Songklanakarin J. Sci. Technol.
46 (5), 430–437, Sep. – Oct. 2024

http://www.sjst.psu.ac.th

Original Article

Effects of seed pelleting with fungicide on seed quality and inhibition of *Fusarium* sp. in Chili (*Capsicum annuum* L.)

Jakkrapong Kangsopa[1, 2*], Aranya Singsopa[1], Nararat Thawong[1], Sukanya Baomeesri[1], Davika Rapeebunyanon[1], and Sirikorn Charoenyai[1]

[1] *Division of Agronomy, Faculty of Agricultural Production, Maejo University, San Sai, Chiang Mai, 50290 Thailand*

[2] *Modern Seed Technology Research Center, Faculty of Agricultural Production, Maejo University, San Sai, Chiang Mai, 50290 Thailand*

Received: 20 May 2024; Revised: 3 July 2024; Accepted: 23 July 2024

Abstract

Yellow wilt disease commonly affects chili seedlings, with the fungal pathogen attacking the roots or stem at the soil surface, leading to significant seedling mortality and severely impacting chili cultivation. Seed pelleting with fungicides can mitigate the risk of fungal infection of seedlings. This study aimed to identify the type and concentration of fungicide that does not affect seed quality while effectively inhibiting *Fusarium* sp. The experiments followed a completely randomized design (CRD) with four replications. The results indicate that fosetyl-aluminium, at all three tested concentrations, inhibits (by 100%) mycelial growth. Furthermore, seeds pelleted with fosetyl-aluminium at 0.5 g.ai. and 1 g.ai. showed higher germination percentage, speed of germination, and shoot length, compared to non-pelleted seeds under laboratory conditions. In greenhouse conditions, seeds pelleted with fosetyl-aluminium at 0.5 g.ai. demonstrated higher germination percentage, speed of germination, and shoot length, compared to non-pelleted seeds. Both laboratory and greenhouse assessments showed that seed pelleting with fosetyl-aluminium at all concentrations significantly inhibited *Fusarium* sp. compared to non-pelleted seeds. Thus, pelleting seeds with fosetyl-aluminium at 1 g.ai. is recommended for enhancing seed quality and effectively inhibiting *Fusarium* sp. in chili seedlings.

Keywords: seed enhancement, seed treatments, Fusarium wilt, seedling disease

1. Introduction

The yellow wilt disease caused by the fungus *Fusarium* sp. impacts both before and after the germination of chili plants (Parihar *et al*., 2022). The spread of this disease originates from fungi that thrive on plant debris, which can grow when chilis are replanted in the same area with suitable environmental conditions. This disease can be transmitted through soil, water, and air, with a higher prevalence in warm and moist soil conditions. Fusarium can spread by the movement of infected soil, plant material, and especially seeds. It is often observed during the rainy season or early winter. When the infection occurs during the seedling stage, symptoms include water-soaked lesions around the soil line, wilting, and a limp appearance (Naik, Rani, & Madhukar, 2008). These symptoms result from the damage to the plant's vascular system caused by the fungus. In severe cases, seedlings may wilt permanently within 2-7 days, leading to rapid plant death. The challenges posed by this disease necessitate farmers to increase the budget for cultivation and hire additional labor to replace the seedlings that die in the planting plot (Shaheen *et al*., 2021). Consequently, the relocation of chili plants within the same area becomes challenging due to the complicated spread of the disease, which poses a high risk of losses in the cultivation process.

*Corresponding author
Email address: jakkrapong_ks@mju.ac.th

Seed pelleting is a method used to increase the size, shape, and weight of seeds, making them easier to handle and protecting them from external threats (Afzal, Javed, Amirkhani, & Taylor, 2020; Kangsopa, Singsopa, Thawong, & Pidtatanao, 2024). This process enhances seed germination and seedling establishment, reducing the quantity of seeds needed for cultivation by up to 30-60%. Seed pelleting also allows the incorporation of fungicides to prevent diseases both before and after germination. Fungicides are designed to control the growth of fungi in plants and come in various types and groups, aiming to prevent or manage diseases caused by fungal infections in crops, ultimately improving plant growth conditions (Siri, 2015). Chemical fungicides such as captan are safe for seeds and seedling stages and exhibit environmental resilience (Tort, & Turkyilmaz, 2003). Similarly, mancozeb and fosetyl-aluminium are fungicides that are safe for seeds and seedlings, effectively controlling diseases like root rot (Gullino *et al*., 2010; Di Marco *et al*., 2011). The prevention of fungal infections in seeds and seedlings involves critical steps, including selecting disease-free seeds, using chemicals to protect and eliminate fungal spores in seeds, and applying fungicides during the seedling stage. These measures reduce the risk of diseases and enhance the chances of achieving robust and productive yields.

Thus, the objective of this research was to pellet seeds with different types and concentrations of fungicides, monitor changes in seed quality, and assess the inhibitory effectiveness against *Fusarium* sp. This aimed to address the yellow wilt disease in chili seedlings and to decrease production costs in Thailand.

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. Organic **'**Pu Meth**'** chili seeds used as experimental seeds were cultivated in 2023 by the Maejo University breeding and producing organic vegetable seeds center. The experiment was conducted from May 2023 to February 2024. The details of the experiment are given below.

2.1 Preparation of *Fusarium* sp.

Sample chili plants exhibiting wilting symptoms were collected from agricultural fields in San Sai District, Chiang Mai Province. The plant samples were washed with clean water to remove any soil. Subsequently, the samples were cut into pieces approximately 1-2 centimeters in size and surface sterilized. The sterilization process involved three steps**:** 1) soaking in 70% ethanol (v/v) for 1 minute, 2) soaking in 2% sodium hypochlorite solution (v/v) for 2 minutes, and 3) rinsing with autoclaved distilled water. The chili pieces were then cut into small sections and placed in a mortar containing 0.50 mL of autoclaved distilled water. The mixture was ground finely and subjected to microbial isolation using the spread plate technique. Specifically, 0.50 mL of the suspension was pipetted onto Potato Dextrose Agar (PDA) plates, with four replicates. A sterile glass spreader was used to evenly distribute the plant extract across the medium. The plates were incubated at 25°C for 7 days, resulting in mixed cultures. Subsequently, the fungi were subcultured to obtain pure cultures using the spot inoculation technique. The isolated *Fusarium* sp. was then cultured on PDA using the dual culture method. This involved cutting a 0.50-centimeter diameter section from the colony edge using a cork borer and placing it on PDA plates, maintaining a distance of 3 cm between each section. The cultures were incubated at room temperature (25±2°C) for 3 days (Chuenchan, Raksasanoy, Yooboriboon, & Kitja, 2019). After isolation, the morphological characteristics of *Fusarium* sp. were observed by examining the hyphae and conidia under a microscope. The hyphae appeared as septate hyphae, which are tube-like structures divided into individual cells. Additionally, the conidia exhibited a rice grain or spindle shape, with tapered and slightly curved ends. The conidia measured 20-50 micrometers (µm) in length and 2-5 micrometers (µm) in width (Harish *et al*., 2023).

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

Testing was conducted using the poisoned food technique, where three types of fungicides—captan (50% WP) (Erawan Ltd., Bangkok, Thailand), mancozeb (80% WP) (Grow Chemical Ltd., Bangkok, Thailand), and fosetyl-aluminum (80% WP) (Sativathai (Thailand) Ltd., Bangkok, Thailand) —were prepared at different concentration levels**:** 0.5 g.ai., 1 g.ai., and 2 g.ai. Each type and concentration were dissolved in 100 mL of distilled water. Subsequently, Potato Dextrose Agar (PDA) was prepared by mixing 80 mL of PDA with 20 mL of the prepared fungicide solution, resulting in a total volume of 100 mL (Grover and Moore, 1962). The mixture was poured into Petri dishes (15x100 mm), left for 24 hours, and then a 0.5 cm cork borer was used to cut *Fusarium* sp. mycelium, which was placed in the center of each PDA Petri dish prepared with the respective fungicide. Afterwards, the percentage inhibition of fungal growth caused by the disease pathogen was calculated, comparing it to the control method specifically designed to manage the pathogenic fungi. The calculation used the formula**:**

Percentage inhibition of fungal growth = [(R1 - R2)/R1 x 100]
where R1 is the average radius of the pathogenic fungi in the control Petri dishes, and R2 is the average radius of the pathogenic fungi in the treated Petri dishes.

2.3 Chili seed pelleting

A 0.3% w/w aqueous solution of carboxymethyl cellulose (CMC, Sigma Aldrich) was prepared as the pellet-binding agent. Calcium sulfate ($CaSO_4$) (Union Chemical Ltd., Bangkok, Thailand) at 50 grams served as the pelleting material (Kangsopa, Hynes, & Siri, 2018). Filler materials were meticulously applied to the seeds using a pipette with the CMC, resulting in five treatments**:** non-pelleted seeds, seeds pelleted with $CaSO_4$, seeds pelleted with $CaSO_4$ + fosetyl-aluminium at 0.5 g.ai., seeds pelleted with $CaSO_4$ + fosetyl-aluminium at 1 g.ai., and seeds pelleted with $CaSO_4$ + fosetyl-aluminium at 2 g.ai. Pelleting was carried out in a Model SKK12 rotary drum (Seeds Processing Plant, Khon Kaen University, Thailand) spinning at 40 rpm. The pelleted seeds were air-dried at room temperature until the moisture content was reduced to 8%.

2.4 Seed quality in laboratory conditions

The quality testing of 50 chili seeds, both pelleted and non-pelleted, was performed in transparent plastic boxes (110 × 110 × 30 mm, length × width × height) using the Top of Paper (TP) method with 4 repetitions. They were placed in a germination incubator at 25°C and 80% relative humidity with 24 hours of light exposure at 180 μE. Chili seed quality was evaluated in various ways, as follows.

Radicle emergence percentage was assessed on day 6 after planting when the seed exhibited root germination at 2 mm. Germination percentage was evaluated in normal seedlings on days 7 (first count) and 14 (final count) (ISTA, 2023). The speed of germination was determined daily by counting the number of normal seedlings from days 7 to 14 after sowing (AOSA, 1983). The mean germination time (MGT) was determined using the formula**:** $MGT = \sum(n \times d) / N$, where n represents the count of germinated seeds each day, d is the number of days elapsed since the start of the test, and N indicates the total count of germinated seeds at the conclusion of the experiment (Ellis, & Roberts, 1981). Ten seedlings were evaluated to determine the mean shoot length and root length on day 14 after sowing.

2.5 Seed quality in greenhouse conditions

Germination testing of chili seeds, both pelleted and non-pelleted, was carried out in seed trays with peat moss (Klasmann-Deilmann GmbH, Ltd., Germany) used as the seeding material. Cotyledon emergence percentage was evaluated by observing cotyledons that emerged from the seeding material on day 6 after sowing. Germination percentage, speed of germination, and mean germination time were assessed using the same methods employed in laboratory conditions. Shoot length was measured on day 14 after sowing. The shoots of 10 selected seedlings were cut close to the planting material and then measured using a ruler (Jeephet, Atnaseo, Hermhuk, & Kangsopa, 2022).

2.6 Testing the inhibition of *Fusarium* sp. in greenhouse conditions

Fusarium sp. isolated in section 2.1 were cultured in sterilized peat moss. The peat moss was sterilized using an autoclave at 121°C for 15 minutes. *Fusarium* sp. cultured on PDA for 7 days, with a spore density of 10^5 spores/mL, were mixed with 1 kilogram of sterilized peat moss. This mixture was placed in 12x18 inch plastic bags, sealed tightly, and incubated at room temperature for 7 days (Norkaew, Khemmuk, McGovern, & To-anun, 2021). Twenty seed samples from each treatment, with four replicates, were randomly selected. Non-pelleted seeds served as the control. The seeds were placed in trays using the *Fusarium* sp.-inoculated peat moss as the growing medium. The survival percentage of the chili seedlings was then calculated following the method of Kunwanlee, Maneerat, & Plodjinda (2023).

2.5 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.

In the preliminary test to identify the type and effectiveness of fungicides inhibiting *Fusarium* sp., three fungicides, namely captan, mancozeb, and fosetyl-aluminum, at three concentration levels were selected. The results revealed distinct variations in the inhibitory effectiveness against *Fusarium* sp. among these fungicides. Tests with captan and mancozeb at all three concentration levels demonstrated inhibitory effects on mycelium growth ranging from 22% to 58% (Table 1). Captan, classified as a phthalimide-type fungicide and dicarboximide, acts as a protectant (Barhate, Musmade, & Nikhate, 2015; Taskeen-Un-Nisa, Bhat, Pala, & Mir, 2011), while mancozeb, similar to captan, exhibits contact fungicidal activity (Allen, Enebak, & Carey, 2004). However, as depicted in Figure 1, the Petri dishes containing captan and mancozeb at all concentration levels still showed the presence of viable *Fusarium* sp. colonies, indicating that these fungicides were not efficient in destroying cells or halting the enzyme synthesis process of the fungus (Allen *et al*., 2004; Ghimire, Adhikari, Puri, Baral, & Basnet, 2022).

On the other hand, fosetyl-aluminum, at all concentration levels, demonstrated 100% effectiveness in inhibiting *Fusarium* sp. Upon examining Figure 1, it is evident that no *Fusarium* sp. colonies were able to grow in PDA at any concentration level of fosetyl-aluminum. This suggests that fosetyl-aluminum is highly efficient in suppressing the growth of *Fusarium* sp. Fosetyl-aluminum, categorized as a phosphonate, possesses complete systemic and rapid penetrative absorption into plant tissues (Silva, Vélez, Hernández, Núñez, & Greslebin, 2016). However, the precise mode of action of fosetyl-aluminum in inhibiting *Fusarium* sp. has not been clearly identified in the literature. Nonetheless, it is suggested that fosetyl-aluminum interferes with spore germination and impedes the development of fungal hyphae. Its mechanisms of action include inhibiting protein synthesis and the synthesis of β-glucan, an essential component of the fungal cell wall. This disruption weakens the cell wall, causing the cells to lyse and die. Additionally, fosetyl-aluminum may interfere with intracellular signaling in fungi, disrupting growth and development processes (Ayesha, Suryanarayanan, Nataraja, Prasad, & Shaanker, 2021; Gormez, Golge, & Kabak, 2021). Another important mechanism is its ability to stimulate the plant's immune system, thereby enhancing resistance to fungal infections. Part of its action involves fosetyl-aluminum competing with or antagonizing phosphite against various controlling enzymes in several pathways, promoting plant resistance and stimulating the production of phytoalexins to combat diseases (Silva *et al.*, 2016). Consequently, fosetyl-aluminum exhibited the highest efficacy in inhibiting the growth of *Fusarium* sp. at 100%. Consistent with previous research findings, fosetyl-aluminum has low antifungal activity against various fungal species in laboratory tests (Fenn, & Coffey, 1985; Saib, Berrebbah, Djebar, & Berredjem, 2015).

Table 1. Inhibition of mycelial growth of *Fusarium* sp.

Treatment	Laboratory condition
	Inhibition of mycelial growth (%)
Control	0 e[1, 2]
Captan 0.5 g.ai.	22 d
Captan 1 g.ai.	23 d
Captan 2 g.ai.	46 c
Mancozeb 0.5 g.ai.	19 d
Mancozeb 1 g.ai.	47 c
Mancozeb 2 g.ai.	58 b
Fosetyl-aluminium 0.5 g.ai.	100 a
Fosetyl-aluminium 1 g.ai.	100 a
Fosetyl-aluminium 2 g.ai.	100 a
F-test	**
CV.%	5.67

** : significantly different at P≤0.01

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

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

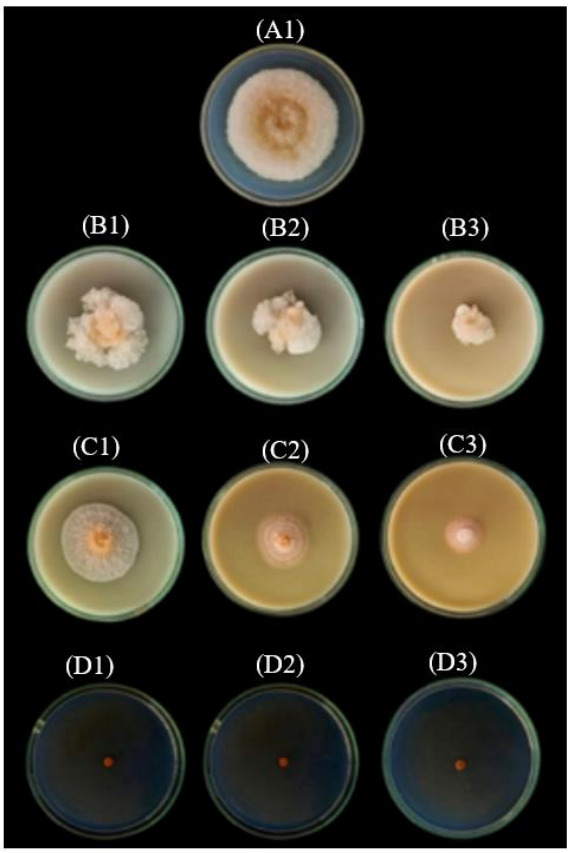

Figure 1. Testing the efficacy of inhibiting *Fusarium* sp. in laboratory conditions with three concentrations of fungicidal chemicals. The growth of *Fusarium* sp. mycelium (A1). The inhibition of *Fusarium* sp. mycelial growth by captan at 0.5 g.ai., 1 g.ai. and 2 g.ai. (B1, B2, B3), mancozeb at 0.5 g.ai., 1 g.ai. and 2 g.ai. (C1, C2, C3), and fosetyl-aluminum at 0.5 g.ai., 1 g.ai. and 2 g.ai. (D1, D2, D3).

Therefore, based on this study, fosetyl-aluminum at concentrations of 0.5 g.ai., 1 g.ai., and 2 g.ai. was selected for use in conjunction with chili seeds to test seed quality and disease control in section 3.2.

3.2 Chili seed quality after seed pelleting with different types and concentrations of fungicide

From Section 3.1, the selected fungicide with inhibitory effects on *Fusarium* sp. is fosetyl-aluminum at concentrations of 0.5 g.ai., 1 g.ai., and 2 g.ai. Subsequently, these concentrations were used in combination with chili seeds to assess changes in seed quality and test the efficacy in inhibiting *Fusarium* sp. in Sections 3.2 and 3.3. The results and discussion follow.

3.2.1 Seed quality after testing in laboratory condition

Pelleting seeds with fosetyl-aluminum at levels of 0.5 g.a., 1 g.ai., and 2 g.ai. demonstrated no hindrance to radicle emergence rate and exhibited differences compared to other treatment methods. This indicates that seed pelleting has no impact on radicle emergence rate when compared to pelleting with $CaSO_4$ alone. As explained by Kangsopa *et al*., (2018), selecting an appropriate pelleting formula will not hinder the germination process. When combined with fungicide at any of the three levels, a high percentage of radicle emergence was maintained, similar to reports on the use of seeds for lettuce (Silva, & Matos, 2016). For seeds pelleted with fosetyl-aluminum at levels of 0.5 g.ai. and 1 g.ai., higher germination rates and speed of germination were observed, showing significant differences compared to other methods (Table 2). This indicates that aluminum, the main active component in this fungicide, has no inhibitory effect on seed germination. This aligns with findings in wheat (*Triticum aestivum*), cowpea (*Vigna ungiculata*), and mung bean (*Vigna radiata*) (Neogy, Datta, Roy, & Mukherji, 2000; Alamgir and Akhter, 2009; James, Sharavanan, & Visvanathan, 2013). Additionally, $CaSO_4$ used for seed pelleting provides large-sized particles containing silica, sulfur, and calcium (Kathpalia, & Bhatla, 2018), enhancing the germination process compared to non-pelleted seeds. However, if fosetyl-aluminum is used at the 2 g.ai. level, it will be observed that the germination percentage of the seeds decreases by 13% compared to non-pelleted seeds. This indicates that using fosetyl-aluminum beyond the 1 g.ai. level in the pelleting of chili seeds can result in seed toxicity, affecting the germination (Silva and Matos, 2016). Seed quality testing over 14 days also reveals that inspecting normal seedlings to evaluate the mean germination time shows no statistical difference between pelleted and non-pelleted seeds. These findings suggest that pelleted and non-pelleted seeds exhibit different seed vigor. Pelleting seeds with fosetyl-aluminum at any of the three concentration levels show significantly higher shoot lengths compared to non-pelleted seeds. As for root length, both non-pelleted seeds and pelleted seeds with fosetyl-aluminum at any of the three concentration levels exhibit significantly higher values than seeds pelleted with $CaSO_4$ alone. The examination of documents does not yet reveal clear data regarding the effect of fosetyl-aluminum on seedlings growth, but it appears to promote clear root and

Table 2. Radicle emergence percentage (RE), germination percentage (GE), speed of germination percentage (SGE), mean germination time (MGT), shoot length (SHL) and root length (RHL) of chili seeds after pelleting with different types of fungicide, tested in laboratory conditions

Treatment	RE (%)	GE (%)	SGE (plant/day)	MGT (day)	SHL (cm)	RHL (cm)
Non-pelleted seed	18 b[1, 2]	59 b	4.10 b	7.03	2.41 b	10.48 a
Pelleted seed + $CaSO_4$ (P)	19 b	54 bc	3.64 bc	7.59	3.18 ab	6.29 b
(P)+Fosetyl-aluminium 0.5 g.ai.	25 a	70 a	5.39 a	7.43	4.13 a	9.33 a
(P)+Fosetyl-aluminium 1 g.ai.	29 a	73 a	5.22 a	7.20	3.89 a	10.33 a
(P)+Fosetyl-aluminium 2 g.ai.	23 a	46 c	3.38 c	7.11	3.85 a	10.15 a
F-test	**	**	**	ns	**	**
CV.%	20.57	6.46	9.61	4.7	17.70	17.80

ns,** : no significant difference and significant difference at P≤0.01, respectively

[1] Data were transformed by the arcsine before statistical analysis and 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.

shoot elongation. In contrast, excessive aluminum, a major component, inhibits the elongation of root and shoot cells (Tahara, Norisada, Yamanoshita, & Kojima, 2008).

3.2.2 Seed quality after testing in greenhouse conditions

The evaluation over a 6-day period reveals that non-pelleted seeds exhibit a significantly higher cotyledon emergence percentage compared to the pelleted seeds. After assessing normal seedlings at the age of 14 days, it was found that seeds subjected to all pelleting methods showed an increased number of normal seedlings, with the method involving fosetyl-aluminum at 0.5 g.ai. demonstrating higher germination than other methods (Table 3). This indicates that seeds processed through pelleting methods may experience a slightly delayed germination during the 6-7 day period. Siri (2015) explains that seeds subjected to pelleting may exhibit delayed germination initially due to factors such as moisture and oxygen having impeded access to the pelleted material. However, once provided with optimal germination conditions, these seeds can germinate and grow into normal seedlings comparable to non-pelleted seeds. This aligns with the assessment of the speed of germination, particularly in the case of pelleted seeds with fosetyl-aluminum at 0.5 and 1 g.ai., showing higher speed of germination than other methods. This information becomes more relevant when evaluating normal seedlings at the 5-14 day stage, suggesting an increasing trend in the number of normal seedlings for seeds subjected to pelleting during this period. This may result from the improved internal moisture treatment within the seeds through the pelleting material (Kangsopa, Singsopa, & Thawong, 2023). Additionally, $CaSO_4$, as a component containing calcium, plays a role in stimulating seed cracking, making germination easier and faster (Liu *et al*., 2011). Moreover, sulfur contributes to amino acid formation, acting as a central element in protein synthesis, leading to enhanced germination and growth of seeds (Guo, Zhao, Wang, Song, & Xia, 2021). Furthermore, pelleting seeds with fosetyl-aluminum at 0.5 and 1 g.ai. also demonstrates a favorable trend in shoot development compared to other methods, as described in the laboratory condition testing section. However, the use of fosetyl-aluminum at 2 g.ai. results in a reduction in shoot length. This could be attributed to the high concentration of fosetyl-aluminum exceeding the seedling's requirements, causing an accumulation of components or excessive aluminum, which might impede cell elongation in the shoots (Tahara *et al*., 2008).

3.3 Effects of inhibiting *Fusarium* sp. after pelleting seeds with fosetyl-aluminum at different concentrations

Under laboratory conditions, non-pelleted seeds exhibited only 5% coverage by *Fusarium* sp. mycelia. It is evident that untreated seeds without fungicidal chemicals are susceptible to *Fusarium* sp. infestation as usual if there are pathogenic agents present. Pelleting seeds with $CaSO_4$ showed a survival rate of 35%, indicating a slight increase in protection against mycelial coverage compared to non-pelleted seeds. Seed pelleting with fosetyl aluminum at concentrations of 0.5 g.ai., 1 g.ai., and 2 g.ai. demonstrated higher inhibition of mycelial coverage compared to other methods. It showed efficacy in inhibiting mycelial coverage up to 1,560, 1,660, and 1,700 percent, respectively, compared to non-pelleted seeds (Table 4). This suggests that adding fungicide to seeds can help protect them from *Fusarium* sp. fungal infestation (Keerio *et al*., 2017). Fosetyl-aluminum, belonging to the phosphonate group, exhibits complete systemic and rapid absorption properties, allowing for efficient penetration into plant tissues. It can translocate swiftly from root tips to shoot and within the plant through both xylem and phloem vessels. It inhibits spore germination and hampers the development of fungal hyphae by competitively antagonizing or displacing phosphates in various enzyme controls. Additionally, it induces plant resistance and stimulates the production of phytoalexins to combat fungal pathogens (Fenn, & Coffey, 1985; Saib *et al*., 2015).

Under greenhouse conditions, observations conducted over a 14-day period clearly illustrate the germination capability of seeds. Table 3 reveals that non-pelleted seeds exhibit an average normal seedling percentage of 59%. However, after inoculation with the pathogen mixed with the growth medium, the percentage of normal seedlings decreases significantly to 13%. It is observed that the seeds exhibit characteristics of decay or abnormal seedling emergence and are unable to emerge from the growing medium. On the other hand, considering seeds treated with fosetyl-aluminum at all three concentration levels, it is evident that the seeds can germinate into seedlings nearly comparable to the seed germination assessment conducted in greenhouse conditions (Table 3). This indicates that the seeds still have

Table 3. Cotyledon emergence percentage (COT), germination percentage (GE), speed of germination (SPE), mean germination time (MGT) and shoot length (SHL) of chili seed after pelleting with different types of fungicide, tested under greenhouse conditions.

Treatment[1/]	COT (%)	GE (%)	SPE (plant/day)	MGT (day)	SHL (cm)
Non-pelleted seed	43 a [1, 2]	61 ab	2.92 ab	7.23 b	7.19 bc
Pelleted seed + $CaSO_4$ (P)	4 b	50 b	2.16 b	9.35 a	7.35 bc
(P)+Fosetyl-aluminium 0.5 g.ai.	10 b	68 a	3.44 a	8.86 a	8.58 a
(P)+Fosetyl-aluminium 1 g.ai.	9 b	63 ab	3.31 a	8.39 a	7.92 b
(P)+Fosetyl-aluminium 2 g.ai.	11 b	61 ab	2.02 b	8.62 a	6.89 c
F-test	*	*	*	*	**
CV.%	29.85	15.44	17.38	7.33	6.50

*, ** : significant difference at P≤0.05 and P≤0.01, respectively.
[1] Data were transformed by the arcsine before statistical analysis and 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.

Table 4. Survival of pelleted chili seeds with fungicide and disease severity score of chili seed after pelleting with different types of fungicide, tested under laboratory and greenhouse conditions.

Treatment	Laboratory		Greenhouse		
	Survival of pelleted chili seeds (%)[1]	(%)	Survival of chili seedlings (%)[2]	(%)	Disease severity score[3]
Non-pelleted seed	5 c [2/]		13 b		5
Pelleted seed + $CaSO_4$ (P)	35 b	(+600)	18 b	(+600)	4
(P)+Fosetyl-aluminum 0.5 g.ai.	83 a	(+1,560)	58 a	(+1,560)	3
(P)+Fosetyl-aluminum 1 g.ai.	88 a	(+1,660)	69 a	(+1,560)	2
(P)+Fosetyl-aluminum 2 g.ai.	90 a	(+1,700)	68 a	(+1,560)	2
F-test	**		**		-
CV.%	19.62		12.82		-

** : significant difference at P≤0.01.
[1] Data were transformed by arcsine before statistical analysis and 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] Disease severity score: 1 = No disease, 2 = Normal seedlings with 25% yellowing cotyledons, 3 = Normal seedlings with 50% yellowing cotyledons, 4 = Normal seedlings with 75% yellowing or wilting cotyledons, and 5 = Normal seedlings with 100% wilting cotyledons.

the capability to germinate into seedlings. Further assessment from disease severity scores reveals that non-pelleted seeds have 100% of seedlings exhibiting wilted cotyledons. As for seeds treated solely with $CaSO_4$, it is found that the cotyledons exhibit yellowing and wilting at 75%. Seeds pelleted with fosetyl-aluminum at concentrations of 1 g.ai. and 2 g.ai. show that 25% of the seedlings have yellow cotyledons, while at the 0.5 g.ai. concentration level, 50% of the seedlings exhibit yellowing cotyledons. Seed pelleting with fosetyl-aluminum demonstrates effective protection against *Fusarium* sp. infestation during both the seed germination and seedling stages up to the 14-day evaluation period (Table 4). Fosetyl-aluminum exhibits induction of defense-related compounds properties that aid in resisting *Fusarium* sp. infestation, which can inhibit the growth of hyphae and conidia of the fungus and reduce the production of toxins that the fungus may release, thereby increasing plant resistance to these toxins (Keerio *et al*., 2017; Silva *et al*., 2016).

4. Conclusions

The efficacy testing of *Fusarium* sp. inhibition in laboratory conditions demonstrated that the application of fosetyl-aluminum at concentrations of 0.5, 1, and 2 g.ai. can effectively inhibit *Fusarium* sp. by 100%. Subsequent seed pelleting with fosetyl-aluminum at all three concentration levels indicated that seed pelleting with fosetyl-aluminum at concentrations of 0.5 and 1 g.ai. promotes higher germination and seedling growth compared to other methods. Seed pelleting with fosetyl-aluminum at all three concentration levels effectively inhibited *Fusarium* sp. both in laboratory and greenhouse conditions.

Consequently, seed pelleting with fosetyl-aluminum at a concentration of 1 g.ai. is a recommended seed treatment for use with chili seeds. This treatment effectively inhibits *Fusarium* sp. without adverse effects on seed germination, seedling vigor, or seedling growth.

Acknowledgements

This work (Grant No. MJ.2-66-037) was supported by Internal institutional budget 2023, Faculty of Agricultural Production, Maejo University. The author would like to offer particular thanks to the Division of Agronomy, Faculty of Agricultural Production, Maejo University for materials and the use of laboratories and research sites.

References

Afzal, I., Javed, T., Amirkhani, M., & Taylor, A. G. (2020). Modern seed technology: seed coating delivery

systems for enhancing seed and crop performance. *Agriculture, 10*, 526. doi:10.3390/agriculture10110526

Alamgir, A. N. M., & Akhter, S. (2009). Effects of aluminium (Al^{3+}) on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *Bangladesh Journal of Botany*, *38*(1), 1–6.

Allen, T. W., Enebak, S. A., & Carey, W. A. (2004). Evaluation of fungicides for control of species of *Fusarium* on longleaf pine seed. *Crop Protection*, *23*(10), 979–982.

AOSA. (1983). *Seed vigor testing handbook*. Ithaca, NY: AOSA. (Contribution to the handbook on seed testing, 32)

Ayesha, M. S., Suryanarayanan, T. S., Nataraja, K. N., Prasad, S. R., & Shaanker, R. U. (2021). Seed treatment with systemic fungicides: time for review. *Frontiers in Plant Science*, *12*, 654512.

Barhate, B. G., Musmade, N. A., & Nikhate, T. A. (2015). Management of Fusarium wilt of tomato by bioagents, fungicides. *International Journal of Plant Protection*, *8*(1), 49–52.

Chuenchan, W., Raksasanoy, S., Yooboriboon, S., & Kitja, W. (2019). Inhibition of *Phytophthora parasitica* by antagonistic molds from soil's Kuiburi Subdistrict, Prachuap Khiri Khan Province. *Journal of Science Ladkrabang*, *28*(1), 52–64.

Di Marco, S., Osti, F., Calzarano, F., Roberti, R., Veronesi, A., & Amalfitano, C. (2011). Effects of grapevine applications of fosetyl-aluminium formulations for downy mildew control on" esca" and associated fungi. *Phytopathologia Mediterranea*, *50*, S285–S299.

Ellis, R. A., & Roberts, E. H. (1980). The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology*, *9*(2), 373–409.

Fenn, M. E., & Coffey, M. D. (1985). Further evidence for the direct mode of action of fosetyl-Al and phosphorous acid. *Phytopathology*, *75*(9), 1064–1068.

Ghimire, M., Adhikari, N., Puri, S., Baral, S., & Basnet, B. (2022). Efficacy of chemical fungicides against the fusarium rhizome rot of Ginger (*Zingiber officinale*). *Archives of Agriculture and Environmental Science*, *7*(3), 402–406.

Gormez, E., Golge, O., & Kabak, B. (2021). Quantification of fosetyl-aluminium/phosphonic acid and other highly polar residues in pomegranates using Quick Polar Pesticides method involving liquid chromatography-tandem mass spectrometry measurement. *Journal of Chromatography A*, *1642*, 462038.

Grover, R. K., & Moore, J. D. (1962). Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. *Phytopathology*, *52*, 876–880.

Gullino, M. L., Tinivella, F., Garibaldi, A., Kemmitt, G. M., Bacci, L., & Sheppard, B. (2010). Mancozeb: Past, present, and future. *Plant Disease*, *94*(9), 1076–1087.

Guo, Z., Zhao, J., Wang, M., Song, S., & Xia, Z. (2021). Sulfur dioxide promotes seed germination by modulating reactive oxygen species production in maize. *Plant Science*, *312*, 111027.

Harish, J., Jambhulkar, P. P., Bajpai, R., Arya, M., Babele, P. K., Chaturvedi, S. K., Kumar, A., & Lakshman, D.K. (2023). Morphological characterization, pathogenicity screening, and molecular identification of *Fusarium* spp. isolates causing post-flowering stalk rot in maize. *Frontiers in Microbiology*, *14*, 1121781. doi:10.3389/fmicb.2023.1121781

International Seed Testing Association. (2023). *International rules for seed testing*. Bassersdorf, Switzerland: Author.

James, S., Sharavanan, P. S., & Visvanathan, P. 2013. Effect of aluminum and lead on the germination and growth of *Vigna ungiculata* (L.) Walp. *International Journal of Current Research*, *2*(1), 38–45.

Jeephet, J., Atnaseo, C., Hermhuk, S., & Kangsopa, J. (2022). Effect of seed pelleting with different matrices on physical characteristics and seed quality of lettuce (*Lactuca sativa*). *International Journal of Agricultural Technology*, *18*(5), 2009–2020.

Kangsopa, J., Hynes, R. K., & Siri, B. (2018). Lettuce seeds pelleting: A new bilayer matrix for lettuce (*Lactuca sativa*) seeds. *Seed Science and Technology*, *46*, 521–531. doi:10.15258/sst.2018.46.3.09

Kangsopa, J., Singsopa, A. and Thawong, N., 2023. Effects of different binder types and concentrations on physical and quality properties in marigold (*Tagetes erecta* L.) seed pelleting. *Songklanakarin Journal of Science and Technology*, *45*(4), 494–500.

Kangsopa, J., Singsopa, A., Thawong, N. and Pidtatanao, J. (2024). Seed encrusting with plant nutrients enhances germination, plant growth and yield of soybean (*Glycine max*). *Legume Research*. doi: 10.18805/LRF-772.

Kathpalia, R., & Bhatla, S.C. (2018).*Plant physiology, development and metabolism*. Singapore: Springer.

Keerio, A., Nizamani, A. Z., Hussain, S., Rafiq, M., Iqbal, S., & Keerio A. D. (2017). Efficacy of some chemical fungicides against fusarium wilt of sunflower in-vitro condition course by *Fusarium oxysporum*. *International Journal of Botany Studies*, *2*, 80–85.

Kunwanlee, P., Maneerat, T., & Plodjinda, K. (2023). Effect of tomato seed priming with *bacillus subtilis* on seed germination, and seedling survival in outbreak bacterial wilt in greenhouse condition. *VRU Agricultural and Food Journal*, *2*(1), 22–28.

Liu, T. W., Wu, F. H., Wang, W. H., Chen, J., Li, Z. J., Dong, X. J., . . . Zheng, H. L. (2011). Effects of calcium on seed germination, seedling growth and photo synthesis of six forest tree species under simulated acid rain. *Tree Physiology*, *31*(4), 402–413.

Naik, M. K., Rani, G. S. D. & Madhukar, H. M. (2008). Identification of resistance sources against wilt of chilli (*Capsicum annuum* L.) resistance caused by *Fusarium solani* (Mart.) Sacc. *Journal of Mycopathological Research*, *46*, 93–96.

Neogy, M., Datta, J., Roy, A. K. & Mukherji, S. (2000). Studies on phytotoxic effect of aluminum on growth and some morphological parameters of *Vigna radiata* L. Wilcz. *Journal of Environmental Biology*, *23*, 411–416.

Norkaew, J., Khemmuk, W., McGovern, J. & To-anun, C. (2021). Selection of antagonistic bacteria against *Fusarium fujikuroi* causing bakanae disease of rice. *Khon Kaen Agriculture Journal*, *49*(1), 144–154.

Parihar, T. J., Sofi, M. Y., Rasool, R. S., Khursheed, S., Bhat, Z. A., Hussain, K., . . . Nehvi, F.A. (2022). *Fusarium chlamydosporum*, causing wilt disease of chili *(Capsicum annum* L.) and brinjal (*Solanum melongena* L.) in Northern Himalayas: A first report. *Scientific Reports*, *12*(1), 20392.

Saib, A., Berrebbah, H., Djebar, M.R. & Berredjem, M. (2015). Fungitoxic evaluation of new modified Amidophosphonates (ap1, ap2) on the in vitro growth of two fungal strains. *Research Journal of Environmental Toxicology*, *9*(4), 196.

Shaheen, N., Khan, U. M., Azhar, M. T., Tan, D. K., Atif, R. M., Israr, M., . . . Rana, I. A. (2021). Genetics and genomics of Fusarium wilt of chilies: A review. *Agronomy*, *11*(11), 2162.

Silva, P., & Matos, M. (2016). Assessment of the impact of Aluminum on germination, early growth and free proline content in *Lactuca sativa* L. *Ecotoxicology and Environmental safety*, *131*, 151–156.

Silva, P.V., Vélez, M.L., Hernández, D., Núñez, C., & Greslebin, A. (2016). Action of fosetyl-al and metalaxyl against *Phytophthora austrocedri*. *Forest Pathology*, *46*, 54–66.

Siri, B. (2015). *Seed conditioning and seed enhancements*. Khon Kaen, Thailand: Klungnanawitthaya Printing.

Tahara, K., Norisada, M., Yamanoshita, T., & Kojima, K. (2008). Role of binding ligands in aluminum resistance of *Eucalyptus camaldulensis* and *Melaleuca cajuputi*. *Plant Soil*, *302*, 175–187.

Taskeen-Un-Nisa, W. A., Bhat, M. Y., Pala, S. A., & Mir, R. A. (2011). In vitro inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. *Journal of Biopesticides*, *4*(1), 53–56.

Tort, N. & Turkyilmaz, B. (2003). Physiological effects of captan fungicide on pepper (*Capsicum annuum* L.) plant. *Pakistan Journal of Biological Sciences*, *6*(24), 2026–2029.