Bokashi leachate as a biopriming on *Basella rubra* L. seed germination and root development

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Abstract

Basella rubra L. is a type of spinach, which is edible with high nutrient composition. It is also known to be an antioxidant. However, initial germination and root growth remain an issue due to hard exterior seed coating. Thus, some may germinate within 10 to 21 days, and some may not work at all. Inhibited growth may lead to vegetative propagation and micropropagation, which fundamentally reduce the growth and yield. *Basella* seed treated with Bokashi leachate was found to improve seed germination and root growth. A study was conducted using food waste EM Bokashi leachate (0:1, 1:1500, 1:1000, 1:500) with biopriming duration (6 and 12 hours). The experiment was conducted in a completely random design (CRD) with 3 replications of 100 seeds, with 24 experimental units. Based on the results, a short biopriming duration (6 hours) significantly enhanced the mean germination rate, germination speed accumulated, and coefficient of germination velocity. However, germination percentage had no significant improvement by leachate. Long priming duration significantly reduced the root development due to the seed may loss of desiccation tolerance. The concentration of leachate and priming duration had no significant interaction. In order to improve the germination and root growth performance, 6 hours of seeds priming duration or 1:500 (0.2%) of food waste Bokashi leachate was recommended to soak the *Basella rubra* seeds.

1.0 Introduction

Basella spp. (Basellaceae) are known to be high in nutrient composition (on a dry weight basis) with 98.7 mg/100 g vitamin C, 5.0% protein, 1.5% crude fat, 0.7% calcium, 250.0 mg/100 g iron and 4.0 mg/100 g fibre (AVRDC, 1985; Lyimo *et al.*, 2003). Basella is also a good source of magnesium (Mg), phosphorus (P), and potassium (K). There are two common spices which are *B. alba* (green stem and leaves) and *B. rubra* (red stem and leaves). Therefore, *B. rubra* had high in betalains (S. Sravan Kumar *et al.*, 2015). *Basella alba* have potential antibacterial and anticancer properties as the methanolic extract has significant growth inhibition on human cancer cell lines and a significant zone of inhibition for microbes studied (Azad *et al.*, 2013; Olaniran Adegoke, 2017; Sushila *et al.*, 2010). *Basella alba* (74 mg) had high vitamin C than *B. rubra* (67 mg) in 100g of fresh leaves (Kumar *et al.*, 2015).

Basella spp. can be propagated by seeds or 20 cm cuttings with the method of direct or transplanting (Lin *et al.*, 2009). The best germination temperature is between the range of 18-24°C (Chaurasiya *et al*., 2021) and the days to emergence are 14 to 21 (Cornell University, 2006). Due to the hard seed coat, germination under *in vitro* or *ex vitro* is a bottleneck (Kumar and Giridhar, 2021). Hence, physically and chemically, pre-treatment is used to improve the germination process. Physically, the seed scarified by using a sharp or sandpaper and cut through the hard seed. *Fusarium* spp. *PPF1* isolated from the roots of bermudagrass (*Cynodon dactylon*) showed a significant high germination percentage and vigour index (Islam *et al.*, 2014). *Basella rubra* L. (Basellaceae) seed will propagate *in vitro* and improve to 70% with 2%

of urea (Kumar and Giridhar, 2021). Also, explant of cotyledon and hypocotyl used in micropropagation (Deping and Bin, 2001).

Biopriming, a mixture of beneficial microorganisms and bioactive molecules, is related to endophytic relationships between plants and specific fungi and bacteria. The relationship improved phytohormones production, improvement of biotic or abiotic stress resistance (Deshmukh *et al.*, 2020; Paparella *et al.*, 2015). Effective microorganisms (EM) consist of predominant lactic acid bacteria, yeasts, minor phototrophic bacteria, filamentous fungi, and actinomycetes (Higa and Parr, 1994). Hence, it consists of a biopriming agent.

Biopriming is an eco-friendly way to promote plant growth and development (Toribio *et al.*, 2021). The biopriming of sunflower has significantly improved seed germination and growth performance (Moeinzadeh *et al.*, 2010). In tomatoes, seed priming with Bokashi leachate increased (13%) stem diameter of transplants, allowing plant nutrients uptake (Olle, 2020). In *Passiflora edulis* L., 16% of Bokashi showed significant initial growth and development (Bócoli *et al.*, 2020). Humic acids extracted from bokashi showed positive effects on the initial performance of maize (Baldotto and Baldotto, 2016).

Bokashi leachate is a sustainable source with no issues of carbon footprint, thus have high potential to be used in urban and rural agriculture production. Thus, this is in line with UN SDG to reduce poverty, reduce hunger and sustainable consumption. The objective of this study was to determine the effect of Bokashi leachate as a biopriming on *Basella rubra* L. seed germination and root development.

2.0 Materials and Methodology

2.1 Study site and experimental design

The experiment was carried at University Putra Malaysia (UPM) (2°59'34.0"N, 101°42'52.3"E). The experiment was conducted using a completely randomized design (CRD) with three (3) replications. There was 4 level of concentration percentage of Bokashi leachate which are 0%, 0.067% (1:1500), 0.1% (1:1000) and 0.2% (1:500) with 2 level of priming duration (6 and 12 hours). In total, 24 experimental units.

2.2 Bokashi leachate preparation

Bokashi preparation method was modified based on Christel (2017) and showed in Figure 1.

Rice bran was purchased from a rice mill and sieved with a 2 mm sieve to remove impurities. One (1) part (20 mL) of EM·1 (EMRO Original; manufactured by EMRO Malaysia Sdn Bhd) and molasses were dissolved in 45 parts (900 mL) water, prepared as a mixture. Subsequently, one (1) part of the mixture was then mixed with two (2) parts (1.8 kg) of rice bran and kept in a garbage bin (50×45cm) and covered with a black garbage bag for two weeks, and sun-dried.

Bokashi leachate buckets were self-made using two (2) buckets (50×45 cm) as Figure 2. Twenty-six (26) holes (2 mm diameter) were drilled at the bottom of the bucket before installing another bucket with a tap at the bottom of the bucket for leachate collection. Height measurement was marked (1 cm and 5 cm) on the stick alternately for easy work. The stick was removed after the finished layering.

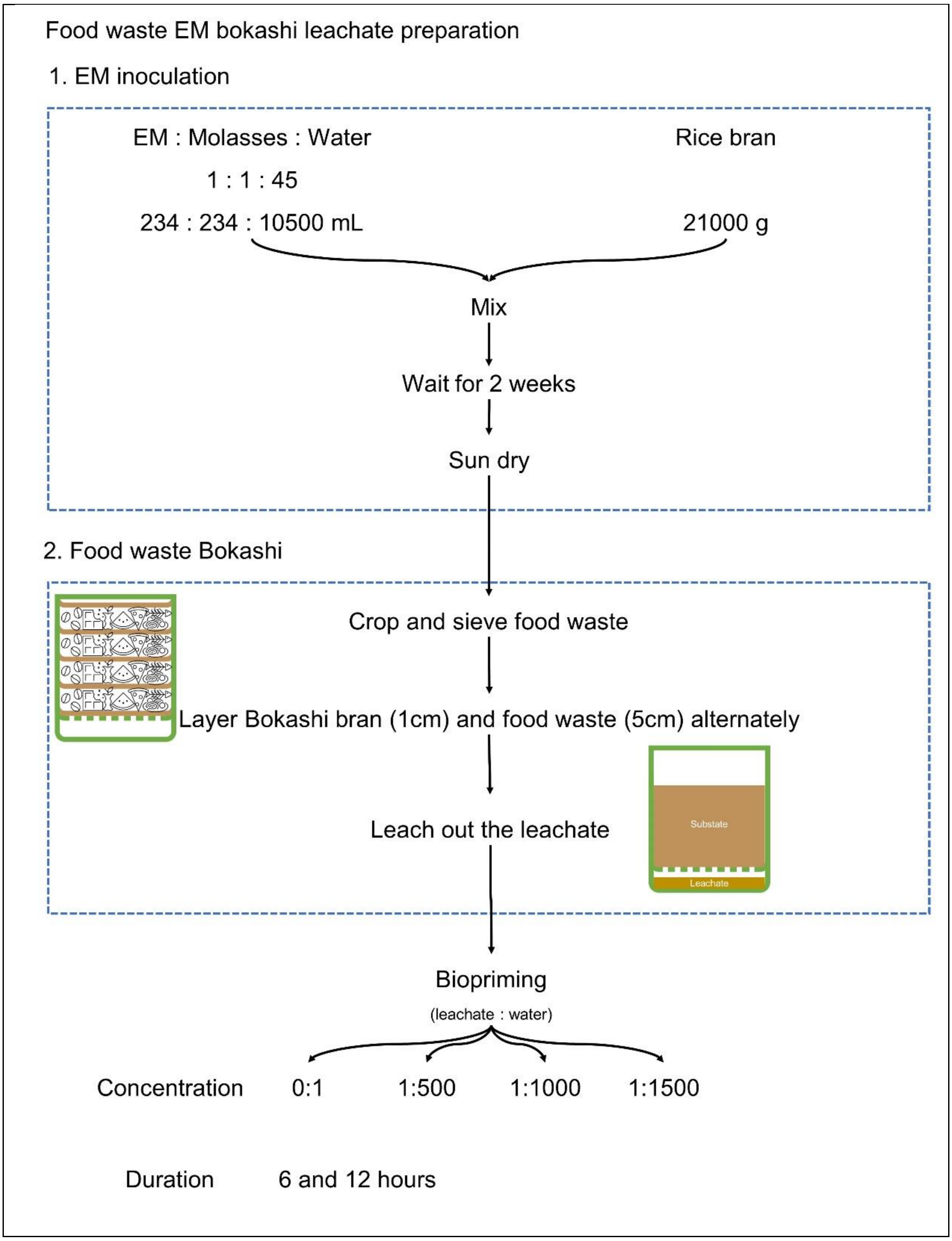

Figure 1. The procedure to prepare Bokashi leachate.

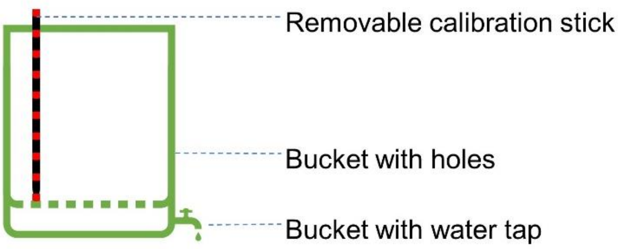

Figure 2. The setup of Bokashi leachate bucket

Food waste was collected from nearby food restaurants and local markets for one pile of the bucket. Collected food waste consisted of raw and cooked plant and animal-based roughly in the ratio of 3:2. The food waste was mixed thoroughly to develop a uniform mixture. Collected food waste was chopped and sieved by using 2 cm wire mesh.

The waste mixture was arranged with alternate layers 1 cm of Bokashi bran and 5 cm of small pieces of food waste until the top of the bucket. Each layer was compacted to eliminate the air as possible, and the cover was covered tightly. The leachate was harvested on day 4 of fermentation.

2.3 Seed treatment and seed germination test

Basella seeds were soaked in Bokashi leachate at four (4) different concentrations, which are 0%, 0.067%, 0.1% and 0.2% under two (2) primming duration (6 and 12 hours). Then, seeds were collected, rinsed with water and dried before germination. Hundred (100) seeds were rolled in a kitchen towel with a food wrapper and supported with 1 cm height of tap water. The water level was maintained at 1 cm and refilled daily.

Daily counting of seedling on germination test was conducted, and data were subjected to R-program statistical software under the package “germinationmetrics”; data obtained were the germination traits: germination percentage, germination time lag (t_0), time for the last germination (t_g), time for the last germination, time spread of germination, the peak period of germination, mean germination time, variance of germination time, standard error of germination time ($S_{\overline{T}}$), mean germination rate ($\overline{V}$), and germination index (GI) (Aravind *et al.*, 2019). Germinated seeds were counted every day for day 7.

2.4 Root morphology

Root morphology was studied on day 7. Fresh roots were washed with distilled water and placed in the root scanner. Total root length (cm), total surface area (cm^2) and root projection area (cm^2) were quantified using Root Scanner (Epson Expression 1680) with root scanning analysis software, version Win-Rhizo 2007d.

2.5 Seedling vigour index

Seedling vigour index was computed by using equation (1):

$$\text{Seedling vigour index} = \text{root length} + \text{shoot length} \times \text{germination \%} \qquad (1)$$

2.6 Statistical Analysis

Data recorded were subjected to two-way Analysis of Variance (ANOVA) using R statistic software. When F values were significant at the $p<0.05$ level, treatment means were compared and separated using Duncan’s Multiple Range Test (DMRT).

3.0 Results and Discussions

3.1 The biopriming concentration on the seed germination and root growth performance

Based on the results, biopriming concentration and biopriming duration had no significant interaction ($p>0.05$) on all the parameters. Similarly, biopriming of *Brassica rapa* had no significant interaction between concentration and biopriming duration (Chin *et al.*, 2021). There is no significant effect on seed biopriming duration and salinity level on seed germination percentage and speed (Seyed and Mahjoobeh, 2016). In contrast, chemical priming and duration had a significant interaction effect on seed germination and root growth performance

(Mohajeri *et al.*, 2016). No significant difference in Bokashi leachate concentration was shown in the seed germination performance. The concentration of Bokashi leachate to soak seeds had no significant effect on the germination percentage (p=0.6284). In contrast, different concentrations of chitosan seed priming significantly affect the germination index and mean germination time but germination percentage (Guan *et al.*, 2009).

Root growth characteristics, including taproot and lateral root in the early phase of plant growth, were improved by seed priming (Blunk *et al.*, 2019). Seed priming with 0.2% of Bokashi leachate showed a significant improvement in root length (p=0.0127012), root projection area (p=0.0173353), root volume (p=0.039321) and root surface area (p=0.0173335) compared to others during the early growth (Figure 3). Tomato seeds soaked in 0.2% Bokashi leachate for 30 minutes significantly affect stem diameter (Olle, 2020). The nutrient concentration does not show a significant difference in shoot nutrient content (Imran *et al.*, 2013). However, the effect of biopriming concentration on root growth was unknown (Singh *et al.*, 2016). EM in the leachate allow the connection between plant soil for nutrient mobility and auxin synthesis for rooting (Kumar Pandey *et al.*, 2017; Ngoma *et al.*, 2013; Sutariati *et al.*, 2019). However, the other concentration of leachate (<0.2%) may be too low for the connection building.

Biopriming also had a positive effect on plant growth and development, not only in initial plant growth. Biopriming with *Piriformospora indica*, a plant growth promoter and abiotic stress alleviator, improved the survival of rice under Cd stress (0.1 mM) as it had the potential to sequester the Cd, decrease the reactive oxygen species (ROS) generation, and cell death in rice roots (Dabral *et al.*, 2019). Therefore, the root-shoot length and biomass had significantly improved after inoculation (Dabral *et al.*, 2019). Besides, biopriming with cyanobacterial extract strengthened the plant from *Pythium ultimum* attack (Toribio *et al.*, 2021).

Food waste Bokashi leachate is believed rich in nutrients and may consider as nutri priming also. Nonetheless, the nutrient analysis should be carried out in advance. Nutri priming has significantly enhanced early seed development and root growth, and nutrients content. For instance, seed priming with Fe and Zn + Mn significantly increased maize plants' biomass production and total root length (Imran *et al.*, 2013), which lined with the studied results. Chitosan seed priming with 0.5% showed significant improvement in shoot and root length and dry weight (Guan *et al.*, 2009).

A low concentration of landfill leachate had the potential to promote the growth of maize. For instance, 10% of landfill leachate to soak seed improved the yield of maize; however, 50% had a deleterious effect on the seed germination due to the phytotoxicity of the leachate (Li *et al.*, 2017). Not all leachate showed a positive effect on plant growth and development. The cigarette butt and ash leachate have suppressed the germination rate and root development in all concentrations (200, 100, 50, 25, 12.5, and 6.25 pc/L) (Mansouri *et al.*, 2020).

The treatment enhanced the root allowed the root for nutrient and water-seeking; however, the results showed that seedling vigour index had no significant difference between the concentration of Bokashi leachate. EM enhanced the seed germination and vigour in carrot, cucumber, pea, beet, and tomato (Alderson, J. Charles & Wall, 1992; Olle and Williams, 2013). Vermicompost and its leachate improved the leaf and root biomass (Kaur *et al.*, 2018).

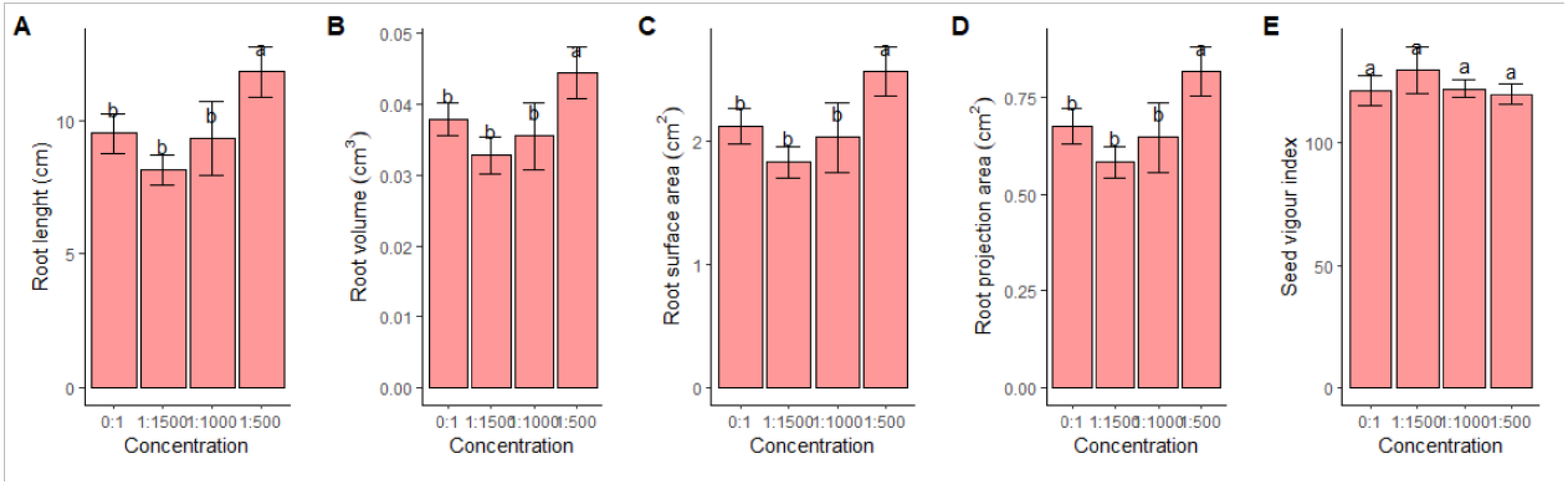

Figure 3. The effect of biopriming concentration (leachate: water) on (A) root length (cm), (B) root volume (cm^3), (C) root surface area (cm^2), (D) root projection area and (E) seed vigour index of seedlings. Means ± standard deviation with different letters is significantly different at $P<0.05$ using DMRT.

3.2 The biopriming duration on the seed germination and root growth performance

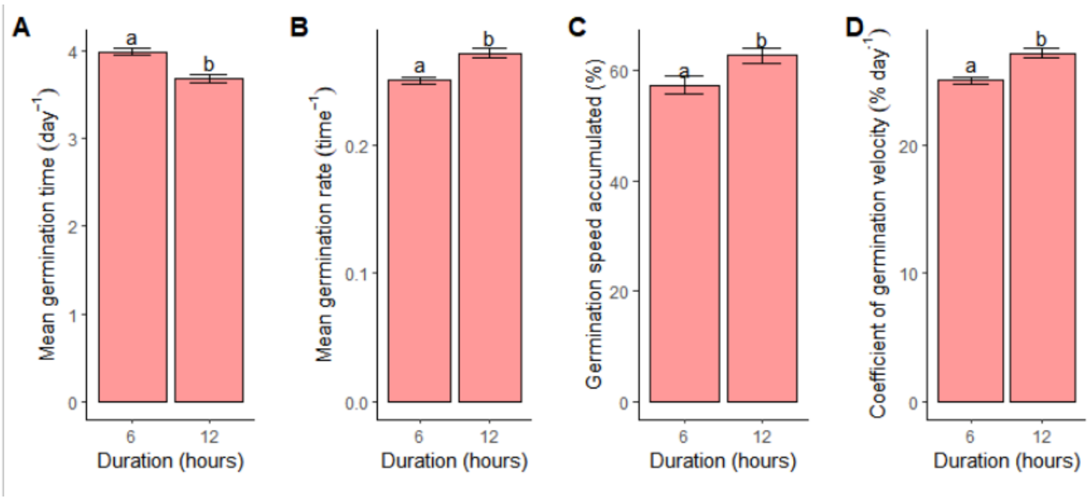

Figure 4 The effect of biopriming duration on (A) mean germination time, (B) mean germination rate, (C) germination speed accumulated, (D) coefficient of germination velocity of the seedlings. Means ± standard deviation with different letters is significantly different at P<0.05 using DMRT.

Seed priming accelerated germination due to the higher initial water concentration, and the seeds were dried back to their original moisture concentration (Pace *et al.*, 2012). Hence, it is crucial to enhanced seed vigour and germination (Paparella *et al*., 2015). Acid and urea seed treatment eliminates the physical dormancy by infusing and cracking the hard coat and thus enhanced the water absorption into the embryo (Harsha *et al.*, 2012). The germination percentage of bitter ground is significantly reduced in a longer priming duration (16 hours) (Saleem *et al.*, 2016). In contrast, long priming duration (48 hours) and high concentration of Gibberellin (200 ppm) have a positive effect on the germination percentage in Christmas Palm (Ruminta *et al.*, 2017). The possible reason was that the osmotic potential in seed was reduced, and the toxic effect occurred (Mohajeri *et al*., 2016).

The 12 hours seed priming duration have significantly enhanced the mean germination time (p=0.0005747). However, 6 hours of seed priming duration were significantly improved the mean germination rate (time^{-1}) (p=0.000768), germination speed accumulated (%) (p=0.03024) and coefficient of germination velocity (% day^{-1}) (p=0.000768) (Figure 4). The long priming duration was prone to imbibition injury (Monalisa *et al.*, 2017). In contrast, 12 hours of priming duration significantly reduce the mean germination time in "Badami" pistachio (Esmaeilpour and Van Damme, 2016). Coefficient of germination velocity in 12 hours priming duration significantly higher than 6 hours one. In bread wheat, 12 and 36 hours priming time of coefficient of germination velocity had significantly higher than 24 hours (Liela *et al.*, 2010). The enhanced coefficient of germination velocity may be due to the cell division increase in the seeds (Ruan *et al.*, 2002). Stress-resistant cultivars depend on cell elongation rather than cell division. Therefore, it does not require DNA duplication for the first germination steps (Pace *et al.*, 2012).

Germination of *Withania somnifera* enhanced by priming in vermicompost leachate or tea with phytohormone and phenolic compounds (Aremu *et al.*, 2015) for 12 hours (Kaur *et al.*, 2018). Also, the combined treatment of vermicompost in seed and growing treatment enhanced germination and seedling growth up to 80% (Kaur *et al.*, 2018). However, plant growth regulators treated with seeds did not significantly increase the germination percentage but aid in the breaking seed dormancy and early radical emergence at day 7 (Small *et al.*, 2019). Gibberellins have the capacity to elongate the cell and stem internode. For instance, gibberellins enhance the shoot growth for the grasses and forb species (Small *et al.*, 2019). Sesame seed soaks with GA_3 for 12 hours increased the germination to nearly 99% (Kyauk *et al.*, 1995). Sesame seed priming for 12 to 14 hours improves the proximate composition and functional properties (Kajihausa *et al.*, 2014).

The stem leachate of *Sonchus arvensis* L. in the ratio of 1:20 (w/v) inhibits maize's seedling emergence and growth (Bashir *et al.*, 2018). Besides, the saltgrass seed soaked with plant growth-promoting bacterial consortium for 2 hours had no significant effect on the germination performance (Xia *et al.*, 2020). The slightly acidic electrolyzed water decontaminates the Enterobacteriaceae in alfalfa seeds growth (Zhang *et al.*, 2021).

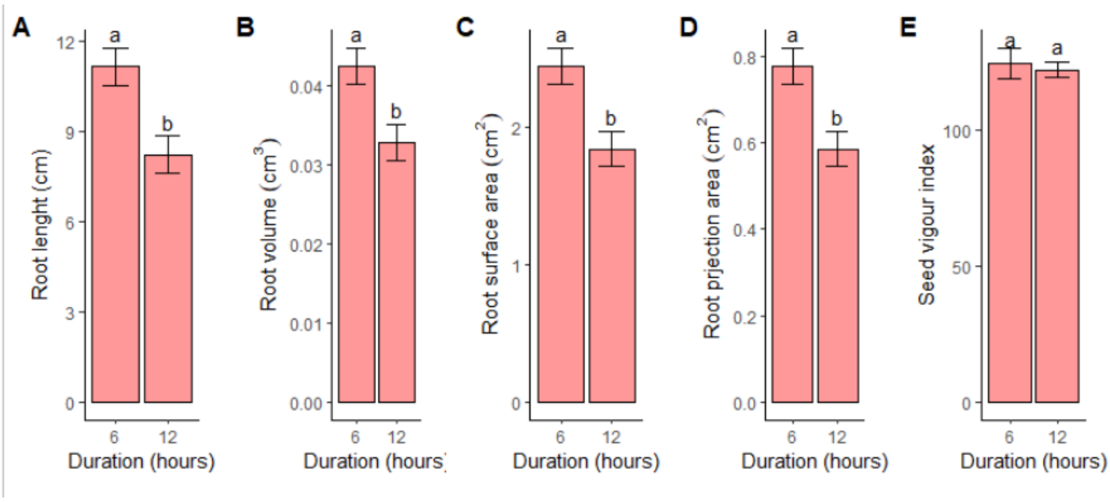

Figure 5 The effect of biopriming duration (hours) on (A) root length (cm), (B) root volume (cm^3), (C) root surface area (cm^2), and (D) root projection area and (E) seed vigour index of seedlings. Means ± standard deviation with different letters are significantly different at $P<0.05$ using DMRT.

Seed priming with 6 hours showed a significant improvement in root length ($p=0.0006091$), root projection area ($p=0.0008063$), root volume ($p=0.002258$) and root surface area ($p= 0.0008062$) compared to others during the early growth (Figure 5). Long priming duration (12 hours) negatively affected the root growth performance and mean germination time. Comparably, 12 hours priming duration of pistachio was reduced the root dry weight (Esmaeilpour and Van Damme, 2016). The seed may cause loss of desiccation tolerance in long priming duration (Paparella *et al.*, 2015). Priming should prevent the seed from fully germinating since it is triggered by 'pre-germinative metabolism' (Paparella *et al.*, 2015). In contrast, long priming duration (12 and 36 hours) significantly increases bread wheat's radicle length (Liela *et al.*, 2010).

4.0 Conclusion

Long biopriming duration (12 hours) significantly affected root growth development. Based on the results, treatment of 6 hours of seeds biopriming duration or 0.2% (1:500) of food waste Bokashi leachate was recommended to soak the *Basella rubra* seeds to enhance the seed germination and root growth performance in the early stage. The Bokashi leachate concentration could be increased to obtain more superior germination and root growth performance for future study. The abiotic and biotic stress could be conducted further to understand the effect of biopriming concentration and duration.

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