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# Modified atmosphere packaging influences germination and seedling growth of organic-coated cucumber (*Cucumis sativus* L.) seeds

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**Abstract:** Organic seed coating is an alternative method for supporting sustainable agriculture. This study investigates the influence of organic coating and seed storability on cucumber seeds using atmosphere-controlling techniques. The seeds were coated with an organic formulation, and a non-coated seed was used as a control. All samples were then packed using modified atmosphere packaging, including normal air, 100% $N_2$, and 100% $CO_2$, and stored under ambient conditions (30 ± 2 °C) for 8 months. Results indicated that the organic seed coating did not significantly affect seed germination compared to the non-coated seeds ($P > 0.05$) throughout the storage period. Moreover, the coating tended to positively influence seedling growth, including root and shoot lengths, seedling growth rate, chlorophyll content, and total phenolic content. Additionally, seeds packed with 100% $CO_2$ showed a slight impact on seedling growth compared to those in normal airbags, but this modified atmosphere packaging technique tended to increase chlorophyll *a* and *b*, as well as the total phenolic content in seedlings. Conversely, seeds packaged with 100% $N_2$ tended to decrease seedling lengths. Therefore, cucumber seeds coated with an organic formulation and packed in a 100% $CO_2$ bag can enhance seedling growth parameters during germination and extend seed storability.

**Keywords:** germination; organic coating ingredients; seedling growth; storability; vigour

Cucumber (*Cucumis sativus* L.) is one of the economic crops normally propagated by seed. Seeds are crucial in agriculture, alongside environmental factors and modern cultivation technologies. Selecting high-quality seeds that are resistant to environmental conditions, diseases, and pests is a key step towards successful production (Meng et al. 2024). Recently, health consciousness among consumers has been growing, increasing demand for organic agricultural products (Suksanniran et al. 2024). Consequently, organic seeds for propagation are required to incentivise the development of organic farming. However, the production of organic vegetable seeds in Thailand remains relatively limited. Furthermore, the application of appropriate technologies to enhance the value of organic vegetable seeds is still restricted.

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Currently, seed coating technology is popular among major seed producers (Siri 2015; Afzal et al. 2020). This technology helps create a unique identity for the seeds and enhances their quality. Therefore, it is essential to consider the raw materials used throughout the entire production process in the organic system. Recently, natural raw materials such as plant-derived smoke solution (SS) and vermicompost extract have emerged as key components in seed coating, providing an alternative approach to enhancing organic seed production. Additionally, further study is needed on techniques that can slow the deterioration of organic seeds, such as modified atmosphere packaging (MAP), which is still relatively under-researched. Plant-derived SS is an extract obtained from the combustion of wood (Noble 2001; Light et al. 2015). The key substances extracted belong to the butanolide group, which includes known bioactive compounds such as karrikins and cyanohydrin. These compounds can stimulate seed germination and promote plant growth. Previous research has shown that soaking seeds in wood vinegar before planting helps break seed dormancy, increases germination rates, and improves seedling growth (Khatoon et al. 2020). Vermicompost extract is another promising biostimulant for promoting seed germination and enhancing nutrient uptake by seedlings during growth. It is derived from the decomposition of organic waste or natural materials by earthworms and contains essential nutrients and growth-promoting substances such as humic acid and phytohormones, including cytokinins, auxins, abscisic acid, gibberellins, brassinosteroids, and fulvic acid (Aremu et al. 2015). Research by Amirkhani et al. (2019) found that using compost and vermicompost extracts as components in broccoli seed pelleting did not significantly increase germination rates compared with the control group, but did significantly improve seedling vigour index and nitrogen uptake.

Therefore, incorporating SS and vermicompost extract as natural bioactive agents in enhancing organic cucumber seed improvement, such as seed coating, while also focusing on preservation techniques, is crucial. One popular post-harvest technology is maintaining optimal temperatures and modifying the atmosphere within packaging (MAP), which helps extend the shelf life of seeds during sales and long-term storage (Gorris, Peppelenbos 2007). Thus, this study aims to contribute to the body of knowledge and advance organic seed production in Thailand, paving the way for future commercial expansion.

## MATERIAL AND METHODS

### Seed coating preparations and packaging for storage conditions

Cucumber seeds were procured from a source as certified by Organic Agricultural Certification Thailand (Certification Alliance, 2019). The ingredients for coating treatments were prepared using substances such as liquid vermicompost (LVC), plant-derived SS (sourced from commercial products in Organic Thailand), and gum arabic (sourced from KemAus™, Australia). The treatments comprised non-coated seeds as a control and 15% (w/w) LVC combined with 5% (w/w) SS in 30% gum arabic (GA). The coating procedure involved coating 50 g of seeds per replication, using a lab seed coater (Ceres KSC-02D, Ceres International Co., LTD, Thailand). The ratio of seeds to coating slurry was maintained at 10 g per 1 mL. Moisture content in the coated seeds was eliminated, ensuring a final moisture content of 9 ± 1% using the hot-air oven method (ISTA 2008). All coating samples were subsequently stored using the MAP technique. In brief, 10 g of seeds per replication were placed in aluminum foil bags (120 × 200 mm) with a thickness of 200 μm, and an oxygen transmission rate [$O_2$TR of < 0.01 $cm^3/(m^2 \cdot d \cdot bar)$ at 25 °C and 50% relative humidity (RH)] as well as a water vapor transmission rate [WVTR of < 0.01 $g/(m^2 \cdot d)$ at 25 °C and 100% RH] (Rosato 2013). All package bags were specified in Table 1 and stored under ambient conditions to mimic shelf life in market conditions. Seed samples were evaluated at 0, 4, and 8 months of storage.

Table 1. Conditions of coating treatment and modified atmosphere packaging (MAP) for storage under ambient conditions (30 ± 2 °C, 70 ± 5% RH)

| Coating treatments: | Modified atmosphere packaging (MAP) conditions: |
|---|---|
| Non-coated seed (control) | Normal air (atmospheric air or control condition) |
| Coated seed (15% LVC + 5% SS) | 100% $N_2$ |
| | 100% $CO_2$ |

Recorded by a data logger (TR-74Ui, T&D Cor., Nagano, Japan)

RH – relative humidity; LVC – liquid vermicompost; SS – smoke solution

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**Analysis measurement**

*Seed germination parameters.* The germination percentage was determined following the standard germination test (ISTA 2008) under laboratory conditions. First, cucumber seeds were germinated using the between-paper method, with 50 seeds per replication, for a total of four replications per treatment. The seeds were then placed in a seed germinator chamber at a temperature of 25 °C. The germination rate was recorded daily by counting the number of seedlings for 8 days. The germination percentage was calculated using the following formula:

Germination (%) = (number of normal seedlings/number of seeds germinated) × 100 (1)

*Speed of germination.* This was measured by calculating the germination index (GI). A higher GI value indicated a greater number of seedlings emerging each day, reflecting stronger seed vigour. The GI was calculated as follows:

GI = Σ (number of normal seedlings emerged each day)/number of days after sowing (2)

Mean germination time (MGT) was measured to assess the speed of germination. The MGT was calculated using the following formula:

$$MGT = \Sigma (Ti \times Ni)/\Sigma Ni \quad (3)$$

Where: $Ni$ – number of normal seedlings that emerged each day after germination; $Ti$ – time after sowing.

*Seedling growth parameters.* Cucumber seeds were germinated using the between-paper method with 25 seeds per replication for four replications. The germinated seeds were then placed in a seed germinator chamber at a temperature of 25 °C without light conditions for 7 days, as represented in Figure 1. Afterwards, the lengths of the shoots and roots of the seedlings were measured and recorded to find the average value. Subsequently, the seedlings were dried to determine the dry weight (DW) at 80 °C for 48–96 hours. A higher DW indicates that the seed batch had a greater accumulation of dry matter. The calculation formula for seedling growth rate (SGR) follows:

SGR = DW of normal seedlings/number of normal seedlings (4)

Seedling vigour index (SVI) was calculated by taking the germination percentage of the seeds germinated in a controlled environment and the measurements of shoot and root lengths of the seedlings, using the following formula:

SVI = (shoot length + root length) × percentage of seeds germinated (5)

*Phytochemical contents.* Seven-day-old seedlings were prepared in coconut coir media and grown in a germination growth chamber at 25 °C. The chlorophyll content was followed from Moran (1982) with slight modifications. Fresh leaf samples (0.5 g) were ground under liquid $N_2$ and mixed with 20 mL of *N,N*-dimethylformamide. The mixture was then allowed to stand in the dark at 4 °C overnight. Afterwards, it was filtered using the Whatman No. 1 paper. The absorbance of the solution was measured using a spectrophotometer (Genesys 10S VIS, Thermo Fisher Scientific, USA) at wavelengths of 664 and 647 nm. The obtained values were then calculated using the following equations:

$$\text{Chlorophyll } a \text{ (µg/mL)} = [(12.64\ OD_{664}) - (2.99\ OD_{647})] \quad (6)$$

$$\text{Chlorophyll } b \text{ (µg/mL)} = [(-5.6\ OD_{664}) + (23.26\ OD_{647})] \quad (7)$$

where: OD – optical density.

To convert the units from µg/mL to mg/100 g FW (fresh weight), the following formulas were used:

Chlorophyll *a* (mg/100 g FW) = [(chlorophyll *a* in µg/mL) × 20.5 × (100/0.5) × (1/1 000)] (8)

Chlorophyll *b* (mg/100 g FW) = [(chlorophyll *b* in µg/mL) × 20.5 × (100/0.5) × (1/1 000)] (9)

Total phenolic content was analysed using the Folin-Ciocalteu assay. The seedling powder (2 g) was extracted with 20 mL of 90% (v/v) methanol containing HCl (90 : 10, v/v), and the mixture was stirred with a magnetic bar for 15 minutes. The mixture was then filtered (Advantec, No.2). For the reaction mixture, 0.2 mL of solution was mixed with 2.6 mL of distilled water, 2 mL of 7% (w/v) sodium carbonate, and 0.2 mL of the Folin-Ciocalteu reagent. The mixed solution was incubated at 30 °C

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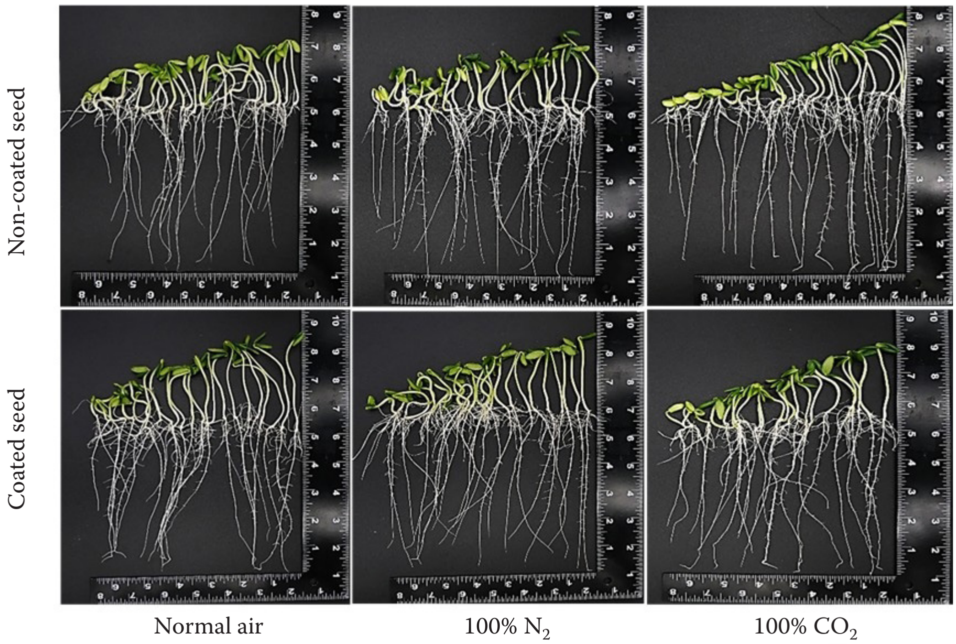


Figure 1. The appearance of 7-day-old seedlings of the non-coated and coated seeds with 15% LVC (liquid vermicompost) + 5% SS (smoke solution) and stored under normal air, 100% $N_2$, and 100% $CO_2$

for 90 minutes. The reaction was measured using a spectrophotometer at 745 nm. The absorbance was compared with values obtained from the gallic acid calibration curve (5-point, 20–100 mg/L) and reported as mg gallic acid equivalents per g fresh weight (mg GAE/g FW).

*Statistical analysis.* A factorial in a completely randomised design with four replicates was performed. The data were subjected to analysis of variance (ANOVA) using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA), and the treatment mean was compared using Tukey's HSD (honestly significant difference) test at a 95% confidence level.

## RESULTS AND DISCUSSION

### Effect of organic seed coating and modified atmosphere packaging on seed germination

The organic coating formulation for cucumber seeds was collected in a prior study (Kaenakham et al. 2023). Results indicated that the 15% (w/w) LVC combined with 5% (w/w) plant-derived SS formulation provided the highest germination and seedling growth in cucumber seeds, both after coating and during the accelerated ageing test. Therefore, this formulation was subsequently selected for packing in combination with MAP and evaluating storability in this study.

In this study, the seed coating used had no significant effect on germination during or after storage (Table 2). The effects of MAP techniques showed that all packaging methods maintained high seed germination, with no statistical differences throughout the storage period. Moreover, there was no interaction between the seed coating and MAP techniques on germination during storage (Figures 2A–2C). After the speed germination evaluation, the coated seeds had lower GI and higher MGT than the non-coated seeds after 8 months of storage (Table 2). Meanwhile, the MAP packages containing both 100% $N_2$ and 100% $CO_2$ exhibited lower germination speed than the normal airbag, as indicated by lower GI and higher MGT. An interaction between the seed coating and MAP techniques in speed germination was observed. Results revealed that the coated seeds packed in 100% $N_2$ tended to show lower GI and higher MGT values, particularly at 4 months of storage (Figures 2D–2I). This finding may be due to the coating forming a thin film that impedes the absorption of water and air into the seed coat. Similarly, various studies have reported that seed-coating techniques or the use of inappropriate materials and conditions for coating can affect water diffusion and seed germination (Taylor et al. 1998; Amirkhani et al. 2016; Qiu et al. 2020). Therefore, coating techniques can slow the emergence of seedlings compared to non-coated seeds. However, the

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Table 2. Germination (G) (%), germination index (GI), and mean germination time (MGT) of cucumber seeds stored in modified atmosphere packaging (MAP) for 8 months under ambient conditions (30 ± 2 °C)

| Specification | G (%) | | | GI | | | MGT (day) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 months | 4 months | 8 months | 0 months | 4 months | 8 months | 0 months | 4 months | 8 months |
| **Coating treatment (C)** | | | | | | | | | |
| Non-coated seed | 99.3 ± 1.3 | 98.8 ± 1.3 | 98.7 ± 1.3 | 13.8 ± 1.4 | 13.9 ± 0.7 | 15.9 ± 2.2$^{a}$ | 3.9 ± 0.4 | 3.9 ± 0.2 | 3.5 ± 0.2$^{b}$ |
| Coated seed | 98.0 ± 2.3 | 97.2 ± 3.1 | 96.3 ± 7.4 | 13.2 ± 1.0 | 13.1 ± 1.1 | 13.5 ± 1.0$^{b}$ | 3.9 ± 0.4 | 3.8 ± 0.3 | 3.7 ± 0.2$^{a}$ |
| **Modified atmosphere packaging (MAP)** | | | | | | | | | |
| Normal air | 99.3 ± 1.0 | 98.5 ± 2.1 | 94.7 ± 8.8 | 14.6 ± 1.1$^{a}$ | 14.1 ± 0.6 | 14.7 ± 2.4 | 3.6 ± 0.1$^{b}$ | 3.7 ± 0.1$^{b}$ | 3.5 ± 0.2$^{b}$ |
| 100% $N_2$ | 98.5 ± 2.8 | 97.7 ± 3.5 | 98.5 ± 1.8 | 12.9 ± 0.9$^{b}$ | 12.5 ± 0.9 | 14.2 ± 1.2 | 4.1 ± 0.4$^{a}$ | 4.2 ± 0.2$^{a}$ | 3.7 ± 0.2$^{a}$ |
| 100% $CO_2$ | 98.3 ± 1.7 | 97.7 ± 2.0 | 99.3 ± 1.0 | 13.0 ± 1.0$^{b}$ | 13.9 ± 0.8 | 15.3 ± 2.6 | 4.1 ± 0.2$^{a}$ | 3.7 ± 0.1$^{b}$ | 3.5 ± 0.2$^{ab}$ |
| **ANOVA** | | | | | | | | | |
| C | 0.09$^{ns}$ | 0.1$^{ns}$ | 0.3$^{ns}$ | 0.2$^{ns}$ | 0.05$^{ns}$ | 0.002$^{**}$ | 0.7$^{ns}$ | 0.3$^{ns}$ | 0.001$^{**}$ |
| MAP | 0.6$^{ns}$ | 0.8$^{ns}$ | 0.2$^{ns}$ | 0.008$^{**}$ | 0.000$^{***}$ | 0.5$^{ns}$ | 0.001$^{**}$ | 0.000$^{***}$ | 0.04$^{*}$ |
| C × MAP | 0.3$^{ns}$ | 0.3$^{ns}$ | 0.2$^{ns}$ | 0.02$^{*}$ | 0.000$^{***}$ | 0.05$^{*}$ | 0.02$^{*}$ | 0.000$^{***}$ | 0.000$^{***}$ |

$^{*}$,$^{**}$, $^{***}$significant levels at $P < 0.05$, 0.01, 0.001, respectively; ns – non-significant

$^{a,b}$means ± SD followed by different letters within the column per parameter and storage time are significantly different according to Tukey's HSD Test ($P < 0.05$)

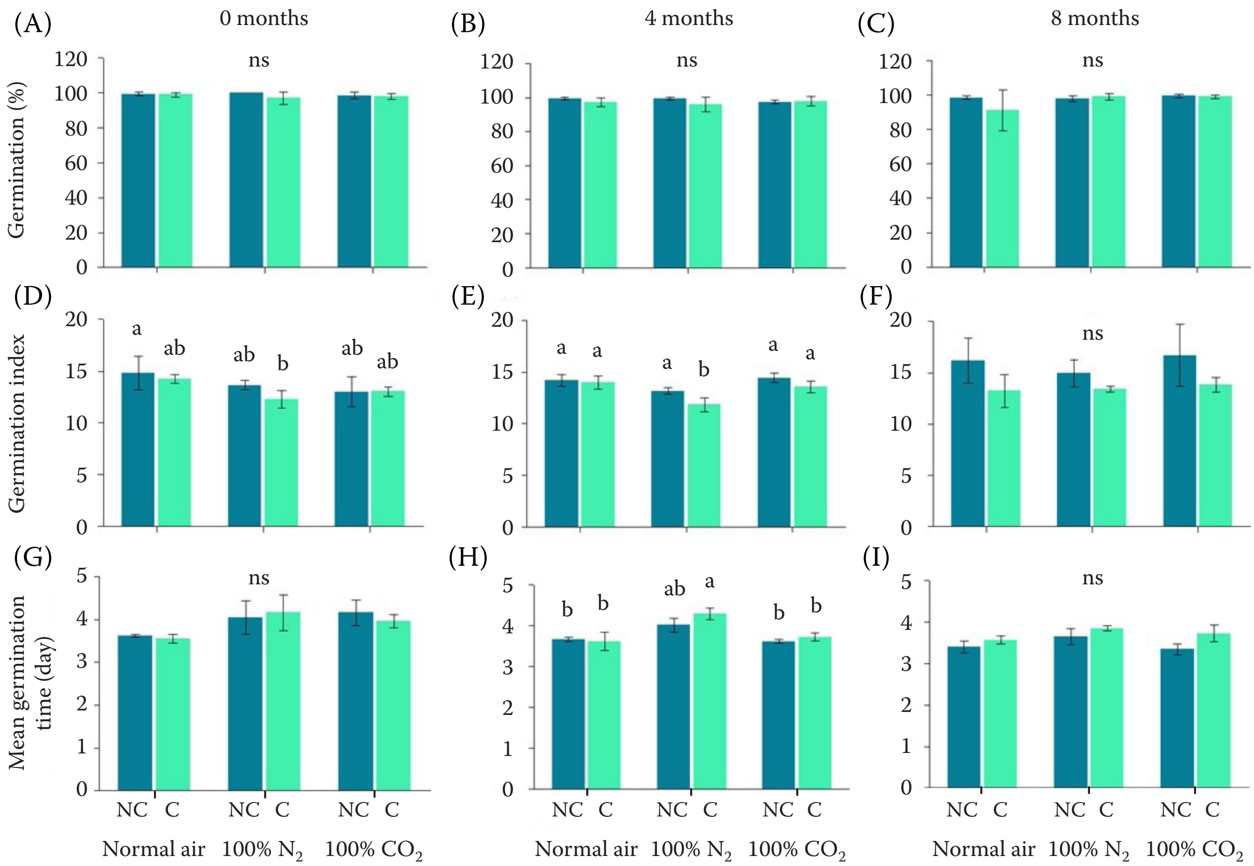


Figure 2. (A–C) Germination percentage, (D–F) germination index, and (G–I) mean germination time at 0 to 8 months of the non-coated (NC) and coated (C) seeds and stored in normal air, 100% $N_2$, and 100% $CO_2$ packaging

$^{a,b}$means ± SD ($n$ = 4) with different lowercase letters within the groups are significantly different according to Tukey's HSD Test ($P < 0.05$); ns – non-significant

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MGT of both seed samples differed by less than one day on average, taking approximately 3.5–3.8 days. In the final germination assessment, the number of normal seedlings from coated seeds was not significantly different from that of non-coated seeds, indicating that the organic seed coating formula can be applied to cucumber seeds without affecting germination percentage during 8 months of storage.

**Effect of organic seed coating and modified atmosphere packaging on seedling growth**

The appearance of 7-day-old seedlings revealed no visually apparent differences among the tested samples (Figure 1). Nevertheless, after measuring seedling growth, the coating formulation promoted cucumber seedling growth during 8 months of storage. Results showed that the coated seeds tended to have longer root and shoot lengths, higher SGR, and higher SVI than the non-coated seeds at the initial period (Table 3). After storage for 4 and 8 months, there were no significant differences in seedling growth parameters between the coated and non-coated seeds. Similar positive effects were reported in studies in which soy flour mixed with micronised vermicompost enhanced various parameters in coated red clover and perennial ryegrass seeds compared to non-coated seeds (Qiu et al. 2020). According to Hussain and Abbasi (2018), the application of vermicompost with improper concentrations may adversely affect plant growth if nutrients are provided in excess of the plant's requirements. Moreover, the resulting salinity from excessive nutrient levels may impede water uptake by seeds, subsequently obstructing their germination, speed of germination, and plant growth (Kaveh et al. 2011).

The results of packaging coated seeds using MAP techniques varied under different atmospheric conditions during storage. Seedlings obtained from seeds stored for 4 months in bags with normal air and 100% $N_2$ exhibited the highest average root and shoot lengths, while the shortest seedlings were obtained from seeds stored in 100% $CO_2$. Conversely, after 8 months of storage, the 100% $CO_2$ bag provided the longest seedling length, followed by normal air packaging, while the 100% $N_2$ bag displayed the shortest average seedling length (Table 3). Additionally, the study evaluated the efficacy of coating and the ability to store seeds using MAP techniques (Figure 3). From this research, both coated and non-coated cucumber seeds packaged in MAP with 100% $N_2$ tended to show improved root and shoot lengths as well as SVI during storage for 4 months (Figures 3B, 3E, and 3K). In contrast, this condition had an adverse impact on root and shoot lengths, as well as SVI during storage for 8 months (Figures 3C, 3F, and 3L). Meanwhile, seed samples packed in 100% $CO_2$ showed seedling growth parameters similar to those in normal air packaging. This indicates that the coating formulation contains active substances and, when combined with MAP techniques, does not adversely affect cucumber germination and seedling growth.

Generally, storing seeds for an extended period adversely impacts seed viability, decreasing seedling growth parameters compared to the beginning of storage. This is because seed quality changes over time at the cytological, physiological, biochemical, and physical levels, leading to a loss of vigour, germination, and viability. The rate of seed deterioration varies based on factors such as individual seed characteristics, cultivar, species, storage duration, seed history, and storage environment (Sundareswaran et al. 2023). Therefore, to delay seed deterioration, MAP technology is recommended as an alternative to chemical treatments, as it is safe and environmentally friendly, helping maintain quality and extend the shelf life of organic seeds. Moreover, the excellent barrier properties of the packaging can reduce seed deterioration, even after more than 8 months of storage under ambient conditions. Additionally, MAP promotes better seedling growth and extends seed shelf life. Seeds can be stored with 100% $N_2$ or 100% $CO_2$ under normal room conditions. For MAP to extend or delay the deterioration of seed quality, Lamani et al. (2020) reported a study on the storage capability of onion (*Allium cepa* L.) seeds (variety 'Arka Kalyan'). The seeds were stored under MAP with mixed $CO_2$, $O_2$, and $N_2$ in 15 different proportions. They found that the onion seeds maintained significantly different qualities throughout the storage period. Seeds stored in cold storage maintained the highest quality in various aspects, with the lowest levels of seed-borne pathogens, seed moisture, and electrical conductivity. Next, the treatment was with 80% $CO_2$, 5% $O_2$, and 15% $N_2$, followed by seeds stored in vacuum packaging. Similarly, Shinde and Hunje (2024) reported a study on Kabuli chickpeas, an important legume crop. One of the limitations reducing the production of Kabuli chickpea seeds is the rapid deterioration process due to their thin and brittle seed coats. The study investigated the effects of modified atmosphere conditions on the germination ability and health of Kabuli chickpea seeds.

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Table 3. Vegetative growth parameters, including root and shoot lengths, seedling growth rate (SGR), and seedling vigour index (SVI) of cucumber seeds stored in modified atmosphere packaging (MAP) for 8 months under ambient conditions (30 ± 2 °C)

| Specification | Root length (cm) | | | Shoot length (cm) | | | SGR (mg/plant) | | | SVI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 months | 4 months | 8 months | 0 months | 4 months | 8 months | 0 months | 4 months | 8 months | 0 months | 4 months | 8 months |
| **Coating treatment (C)** | | | | | | | | | | | | |
| Non-coated seed | 14.2 ± 0.3$^{b}$ | 13.7 ± 2.4 | 9.6 ± 2.9 | 6.1 ± 0.6$^{b}$ | 5.4 ± 1.2 | 5.6 ± 1.8 | 6.4 ± 0.5$^{b}$ | 13.2 ± 0.7 | 13.6 ± 0.7 | 2 002.5 ± 92.5 | 1 856.5 ± 98.2 | 1 428.8 ± 93.8 |
| Coated seed | 14.7 ± 0.5$^{a}$ | 13.9 ± 1.3 | 11.2 ± 1.9 | 6.7 ± 0.6$^{a}$ | 5.2 ± 0.4 | 5.9 ± 1.1 | 7.0 ± 0.6$^{a}$ | 13.4 ± 0.8 | 13.4 ± 0.8 | 2 021.8 ± 97.1 | 1 784.9 ± 85.9 | 1 549.4 ± 91.6 |
| **Modified atmosphere packaging (MAP)** | | | | | | | | | | | | |
| Normal air | 14.5 ± 0.5 | 14.9 ± 1.2$^{a}$ | 11.5 ± 1.5$^{a}$ | 6.4 ± 0.7 | 5.5 ± 0.4$^{a}$ | 7.1 ± 0.8$^{a}$ | 6.7 ± 0.5 | 12.8 ± 0.7 | 13.2 ± 0.7 | 1 992.3 ± 94.5 | 1 924.9 ± 90.0$^{a}$ | 1 658.8 ± 90.1$^{a}$ |
| 100% $N_2$ | 14.5 ± 0.5 | 14.4 ± 1.4$^{a}$ | 7.5 ± 1.6$^{b}$ | 6.4 ± 0.7 | 5.9 ± 0.7$^{a}$ | 4.1 ± 0.6$^{c}$ | 7.0 ± 0.6 | 13.7 ± 0.9 | 13.8 ± 0.6 | 2 024.5 ± 90.9 | 1 937.7 ± 83.9$^{a}$ | 1 092.6 ± 79.3$^{b}$ |
| 100% $CO_2$ | 14.5 ± 0.5 | 12.0 ± 1.7$^{b}$ | 12.1 ± 1.3$^{a}$ | 6.4 ± 0.7 | 4.4 ± 0.5$^{b}$ | 5.6 ± 0.7$^{b}$ | 6.4 ± 0.7 | 13.5 ± 0.4 | 13.5 ± 0.9 | 2 019.6 ± 94.4 | 1 599.6 ± 90.1$^{b}$ | 1 716.0 ± 77.8$^{a}$ |
| **ANOVA** | | | | | | | | | | | | |
| C | 0.01* | 0.7$^{ns}$ | 0.1$^{ns}$ | 0.02* | 0.5$^{ns}$ | 0.6$^{ns}$ | 0.02* | 0.5$^{ns}$ | 0.5$^{ns}$ | 0.6$^{ns}$ | 0.5$^{ns}$ | 0.4$^{ns}$ |
| MAP | 1.0$^{ns}$ | 0.001** | 0.000*** | 1.0$^{ns}$ | 0.000*** | 0.000*** | 0.1$^{ns}$ | 0.04$^{ns}$ | 0.3$^{ns}$ | 0.8$^{ns}$ | 0.01* | 0.000*** |
| C × MAP | 0.3$^{ns}$ | 0.000*** | 0.000*** | 0.5$^{ns}$ | 0.000*** | 0.000*** | 0.04* | 0.1$^{ns}$ | 0.5$^{ns}$ | 0.9$^{ns}$ | 0.000*** | 0.000*** |

*,**, ***significant levels at $P < 0.05$, 0.01, 0.001, respectively; ns – non-significant

[a,b,c]means ± SD followed by the different letters within the column per parameter and storage time are significantly different according to Tukey's HSD Test ($P < 0.05$)

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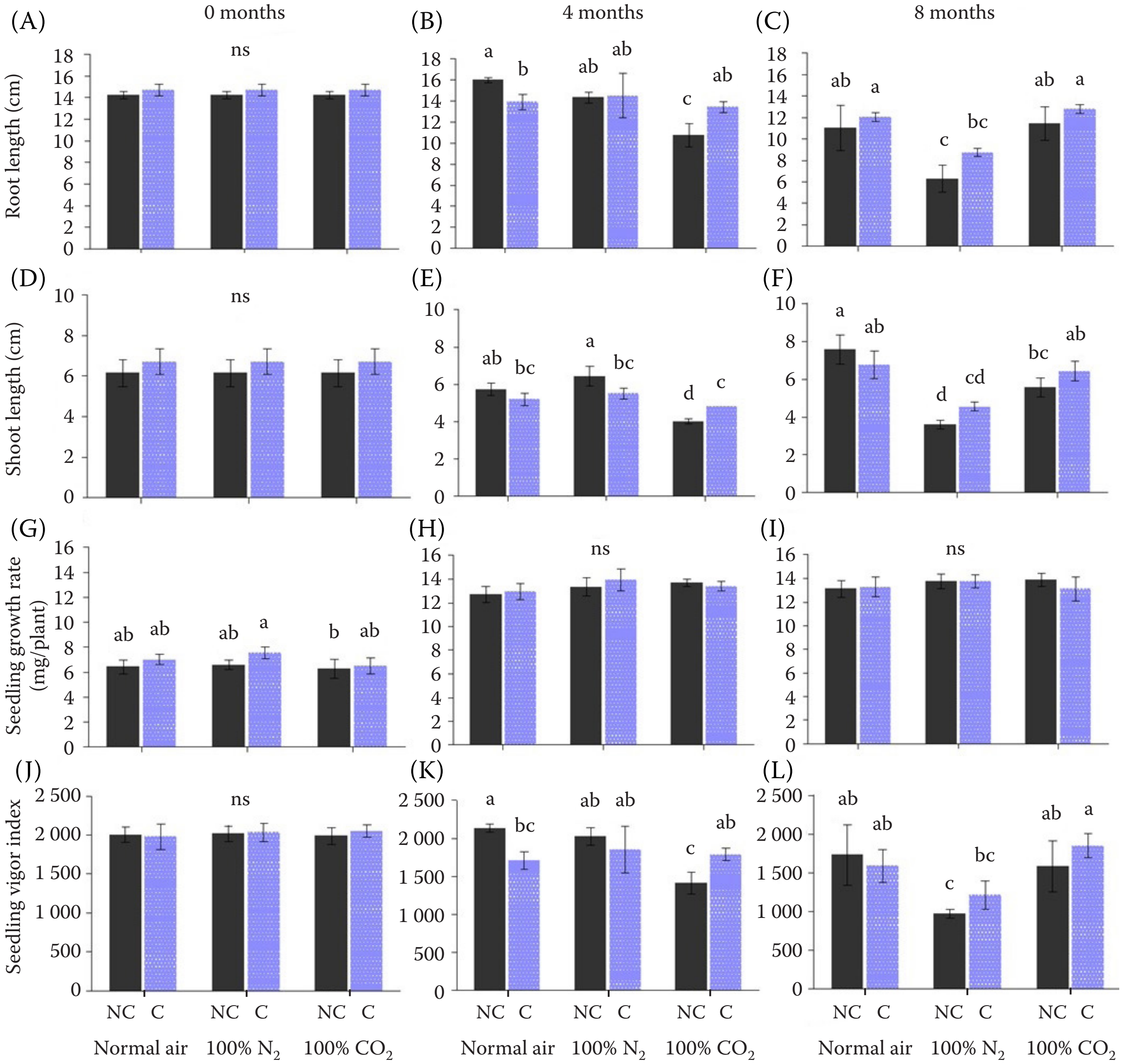


Figure 3. Vegetative growth of the 7-day-old seedlings, including (A–C) root length, (D–F) shoot length, (G–I) seedling growth rate, and (J–L) seedling vigour index at 0 to 8 months of the non-coated (NC) and coated (C) seeds and stored in normal air, 100% $N_2$, and 100% $CO_2$ packaging

[a–d]means ± SD ($n$ = 4) with different lowercase letters within the groups are significantly different according to Tukey's HSD Test ($P < 0.05$); ns – non-significant

**Effect of organic seed coating and modified atmosphere packaging on phytochemical contents in seedlings**

Coating cucumber seeds with the organic formulation, which included 15% LVC and 5% SS, tended to increase chlorophyll content and total phenolic compounds in seedlings compared to non-coated seeds (Table 4). Meanwhile, the MAP containing 100% $CO_2$ tended to increase chlorophyll content, particularly at 4 months of storage. Notably, the coated seeds packed in 100% $CO_2$ revealed the highest levels of chlorophyll *a* and *b,* as well as total phenolic compounds (Figures 4B, 4E, 4H, and 4I).

The application of vermicompost for coating cucumber seeds is beneficial as it is derived from animal manure composted by earthworms, providing essential nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, and micronutrients. Additionally, it contains plant hormones, enzymes, and beneficial microorganisms that promote plant growth (Aremu et al. 2015; Hagh et al. 2016; Ozyazici, Turan 2021). According to Aremu

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Table 4. Phytochemical compounds, including chlorophyll *a*, chlorophyll *b*, and total phenolic content of cucumber seeds stored in modified atmosphere packaging (MAP) for 8 months under ambient conditions (30 ± 2 °C)

| Specification | Chlorophyll *a* (mg/100 g FW) | | | Chlorophyll *b* (mg/100 g FW) | | | Total phenolic content (mg GAE/g FW) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 months | 4 months | 8 months | 0 months | 4 months | 8 months | 0 months | 4 months | 8 months |
| **Coating treatment (C)** | | | | | | | | | |
| Non-coated seed | 10.4 ± 0.7$^{b}$ | 15.5 ± 1.5 | 15.9 ± 1.8 | 3.6 ± 0.2 | 5.4 ± 1.5 | 5.9 ± 0.6 | 0.07 ± 0.0$^{b}$ | 0.08 ± 0.0$^{b}$ | 0.08 ± 0.0$^{b}$ |
| Coated seed | 11.4 ± 0.5$^{a}$ | 15.7 ± 2.3 | 14.5 ± 1.6 | 3.7 ± 0.5 | 5.4 ± 1.7 | 5.4 ± 1.1 | 0.08 ± 0.0$^{a}$ | 0.10 ± 0.0$^{a}$ | 0.09 ± 0.0$^{a}$ |
| **Modified atmosphere packaging (MAP)** | | | | | | | | | |
| Normal air | 10.9 ± 0.8 | 14.8 ± 1.0$^{b}$ | 14.6 ± 1.7 | 3.7 ± 0.4 | 4.8 ± 0.4$^{b}$ | 5.5 ± 1.4 | 0.07 ± 0.0 | 0.08 ± 0.0 | 0.08 ± 0.0 |
| 100% $N_2$ | 10.9 ± 0.8 | 14.7 ± 1.0$^{b}$ | 15.9 ± 2.2 | 3.7 ± 0.4 | 4.1 ± 0.5$^{b}$ | 5.9 ± 0.8 | 0.08 ± 0.0 | 0.09 ± 0.0 | 0.07 ± 0.0 |
| 100% $CO_2$ | 10.8 ± 0.8 | 17.4 ± 2.2$^{a}$ | 15.1 ± 1.4 | 3.7 ± 0.4 | 7.4 ± 0.8$^{a}$ | 5.6 ± 0.5 | 0.08 ± 0.0 | 0.10 ± 0.0 | 0.08 ± 0.0 |
| **ANOVA** | | | | | | | | | |
| C | 0.000*** | 0.8$^{ns}$ | 0.05$^{ns}$ | 0.7$^{ns}$ | 0.7$^{ns}$ | 0.1$^{ns}$ | 0.006** | 0.01* | 0.03* |
| MAP | 1.0$^{ns}$ | 0.002** | 0.3$^{ns}$ | 1.0$^{ns}$ | 0.000*** | 0.8$^{ns}$ | 1.0$^{ns}$ | 0.1$^{ns}$ | 0.09$^{ns}$ |
| C × MAP | 0.04* | 0.01* | 0.2$^{ns}$ | 0.9$^{ns}$ | 0.000*** | 0.6$^{ns}$ | 0.2$^{ns}$ | 0.002** | 0.004** |

*,**, ***significant levels at $P < 0.05$, 0.01, 0.001, respectively; ns – non-significant

[a,b]means ± SD followed by the different letters within the column per parameter and storage time are significantly different according to Tukey's HSD Test ($P < 0.05$)

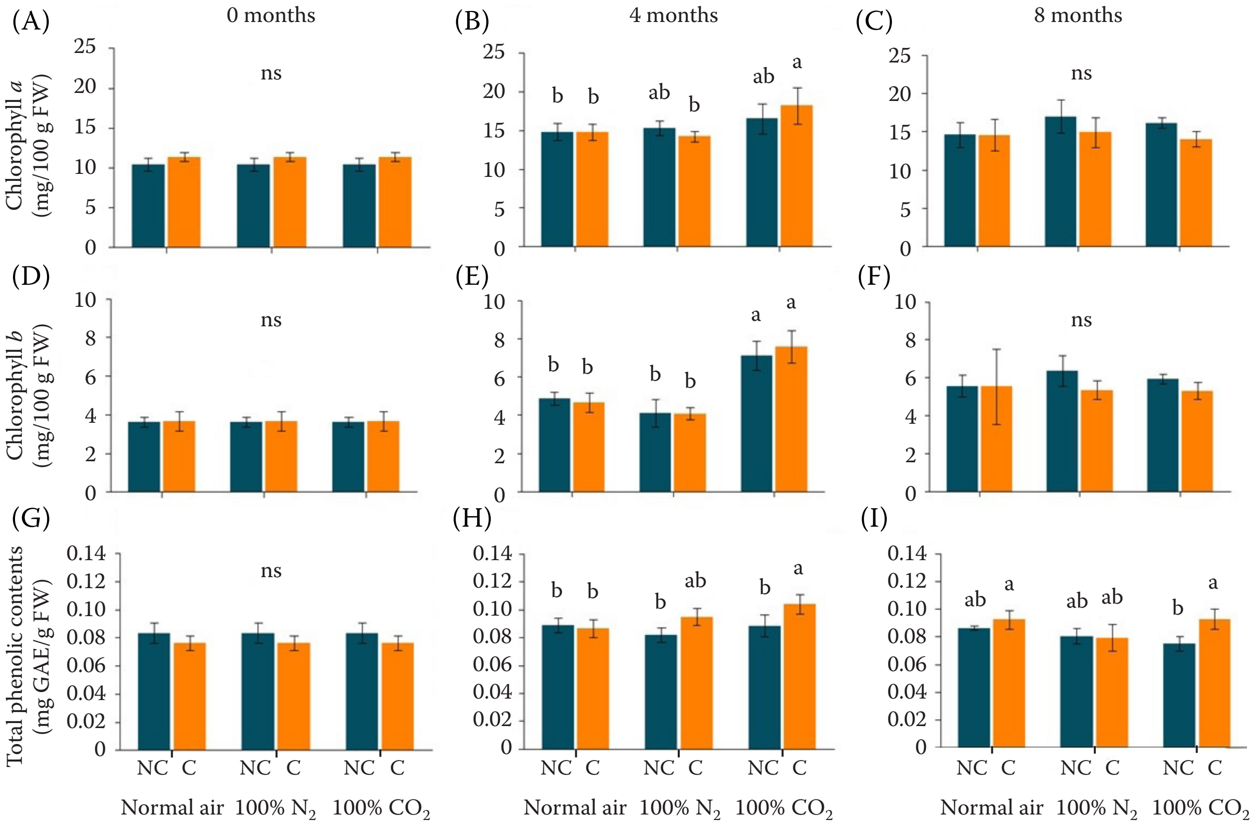


Figure 4. Phytochemical compounds of the 7-day-old seedlings, including (A–C) chlorophyll *a*, (D–F) chlorophyll *b*, and (G–I) total phenolic content at 0 to 8 months of the non-coated (NC) and coated (C) seeds and stored in normal air, 100% $N_2$, and 100% $CO_2$ packaging

[a,b]means ± SD ($n = 4$) with different lowercase letters within the groups are significantly different according to Tukey's HSD Test ($P < 0.05$); ns – non-significant

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et al. (2012), smoke-water (SW) and smoke-derived karrikinolide (KAR1) enhance plant defence systems by increasing the amounts of flavonoids, tannins, and phenolic compounds in the tissue culture of banana. This outcome may be due to the active compounds in SW, especially at low concentrations, acting similarly to cytokinins and auxins, which play roles in growth and pigment synthesis, including chlorophyll *a* and *b*, and in phenolic content. The roles of substances in SW and KAR1, which elicit plant responses similar to those triggered by plant growth regulators, likely result from their induction of the synthesis of bioactive compounds (Jain et al. 2008; Chiwocha et al. 2009). Recent studies indicate that genes related to the synthesis of phenylpropanoids and flavonoids, which are derivatives of the phenolic compound synthesis pathway, are upregulated following the application of SW and KAR1 (Soós et al. 2010). Moreover, previous research has reported that vermicompost leachate affects chlorophyll accumulation in seedlings, as it contains essential nutrients for chlorophyll synthesis, such as nitrogen, magnesium, and iron. These nutrients stimulate seedlings to produce more chlorophyll *a* and *b*, enhancing photosynthesis. Furthermore, the plant hormones in vermicompost leachate promote seedling growth by stimulating cell division and elongation, resulting in larger leaves and stronger stems. Beneficial microorganisms decompose organic matter in the soil, releasing nutrients plants need, thereby supporting the robust growth of seedlings with green leaves and strong stems. According to Voko et al. (2022), the effects of different biostimulant formulations under various irrigation regimes on the photosynthetic pigment content of *Vigna unguiculata* over 13 weeks indicated that biostimulant formulations consistently maintained higher chlorophyll *a* levels.

## CONCLUSION

In summary, the research results indicate that all treatments applied to cucumber seeds during storage can be utilised without affecting seed germination. However, to promote good seedling growth during germination, seeds should be coated with an organic coating formulation. Additionally, to extend the shelf life of the seeds, they can be packaged with 100% $CO_2$ gas, which can be stored at ambient temperature. Nevertheless, testing the efficiency of such packaging over a longer period is advisable to obtain more precise conclusions on the effects of organic coating and MAP.

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