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Research article

Seed Coating with Fungicidal Agents: Enhancing Quality, Storability, and *Fusarium* sp. Inhibition in Vegetable Soybean Seeds

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Abstract

This study evaluated the effects of fungicidal seed coating on *Fusarium* sp. inhibition, seed quality, and seedling growth in vegetable soybeans. Laboratory tests showed that mancozeb, prochloraz, and carboxin completely inhibited fungal growth for nine days, whereas thiram, captan, and metalaxyl-M provided only partial suppression. Among the tested fungicides, prochloraz was most effective in maintaining seed germination and seedling vigor. In seed coating trials, prochloraz applied at 6 g of active ingredient (g.ai.) increased shoot length to 26.5 cm and root length to 21.3 cm under sand test conditions, while mancozeb and carboxin produced similar but slightly lower values. Seedling survival reached up to 87% with prochloraz at 4 to 6 g.ai., compared with only 65% in uncoated seeds. Storage experiments demonstrated that prochloraz-coated seeds maintained germination above 85% for up to 4 months under controlled conditions, whereas uncoated seeds dropped below 70%. Under ambient conditions, germination of all treatments declined after 4 months, but coated seeds still performed better than untreated ones. Overall, prochloraz at 6 g.ai. was identified as the most effective treatment for improving germination, seedling vigor, and disease resistance, particularly in the short-term storage of vegetable soybean seeds. These findings highlight the practical value of fungicidal seed coating as a cost-effective strategy for protecting soybean seeds from early infection and improving seedling establishment in pathogen-prone environments.

Keywords: Legume, seed enhancement, seed treatments, seedling disease

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1. Introduction

Vegetable soybean (*Glycine max* (L.) Merrill) is an important economic crop renowned for its high nutritional value, being rich in high-quality protein, essential amino acids, dietary fiber, vitamins A, B, and C, as well as essential minerals such as calcium, iron, and potassium. Moreover, it contains antioxidant compounds that contribute to health promotion and the reduction of chronic disease risks, making it widely utilized in the food industries for both human and animal consumption (Nair et al., 2023).

In Thailand, vegetable soybean cultivation is predominantly concentrated in the provinces of Chiang Mai, Lamphun, Lampang, and Chiang Rai. However, a major challenge faced by growers is the outbreak of *Fusarium* sp., which causes root and stem rot diseases. This pathogen severely compromises seedling quality and survival rates by inducing root browning, epidermal peeling, tissue decay, and eventual plant death (Zakaria, 2023; Liu et al., 2025). The prevalence of such infections significantly increases farmers' costs by necessitating higher seeding rates and intensive field management practices. *Fusarium* sp. is both a seed-borne and soil-borne pathogen, capable of infecting seeds during germination. To address this, seed coating technology has been developed, wherein seeds are encapsulated with a polymer film that carries active ingredients. This approach provides targeted delivery of fungicidal agents, reduces the volume of chemicals required, and enhances disease prevention efficiency (Kangsopa & Atnaseo, 2022; Paravar et al., 2023; Zaim et al., 2023).

Applying fungicidal agents via seed coating has proven effective in mitigating infection risks during the germination stage. Commonly used fungicides include mancozeb, prochloraz, carboxin, thiram, metalaxyl, and captan, each exhibiting distinct mechanisms of action (Buttar et al., 2023). Mancozeb disrupts enzymes containing sulfhydryl (-SH) groups, leading to protein denaturation and cellular dysfunction. Prochloraz inhibits ergosterol biosynthesis, compromising fungal cell membrane integrity (Ma et al., 2022). Carboxin impedes succinate dehydrogenase activity, disrupting energy production through the citric acid cycle (Rupe, 1992). Thiram interferes with spore germination by targeting sulfhydryl group-dependent enzymes, impairing metabolic processes (Rose et al., 2003). Additionally, seed coating with captan has been reported to effectively reduce the incidence and severity of *Fusarium* wilt in tomato plants under field conditions, with disease incidence and severity indices significantly lower in coated seeds (25% incidence and 0.25 severity index) compared to uncoated seeds (Koohakan et al., 2020).

Moreover, seed coating introduces challenges during storage because coated seeds are more sensitive to environmental conditions such as temperature and relative humidity. These factors directly influence seed moisture balance, metabolic activity, and pathogen development, which in turn affect seed quality and the residual efficacy of fungicidal agents (Capo et al., 2020). Under unfavorable ambient conditions, coated seeds may deteriorate faster than uncoated seeds due to impaired gas exchange and chemical degradation of active ingredients, whereas controlled storage can prolong their viability and maintain treatment effectiveness (Gorim & Asch, 2017). Thus, understanding the interaction between storage conditions and fungicide performance is critical for practical seed technology applications. Despite considerable research on the use of fungicides in seed coating, limited attention has been paid to how different concentrations of fungicidal agents influence seed storability and germination across varying storage durations and environments. Few studies have systematically compared the performance of coated seeds under controlled storage versus ambient conditions, leaving a research gap in optimizing fungicide concentration for both immediate disease protection and long-term seed quality (Xing et al., 2025).

Therefore, the aim of this study was to evaluate the effects of coating vegetable soybean seeds with fungicidal agents at varying concentrations by assessing fungal inhibition, seed quality maintenance, and storability. The experiments were conducted under sand and top of paper covered with sand conditions, seeking to identify effective seed coating strategies that could enhance germination efficiency and seedling vigor, thereby contributing to the sustainable cultivation of vegetable soybean.

2. Materials and Methods

This experiment was conducted at the Seed Technology Laboratory and Plant Disease Laboratory of the Agronomy Program, Faculty of Agricultural Production, Maejo University. Vegetable soybean seeds of the CK-024 variety, provided by Lanna Agro-Industry Co., Ltd., were used, with an initial germination rate of 89% and a moisture content of 10%. The experiment was conducted from February 2024 to December 2024. The details of the experiment are as follows:

2.1 Source and preparation of *Fusarium* sp. for seed coating

In January 2024, symptomatic vegetable soybeans were collected from a farmer’s field located in Chiang Muan District, Phayao Province. The samples were initially rinsed with clean water to eliminate adhering soil particles. Small tissue sections (approximately 0.5 × 0.5 cm) were then excised and subjected to surface sterilization using a three-step protocol: immersion in 70% ethanol for 1 min, followed by soaking in 2% sodium hypochlorite solution for 5 min, and finally rinsing three times with sterilized distilled water. Approximately 1 g of sterilized tissues was finely chopped and placed in a mortar with 0.5 mL of sterile distilled water. The mixture was thoroughly macerated, and 0.5 mL of the resulting suspension was spread on Potato Dextrose Agar (PDA) using the spread plate technique. Each treatment was replicated four times. A sterile glass rod was used to distribute the suspension evenly on the surface of the agar. Plates were incubated at 25°C for 10 days to allow fungal colonies to develop. To obtain pure cultures, fungal colonies were subcultured using spot inoculation. For dual culture assays, *Fusarium* sp. was cultured by excising a 0.5 cm diameter agar plug from the growing edge of the colony and transferring it to fresh PDA plates, maintaining a 3 cm distance between inoculation points. Cultures were incubated at ambient conditions (25±2°C) for three days (Chuenchan et al., 2019). Morphological identification of *Fusarium* sp. was carried out under a light microscope. The hyphae were septate, forming tubular structures separated into compartments. Conidia were observed to be spindle- or rice grain-shaped with slightly curved, tapering ends. Their dimensions ranged from 20-50 μm in length and 2-5 μm in width (Harish et al., 2023).

2.2 Testing various fungicides for inhibiting *Fusarium* sp. in the laboratory

Antifungal activity was assessed using the poisoned food technique. Seven fungicides were tested: metalaxyl (25% WP; Elefante Agro Chemical Co., Ltd., Bangkok, Thailand), mancozeb (80% WP; Grow Chemical Ltd., Bangkok, Thailand), prochloraz (45% W/V EC; Max Ag Co., Ltd., Bangkok, Thailand), carboxin (75% WP; Thepwatana Ltd., Bangkok, Thailand), thiram (80% WP; Farm Protection Ltd., Bangkok, Thailand), captan (50% WP;

Erawan Ltd., Bangkok, Thailand), and metalaxyl-M (68% WG; Multilink Park Ltd., Bangkok, Thailand). Each fungicide was diluted in 100 mL of distilled water at the desired concentration. To prepare the fungicide-amended medium, 80 mL of melted PDA was mixed with 20 mL of the fungicide solution, resulting in a final volume of 100 mL per treatment. Since the fungicide solution was prepared in sterile distilled water without additional nutrients or inhibitory substances, this dilution did not affect the PDA composition or fungal growth apart from the intended fungicidal effect. The mixture was poured into sterile 15 × 100 mm Petri dishes and left to solidify for 24 h. A 0.5 cm agar disc containing actively growing *Fusarium* sp. mycelium was then cut using a sterile cork borer and centrally placed on each fungicide-amended plate. The efficacy of each fungicide was evaluated by measuring the radial growth of *Fusarium* colonies after incubation, compared to untreated control. The percentage inhibition of mycelial growth was calculated using the following formula:

$$\text{Inhibition (\%)} = [(R1 - R2) / R1] \times 100$$

where R1 is the mean colony radius in the control treatment, and R2 is the mean colony radius in the fungicide-treated plates.

2.3 Vegetable soybean seed coating

The seed coating formulation used in this study was a commercial seed coating formula developed by the Modern Seed Technology Research Center, Maejo University. A total of eleven treatments were prepared as follows: uncoated seeds (control), polymer-coated only, mancozeb at 2, 4, and 6 g of active ingredient (g.ai.), prochloraz at 2, 4, and 6 g.ai., and carboxin at 2, 4, and 6 g.ai. Seed coating was performed using a Model KSC-02D rotary coater operating at 30 rpm. After coating, seeds were air-dried at ambient temperature until they reached a final moisture content of approximately 10%.

2.4 Sand testing

Fine sand with a particle size smaller than 0.05 mm was sterilized by dry heat at 200°C for 2 h and subsequently used as a growth substrate for seed quality assessment. The germination experiment was conducted in plastic containers (dimensions: 180 mm × 140 mm × 90 mm, L × W × H), with each treatment replicated four times using 50 seeds per replicate. The sand was adjusted to approximately 60% moisture content and added to each container to a depth of 3 cm. Seeds were evenly placed on the surface, followed by an additional 2 cm layer of sand to cover them. All containers were transferred into a controlled germination chamber maintained at 25°C, 80% relative humidity, and continuous illumination at 180 µE for 24 h. Germination counts were performed on days 5 and 8 after sowing according to the ISTA (2023). Speed of germination was determined by counting the number of normal seedlings emerging between days 5 and 8, following the methodology described by AOSA (1983). Mean germination time (MGT) was calculated using the formula $MGT = \sum(n \times d) / N$, where n represents the number of seeds germinated on a given day, d denotes the number of days since sowing, and N is the total number of germinated seeds by the end of the test (Ellis & Roberts, 1980). On day 8, ten seedlings per treatment were randomly selected for measuring shoot and root lengths. Shoot length was recorded from the base to the apex, and fresh weights were measured immediately after excision (Kangsopa et al., 2023).

2.5 Top of paper covered with sand testing (TPS)

TPS method followed the same setup as the sand test, utilizing identical plastic containers and pre-treated sand. In this procedure, a sheet of germination paper was placed at the base of each box, and 50 seeds were evenly distributed on the surface. A 2 cm layer of sand was then used to cover the seeds (ISTA, 2023). The boxes were sealed and incubated under the same environmental conditions as those used in the sand method. All seed quality parameters were assessed using the same criteria as applied in the sand-based evaluation.

2.6 Evaluation of *Fusarium* sp. suppression under greenhouse conditions

The *Fusarium* sp. isolate obtained from Section 2.1 was propagated in sterilized peat moss to prepare an inoculum. Prior to inoculation, the peat moss was autoclaved at 121°C for 15 min to eliminate any contaminating microorganisms. A spore suspension of *Fusarium* sp., previously cultured on PDA for 10 days, was prepared and adjusted to a concentration of 10^5 spores/mL using a hemocytometer. This spore suspension was thoroughly mixed with 1 kg of sterilized peat moss. The inoculated substrate was transferred into sterile 12 × 18-inch polyethylene bags, sealed, and incubated at ambient temperature (~25°C) for seven days to allow fungal colonization (Norkaew et al., 2021). For the pathogenicity assay, twenty seeds from each treatment group were randomly selected, with four replications per treatment. Uncoated seeds served as the negative control. Seeds were sown in trays filled with the *Fusarium*-infested peat moss as the growing medium. The percentage of surviving vegetable soybean seedlings was recorded according to the method described by Kunwanlee et al. (2023).

2.7 Seed storages

Seeds from each treatment were packed in aluminum foil bags measuring 10 by 15 cm and kept under two storage regimes: ambient conditions at 27±2°C with 80±5% relative humidity, and controlled conditions at 4°C with 50% relative humidity. At two-month intervals over a six-month period, seed quality was evaluated using randomly selected samples from each treatment group.

2.8 Statistical analysis

The percentage of germination was arcsine-transformed to normalize the data before statistical analysis. All data were analyzed by one-way analysis of variance (ANOVA) (completely randomized design), and the difference between the treatments was tested by Duncan’s multiple range test (DMRT).

3. Results and Discussion

3.1 Effects of fungicide on inhibition of *Fusarium* sp.

The evaluation of the efficacy of chemical treatments on the inhibition of *Fusarium* sp. growth revealed that mancozeb, prochloraz, and carboxin exhibited the highest levels of antifungal activity, maintaining 100% inhibition consistently across all incubation periods from day 1 to day 9. In contrast, thiram and captan showed a gradual decline in efficacy, with inhibition percentages of 83% and 72%, respectively, by day 9. The lowest levels of inhibition were observed with metalaxyl and metalaxyl-M, at only 20% and 17% on day 9, respectively. Statistical analysis confirmed that the differences in inhibition among chemical treatments were highly significant (P≤0.01). These results correspond closely with the colony images shown in Figure 1, where the mancozeb, prochloraz, and carboxin plates (Figures 1C-E) remained free of visible mycelial growth, while partial colony development was evident in thiram and captan plates (Figure 1F). By contrast, substantial fungal growth was visible in metalaxyl and metalaxyl-M plates (Figures 1B, 1G and 1H), consistent with the low inhibition values in Table 1.

The complete inhibition of *Fusarium* sp. growth by mancozeb, prochloraz, and carboxin over the 9-day incubation period demonstrates their stable and prolonged antifungal efficacy. Mancozeb, a contact fungicide in the dithiocarbamate group, inhibits sulfhydryl (-SH) group-containing enzymes essential for fungal respiration, protein synthesis, and cell wall formation, thereby disrupting cell division and growth (Ma et al., 2022). Prochloraz, a systemic imidazole fungicide, interferes with ergosterol biosynthesis, compromising membrane integrity and leading to cellular leakage, disrupted homeostasis, and cell death (Shen et al., 2024). Carboxin, a systemic fungicide in the anilide group, inhibits succinate dehydrogenase (SDH), a key enzyme in the Krebs cycle, resulting in ATP depletion and inhibition of fungal reproduction (Rupe, 1992).

In contrast, captan exhibited a marked decline in antifungal efficacy over time, with inhibition decreasing to 22% by day 9, despite a transient increase on day 7. This reduction may be associated with limited persistence or degradation of captan under prolonged incubation and moist conditions (Ellis et al., 2011). Thiram's diminishing performance may be attributed to the adaptive response of *Fusarium* to dithiocarbamate exposure (Rose et al., 2003). The discrepancy between partial inhibition values and visible colony size in Figure 1F may reflect differences in colony density versus radial growth, indicating that visual inspection alone may underestimate sublethal inhibition effects (Paulitz & Bélanger,

Figure 1. *In vitro* efficacy of seven fungicides in inhibiting *Fusarium* sp. after 9 days of incubation. Treatments include: A: Untreated control, B: Metalaxyl (25% WP), C: Mancozeb (80% WP), D: Prochloraz (50% WP), E: Carboxin (75% WP), F: Thiram (80% WG), G: Captan (80% WG), and H: Metalaxyl-M (35% W/V ES).

Table 1. Inhibition (%) of *Fusarium* sp. mycelial growth by fungicides over a 9-day incubation period.

Treatment	Inhibition on the growth of mycelium[1] (%)				
	1 Day	3 Day	5 Day	7 Day	9 Day
metalaxyl	100	36^{b}	18^{d}	34 d	20 ^{c}
mancozeb	100	100^{a}	100^{a}	100^{a}	100^{a}
prochloraz	100	100^{a}	100^{a}	100^{a}	100^{a}
carboxin	100	100^{a}	100^{a}	100^{a}	100^{a}
thiram	100	100^{a}	40^{c}	82^{b}	83^{b}
captan	100	100^{a}	46^{b}	72^{c}	22^{c}
metalaxyl-M	100	19^{c}	14^{e}	23^{e}	17^{d}
F-test	-	**	**	**	**
CV. %	0	2.48	2.42	5.14	2.15

**: significantly different at $P \leq 0.01$.

[1] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ by DMRT.

2001). Metalaxyl and metalaxyl-M exhibited the lowest inhibition rates (20% and 17%, respectively, on day 9), suggesting that these phenylamide fungicides, which primarily target Oomycetes such as *Pythium* and *Phytophthora*, are less effective against true fungi like *Fusarium* (Nagashima et al., 2020). This mode-of-action specificity explains the weak performance observed in both Table 1 and Figure 1, where extensive colony growth was evident in the metalaxyl-, captan-, and metalaxyl-M-treated plates. The integration of visual and quantitative data therefore reinforces the conclusion that these fungicides are not suitable for effective control of *Fusarium*.

3.2 Effects of seed coating with fungicides on seed quality and seedling growth

Based on fungal inhibition assays, mancozeb, prochloraz, and carboxin were identified as the most effective fungicides, showing complete inhibition of *Fusarium* mycelial growth. These fungicides were selected for seed coating trials on vegetable soybean to evaluate their effects on seed quality and seedling growth.

Under sand test conditions, seeds coated with prochloraz at 6 g.ai. and carboxin at 4 g.ai. had high germination percentages, but not significantly different from uncoated seeds. However, treatment with mancozeb at 6 g.ai. and carboxin at 6 g.ai. resulted in the slowest germination speeds. In contrast, prochloraz at 6 g.ai. and mancozeb at 4 g.ai. significantly enhanced speed of germination over the uncoated seeds. For seedling growth, prochloraz at 6 g.ai. yielded significantly greater shoot length, and prochloraz at 2 g.ai. increased root length compared to uncoated seeds (Table 2). In the top-of-paper-covered-with-sand test, carboxin at 6 g.ai. caused the lowest germination rate and speed of germination, possibly due to phytotoxicity at high concentrations. Treatments with mancozeb at 2 and 6 g.ai., prochloraz at 4 g.ai., and carboxin at 4 g.ai. exhibited slightly delayed mean germination times. The polymer-only coating showed no adverse effects and even resulted in the highest shoot length among all treatments. In contrast, mancozeb at 2 g.ai. and the control had the shortest root lengths (Table 3).

Table 2. Seed quality and seedling growth of vegetable soybean after coating with different fungicide types and concentrations under sand test conditions.

Treatment	Sand Test				
	Seed Quality			Seedling Growth	
	GE^{3} (%)	SGE^{3} (%)	MGT^{3} (day)	Shoot Length (cm)	Root Length (cm)
uncoated	89$^{ab1, 2}$	5.13^{a}	5.86^{a}	23.88^{b}	19.72 ^{e}
coated only polymer	85^{b}	3.98^{ab}	5.72^{ab}	25.00^{ab}	20.01$^{c\text{-}e}$
mancozeb 2 g.ai	85^{b}	3.82^{ab}	5.58^{ab}	24.94^{ab}	19.87^{de}
mancozeb 4 g.ai	87^{ab}	2.58^{ab}	5.29^{b}	24.26^{ab}	21.16$^{a\text{-}e}$
mancozeb 6 g.ai	83^{b}	3.33^{b}	5.60^{ab}	24.40^{ab}	21.66$^{a\text{-}c}$
prochloraz 2 g.ai	92^{ab}	4.06^{ab}	5.70^{ab}	25.06^{ab}	22.72^{a}
prochloraz 4 g.ai	92^{ab}	4.28^{ab}	5.65^{ab}	25.15^{ab}	21.14$^{a\text{-}e}$
prochloraz 6 g.ai	95^{a}	3.77^{ab}	5.21^{b}	26.54^{a}	21.28$^{a\text{-}e}$
carboxin 2 g.ai	92^{ab}	3.97^{ab}	5.63^{ab}	25.55^{ab}	21.80^{ab}
carboxin 4 g.ai	94^{a}	3.89^{ab}	5.62^{ab}	24.29^{ab}	20.37$^{b\text{-}e}$
carboxin 6 g.ai	85^{b}	3.12^{b}	5.55^{ab}	23.93^{b}	21.52$^{a\text{-}d}$
F-test	**	**	**	**	**
CV.%	7.07	27.93	5.15	7.07	5.05

** : significantly different at P≤0.01.

[1] Data were arcsine-transformed before analysis, and the back-transformed data are presented.

[2] Means within a column followed by the same letter are not significantly at P≤0.05 by DMRT.

[3] Abbreviations: GE=germination percentage, SGE=speed of germination and MGT=mean germination time.

Table 3. Seed quality and seedling growth of vegetable soybean after coating with different fungicide types and concentrations under top of paper covered with sand test condition.

Treatment[1]	Top of Paper Covered with Sand				
	Seed Quality			Seedling Growth	
	GE^{3} (%)	SGE^{3} (%)	MGT^{3} (day)	Shoot Length (cm)	Root Length (cm)
uncoated	93^{ab1,2}	3.40$^{a\text{-}d}$	5.61^{b}	19.66^{bc}	22.03^{bc}
coated only polymer	86$^{a\text{-}c}$	1.37^{e}	4.98^{c}	22.79^{a}	24.43^{ab}
mancozeb 2 g.ai	86$^{a\text{-}c}$	4.91^{a}	6.05^{a}	17.52^{c}	21.73^{c}
mancozeb 4 g.ai	94^{a}	3.66$^{a\text{-}d}$	5.49^{b}	18.27^{cd}	25.57^{a}
mancozeb 6 g.ai	89$^{a\text{-}c}$	3.36$^{a\text{-}d}$	5.65^{ab}	18.58^{cd}	24.82^{a}
prochloraz 2 g.ai	87$^{a\text{-}c}$	2.75$^{b\text{-}e}$	5.43^{b}	18.90$^{b\text{-}d}$	25.90^{a}
prochloraz 4 g.ai	93^{ab}	4.10^{ab}	5.67^{ab}	18.09^{cd}	25.71^{a}
prochloraz 6 g.ai	93^{ab}	3.51$^{a\text{-}d}$	5.51^{b}	18.55^{cd}	25.01^{a}
carboxin 2 g.ai	89$^{a\text{-}c}$	2.00$^{c\text{-}e}$	5.34^{bc}	19.68^{bc}	25.32^{a}
carboxin 4 g.ai	86$^{a\text{-}c}$	3.87$^{a\text{-}c}$	5.73^{ab}	18.29^{cd}	23.64$^{a\text{-}c}$
carboxin 6 g.ai	84^{c}	1.83^{de}	5.31^{bc}	20.34^{b}	24.76^{a}
F-test	**	**	**	**	**
CV.%	8.05	37.25	4.76	5.41	6.92

** : significantly different at P≤0.01.

[1] Data were arcsine-transformed before analysis, and the back-transformed data are presented.

[2] Means within a column followed by the same letter are not significantly different at P≤0.05 by DMRT.

[3] Abbreviations: GE=germination percentage, SGE=speed of germination and MGT=mean germination time.

The results indicated that seed coating with prochloraz and carboxin, particularly at 4-6 g.ai., effectively maintained seed quality and enhanced seedling growth. These findings align with previous studies showing that systemic fungicides suppress early-stage soil-borne pathogens, promoting uniform seedling establishment (Panth et al., 2020). The superior performance of prochloraz at 6 g.ai. is likely due to its inhibition of ergosterol biosynthesis, reducing pathogen pressure and improving germination uniformity (Shen et al., 2024). While carboxin also supported high germination under sand conditions, its application at 6 g.ai. under paper-covered conditions reduced germination, possibly due to physiological stress from excessive concentrations (Rupe, 1992; Rogério et al., 2012). Mancozeb at 6 g.ai. resulted in the slowest germination, consistent with reports that high levels may disrupt enzymes critical for early development (Ergin et al., 2021). Notably, polymer-only coatings enhanced shoot elongation, likely due to improved moisture regulation at the seed surface during early germination (Halmer, 2008). Overall, prochloraz at 6 g.ai. was identified as the most effective treatment for improving germination and seedling vigor in vegetable soybean under laboratory conditions.

3.3 Effects of seedling survival of vegetable soybean

All seed coating treatments resulted in noticeably higher seedling survival rates compared to the uncoated seeds. Notably, seed coating with prochloraz at 4 and 6 g.ai. produced high seedling survival, although not significantly different from coatings with mancozeb at 2-6 g.ai., prochloraz at 2 g.ai., and carboxin at 6 g.ai. In contrast, the uncoated seeds exhibited the lowest average survival rate (Figure 2).

The results showed that all fungicidal seed coatings significantly improved seedling survival compared to untreated seeds. Prochloraz at 4 and 6 g.ai. yielded the highest survival rates, highlighting its systemic activity in protecting emerging seedlings from early-stage pathogens such as *Fusarium* spp., which cause damping-off and root rot (Shen et al., 2024). Early pathogen suppression preserves seed energy for growth, enhancing establishment (Wolny et al., 2018). Mancozeb, despite being a contact fungicide, promoted survival rates comparable to prochloraz and carboxin, indicating strong surface protection during the vulnerable germination phase. It inhibits sulfhydryl (-SH) group enzymes critical to fungal metabolism, limiting infection (Ma et al., 2022). Though non-systemic, its barrier effect is particularly effective immediately after sowing. In contrast, uncoated seeds had the lowest survival, underscoring the importance of early fungal protection especially under moist conditions or high pathogen loads, where damping-off risk is elevated (Allen et al., 2004; Kangsopa et al., 2024). These findings affirm the role of fungicidal seed treatments in enhancing seedling establishment and managing soil-borne diseases in crop production systems.

Beyond *Fusarium*, seedborne pathogens such as *Phomopsis longicolla*, *Macrophomina phaseolina*, and *Cercospora kikuchii* also represent major threats to soybean seed quality and early vigor (Blanco & Aveling, 2016). Previous studies have shown that fungicidal coatings, particularly with systemic or broad-spectrum fungicides, can suppress infection and improve field performance under pathogen pressure (Mancini & Romanazzi, 2014; Ibrahim, 2015). This suggests that the protective effect observed in our study is not limited to *Fusarium* spp. but could also be relevant for other economically important seedborne fungi affecting soybeans.

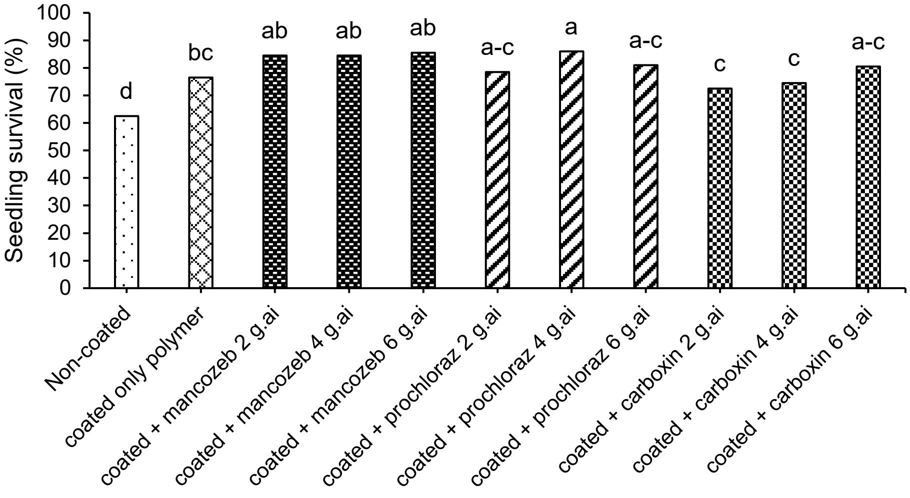

Figure 2. Survival rate (%) of vegetable soybean seedlings 9 days after seed coating with fungicidal treatments for root rot prevention.

3.4 Seed quality and seedling growth following storage under controlled and ambient conditions

Under controlled conditions, seed coating with prochloraz at 2, 4, and 6 g.ai. significantly enhanced germination percentage throughout the 6-month storage period compared to uncoated seeds, as evidenced by the sand test. Although polymer-only treatment also resulted in improved germination relative to the control, its efficacy declined more rapidly than prochloraz-coated seeds. In the TPS test, all coated treatments (T2-T5) consistently maintained higher germination rates than uncoated seeds over the entire storage duration (Table 4). In terms of shoot development, prochloraz-treated seeds exhibited significantly greater shoot length than uncoated seeds throughout storage in the sand test. Specifically, prochloraz at 6 g.ai. also produced significantly longer shoots under TPS conditions (Table 5). Similarly, root length was significantly greater in all prochloraz treatments compared to both uncoated and polymer-only seeds during the first four months of storage under sand test conditions. In TPS, all coated treatments outperformed the uncoated control in root length across all time points (Table 6).

Under ambient conditions, germination rates in both sand and TPS tests declined sharply after 2 months for all treatments, except seeds coated with prochloraz at 6 g.ai., which retained high germination at 2 months and remained comparable to the control at 4 months. However, by six months, all coated treatments showed greater reductions in germination than uncoated seeds (Table 4). For shoot length, coated seeds maintained significantly longer shoots than the control during the initial 4 months under sand test conditions. By the sixth month, however, all coatings exhibited reduced shoot length below that of uncoated seeds. In TPS, prochloraz at 6 g.ai. resulted in significantly greater shoot length at four months, but this benefit diminished by 6 months (Table 5). Root length under both testing conditions remained significantly higher in seeds coated with prochloraz at 4 and 6 g.ai. after 2 months. Although root length decreased across all treatments by months 4 and 6, prochloraz at 6 g.ai. continued to perform comparably to uncoated seeds (Table 6).

Table 4. Germination percentage of vegetable soybean seeds after coating with different fungicide types and concentrations during a 6-month storage period under controlled and ambient conditions.

Treatment[1]	**Sand Test**				**Top of Paper Covered with Sand**			
	0	**2**	**4**	**6**	**0**	**2**	**4**	**6**
Controlled conditions								
T1	$45^{b2,3}$	86^{b}	75^{b}	79^{b}	55^{b}	78^{b}	79	76^{b}
T2	67^{a}	90^{a}	77^{b}	83^{ab}	71^{a}	87^{a}	82	80^{ab}
T3	75^{a}	91^{a}	86^{ab}	86^{a}	73^{a}	85^{ab}	81	80^{ab}
T4	74^{a}	92^{a}	89^{a}	79^{b}	69^{a}	83^{ab}	79	85^{a}
T5	64^{a}	92^{a}	85^{ab}	86^{a}	54^{b}	85^{ab}	80	83^{ab}
F-test	**	**	*	*	**	*	ns	**
CV.%	10.71	8.10	7.99	6.26	7.29	5.76	8.42	5.9
Ambient conditions								
T1	45^{b}	50^{b}	51^{a}	30^{a}	55^{b}	58^{a}	46^{ab}	64^{a}
T2	67^{a}	43^{b}	33^{b}	14^{bc}	71^{a}	46^{b}	36^{bc}	24^{b}
T3	75^{a}	44^{b}	27^{b}	10^{c}	73^{a}	46^{b}	22^{d}	14^{b}
T4	74^{a}	43^{b}	34^{b}	8^{c}	69^{a}	54^{ab}	30^{cd}	11^{b}
T5	64^{a}	67^{a}	52^{a}	23^{ab}	54^{b}	58^{a}	53^{a}	32^{ab}
F-test	**	**	**	**	**	*	**	**
CV.%	10.71	11.62	10.63	22.78	7.29	7.72	14.60	36.64

ns,*,** : no-significantly and significantly different at P≤0.05 and P≤0.01, respectively.

[1] T1 = uncoated, T2 = coated only with polymer, T3= coated + prochloraz 2 g.ai., T4= coated + prochloraz 4 g.ai. and T5= coated + prochloraz 6 g.ai.

[2] Data were arcsine-transformed before analysis, and the back-transformed data are presented.

[3] Means within a column followed by the same letter are not significantly different at P≤0.05 by DMRT.

Table 5. Shoot length (cm) of vegetable soybean seeds after coating with different fungicide types and concentrations during a 6-month storage period under controlled and ambient conditions.

Treatment[1]	**Sand Test**				**Top of Paper Covered with Sand**			
	0	**2**	**4**	**6**	**0**	**2**	**4**	**6**
Controlled conditions								
T1	12.34^{b2}	15.25^{b}	6.82^{ab}	8.97^{c}	14.70^{b}	15.94^{c}	8.42	12.73^{b}
T2	15.97^{a}	18.12^{a}	7.11^{a}	13.35^{a}	14.69^{b}	16.72^{bc}	8.68	12.41^{b}
T3	16.59^{a}	17.82^{a}	5.95^{c}	13.05^{a}	16.34^{ab}	17.54^{ab}	8.41	12.66^{b}
T4	16.91^{a}	17.41^{a}	6.33^{bc}	11.07^{b}	16.52^{ab}	17.52^{ab}	8.35	14.41^{a}
T5	15.64^{a}	17.31^{a}	6.08^{c}	12.54^{a}	17.76^{a}	17.99^{a}	7.75	12.97^{ab}
F-test	*	**	*	**	**	*	ns	*
CV.%	11.10	3.81	5.81	6.90	17.43	4.44	8.79	7.84
Ambient conditions								
T1	12.34^{b}	11.73^{c}	6.54^{a}	9.66^{a}	14.70^{b}	14.98	6.76^{ab}	8.84^{a}
T2	15.97^{a}	13.65^{b}	5.94^{bc}	7.28^{b}	14.69^{b}	14.97	6.99^{a}	7.23^{ab}
T3	16.59^{a}	13.53^{b}	6.31^{ab}	7.37^{b}	16.34^{ab}	14.82	6.52^{ab}	5.78^{b}
T4	16.91^{a}	12.80^{b}	5.67^{c}	7.13^{b}	16.52^{ab}	14.23	6.25^{b}	4.99^{c}
T5	15.64^{a}	17.30^{a}	6.65^{a}	8.31^{b}	17.76^{a}	14.61	7.09^{a}	7.18^{ab}
F-test	*	**	**	**	**	ns	*	*
CV.%	11.10	4.50	5.08	9.78	17.43	4.27	5.95	35.66

ns,*,** : no-significantly and significantly different at P≤0.05 and P≤0.01, respectively.

[1] T1 = uncoated, T2 = coated only with polymer, T3= coated + prochloraz 2 g.ai., T4= coated + prochloraz 4 g.ai. and T5= coated + prochloraz 6 g.ai.

[2] Means within a column followed by the same letter are not significantly different at P≤0.05 by DMRT.

Table 6. Root length (cm) of vegetable soybean seeds after coating with different fungicide types and concentrations during a 6-month storage period under controlled and ambient conditions

Treatment[1]	**Sand Test**				**Top of Paper Covered with Sand**			
	0	**2**	**4**	**6**	**0**	**2**	**4**	**6**
Controlled conditions								
T1	17.20^{b}[2]	23.08^{bc}	13.60	19.26^{c}	15.55^{b}	19.95^{b}	17.02	21.01^{b}
T2	21.23^{a}	26.68^{a}	13.92	19.78^{bc}	21.07^{a}	25.58^{a}	18.62	21.42^{b}
T3	19.81^{ab}	20.50^{c}	13.42	21.14^{a}	18.13^{b}	25.65^{a}	17.83	21.07^{b}
T4	19.71^{ab}	23.76^{bc}	13.58	20.67^{ab}	18.23^{b}	25.87^{a}	18.30	24.02^{a}
T5	20.65^{a}	22.14^{bc}	13.29	18.90^{c}	15.64^{b}	22.23^{ab}	17.84	22.69^{ab}
F-test	*	**	ns	**	**	**	ns	*
CV.%	9.35	8.58	4.72	4.10	9.76	9.64	6.18	7.36
Ambient conditions								
T1	17.20^{b}	17.43^{b}	14.06^{a}	17.59^{a}	15.55^{b}	16.82^{ab}	13.00^{a}	9.73^{a}
T2	21.23^{a}	17.61^{b}	12.45^{b}	12.19^{bc}	21.07^{a}	14.84^{b}	10.27^{b}	7.27^{ab}
T3	19.81^{ab}	18.06^{b}	10.69^{c}	12.15^{bc}	18.13^{b}	15.03^{ab}	9.70^{b}	4.61^{b}
T4	19.71^{ab}	21.26^{a}	12.21^{bc}	10.83^{c}	18.23^{b}	18.03^{a}	10.16^{b}	4.59^{b}
T5	20.65^{a}	21.09^{a}	15.26^{a}	14.57^{ab}	15.64^{b}	18.28^{a}	14.32^{a}	8.69^{a}
F-test	*	*	**	**	**	**	**	*
CV.%	9.35	9.54	8.23	15.27	9.76	14.31	14.72	34.35

ns,*,** : no-significantly and significantly different at P≤0.05 and P≤0.01, respectively.

[1] T1 = uncoated, T2 = coated only with polymer, T3= coated + prochloraz 2 g.ai., T4= coated + prochloraz 4 g.ai. and T5= coated + prochloraz 6 g.ai.

[2] Means within a column followed by the same letter are not significantly different at P≤0.05 by DMRT.

The study demonstrated that seed coating with prochloraz at 2, 4, and 6 g.ai. significantly improved germination of vegetable soybean seeds under controlled storage for up to six months, particularly in sand tests. Prochloraz, a systemic fungicide, inhibits ergosterol biosynthesis, disrupting fungal membrane integrity and preventing infection without compromising germination (Shen et al., 2024). Interestingly, germination percentages at the initial assessment (0 months) were relatively low across all treatments, including the control, but increased after two to six months of controlled storage. This pattern may be attributed to an after-ripening effect, in which seeds undergo physiological adjustments that alleviate residual dormancy or repair subcellular damage during short-term storage, thereby improving germination performance (Bewley & Black, 2013). Such a trend highlights the importance of considering both immediate and time-dependent seed responses when evaluating coating efficacy. Notably, prochloraz at 6 g.ai. enhanced shoot and root growth, especially during the first 2-4 months, likely due to early fungal suppression reducing stress and enabling energy allocation toward growth (Rétif et al., 2023). This early protection may also enhance rhizosphere conditions, supporting root development, consistent with Halmer (2008), who emphasized the role of uniform water uptake in promoting elongation.

Under ambient storage with fluctuating temperature and humidity, coating efficacy declined more rapidly. Germination dropped markedly after two months in all treatments except prochloraz at 6 g.ai., which maintained high viability until month four. The faster deterioration under ambient conditions can be explained by the combined influence of high temperature (27±2°C), elevated relative humidity (80±5%), and the semi-permeable nature of the foil packaging, which likely permitted gradual moisture exchange. These factors accelerate seed aging, reduce fungicide stability, and may promote the accumulation of toxic byproducts, thereby limiting both seed longevity and coating effectiveness (Ellis & Roberts, 1980; Walters et al., 2010; Corbineau, 2024).

By month six, performance decreased across all coatings, indicating that seed longevity depended not only on fungicidal efficacy but also on active ingredient stability and environmental stress (Ramtekey et al., 2022). Initially, all coated treatments promoted greater shoot and root growth than the control, but by month six, all showed declines, especially in root length. This may result from structural seed deterioration or phytotoxic byproducts accumulating under high humidity (Gubišová et al., 2024). Overall, these findings emphasize that while prochloraz coating is effective in the short term, especially under controlled conditions, its long-term benefits are highly dependent on storage environment, which represents a critical factor for practical application in seed supply chains.

4. Conclusions

This study confirmed that seed coating of vegetable soybean with prochloraz, carboxin, and mancozeb at 4-6 g.ai. effectively inhibited *Fusarium* sp. under laboratory conditions. Prochloraz at 6 g.ai. showed superior seed quality by maintaining the highest germination percentage and speed during 2-4 months of storage, with no adverse effects on viability. Across both controlled and ambient environments, prochloraz-treated seeds consistently exhibited significantly longer shoot and root lengths than uncoated seeds, particularly in the early storage phase. Controlled conditions preserved treatment efficacy better than ambient conditions, with prochloraz remaining effective for up to four months. Additionally, all coatings improved seedling survival, with prochloraz at 4-6 g.ai. achieving the highest rates. Therefore, seed coating with prochloraz at 6 g.ai. is recommended as the most effective formulation for enhancing the quality of vegetable soybean seeds.

These findings confirm that fungicidal coatings, particularly prochloraz, effectively suppress *Fusarium* while maintaining seed quality and seedling vigor during storage. For vegetable soybean production, prochloraz offers practical benefits by reducing seedling losses, promoting uniform establishment, and preserving seed quality under controlled storage. Nonetheless, efficacy declines under ambient conditions, and field validation remains limited. Future studies should explore long-term storage stability, packaging interactions, and integration with biological seed treatments to strengthen sustainable disease management.

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6. Authors' Contributions

Davika Rapeebunyanon conducted laboratory experiments, including seed coating procedures, germination testing, and microbial inhibition assays; Chatsuda Phuakjaiphaeo managed field and storage trials, collected quantitative data, and performed statistical analyses; Vassana Viroonrat contributed to the formulation of fungicidal seed coating agents, evaluated seed quality and storability, and reviewed the manuscript for technical coherence; and Jakkrapong Kangsopa conceptualized and supervised the entire study, provided guidance on experimental design and data interpretation, and finalized the manuscript for submission. All authors have read and approved the final version of the manuscript.

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7. Conflicts of Interest

The authors declare that there is no conflict of interest.

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