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EFFECT OF COMPOSTABLE PLASTIC-DERIVED COMPOST AND COMPOST EXTRACT ON GERMINATION INDEX OF MUSTARD (*Brassica nigra*), CUCUMBER (*Cucumis sativus*), AND DWARF PAK CHOY (*Brassica rapa*)

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ABSTRACT

The environmental impact of plastic waste has encouraged the integration of biopolymer-based materials into vermicomposting systems to harness their biodegradability and reduce waste generation. Inefficient plastic disposal remains a major environmental concern, underscoring the value of biopolymer recycling as a sustainable approach to minimise pollution and support eco-friendly waste management. However, the potential use of biopolymer-amended vermicast as an agricultural fertiliser or soil amendment remains insufficiently explored. To address this, a 180-day composted vermicast was prepared using bedding, earthworms, kitchen waste, and biopolymer bags. The resulting solid compost and its extract were evaluated alongside three control treatments: Garden soil and its extract as positive controls, and distilled water as a negative control. Furthermore, germination bioassays were performed using *Cucumis sativus*, *Brassica rapa*, and *Brassica nigra* due to their high sensitivity, rapid germination, and distinct root morphology. Five seeds of each species were incubated per treatment on Petri dishes at 25°C for 72 hours, with germination and root elongation recorded every 24 hours. In addition, the Germination Index (GI) values were 82% ± 18.9%, 89% ± 30.6%, and 82% ± 84.2% in solid compost, and 114% ± 23.4%, 77% ± 33.2%, and 37% ± 32.4% in compost extract for *C. sativus*, *B. rapa*, and *B. nigra*, respectively. Overall, no significant differences were observed between treatments and controls, indicating that biopolymer-based vermicast did not inhibit seed germination. These findings also highlight the potential of biopolymer-integrated vermicompost for agricultural use and its contribution to sustainable environmental management.

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Introduction

One major global concern is the accumulation of plastic waste that persists in the environment. In 2019 alone, global plastic production was estimated at approximately nine billion metric tons, and this amount is projected to increase nearly sixfold by the mid-21st century (Zalasiewicz *et al.*, 2019). The growing plastic-disposal issue has further intensified environmental challenges worldwide. Consequently, biopolymer technology has emerged as a sustainable alternative to conventional petroleum-based plastics, offering a potential pathway to reduce the persistence of non-degradable plastic waste (Kabir *et al.*, 2020). Biopolymers are synthesised from monomeric units derived from renewable biological resources to form polymeric materials (Inamuddin *et al.*, 2019). In this regard, composting is a sustainable method for disposing of these biopolymer plastics.

Vermicomposting is the biological degradation of organic matter mediated by earthworms and indigenous microorganisms, resulting in a complex, and diverse microbial community and ultimately yielding nutrient-enriched vermicast (Pathma & Sakthivel, 2012). The bacterial and fungal communities in the system break down polymer chains into simpler molecules such as monomers and oligomers, through enzymatic activity (Enebe & Erasmus, 2023). Under aerobic conditions, available carbon sources convert into carbon dioxide (CO_2), humic substances, and water (Nizamuddin *et al*., 2024), thereby increasing the rate of degradation. This process supports a circular green economy by enabling vermicompost as an effective biofertiliser and a sustainable approach to environmental restoration (Ge *et al*., 2016; Liang *et al*., 2017; Luo *et al.*, 2018). According to Wang *et al.* (2024), biopolymer bags are degraded effectively when introduced as composting substrates. However, the potential phytotoxicity of such materials in composting systems remains insufficiently explored.

The most widely used highly biodegradable and renewable biopolymers are polylactic acid (PLA), polyhydroxyalkanoates (PHA), and starch-derived bioplastics (Fan & Fu, 2016). Despite their advantages, the use of biopolymer-based vermicompost in agricultural applications remains limited. Accordingly, this study aims to evaluate the phytotoxicity of these materials to assess and minimise potential environmental risks before their direct application in agricultural fields (Wang *et al.*, 2022). In this regard, Unmar and Mohee (2008) reported no detrimental effects on plant growth when composts amended with biopolymer plastics were introduced. In contrast, Kopeć *et al.* (2013) classified biopolymers as slightly toxic (toxicity class III) based on experimental observations. Similarly, Bandini *et al.* (2022) demonstrated that using biopolymer-based vermicompost could negatively affect soil quality.

Various techniques can assess compost stability and maturity. Among these, Germination Index (GI) is a reliable approach commonly employed as a biological indicator (Antil *et al.*, 2014). This technique measures seed germination and root elongation to evaluate compost maturity. A GI value greater than 80% indicates non-toxicity and suitability for agricultural application (Zucconi *et al.*, 1981). However, the recent organic fertiliser standard (NY/T525-2021) specifies a minimum acceptable value of 70% (Du *et al.*, 2024b; Kong *et al.*, 2024). Given that this study involves the degradation of biopolymer plastics within vermicomposting systems, maintaining compost quality,

particularly the maturity, and safety of the final product for agricultural use, is essential.

To address this, evaluating the phytotoxicity of biopolymer-based vermicompost using the GI is important. For example, suitable seed selection is essential for phytotoxicity assays since different plant species vary in sensitivity (Yang *et al.*, 2021), germination rate, and availability. In a previous study by Yang *et al.* (2021), *Cucumis sativus* seeds recorded the highest sensitivity among the 17 seed species tested. Meanwhile, *Brassica rapa* and *Brassica nigra* achieved GI values above 80%, displayed distinctive root morphologies, and exhibited fast growth rates, making them suitable indicator species. Therefore, this study aims to determine GI using solid and compost extracts of biopolymer-based vermicompost, with *C. sativus*, *B. rapa*, and *B. nigra* selected as indicator species. It is hypothesised that the compost would not inhibit seed germination, although phytotoxicity may vary between solid and liquid compost treatments.

Materials and Methods

Compost Materials

The treatment consists of earthworms, biopolymer bags, bedding, and kitchen waste. *Eudrilus eugeniae* were procured from Growing Bean Worm Farm 1 (Melaka, Malaysia). Earthworms were acclimatised for seven days before being introduced into the composting tank: 150 worms were introduced at the initial stage of composting, and an additional 30 worms were introduced on day 120. The biopolymer bags were bought from Winstar Material Sdn. Bhd. Johor, Malaysia, and are certified under SIRIM ECO 001:2018 (license number EL000145). Three biopolymer bags (45 cm × 25 cm) were cut into six pieces each before being placed in the middle layer of the compost. The bedding comprised corrugated cardboard, cocopeat, cow dung, spent mushroom substrate, and dry leaves (*Agathis robusta*). The selected kitchen wastes were green bell pepper, cabbage, and coffee waste. Prior to introducing the kitchen waste into the composting tank, it was chopped, frozen, thawed, and squeezed to remove excess moisture. The compost underwent composting for 180 days, with an initial ratio of 30–40:1. Following this, the temperature was maintained between 21°C and 29°C, and moisture content was regulated between 40% and 60%. The composting substrate was manually turned every two days to aerate the tanks. Accordingly, Electrical Conductivity (EC) was 1.03 mS cm^{-1}, pH was 6.93, carbon content was 36.66%, nitrogen content was 2.07%, and the C/N ratio was 17.69. These were recorded at the end of the composting period, suggesting that the vermicast had reached a mature, stable stage.

Seed Morphology and Germination Test

The compost phytotoxicity was assessed using solid compost and its extract, prepared at a 1:10 (w/v) dilution in distilled water, to provide a more comprehensive evaluation of compost quality. This provides a clearer understanding of compost behaviour under practical conditions, whether applied as solid amendments or liquid biofertilisers (Briton & Tränkner, 1996). Three crop seeds (*C. sativus*, *B. rapa*, and *B. nigra*) were selected for the germination assay due to their sensitivity to phytotoxic compounds (Hase & Kawamura, 2012; Jankauskienė *et al.*, 2022), root morphology, and ease of availability.

The seed germination assay was performed following the method suggested by Pei *et al.* (2023), with slight modifications. For the negative control, five seeds of each plant species were incubated in each Petri

dish containing 10 mL of distilled water. Each treatment was conducted in triplicate and incubated in the dark at 25°C for 72 hours (Memmert Incubator IN110, Memmert GmbH, Germany). Seed germination and root growth length were measured every 24 hours for three continuous days using a Zeiss Stemi 305 microscope (Carl Zeiss AG, Oberochen, Germany) equipped with a 1.5-megapixel Scope Pad-500 digital camera. The protrusion of the radicle from the seeds was considered a germinated seed (Luo *et al.*, 2018). Meanwhile, root elongation was measured from the tip to the root hair using ImageJ software (Yang *et al.*, 2021).

For the solid compost experiment, 4 g of vermicast was placed in the Petri dishes. Five seeds from each seed species were placed on the vermicast and lightly covered to ensure uniform contact and adequate spacing for proper germination within each Petri dish. All Petri dishes, prepared in triplicate, were subsequently incubated at 25°C for 72 hours in a dark environment (Memmert Incubator IN110, Memmert GmbH, Germany). Seed germination and root elongation measurements were recorded daily. The same process was applied for garden soil treatments, which served as a positive control.

To prepare the compost extract, 4 g of compost was mixed with distilled water at a 1:10 ratio (w/v), shaken at 200 strokes per minute for 10 minutes, and centrifuged at 4,000 rpm for 15 minutes. Then, 10 mL of the resulting supernatant was added to each Petri dish. As with the other treatments, five seeds of each plant species were added, with three replicates per treatment, and incubated at 25°C for 72 hours in dark conditions. Seed germination and root length were measured, and the GI was calculated using the standard formula provided:

$$GI = \frac{num.\,of\ germinated\ seeds\ in\ treatment}{num.\,of\ germinated\ seeds\ in\ control} \times \frac{root\ length\ in\ treatment\ (mm)}{root\ length\ in\ control\ (mm)} \times 100\%.$$

Additionally, seed morphology was observed for signs of phytotoxicity, based on European and Mediterranean Plant Protection Organisation (2014) guidelines.

Statistical Data Analysis

The root length data were measured using ImageJ software and reported as mean and Standard Deviation (SD). A t-test was used in PAST version 5.9 to analyse significance between the treatment and control groups. The calculated t-values for both treatment groups were compared with the t-critical value to determine significant differences between the treatment and control groups. The critical t-value represents the significance threshold, whereas the t-value represents the calculated difference in units of standard error. Statistical significance is noted as $p < 0.05$.

Results and Discussion

Seed Germination Index

After 72 hours of incubation, the GI for each plant species was calculated using total root length (mm) and seed germination frequency (Figure 1). The GI values for *C. sativus* in all controls and both treatments were 80% or higher, indicating strong germination and low phytotoxicity. Likewise, GI values in *B. rapa* were 80% or higher in all treatment and control groups, except in compost extract. In comparison, the slightly lower GI value observed for *B. rapa* and *B. nigra* in compost extract may be associated with dilution effects. For *B. nigra*, GI values were higher in distilled water and compost, and lower in

garden soil and garden soil extract (32.9% and 32.1%, respectively). Nevertheless, no significant differences were observed across controls and treatments.

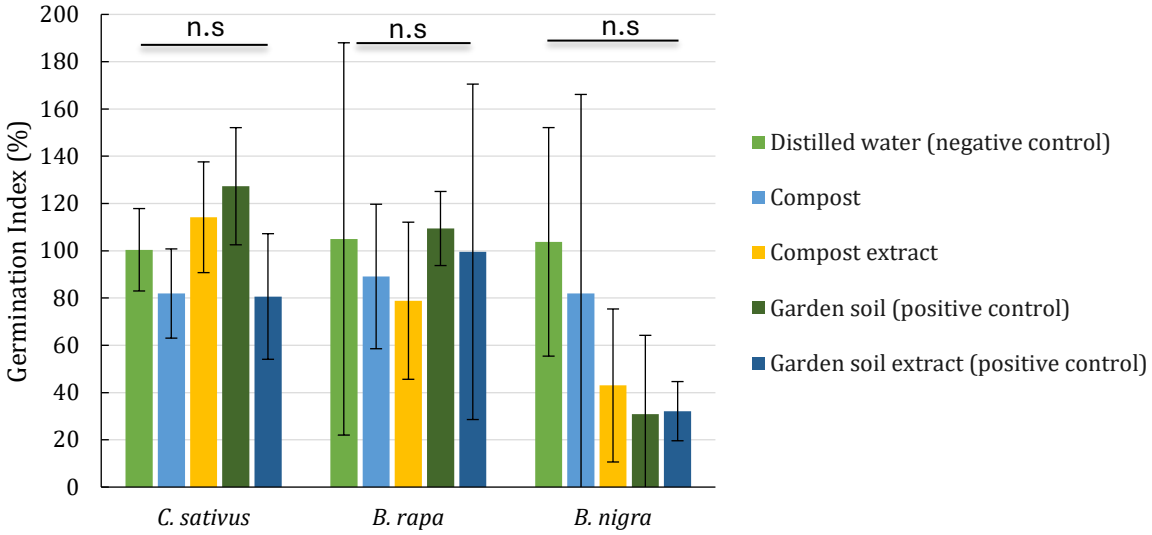

Figure 1: GI (%) of *C. sativus*, *B. rapa*, and *B. nigra* after three days of incubation
Note: Garden soil and garden soil extract are the positive controls; distilled water is the negative control (n = 3); error bars represent the SD; n.s.: not significant

Each species responded differently to compost and compost extract. In *C. sativus*, GI was higher in compost extract than in compost, whereas *B. rapa* and *B. nigra* had higher GI values in compost compared to the extract. According to Muhammad *et al.* (2015), nutrient concentration correlates with germination success. Compost generally contains higher nutrient concentrations than compost extract. Still, excessive concentration may inhibit germination, while lower nutrient levels can promote it (Gassama *et al.*, 2015), which could explain why *C. sativus* indicated better germination in compost extract than in rich compost. In contrast, *B. rapa* responded positively to nutrient-rich compost. The differences in GI value among seed species indicate that each plant has specific nutritional requirements and varying sensitivities to compost components (Milinković *et al.*, 2019). For *B. nigra*, GI values remained low even in the positive control treatment (garden soil), suggesting that this seed species is particularly sensitive to certain compounds present in the compost. On the other hand, strong germination was demonstrated by *C. sativus* and *B. rapa* in solid vermicast and its extract, compared with *B. nigra*. This supports the previous findings by Zafar *et al.* (2016) that highlight the high sensitivity of *B. nigra* to phytotoxic compounds (Zafar *et al.*, 2016), likely due to the presence of small compounds in the extract that might affect the presence of soluble nitrogen, carbon, and inorganic salts, thereby influencing GI values (Mojarad *et al.*, 2014; Du *et al.*, 2024a).

Furthermore, the *C. sativus* and *B. rapa* seeds recorded GI values above 80% in most treatments, suggesting that the compost was mature, non-toxic, and suitable for agricultural use, whether applied as solid compost or compost extract (Zucconi *et al.*, 1981; Kong *et al.*, 2024). Notably, *C. sativus*

consistently indicated the highest GI values across treatments, highlighting its potential as a reliable indicator species in compost phytotoxicity assays. Conversely, *B. nigra* recorded lower GI values in the compost extract treatment, including in the positive controls. This suggests that *B. nigra* seeds may be more sensitive during early germination, likely due to water-soluble inhibitory compounds and glucosinolates (Mazzini *et al.*, 2025). In general, the inhibition of radicle emergence observed in this species highlights the importance of using multiple plant species with different sensitivity levels to obtain a more robust and comprehensive assessment of compost quality.

The GI values from the four treatments tested on three plant species (*C. sativus*, *B. rapa*, *and B. nigra*) were analysed using a t-test (Table 1). A calculated t-value that exceeded the critical value of 2.7764 ($\alpha = 0.05$, df = 2) was considered statistically significant. However, all computed t-values were below this threshold, indicating no significant differences between the treatments and the control groups ($p > 0.05$).

Table 1: T-test analysis comparing the means of GI values (%) for the three seeds: *C. sativus*, *B. rapa*, and *B. nigra*

GI (%)	***Cucumis sativus***		***Brassica rapa***		***Brassica nigra***	
	t-value	**t-critical**	**t-value**	**t-critical**	**t-value**	**t-critical**
C/DW (-)	1.2478	2.7764	0.3113	2.7764	0.3892	2.7764
CE/DW (-)	0.8161	2.7764	0.5068	2.7764	1.8088	2.7764
C/GS (+)	0.0698	2.7764	1.0233	2.7764	0.9771	2.7764
CE/GSE (+)	0.6417	2.7764	0.4574	2.7764	0.5425	2.7764

Note: C: Compost; CE: Compost Extract; DW: Distilled Water; GS: Garden Soil; GSE: Garden Soil Extract

In addition, no significant difference in GI values was recorded between the control and treatment for *C. sativus*, *B. rapa*, and *B. nigra* (Table 1). The findings suggest that vermicast treated with biopolymer bags, whether applied as solid or extract, did not exhibit any phytotoxic effects on seed germination. This supports the hypothesis that the compost is safe for seed germination and could be used as a soil amendment or liquid fertiliser with minimal or no negative impact on plant development.

Seed morphology was evaluated to detect any potential phytotoxic effects, as seeds are especially sensitive to toxic compounds that can be absorbed through water uptake via seed pores (Parkpian *et al.*, 2002; Li *et al.*, 2021). Abnormalities observed in crop species were considered indicators of phytotoxic effects when exposed to harmful chemicals (Mitchell *et al.*, 1988; De Barro, 2008; Balestri *et al.*, 2019). Common phytotoxic symptoms include deformed cotyledons, twisted hypocotyls, missing radicles, and overall seedling deformity (Balestri *et al.*, 2019). However, this study detected no major abnormalities in

any seeds. Some seedlings had slightly bent hypocotyls, likely due to space limitations in the Petri dishes rather than phytotoxic effects (Figure 2).

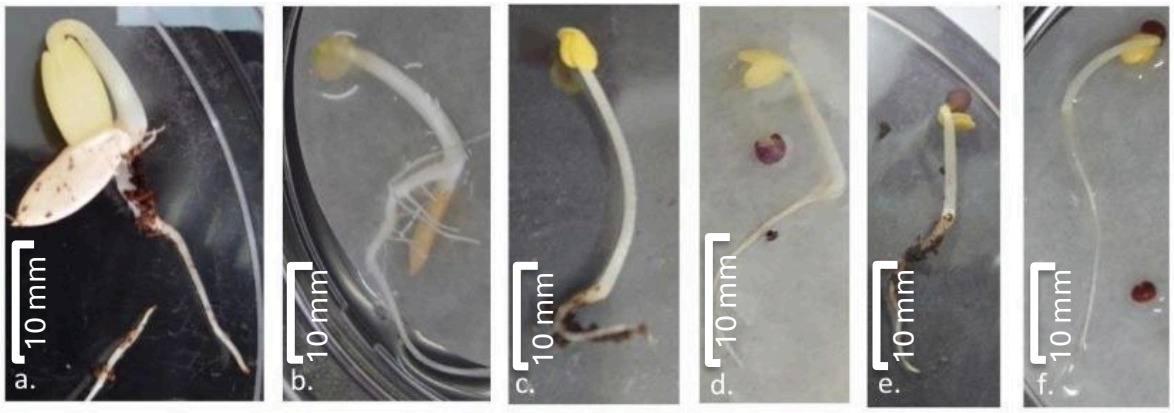

Figure 2: Seed morphology in compost and compost extract; (a) *C. sativus* in compost, (b) *C. sativus* in compost extract, (c) *B. rapa* in compost, (d) *B. rapa* in compost extract, (e) *B. nigra* in compost, and (f) *B. nigra* in compost extract

Combined with morphological observations, the biopolymer-based vermicompost indicated minimal to no phytotoxic impact on seed germination and root development. Both the solid and extract forms of the biopolymer-based vermicompost were safe for seed germination. Nevertheless, their effectiveness may differ depending on each crop species' specific nutrient demands. Overall, the findings suggest that the compost poses minimal phytotoxic risk to *C. sativus* and *B. rapa*, with performance comparable to standard garden soil. In contrast, *B. nigra* indicated a weaker response, suggesting that it may be sensitive to certain compost characteristics, which warrants further investigation. A limitation of this study was the small sample size and the restricted growth environment, which may have indirectly influenced seedling morphology. Therefore, future studies should increase replicates, expand the dataset, and include a wider range of compost extract concentrations. It is also recommended to conduct long-term experiments on plant growth and nutrient assimilation under greenhouse or field conditions.

Conclusions

The GI values effectively reflected variations among the three seed species tested, corresponding to their specific nutritional requirements for germination. The results indicate no toxic effect on seed germination with biopolymer-based vermicompost, whether applied as solid compost or extract, and its performance was comparable to garden soil and distilled water. This ultimately supports the use of biopolymer-based composts in agriculture.

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Author Contributions Statement

Huda Faqihah Rozman carried out the research and wrote the first draft of manuscript. Khooseelaa Vijaya Deventhiran provided resource, validated the finding and

reviewed the manuscript. Amirah Alias conceptualised the research, supervised the research progress, and reviewed the manuscript. All authors approved the manuscript submission.

Conflict of Interest Statement

The authors declare that no conflict of interest could have influenced the reporting of this work.

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