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Effect of Priming on *Brassica rapa* subsp. *chinensis* (Bok Choy) Seeds Germination

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ABSTRACT

Brassica rapa subsp. *Chinensis,* commonly known as Bok Choy, is a nutrient-rich vegetable with substantial antioxidant content. Therefore, this study aimed to evaluate the effect of hydropriming and bio-nutri-priming using Sandwich compost leachate on seed germination, SPAD reading, and dry matter accumulation in 280 dwarf variants of Bok Choy seeds sourced from Green Eagle. The experimental process involved hydropriming with tap water, bio-nutri-priming using 0.2% Sandwich compost leachate, and a control group cultivated in soil without priming. A complete randomization design (CRD) with three replications assessed seed germination performance, SPAD, root and shoot dry matter, and root-to-shoot ratio. While there was no significant difference in the germination percentage (88.35±1.13%), the entire priming seeds exhibited a 2-day peak germination period, compared to 3 days for the non-priming counterparts. Bio-nutri-priming seeds showed faster median and mean germination times due to enhanced nutrient uptake. They further displayed high SPAD readings, suggesting a lack of toxic compounds. The dry matter production of all treated Bok Choy was similar because administered treatments did not interfere with plant growth and development. Therefore, applying bio-nutri-priming using Sandwich compost leachate positively affected seed germination performance, warranting its recommendation as a seeds priming solution.

Keywords: Animal and plant-based food waste; biopriming; bokashi; growth; leachate; seeds soaking; raw and cooked food waste

INTRODUCTION

Food waste is a persistent challenge worldwide, specifically in Malaysia, necessitating effective management strategies. Addressing food waste disposal requires optimizing its use at the source. Digestion methods, including fermentation, offer a promising approach (Usmani et al., 2021). Sandwich compost, derived from Bokashi, has positively affected plant initial growth. Employing raw and cooked food waste in preparing Sandwich compost presents an alternative for waste management.

The potential of Sandwich compost leachate extends to enhancing seed germination and substantial growth, contributing to food security. As a sustainable resource, Sandwich compost leachate holds promising applications in food production. For example, priming tomato seeds with Sandwich compost leachate led to a 13% increase in transplant stem diameter, facilitating nutrient uptake (Olle, 2020). Similarly, passion fruit treated with 16% Sandwich compost priming solution exhibited superior initial growth (Bócoli et al., 2020). Humic acids extracted from Sandwich compost proved advantageous for the initial growth of maize (Baldotto & Baldotto, 2016).

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Seeds priming has been recognized for improving germination rates by facilitating rapid water absorption to activate messenger ribonucleic acid (mRNA) and promote germination (Johnson & Puthur, 2021). For instance, biopriming of corn triggers DNA, RNA, and protein production (Nciizah et al., 2020). Timely germination is a critical concern for farmers, leading to the adoption of various priming techniques such as hydropriming, chemo-priming, osmo-priming, hormo-priming, solid matrix priming, and nutri-priming to mitigate abiotic stress (Deshmukh et al., 2020; Lutts et al., 2016).

Biopriming, involving beneficial microbes and bioactive molecules, fosters endophytic interactions between plants, specific fungi, and bacteria. This interaction enhances phytohormone synthesis, promoting resistance to biotic and abiotic stress (Paparella et al., 2015). It also offers an environmentally friendly method to augment plant growth, development, and stress resistance (Mahmood, Turgay, Farooq, & Hayat, 2016; Toribio et al., 2021). For instance, biopriming with plant growth-promoting rhizobacteria improves seed germination, growth performance, and nutrient and stress management (Mitra et al., 2021).

Brassica sp. possesses high sulfur- and nitrogen-containing secondary metabolites (Miao et al., 2021; Park et al., 2020), which are vital for plant defence against microbial pathogens and herbivorous insects (Chhajed et al., 2020). In Malaysia, *Brassica* sp. production reaching 0.15 Mt, accounted for 15% of total vegetable production in 2019 (Jabatan Pertanian Malaysia, 2019). Considering the high demand for Bok Choy, the application of priming is essential to help enhance seed germination, uniformity, and final stand (Ruttanaruangboworn et al., 2017).

To address food waste challenges, this study employed Sandwich compost leachate for seed priming, investigating seeds emergence and subsequent growth. Additionally, this study provided insight into agricultural food waste management. The inefficiently used potential of Sandwich compost leachate prompted the examination of its impact on Bok Choy seed germination, SPAD reading, and dry matter accumulation compared to hydropriming and dry seed germination. The major aim was to evaluate the effect of hydropriming and bio-nutri-priming using Sandwich compost leachate on seed germination, SPAD reading, and dry matter accumulation in 280 dwarf Bok Choy seeds.

METHODS

Study Site and Experimental Design

The seeds germination tray study was conducted in Field 10 net house at the University of Putra Malaysia (UPM), located at the coordinates with latitude 2°59’34.0”N and longitude 101°42’52.3”E. The experimental process employed a completely randomized design (CRD) with three replications, each consisting of 104 seeds.

Table 1. The physicochemical properties of sandwich compost leachate and tap water

Physiochemical parameter	Sandwich compost leachate	Tap water
pH	4.78±0.011	6.98±0.02
Electric conductivity (dS m^{-1})	0.3357±0.0003	0.134±0.00
Total dissolved salt (mg L^{-1})	22.19±0.2133	85.76±0.00
Osmotic potential (bar)	0.0125±0.0001	0.04824±0.00
N (%)	0.2135±0.0052	$0.0006\pm1.63\times10^{-18}$
P (ppm)	5833±223	0.0447±0.0197
K (ppm)	3941±131	3.64±0.0415
Ca (ppm)	528±18.6	13.8±0.150
Na (ppm)	332±151	Not detected
Mn (ppm)	72.0±2.67	Not detected
Al (ppm)	770±510	Not detected
Fe (ppm)	160±42.5	0.306±0.015

*mean ± standard error.

The Sandwich compost preparation method uses accumulated food waste (Phooi et al., 2022). A Sandwich taster was created by inoculating effective microbe (EM)-1 (commercially available) into rice bran and fermenting it anaerobically for two weeks. EM-1 comprised various microbes in a liquid culture with a pH below 3.5 (Higa, 2001; Higa & Parr, 1994). Cooked and raw food waste was alternately layered with the bran to create the compost.

On the 34th day of fermentation, leachate was collected to serve as a bio-nutri-priming solution. Commercial 280 dwarf Bok Choy seeds were purchased from Green Eagle (Green Eagle Seeds, 2022). The seeds were bio-nutri-priming with 0.2% Sandwich compost leachate following a previously described method (Phooi et al., 2022), where 1 g seeds were soaked in 250 mL solution for 3 hours. A separate treatment involved hydropriming the seeds with tap water for 3 hours (Alias et al., 2018).

The control group was left unprimed, while the seeds were sown in 104-hole germination trays on moist peat moss. The diameter of the holes was 3.7 cm, and the peat moss was procured from Kekkila Professional. Top irrigation was carried out twice daily using tap water delivered through a hose to prevent soil water from drying out. The seeds were subjected to a 16:8 hours light/dark photoperiod at 32 °C (day) and 28 °C (night) and cultivated until day 30. Additionally, the samples were collected in two holes away from the tray margin.

The physicochemical properties of the priming solutions (tap water and Sandwich compost leachate) were determined, as presented in Table 1. Kjeldahl methods determined total nitrogen (Campbell & Hanna, 1937). A total of 1 g of 2 mm-sized soil and 5 mL of concentrated sulphuric acid with 5% salicylic acid were put in a digestion tube overnight. Subsequently, 0.3 g $Na_2S_2O_3$ and a single Kjeldahl tablet were added and digested until the mixture turned greyish-white. The resulting digest was diluted with distilled water up to 100 mL. A mixture of 10 mL of 30% NaOH and 10 mL extractant was subjected to distillation with 10 mL of 2% boric acid containing an indicator. The initial purple colour of the boric acid-indicator mixture changed to green. The green solution was collected after distilling it to a volume of 50 mL for titration with 0.01 N HCl.

Available phosphorous (P) was evaluated with the colourimetric method described by Murphy and Riley (Murphy & Riley, 1962). The extracting solution for available P was prepared by combining 60 mL of 1 N H_4F and 200 mL of 1 N HCl in a 2000 mL volumetric flask. Reagent A was created by stirring with 12 g of ammonium molybdate and 148 mL H_2SO_4, following cooling, 0.2908 g antimony K tartaric was added. Reagent B was prepared by dissolving 1.32 g ascorbic acid and combining it with reagent A up to a volume of 250 mL. In this process, 2 g air-dried soil featuring a particle size <2 mm was mixed with 14 mL extracting solution in a 50 mL falcon tube, which was then shaken for 45 seconds at a constant speed. The resulting filtrate (2 to 5 mL) was added to 8 mL reagent B and made to reach 50 mL using distilled water in a volumetric flask. The solution was allowed to stand for 15 minutes and read spectrophotometrically at 885 nm. The nutrient contents, including (Ca), sodium (Na), manganese (Mn), iron (Fe), and zinc (Zn), were subsequently extracted (Mehlich, 1984; Minca et al., 2013) and analyzed with Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Optima 8300 ICP-OES, Perkin Elmer, Waltman, MA, USA).

Seeds Germination Test

Seed germination was identified when a visible protrusion with at least 0.2 cm length of the seedling radicle emerged. Germinated seeds were counted daily for seven days. The data obtained were analyzed using the "germinationmetrics" package in R statistical software by comparing germination traits (Aravind et al., 2019). Parameters assessed included germination percentage (GP), peak germination time, median germination time (t_{50}) (Coolbear), mean germination time ($\overline{T}$), variance germination time (S^2_T), standard error of germination time ($s_{\overline{T}}$), coefficient of variation of the germination time (CVT), speed of accumulated germination ($S_{accumulated}$), mean germination percentage (%), Geoge's index (GR), and germination index (GI) (Melville).

The germination percentage was employed to measure the germination capacity (ISTA, 2015), as shown in Equation 1.

$$Germination\ percentage\ = \frac{the\ number\ of\ germinated\ seeds}{the\ total\ number\ of\ seeds} \times 100\% \qquad (1)$$

Peak germination represents when the highest frequency of germinated seeds is observed (Ranal & Santana, 2006). Median germination time (t_{50}) (Coolbear) signifies the duration required to reach 50% of the maximum germination rate (Coolbear, Francis, & Grierson, 1984), as expressed in Equation 2.

$$t_{50}\ =\ T_i + \frac{(\frac{N+1}{2} - N_i)(T_j - T_i)}{N_j - N_i} \qquad (2)$$

Where t_{50} denotes the median germination time, N is the final number of germinated seeds, and N_i and N_j stand for the total numbers of germinated seeds identified during adjacent counts at times T_i and T_j, respectively when $N_i < \frac{N+1}{2} < N_j$.

Mean germination time ($\overline{T}$) is an estimation of the average time needed for the complete germination of a batch of seeds (Czabator, 1962; Ranal & Santana, 2006), as indicated in Equation 3.

$$\bar{T} = \frac{\sum_{i=1}^{k} NiTi}{\sum_{i=1}^{k} Ni} \qquad (3)$$

Where T_i represents the time from the beginning of the experiment to the i th interval, Ni is the number of seeds germinated within the i th time interval (not the count, but the count corresponding to the i th interval), and k signifies the total number of time intervals. It is the reciprocal of the mean germination rate ($\bar{T}$) indicated in Equation 4.

$$\bar{T} = \frac{1}{\bar{v}} \qquad (4)$$

Variance germination time (S^2_T), standard error of germination time ($s_{\bar{T}}$), and the coefficient of variation of the germination time (CV_T) indicates the germination rate. Speed of accumulated germination ($S_{accumulated}$) denotes the rate of cumulative total germinated seeds during the time interval (Santana & Ranal, 2004). Mean germination percentage ($\bar{G}$) represents the final percentage of germinated seeds per the total time intervals required for final germination time (Czabator, 1962). Geoge's index (GR) quantifies the number of germinated seeds within a specific time interval (George, 1961). The germination index (GI) measures the timing of germinated seeds numbers from the starting day to the interval per the total number of tested seeds (Melville et al., 1980).

SPAD Reading

The chlorophyll concentration was non-destructively measured with a SPAD meter (Konica Minolta SPAD-502 Plus). SPAD reading was used to monitor plant growth and optimize nitrogen fertilization. Furthermore, its mean was immediately calculated before harvesting on day 30. Three mean readings per plant were recorded from the rib of the leaf at the same spot.

Dry Matter and Root-to-Shoot Ratio

Fresh root and shoot samples were washed, air-dried, weighed, and then oven-dried at 45°C for 60 hours to determine the dry weight. The dry matter was expressed as a percentage using the formula: (fresh weight - dry weight)/dry weight x 100. The root-to-shoot ratio was computed by dividing root dry matter with dry shoot matter.

Statistical Analysis

Recorded data were subjected to the Shapiro-Wilk normality test ($p>0.05$). Subsequently, a one-way Analysis of Variance (ANOVA) was performed with the "agricolae" package in R statistical software (Mendiburu & Yaseen, 2020). When F values were significant at a *p*-level of 0.05, treatment means were compared and separated using the Least Significant Difference (LSD) (Mendiburu & Yaseen, 2020).

RESULTS AND DISCUSSION

No significant differences in seeds germination percentage (88.35±1.13%) were observed among the two priming treatments and the control, as presented in Table 2. In this study, seeds treated with bio-nutri-priming and hydropriming exhibited a peak germination time of 2 days, while their counterparts spent 3 days to reach the peak stage. Median germination time was the shortest (1.75 days) for bio-nutri-priming seeds, and the mean time ranged from 2.52 to 2.79 days for priming seeds, which was faster than non-priming seeds. SPAD reading of bio-nutri-priming seeds showed significant differences among treatments in this study, but no substantial variations were found in the dry matter of the plants.

Seeds Emergence Performance in Bio-Nutri-Priming Seeds

The significantly elevated germination rate observed in priming seeds, compared to none- priming seeds, could be attributed to metabolic repairing and increased production of metabolites needed for germination during the imbibition process (Afzal et al., 2016). Hydropriming and bio-nutri-priming facilitated the dehydration of seeds post-priming, followed by re-initiation under favorable conditions for cell elongation and radicle protrusion (Lutts et al., 2016). Moreover, protein expression post-priming improved seed emergence (Afzal et al., 2016).

Bio-nutri-priming outperformed hydropriming, probably due to the leachate nutrient storage within the seeds aided by microbes during seed germination phase III, namely cell elongation, leading to radicle protrusion (Afzal et al., 2016). Similarly, previous studies showed that Agnihotra ash, compost tea, and hot water priming significantly enhanced germination rates in treated rosemary seeds compared to dry seeds (Sharma et al., 2019). The examined seeds stored a substantial amount of carbohydrates to endure low oxygen stress conditions, conferring improved germination in those subjected to bio-nutri-priming (Deshmukh et al., 2020; Paparella et al., 2015).

The leachate, rich in humic acid and low in total organic carbon and organic acids, increased the germination index of bio-nutri-priming seeds (Wang et

Table 2. Germination performance, root-to-shoot ratio, and root and shoot dry matter based on seeds treatments including bio-nutri-priming, hydropriming, and no priming

Treatment	GP* (%)	Peak germination time (day)	t_{50} (Coolbear) (day)	$\bar{T}$ (day)	S^2_T (day)	S_T	CV_T	$S_{accumulated}$ (% day^{-1})	$\overline{GP}$ (%)	GR (day^{-1})	GI	SPAD	RSR	RDM (g)	SRM (g)
Bio-nutri-priming	88.14^{a}	2^{a}	1.75^{a}	2.52^{a}	1.18^{a}	0.11^{a}	0.43^{a}	125.48^{a}	12.59^{a}	146.33^{a}	3.95^{a}	29.13333^{a}	0.745847	8.984069	12.04685
Hydropriming	87.50^{a}	2^{a}	2.09^{b}	2.79^{a}	1.03^{a}	0.11^{a}	0.36^{a}	109.97^{ab}	12.50^{a}	119.33^{a}	3.68^{ab}	23.23333^{b}	0.65107	8.199364	12.4955
None	89.42^{a}	3^{b}	2.62^{c}	3.28^{b}	0.77^{a}	0.09^{a}	0.26^{b}	92.29^{b}	12.77^{a}	80.00^{b}	3.32^{b}	22.65^{b}	0.700485	9.950556	14.74962
Mean	88.35	2.33	2.15	2.86	0.99	0.1	0.35	109.25	12.62	115.22	3.65	25.00556	0.699134	9.044663	13.09732
P-value	0.866 ns	< 2.2e-16 ***	0.002207 **	0.01028 *	0.2902	0.241	0.007538 **	0.01096 *	0.866	0.003211 **	0.03181 *	0.01327*	0.7971	0.5119	0.1446
CV	5	6.15E-15	7.87	7.06	28.737	16.23	12.26	8.12	5	12.04	5.85	7.987941	24.19637	19.39461	11.62325
R^2	0.047	1	0.86	0.783	0.338	0.378	0.804	0.778	0.047	0.852	0.683	0.763	0.073	0.2	0.475

Means with the same letter were not significantly different between treatments using LSD. *** $p<0.00$, ** $p<0.001$, * $p<0.01$, and $p>0.05$: not significant.
*Germination percentage (GP), peak germination time, median germination time (t50) (Coolbear), mean germination time ($\bar{T}$), variance germination time (S2T), standard error of germination time (ST), coefficient of variation of the germination time (CVT), speed of accumulated germination (Saccumulated), mean germination percentage ($\overline{GP}$), Geoge's index (GR), germination index (GI) (Melville), root-to-shoot ratio (RSR), root dry matter (RDM), shoot dry matter (SDM)

al., 2022). Low concentrations of compost leachate could induce a hormesis effect for seed germination enhancement (Bona et al., 2022). For instance, compost and vermicompost tea improved germination, seedling vigour, and characteristics (Ali et al., 2018). The compost particle size of compost also influenced lentils' germination and seedling traits by promoting their cell division (Ali et al., 2018). Biochar leachate improved germination performance (Bieser et al., 2022), and vermicompost leachate boosted the germination percentage of aged rapeseed seeds without NaCl (Benazzouk et al., 2019). Leachate priming facilitated a rapid connection between soil and plants, promoting nutrient mobility and auxin synthesis for rooting (Ngoma et al., 2013; Pandey et al., 2017; Sutariati et al., 2019). The superiority of bio-nutri-priming over hydropriming could be attributed to enhanced microbial connections with soil plants. Low concentrations of vermicompost tea (1%) improved seed germination, while higher concentrations (5%) significantly promoted plant growth (Arancon et al., 2012). In contrast, concentrated compost tea (20%) suppressed weed germination due to its phytotoxicity (Ibrahim & Balah, 2018). The phytotoxicity of leachate was found to be concentration-dependent (Sakit ALHaithloul et al., 2022; Šourková et al., 2020).

The seed germination index of *Brassica napus* L. was amplified by biopriming with bacteria (*Bacillus subtilis*) and fungus (*Macrophomina phaseolina*) (Mousavi & Omidi, 2019). Bacteria priming has been observed to increase seed germination percentage in saline conditions (Chu et al., 2019; Ghorbanpour & Hatami, 2014) and shorten the mean germination time to 1.28-4.25 days (Ghorbanpour & Hatami, 2014; Mousavi & Omidi, 2019). This impact was assumed to be triggered by the completion of pre-germination metabolite activity and reorganization of membrane structure (Muhammad et al., 2015). Dry seeds coated with *Pseudomonas fluorescens* AB254 facilitated germination through improved water imbibition, leading to a moisture content reaching 35–40% (Callan, 1990; Sisodia et al., 2018). Furthermore, biopriming with bacterial antagonists boosted the antagonist population, protecting the rhizosphere from plant pathogen infection (Mahmood & Kataoka, 2018). The effectiveness of biopriming using plant growth-promoting rhizobacteria varied with bacterial strain species (Ghorbanpour & Hatami, 2014).

Enhancement of SPAD Reading through Bio-Nutri-Priming

Biopriming has been found to elevate SPAD values significantly (Roslan et al., 2020), corroborating the results of this study. Based on previous investigations, SPAD reading showed a high correlation (r^2=0.94) with chlorophyll content (Chu et al., 2019; James et al., 2002). High chlorophyll content was observed in a plant grown from biopriming seeds (Singh et al., 2020). The improved nutrient uptake in bio-nutri-priming plants supported root development (Sarkar, Sankar, et al., 2021). Bio-nutri-priming also enhanced soil microbe activity, mineralising organic nutrients into inorganic forms that could be retained as soil solution (Sarkar, Sankar, et al., 2021). Superior assimilation in plants contributed to higher SPAD readings (Sarkar, Sankar, et al., 2021). Therefore, bio-nutri-priming enhanced nitrogen use efficiency (Sarkar, Rakshit, et al., 2021). Another contributing factor might be the non-involvement of indirect mechanisms in the synthesis and activity of antioxidant enzymes such as peroxidase, catalase, and superoxide dismutase in host tissues capable of harming the plant (Singh et al., 2020). These observations aligned with previous studies indicating significant improvements in chlorophyll biosynthesis and photosynthetic activity (Chitra & Jijeesh, 2021; Zulueta-Rodríguez et al., 2015). Further nutrient analysis could improve the priming method, specifically regarding nutrient content, as SPAD reading correlated with ascorbic acid content (Yaseen & Takacs-Hajos, 2022).

Shoot and Root Dry Matter

Biopriming has been shown to augment plant biomass (Sivakumar et al., 2017). The lack of significant influence from biopriming on plant dry matter in this study might be due to the plants being in a similar growth phase. Therefore, further exploration should be conducted on aspects including plant growth performance, omics (e.g., metabolomics and proteomics), and responses to biotic and abiotic stressors (e.g., pest infection, saline, and drought). Investigations related to destructive sampling could enhance the understanding of dry matter accumulation across different growth cycle stages.

CONCLUSION

In conclusion, bio-nutri-priming using Sandwich compost leachate significantly enhanced mean germination time and SPAD reading. The addition of this leachate as a bio-nutri-priming solution held promising potential for addressing food waste challenges while concurrently promoting plant growth. The substantial improvements in germination index and median germination time observed in bio-nutri-priming seeds were attributed to improved nutrient storage within seeds facilitated by microbial assistance. Furthermore, increased SPAD reading could reduce fertilizer requirements due to augmented nutrient use efficiency post bio-nutri-priming. This observation indicated the advantageous role of Sandwich compost leachate in priming, such as the enhancement of seed germination and leaf greenness. The use of this approach aligned with the demand for leafy vegetables in Malaysia, contributing to food and nutritional security promotion.

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CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Afzal, I., Rehman, H. U., Naveed, M., & Basra, S. M. A. (2016). Recent advances in seed enhancements. In *New Challenges in Seed Biology - Basic and Translational Research Driving Seed Technology*. InTech. https://doi.org/10.5772/64791

Ali, O., El-Tahlawy, Y., & Abdel-Gwad, S. (2018). Impact of compost tea types application on germination, nodulation, morphological characters, and yield of two lentil cultivars. *Egyptian Journal of Agronomy*, *0*(0), 1–19. https://doi.org/10.21608/agro.2018.5678.1126

Alias, N. S. B., Billa, L., Muhammad, A., & Singh, A. (2018). Priming and temperature effects on germination and early seedling growth of some Brassica spp. *Acta Horticulturae*, *1225*, 407–414. https://doi.org/10.17660/ACTAHORTIC.2018.1225.57

Arancon, N. Q., Pant, A., Radovich, T., Hue, N. v., Potter, J. K., & Converse, C. E. (2012). Seed germination and seedling growth of tomato and lettuce as affected by vermicompost water extracts (teas). *HortScience*, *47*(12), 1722–1728. https://doi.org/10.21273/hortsci.47.12.1722

Aravind, J., Vimala Devi, S., Radhamani, J., Jacob, S. R., & Kalyani, S. (2019). *germinationmetrics: Seed germination indices and curve fitting*. Retrieved from https://aravind-j.github.io/germinationmetrics/articles/Introduction.html

Baldotto, M. A. & Baldotto, L. E. B. (2016). Initial performance of corn in response to treatment of seeds with humic acids isolated from bokashi. *Revista Ceres*, *63*(1–62), 62–67. https://doi.org/10.1590/0034-737X201663010009

Benazzouk, S., Djazouli, Z.-E., & Lutts, S. (2019). Vermicompost leachate as a promising agent for priming and rejuvenation of salt-treated germinating seeds in *Brassica napus. Communications in Soil Science and Plant Analysis*, *50*(11), 1344–1357. https://doi.org/10.1080/00103624.2019.1614608

Bieser, J. M. H., Al-Zayat, M., Murtada, J., & Thomas, S. C. (2022). Biochar mitigation of allelopathic effects in three invasive plants: evidence from seed germination trials. *Canadian Journal of Soil Science*, *102*(1), 213–224. https://doi.org/10.1139/CJSS-2020-0160/SUPPL_FILE/CJSS-2020-0160SUPPLA.DOCX

Bócoli, F. A., Marcon, J. A., Izidoro, M., Bortolon, P. de T., de OLIVEIRA, S. E. R., Spalevic, V., & de SOUZA, P. S. (2020). Bokashi use in the passionfruit (*Passiflora edulis* l.) germination and initial growth. *Agriculture and Forestry*, *66*(4), 101–111. https://doi.org/10.17707/AgricultForest.66.4.08

Bona, D., Scrinzi, D., Tonon, G., Ventura, M., Nardin, T., Zottele, F., … Silvestri, S. (2022). Hydrochar and hydrochar co-compost from OFMSW digestate for soil application: 2. agro-environmental properties. *Journal of Environmental Management*, *312*, 114894. https://doi.org/10.1016/J.JENVMAN.2022.114894

Callan, N. W. (1990). Bio-priming seed treatment for biological control of *Pythium ultimum* preemergence damping-off in sh2 sweet corn. *Plant Disease*, *74*(5), 368. https://doi.org/10.1094/PD-74-0368

Campbell, W. R. & Hanna, M. I. (1937). The determination of nitrogen by modified Kjeldahl methods. *Journal of Biological Chemistry*, *119*(1), 1–7. https://doi.org/10.1016/S0021-9258(18)74426-8

Chhajed, S., Mostafa, I., He, Y., Abou-Hashem, M., El-Domiaty, M., & Chen, S. (2020). Glucosinolate biosynthesis and the glucosinolate–myrosinase system in plant defense. *Agronomy*, *10*(11), 1786. https://doi.org/10.3390/agronomy10111786

Chitra, P., & Jijeesh, C. M. (2021). Biopriming of seeds with plant growth promoting bacteria Pseudomonas fluorescens for better germination and seedling vigour of the East Indian sandalwood. *New Forests*, *52*(5), 829–841. https://doi.org/10.1007/s11056-020-09823-0

Chu, T. N., Tran, B. T. H., van Bui, L., & Hoang, M. T. T. (2019). Plant growth-promoting rhizobacterium Pseudomonas PS01 induces salt tolerance in Arabidopsis thaliana. *BMC Research Notes*, *12*(1), 11. https://doi.org/10.1186/s13104-019-4046-1

Coolbear, P., Francis, A., & Grierson, D. (1984). The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *Journal of Experimental Botany*, *35*(11), 1609–1617. https://doi.org/10.1093/jxb/35.11.1609

Czabator, F. J. (1962). Germination value: an index combining speed and completeness of pine seed germination. *Forest Science*, *8*(4), 386–396. https://doi.org/10.1093/forestscience/8.4.386

Deshmukh, A. J., Jaiman, R. S., Bambharolia, R. P., & Patil, V. A. (2020). Seed biopriming– a review. *International Journal of Economic Plants*, *7*(1), 038–043. https://doi.org/10.23910/2/2020.0359

George, D. W. (1961). Influence of germination temperature on the expression of post-harvest dormancy in wheat. *Crop Science Abstracts*, *1961*(Western Society of Crop Science Annual Meeting), 15.

Ghorbanpour, M., & Hatami, M. (2014). Biopriming of *Salvia officinalis* seed with growth promoting Rhizobacteria affects invigoration and germination indices. *J. Biol. Environ. Sci*, *8*(22), 29–36.

Green Eagle Seeds. (2022). Pak choy. Retrieved May 23, 2022, from Green Eagle Seeds website: http://greeneagle.com.my/

Higa, T. (2001). Effective microorganisms in the context of Kyusei nature farming – a technology for the future. *International Conference on Kyusei Nature Farming*.

Higa, T. & Parr, J. F. (1994). Beneficial and effective for a sustainable agriculture. *Agriculture*, (808), 1–16.

Ibrahim, H. A. K. & Balah, M. A. aziz. (2018). Study the use of compost tea in weed suppression. *International Journal of Environmental Research*, *12*(5), 609–618. https://doi.org/10.1007/s41742-018-0119-6

ISTA. (2015). Chapter 5: The germination test. *International Rules for Seed Testing*, *2015*(1), 5-1-5–56. https://doi.org/10.15258/istarules.2015.05

Jabatan Pertanian Malaysia. (2019). *Statistik tanaman sayur-sayuran dan tanaman ladang*.

James, R. A., Rivelli, A. R., Munns, R., & Caemmerer, S. von. (2002). Factors affecting CO_2 assimilation, leaf injury and growth in salt-stressed durum wheat. *Functional Plant Biology*, *29*(12), 1393. https://doi.org/10.1071/FP02069

Johnson, R. & Puthur, J. T. (2021). Seed priming as a cost effective technique for developing plants with cross tolerance to salinity stress. *Plant Physiology and*

Biochemistry, *162*, 247–257. https://doi.org/10.1016/j.plaphy.2021.02.034

Lutts, S., Benincasa, P., Wojtyla, L., Kubala, S., Pace, R., Lechowska, K., … Garnczarska, M. (2016). Seed priming: new comprehensive approaches for an old empirical technique. In *New Challenges in Seed Biology - Basic and Translational Research Driving Seed Technology*. InTech. https://doi.org/10.5772/64420

Mahmood, A. & Kataoka, R. (2018). Potential of biopriming in enhancing crop productivity and stress tolerance. *Advances in Seed Priming*, 127–145. https://doi.org/10.1007/978-981-13-0032-5_9/TABLES/3

Mahmood, A., Turgay, O. C., Farooq, M., & Hayat, R. (2016). Seed biopriming with plant growth promoting rhizobacteria: a review. *FEMS Microbiology Ecology*, *92*(8), 112. https://doi.org/10.1093/FEMSEC/FIW112

Mehlich, A. (1984). Mehlich 3 soil test extractant: a modification of mehlich 2 extractant. *Communications in Soil Science and Plant Analysis*, *15*(12), 1409–1416. https://doi.org/10.1080/00103628409367568

Melville, A. H., Galletta, G. J., Draper, A. D., & Ng, T. J. (1980). Seed germination and early seedling vigor in progenies of inbred strawberry selections. *HortScience*.

Mendiburu, F. de, & Yaseen, M. (2020). *agricolae: Statistical Procedures for Agricultural Research*.

Miao, H., Zeng, W., Wang, J., Zhang, F., Sun, B., & Wang, Q. (2021). Improvement of glucosinolates by metabolic engineering in Brassica crops. *ABIOTECH*, *2*(3), 314–329. https://doi.org/10.1007/s42994-021-00057-y

Minca, K. K., Basta, N. T., & Scheckel, K. G. (2013). Using the mehlich-3 soil test as an inexpensive screening tool to estimate total and bioaccessible lead in urban soils. *Journal of Environmental Quality*, *42*(5), 1518–1526. https://doi.org/10.2134/jeq2012.0450

Mitra, D., Mondal, R., Khoshru, B., Shadangi, S., das Mohapatra, P. K., & Panneerselvam, P. (2021). Rhizobacteria mediated seed bio-priming triggers the resistance and plant growth for sustainable crop production. *Current Research in Microbial Sciences*, *2*, 100071. https://doi.org/10.1016/j.crmicr.2021.100071

Mousavi, M. & Omidi, H. (2019). Seed priming with bio-priming improves stand establishment, seed germination and salinity tolerance in canola cultivar (Hayola 401). *Iranian Journal of Plant Physiology*, *9*(3), 2807–2817.

Muhammad, I., Kolla, M., Volker, R., & Günter, N. (2015). Impact of nutrient seed priming on germination, seedling development, nutritional status and grain yield of maize. *Journal of Plant Nutrition*, *38*(12), 1803–1821. https://doi.org/10.1080/01904167.2014.990094

Murphy, J. & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, *27*(C), 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5

Nciizah, A. D., Rapetsoa, M. C., Wakindiki, I. I., & Zerizghy, M. G. (2020). Micronutrient seed priming improves maize (Zea mays) early seedling growth in a micronutrient deficient soil. *Heliyon*, *6*(8), e04766. https://doi.org/10.1016/j.heliyon.2020.e04766

Ngoma, L., Esau, B., & Babalola, O. O. (2013). Isolation and characterization of beneficial indigenous endophytic bacteria for plant growth promoting activity in Molelwane Farm, Mafikeng, South Africa. *African Journal of Biotechnology*, *12*(26), 4105–4114. https://doi.org/10.4314/ajb.v12i26.

Olle, M. (2020). Short communication: The improvement of the growth of tomato transplants by bokashi tea. *Agraarteadus*, *31*(1), 70–73. https://doi.org/10.15159/JAS.20.10

Pandey, P. K., Singh, S., Singh, M. C., Singh, A. K., Pandey, P., Pandey, A. K., … DPatidar, R. K. (2017). Inside the Plants: Bacterial Endophytes and their Natural Products. *International Journal of Current Microbiology and Applied Sciences*, *6*(6), 33–41. https://doi.org/10.20546/ijcmas.2017.606.003

Paparella, S., Araújo, S. S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A. (2015). Seed priming: state of the art and new perspectives. *Plant Cell Reports*, *34*(8), 1281–1293. https://doi.org/10.1007/s00299-015-1784-y

Park, C. H., Park, Y. E., Yeo, H. J., Kim, J. K., & Park, S. U. (2020). Effects of light-emitting diodes on the accumulation of phenolic compounds and glucosinolates in *Brassica juncea* sprouts. *Horticulturae*, *6*(4), 77. https://doi.org/10.3390/horticulturae6040077

Phooi, C. L., Azman, E. A., Ismail, R., & Shahruddin, S. (2022). Effect of Sandwich compost leachate on *Allium tuberosum* seed germination. *Pertanika Journal of Tropical Agricultural Science*, *45*(2), 481–490. https://doi.org/10.47836/pjtas.45.2.09

Ranal, M. A. & Santana, D. G. de. (2006). How and why to measure the germination process? *Revista Brasileira de Botânica*, *29*(1), 1–11. https://doi.org/10.1590/S0100-84042006000100002

Roslan, M. A. M., Zulkifli, N. N., Sobri, Z. M., Zuan, A. T. K., Cheak, S. C., & Abdul Rahman, N. A. (2020). Seed biopriming with P- and K-solubilizing Enterobacter hormaechei sp. improves the early vegetative growth and the P and K uptake of okra (*Abelmoschus esculentus*) seedling. *PLOS ONE*, *15*(7), e0232860. https://doi.org/10.1371/journal.pone.0232860

Ruttanaruangboworn, A., Chanprasert, W., Tobunluepop, P., & Onwimol, D. (2017). Effect of seed priming with different concentrations of potassium nitrate on the pattern of seed imbibition and germination of rice (*Oryza sativa* L.). *Journal of Integrative Agriculture*, *16*(3), 605–613. https://doi.org/10.1016/S2095-3119(16)61441-7

Sakit ALHaithloul, H. A., Khan, M. I., Musa, A., Ghoneim, M. M., Aysh ALrashidi, A., Khan, I., … Soliman, M. H. (2022). Phytotoxic effects of *Acacia saligna* dry leachates on germination, seedling growth, photosynthetic performance, and gene expression of economically important crops. *PeerJ*, *10*, e13623. https://doi.org/10.7717/peerj.13623

Santana, D. G., & Ranal, M. A. (2004). Análise Da Germinação: Um Enfoque Estatístico. *Brasília: Universidade de Brasília*.

Sarkar, D., Rakshit, A., Al-Turki, A. I., Sayyed, R. Z., & Datta, R. (2021). Connecting bio-priming approach with integrated nutrient management for improved nutrient use efficiency in crop species. *Agriculture*, *11*(4), 372. https://doi.org/10.3390/AGRICULTURE11040372

Sarkar, D., Sankar, A., Devika, O. S., Singh, S., Shikha, Parihar, M., … Datta, R. (2021). Optimizing nutrient use efficiency, productivity, energetics, and economics of red cabbage following mineral fertilization and biopriming with compatible rhizosphere microbes. *Scientific Reports*, *11*(1), 1–14. https://doi.org/10.1038/s41598-021-95092-6

Sharma, Y., Fagan, J., & Schaefer, J. (2019). Influence of organic pre-sowing seed treatments on germination and growth of rosemary (*Rosmarinus officinalis* L.). *Biological Agriculture & Horticulture*, *36*(1), 35–43. https://doi.org/10.1080/01448765.2019.1649193

Singh, S., Singh, U. B., Malviya, D., Paul, S., Sahu, P. K., Trivedi, M., … Saxena, A. K. (2020). Seed biopriming with microbial inoculant triggers local and systemic defense responses against *Rhizoctonia solani* causing banded leaf and sheath blight in maize (*Zea mays* L.). *International Journal of Environmental Research and Public Health*, *17*(4), 1396. https://doi.org/10.3390/ijerph17041396

Sisodia, A., Padhi, M., Pal, A. K., Barman, K., & Singh, A. K. (2018). Seed priming on germination, growth and flowering in flowers and ornamental trees. In *Advances in Seed Priming* (pp. 263–288). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-13-0032-5_14

Sivakumar, T., Ambika, S., & Balakrishnan, K. (2017). Biopriming of rice seed with phosphobacteria for enhanced germination and vigour. *ORYZA- An International Journal on Rice*, *54*(3), 346. https://doi.org/10.5958/2249-5266.2017.00048.0

Šourková, M., Adamcová, D., Zloch, J., Skutnik, Z., & Vaverková, M. D. (2020). Evaluation of the phytotoxicity of leachate from a municipal solid waste landfill: the case study of bukov landfill. *Environments*, *7*(12), 111. https://doi.org/10.3390/environments7120111

Sutariati, G. A. K., Khaeruni, A., Muhidin, Madiki, A., Rakian, T. C., Mudi, L., & Fadillah, N. (2019). Seed biopriming with indigenous endophytic bacteria isolated from Wakatobi rocky soil to promote the growth of onion (*Allium ascalonicum* L.). *IOP Conference Series: Earth and Environmental Science*, *260*(1), 012144. IOP Publishing. https://doi.org/10.1088/1755-1315/260/1/012144

Toribio, A. J., Jurado, M. M., Suárez-Estrella, F., López, M. J., López-González, J. A., & Moreno, J. (2021). Seed biopriming with cyanobacterial extracts as an eco-friendly strategy to control damping off caused by *Pythium ultimum* in seedbeds. *Microbiological Research*, *248*, 126766. https://doi.org/10.1016/j.micres.2021.126766

Usmani, Z., Sharma, M., Awasthi, A. K., Sharma, G. D., Cysneiros, D., Nayak, S. C., … Gupta, V. K. (2021). Minimizing hazardous impact of food waste in a circular economy – Advances in resource recovery through green strategies. *Journal of Hazardous Materials*, *416*, 126154. https://doi.org/10.1016/j.jhazmat.2021.126154

Wang, G., Yang, Y., Kong, Y., Ma, R., Yuan, J., & Li, G. (2022). Key factors affecting seed germination in phytotoxicity tests during sheep manure composting with carbon additives. *Journal of Hazardous Materials*, *421*, 126809. https://doi.org/10.1016/j.jhazmat.2021.126809

Yaseen, A. A., & Takacs-Hajos, M. (2022). Evaluation of moringa (*Moringa oleifera* Lam.) leaf extract on bioactive compounds of lettuce (*Lactuca sativa* L.) grown under glasshouse environment. *Journal of King Saud University - Science*, *34*(4), 101916. https://doi.org/10.1016/J.JKSUS.2022.101916

Zulueta-Rodríguez, R., Hernández-Montiel, L. G., Murillo-Amador, B., Rueda-Puente, E. O., Capistrán, L. L., Troyo-Diéguez, E., & Córdoba-Matson, M. V. (2015). Effect of hydropriming and biopriming on seed germination and growth of two Mexican fir tree species in danger of extinction. *Forests*, *6*(9), 3109–3122. https://doi.org/10.3390/f6093109