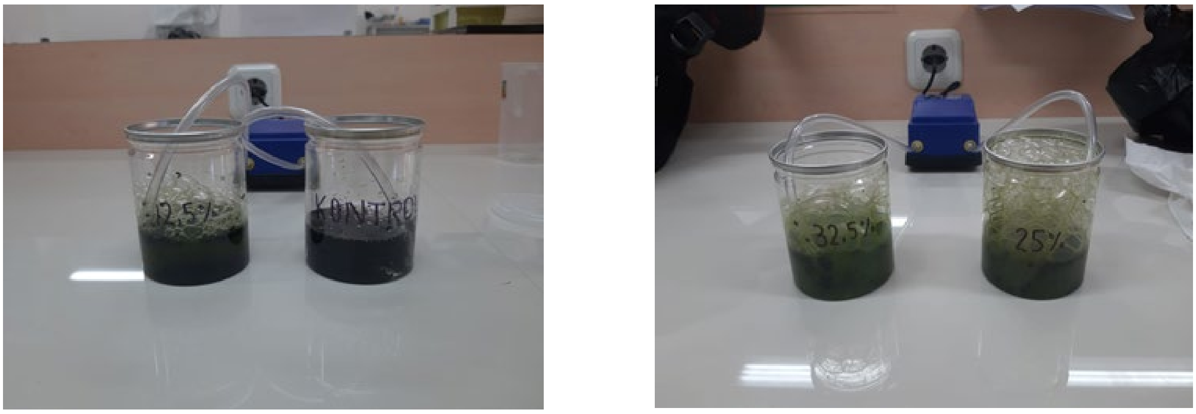
(a) (b)

Figure 1. Process of compost preparation from *Tithonia*. (a) Chopped fresh biomass; (b) thirty days after composting.

Priming materials

Moringa leaf extract was used as priming material. The procedure to develop the extract followed Yaseen and Hájos (2020). In brief, moringa leaves were obtained from the local market in Makassar City. Selected moringa leaves were separated from the petioles and primary veins, and it was washed thoroughly with running water. The leaves were crushed using an electric blender by adding distilled water with a ratio of 1:1 (v/w). The solution was filtered using filter paper and poured into a sterile bottle.

Shallots seeds were soaked in moringa leaf extract (MLE), with a ratio of seed to MLE solution was 1:5 (W/V). The weight of seeds for each treatment was about 20 g. The soaking was carried out for 20 hours at room temperature, and the supplement aeration was added using a bubbling pump (Figure 2). After the priming process, the seeds were dried at room temperature.



(a) (b)

Figure 2. Priming process of true shallots seeds supplemented with aeration. Treatment of 12.5% MLE and hydropriming (a) and treatment of 37.5% and 25% MLE (b).

Seedling preparation

Seeds after priming were sown in seedling beds of fine soil. Maintenance during seedling was watering. Here, seeds from different priming treatments were planted separately. After 40 days, the seedlings were collected and transferred to cultivation beds. Only healthy seedling was collected in this stage.

Cultivation process

The planting bed was sized 100 cm × 150 cm, with a height of 30 cm and a distance between beds of 50 cm. The compost was spread on the bed at seven days before transplanting. The beds were situated in the open field.

After transplanting, watering was done twice a day. Fertilizers were applied before and after transplanting. Seven days before transplanting, P_2O_5 was applied at a level of 62.5 kg ha^{-1}. After transplanting, K_2O and N at rates of 60 kg ha^{-1} and 90 kg ha^{-1}, respectively were applied. The potassium and nitrogen fertilizers were split into three times of application at 15, 30, and 45 days after transplanting (DAT). Each application time used one-third of total fertilizer levels. Weeding was done manually. Pest control was carried out by manual and biological agents of *Beauveria bassiana*.

Harvesting is done at 70 days after transplanting. The shallots bulb was cleaned and separated from the leaves. Fresh and dry masses were measured after harvest. A dry mass was obtained after drying under sunshine for seven days.

Measurement and data analysis

Observed traits included plant height (cm), number of leaves, leaf weight (g), bulb weight (g), leaf and bulb ratio, stomatal opening area, and leaf chlorophyll index. Plant height and the number of leaves were observed at 20, 40, and 60 days after transplanting. The weight of the leaves and bulbs was measured after harvest. The stomatal opening area was calculated using a formula based on Song et al. (2020) as follows: Stomatal Opening Area $= \pi \times a \times b$; a = ½ length of stomata and b = ½ wide of stomata. The chlorophyll index was measured using CCM-200 Plus at 50 DAT.

The data obtained were then analyzed for variance (ANOVA). For any significant effect, a further Tukey test was performed with $\alpha = 0.05$. Data processing used RStudio software version 4.2.1.

RESULTS AND DISCUSSION

Plant height and number of leaves

Variance analysis showed no interaction effect between priming and *Tithonia* compost application on plant height at 20, 40, and 60 DAT (data not shown). However, priming with MLE and applying *Tithonia* compost solely affected plant height (Table 1). In general, increasing the concentration of MLE increased plant height in all observation periods. Priming with 37.5% MLE recorded the highest average plant height as compared to other treatments. The plant height significantly increased in the 37.5% MLE treatment by 20.47%, 12.01%, and 10.07% at 20, 40, and 60 DAT, respectively, as compared to unprimed ones. It is known that MLE contains hormones such as gibberellic acid, zeatin, and indole acetic acid (Ali et al., 2018; Alkuwayti et al., 2020; Yap et al., 2021) that can regulate plant growth and development. According to Castro-Camba et al. (2022), gibberellin promotes plant elongation.

Tithonia compost treatment increased plant height (Table 1). Increasing the level of *Tithonia* compost increased plant height. Application of 15 tons ha^{-1} significantly increased the average plant height by 9.04%, 6.62%, and 3.52% at 20, 40, and 60 DAT, respectively as compared to control (0 tons ha^{-1}).

Table 1. Effect of priming and *Tithonia* compost on plant height of shallots.

Treatment	Plant height (cm)		
	20 DAT	40 DAT	60 DAT
Seed priming			
Unprimed	20.51c	29.21b	34.73c
Hydropriming	21.88bc	30.91ab	36.04bc
12.5% MLE	22.68abc	31.10ab	36.61b
25% MLE	23.63ab	32.30a	37.16ab
37.5% MLE	24.71a	32.72a	38.23a
Tithonia compost			
0 tons ha^{-1}	21.79b	30.21b	36.05b
5 tons ha^{-1}	22.23ab	31.01ab	36.28b
10 tons ha^{-1}	22.94ab	31.67a	36.65ab
15 tons ha^{-1}	23.76a	32.21a	37.32a

Note: Means followed by the same letter in the same column are not significantly different at ($p > 0.05$) according to Tukey test. DAT = days after transplanting.

Analysis of variance showed no interaction effect between priming and application of *Tithonia* compost on the average number of leaves at the age of 20, 40, and 60 DAT (data not shown). However, priming and *Tithonia* compost treatment individually affected the average number of leaves (Table 2). At 60 DAT, shallots from the treatment of priming 12.5% MLE and 37.5% MLE had an about single leaf in different, indicating the seed priming had a significant effect. It is probable that the cytokinin content of MLE (Alkuwayti et al., 2020) might affect leaf number. Sakakibara (2021) states that cytokinin regulates shoot growth.

Table 2. Effect of priming and *Tithonia* compost on average number of shallots leaves.

Treatment	Average number of leaves		
	20 DAT	40 DAT	60 DAT
Seed priming			
Unprimed	4.00b	6.28d	8.14d
Hydropriming	4.01b	7.01cd	8.82cd
12.5% MLE	4.20b	7.40bc	9.36bc
25% MLE	4.20b	8.03ab	9.88ab
37.5% MLE	4.83a	8.38a	10.55a
Tithonia compost			
0 tons ha^{-1}	4.06b	7.11b	8.94b
5 tons ha^{-1}	4.13ab	7.35ab	9.30ab
10 tons ha^{-1}	4.33ab	7.50ab	9.42ab
15 tons ha^{-1}	4.46a	7.72a	9.74a

Note: Means followed by the same letter in the same column are not significantly different ($p > 0.05$) according to Tukey test. DAT = days after transplanting.

Application of *Tithonia* compost at a level of 15 tons ha^{-1} significantly increased the number of leaves (Table 2), irrespective of plant age, as compared to control no compost application. The laboratory analysis showed that *Tithonia* compost contained 0.95% N, 0.14% P, and 0.32% K. Therefore, a higher level of *Tithonia* application likely increases the availability of NPK nutrients. According to Fathi (2022), sufficient nitrogen in plants ensures high performance in the photosynthetic process. Nevertheless, providing 15 tons ha^{-1} compost required 37.5 tons of fresh *Tithonia* biomass. As shown in Table 2 application of 5 tons ha^{-1} *Tithonia* compost had no significant difference in leaf number at 60 DAT to 15 tons ha^{-1}; thus 5 tons ha^{-1} could be considered.

Leaf and bulb weight

Interaction between seed priming and *Tithonia* compost did not show any significant effect (data not shown). The application of priming treatment significantly affected leaf and bulb weight (Table 3). Priming treatment at a concentration of 37.5% significantly increased leaf and bulb weight by 61.88% and 53.34%, compared to unprimed. Likewise, treating the *Tithonia* compost application affected both parameters (Table 3). Application at a dose of 15 tons ha^{-1} increased leaf and bulb weight by 27.01% and 22.49%, compared to 0 tons ha^{-1}.

Table 3. Effect of priming and *Tithonia* compost on leaf and bulb weight of shallots at 60 DAT.

Treatment	Leaf weight (g)	Bulb weight (g)	Leaf and bulb ratio	Stomatal opening area (μm^2)
Seed priming				
Unprimed	9.68b	19.31b	0.043	136.59
Hydropriming	11.04b	21.13b	0.045	164.52
12.5% MLE	12.36ab	25.48a	0.042	164.72
25% MLE	13.18ab	26.19a	0.041	174.40
37.5% MLE	15.67a	29.91a	0.039	200.83
Tithonia compost				
0 tons ha^{-1}	10.77b	22.05b	0.041	144.49
5 tons ha^{-1}	12.37ab	24.18ab	0.043	157.26
10 tons ha^{-1}	12.71ab	24.36ab	0.043	179.03
15 tons ha^{-1}	13.68a	27.01a	0.041	192.06

Note: Means followed by the same letter in the same column are not significantly different ($p > 0.05$) according to Tukey test.

It was evident that shallot leaf and bulb weight was heavier after priming than that without priming ones (Table 3). Such phenomenon might correlate with increasing plant height and leaf number (Table 1 and 2), which produced more assimilates. Kirschbaum (2011) stated that some portion of assimilate from photosynthesis is allocated to support plant growth and development, while Paat (2011) stated that assimilate is used to form new cells. In the present study, the application of *Tithonia* compost increased the weight of the leaf and shallots bulb. The finding is in line with Pelu et al. (2020) where pak choi vegetable grows more vigorously and produces more biomass after the application of *Tithonia* compost.

Applications of seed priming and *Tithonia* compost had no effect on the leaf bulb ratio (Table 3). The interaction of the two treatment factors did not significant (data not shown). The average leaf-to-bulb ratio ranged from 0.039 to 0.043. This means that assimilates allocation to leaf and bulb is constant, irrespective of priming and compost applications. According to Irinato et al. (2017), a high ratio of leaf to bulb indicates more assimilates are allocated to vegetative organs especially leaves.

Stomatal opening area

Seed priming treatment and *Tithonia* compost application did not significantly affect the stomatal opening area (Table 3). There is no interaction between the two factors (data not shown). The area of stomatal openings in both treatments ranged from 126.59 μm^2 to 200.83 μm^2. The stomatal opening area tended to be higher in the 37.5% MLE treatment than in the unprimed treatment. *Tithonia* compost application at a dose of 15 tons ha^{-1} tended to have a larger stomatal opening area than without *Tithonia* compost application.

The insignificant effect of priming and compost applications on the stomatal opening area is likely due to the stomatal opening being strongly affected by environmental factors. According to Driesen et al. (2020), guard cells that regulate stomata opening are controlled by environmental conditions including light, carbon dioxide concentration, temperature, and air relative humidity.

Clorophyll index

Observation of shallot leaf chlorophyll index was influenced by the interaction between priming treatment with MLE and the application of *Tithonia* compost (data not shown). The interaction between seed priming with 37.5% MLE and the application of 15 tons ha^{-1} *Tithonia* compost recorded the highest chlorophyll index value (Table 4). The increase in the chlorophyll index value was 158.92%, compared to the lowest in the interaction between unprimed and without *Tithonia* compost application and the interaction between hydropriming and without *Tithonia* compost application.

Table 4. Effect of priming and *Tithonia* compost on chlorophyll index of shallots at 50 DAT.

Seed priming	*Tithonia* compost			
	0 tons ha^{-1}	5 tons ha^{-1}	10 tons ha^{-1}	15 tons ha^{-1}
Unprimed	10.03f	11.07ef	11.50def	11.93def
Hydropriming	10.03f	10.10f	16.00abcde	16.17abcd
12.5%	11.63def	15.43abcde	17.37abc	17.87abc
25%	11.30def	14.50bcdef	17.17abc	20.47b
37.5%	12.63cdef	15.50abcde	14.10bcdef	25.97a

Note: Means followed by the same letter are not significantly different (p > 0.05) according to Tukey test.

The present study envisages the prospect of MLE and *Tithonia* compost to enhance shallot growth. Research on maize conducted by Bakhtavar et al. (2015) revealed that seed priming using MLE increases the leaf chlorophyll. It is likely that the high nitrogen content of *Tithonia* compost might contribute to chlorophyll formation in shallot leaves. According to Anas et al. (2020), nitrogen is an essential macronutrient for plants, and one of its functions is as a constituent of green leaf substance or chlorophyll. Nevertheless, it needs further study the availability of nutrients after the application of the *Tithonia* compost because nutrient analysis after the application of the compost was absent in the present experiment.

CONCLUSIONS

Combination of TSS priming with MLE at a concentration of 37.5% and the application of *Tithonia* compost of 15 tons ha^{-1} increased the chlorophyll index by 158.92% as compared to the combination of no priming and no application of compost. Priming shallot seeds using MLE at a concentration of 37.5% increased plant height (24.71, 32.72, and 38.23 cm) and the number of leaves (4.83, 8.38, and 10.55) at the age of 20, 40, and 60 DAT, respectively as compared to unprimed treatment. Likewise, the leaf weight (15.67 g) and bulb weight (29.92 g) increased significantly compared to the control. In addition, applying *Tithonia* compost of 15 tons ha^{-1} increased the average plant height (23.76, 32.21, and 37.72 cm) and the number of plant leaves (4.46, 7.72, and 9.74) at the age of 20, 40, and 60 DAT, respectively, as compared to no application of compost. Leaf weight (13.68 g) and bulb weight (27.01 g) were recorded as the heaviest in applying *Tithonia* compost at a level of 15 tons ha^{-1}.

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