Research Article

Potential of Seed Bulbs as an Inoculum Source of Molar Pathogens and the Effectiveness of Various Fungicides in Reducing Infection Index

Salim Widono[1*], Supyani[2], S.H. Poromarto[2] and Hadiwiyono[2]

[1]Doctoral program of Agriculture Sciences, Faculty of Agriculture, Sebelas Maret University, Surakarta, Central Java, Indonesia; [2]Department of Agrotechnology, Faculty of Crop Agriculture, Sebelas Maret University, Surakarta, Central Java, Indonesia.

Abstract |Basal rot caused by *Fusarium oxysporum* f.sp. *cepae* is the most detrimental disease in all world shallot producers. Diseased shallots show rotting at the base of the bulbs, and the leaves turn yellow and twist, so in Java, it is known as "moler disease." The moler pathogen causes yield losses of up to 45% in the field, and bulb losses in the storage of up to 12-30%. Seed management is an important aspect in controlling basal rot of shallot disease because this disease is soil-borne and seed-borne. This study examines five shallot varieties as potential moler pathogen carriers and evaluates fungicide effectiveness. Five varieties of shallot bulbs (Thailand, Bima, Bauji, Manjung, and Super Phillips) were tested and calculated for pathogen infection index by observing the growth of pathogenic *Fusarium* spp. colonies on shallot bulbs tested on PDA medium after incubation for 7 days at room temperature. Meanwhile, to study the effect of seed treatment, the seeds of the varieties currently widely planted by Brebes farmers, the Bima variety, were treated with contact fungicides (Chlorothalonil, Mancozeb, and Dithiocarbamate) and systemic fungicides (Carbendazim) before planting. The results showed that seed bulbs had the potential as an inoculum source of the moler pathogen. Varieties and fungicide treatment significantly affect the infection index of bulbs. Pre-planting seed treatment with systemic and contact fungicides can reduce yield loss by more than 25%, with control effectiveness reaching more than 70%.

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***Correspondence** | Salim Widono, Department of Agrotechnology, Faculty of Agriculture, Sebelas Maret University, Surakarta, Central Java, Indonesia. **Email:** salimwidono@staff.uns.ac.id
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Introduction

Shallot (*Allium cepa* L.) is a commodity that is a source of economic and employment opportunities for the community and contributes to regional economic development. The community also widely uses shallots as a cooking spice and traditional medicinal ingredient. *Allium* species (shallot and garlic) contain many vitamins, carotene, minerals, antioxidants, and antibiotics (Sharifi-Rad *et al*., 2016). Indonesia's average shallot productivity is 12.11 t.ha^{-1} (BPS, 2025). This fact is still far below the productivity of the three highest world shallot-supplying countries, including New Zealand, Germany, and China, with successive productivity of 42 $t.ha^{-1}$, 41.43 $t.ha^{-1}$, and 38.81 $t.ha^{-1}$, respectively (Susanti, 2020). Pathogen attack is one of the causes of low shallot productivity in the field. In addition, the low productivity of shallots is also influenced by environmental conditions and cultivation activities carried out by farmers, especially seed management (Cahyaningrum *et al*., 2023; Saidah *et al*., 2020). Utilizing healthy or pathogen-free seeds will reduce the risk of yield loss due to seed-borne pathogens. Nevertheless, basal rot of shallot can be managed effectively by seed bulb dressing or dip treatment in fungicide (Sintayehu *et al*., 2014).

Basal rot, caused by *Fusarium oxysporum* f.sp. *cepae,* is a major global shallot disease (Cramer, 2000; Edel-Hermann and Lecomte, 2019). Detected species included *F. proliferatum* and *F. oxysporum* f.sp. *cepae*, *F. acutatum*, and *F. anthophilium* (Le *et al*., 2021a). *F. oxysporum* was most commonly found (81%) in bulbs showing basal rot symptoms of shallot, followed by *F. solani* (15%), and *F. proliferatum* (4%) (Le *et al*., 2021a). Diseased shallots show decay symptoms at the bulbs' base; leaves turn yellow and curl (Le *et al*., 2021b). This disease in Java is known as shallot "moler" disease. This disease can cause yield losses ranging between 2.13-4.38% for resistant varieties and 27.26-40.04% for moderately resistant varieties, respectively (Hadiwiyono *et al*., 2020). Basal rot of shallot also affects 50% of shallot seedlings in African fields (Dauda *et al*., 2018). Moler disease pathogens cause yield losses of up to 45% in the field and up to 12-30% of bulb losses in storage (Sintayehu *et al*., 2011). *Fusarium* spp. is a soil-borne (Bahadur, 2022; Le *et al*., 2021b; Patil *et al*., 2018) and seed-borne pathogen (Adongo *et al*., 2015; Ozer and Koycu, 2004; Sintayehu *et al*., 2011).

Various methods can be used to control shallot moler disease of shallot, including fertilizing with compost, planting healthy seeds, treating seeds, using biological agents, and chemical control (Gajendran *et al*., 2016). In addition, plant resistance is also crucial in moler disease control (Galván *et al*., 2008; Mandal and Cramer, 2021). Pathogen-free seeds minimize the source of primary inoculum, especially for soil-borne pathogens. Knowing the health level of shallot bulbs before planting and treating them to eliminate or reduce seed-borne pathogens is also necessary. Tebuconazole was the most effective for reducing disease severity, giving a 26.5% reduction of basal plate rots and 44% reduction of bulb rots on garlic dry rot caused by *F. proliferatum* (Mondani *et al*., 2022). Farmers use chemicals to control shallot root rot or moler disease, working systemically or in contact as a protectant. This research evaluates the potential of bulbs of five varieties of shallots as agents of transmission of the moler pathogen and studies the effectiveness of seed treatment with fungicides used by farmers in controlling shallot moler disease in the field.

Materials and Methods

Seed infection test-1

The experiment was conducted in the Pest Laboratory of the Faculty of Agriculture, Universitas Sebelas Maret in Surakarta, using a Completely Randomized Design with five replications. The main ingredients were five varieties of shallot bulbs, including Thailand, Bima, Bauji, Manjung, and Super Phillip. One treatment unit consisted of 25 pieces of the base of shallot bulbs, which were incubated on 40 mL PDA media in a Petri dish with a diameter of 20 cm. Observations were made after the bulbs were incubated for seven days at room temperature. Measurement of the Pathogenic Infection Index of *Fusarium* spp. was based on observing fungal colonies growing from bulb tissue. The growing fungal colonies were verified microscopically to prove the presence of specific structures in the form of *Fusarium* micro and macroconidia (Mondani *et al*., 2022).

Seed treatment test-2

Seed treatment tests with fungicide were conducted in Sidamulya village (6°54'18.9" S 109°01'09.1" E, at 3 meters above sea level, and alluvial soil), Wanasari sub-district, Brebes district, Central Java. The types of fungicides used for treatment included the contact fungicide group, represented by Chlorothalonil, Mancozeb, and Dithiocarbamate, and the systemic fungicide represented by Carbendazim. The three selected fungicides are the most widely used types by shallot farmers in Brebes. The experiment was conducted by cultivating shallots according to the habits of Brebes farmers in a completely randomized design with three replications. The treatment unit is a bed measuring $8x1m^2$. Seed treatment was carried out with the same dose of all types of fungicide tested, as much as 20 g formulation (equal to 1.5 $g.L^{-1}$ Chlorothalonil; 0.1 $g.L^{-1}$ Carbendazim + 1,5 $g.L^{-1}$

Mancozeb; 1.4 g.L^{-1} Dithiocarbamate respectively) in 10 L of water to soak 8 kg of bulbs for 5 minutes. Disease intensity of shallots harvested 60 days after planting was the primary variable observed in this experiment. The intensity of moler disease is measured by the following formula.

$$D = \frac{\sum (n \times v)}{N \times Z} \times 100\%$$

With *DI* = Disease intensity; *n* = the number of plants observed shows a specific score; v = Attack percentage score. With the following score. *0* = beds with shallots showing no MDS symptom, 1 = beds with symptoms of 1-25% MDS, 2 = beds with 26-50% symptoms of MDS, 3 = beds with 51-75% symptoms of MDS, and 4 = the bed has shallots with symptoms of MDS 71-100%.

The intensity of moler disease was measured on 10 sample plants, which were determined systemically randomly (Sampling was carried out at five points, with two sample units taken from each point, excluding the outermost plants. One point was located in the center of the plot, and four points were placed at each corner of the plot). The supporting variables observed included shallot yield per hectare unit. Yield measurements were carried out on three sample plots measuring one m^2 for each treatment unit. The effectiveness of controlling shallot moler disease is calculated based on the difference between the intensity of the disease in the treatment and the control, divided by the intensity of the disease in the plot without control, multiplied by 100%. The yield return effectiveness is determined by calculating the difference between the shallot yield in the treatment plot and the shallot yield in the plot without control, then dividing it by the shallot yield in the plot without control, then multiplying by 100%.

All data were subjected to analysis of variance (ANOVA) to evaluate the level of *Fusarium* infection index of the bulb of shallot varieties tested and evaluate the effectiveness of fungicide treatments in suppressing the development of moler disease of shallot. The differences between treatments were tested with the DMRT (Duncan Multiple Range Test) at 5%.

Results and Discussion

Seed infection test-1

Moler pathogen infestation and infection detected in all tested shallot varieties can be seen in Figure 1. The value of the *Fusarium* infection index, from the highest to the lowest, respectively, was shown by the varieties Bauji, Manjung, Thailand, Bima, and Super Phillip. Brebes shallot farmers prefer the Bima variety over introduced varieties. Although the yield and tuber size of the Bima variety were smaller than the imported variety, Brebes shallot farmers preferred the characteristics of the number of tillers, shape, color, and aroma of the bulbs (Mathivanan and Prabavathy, 2007).

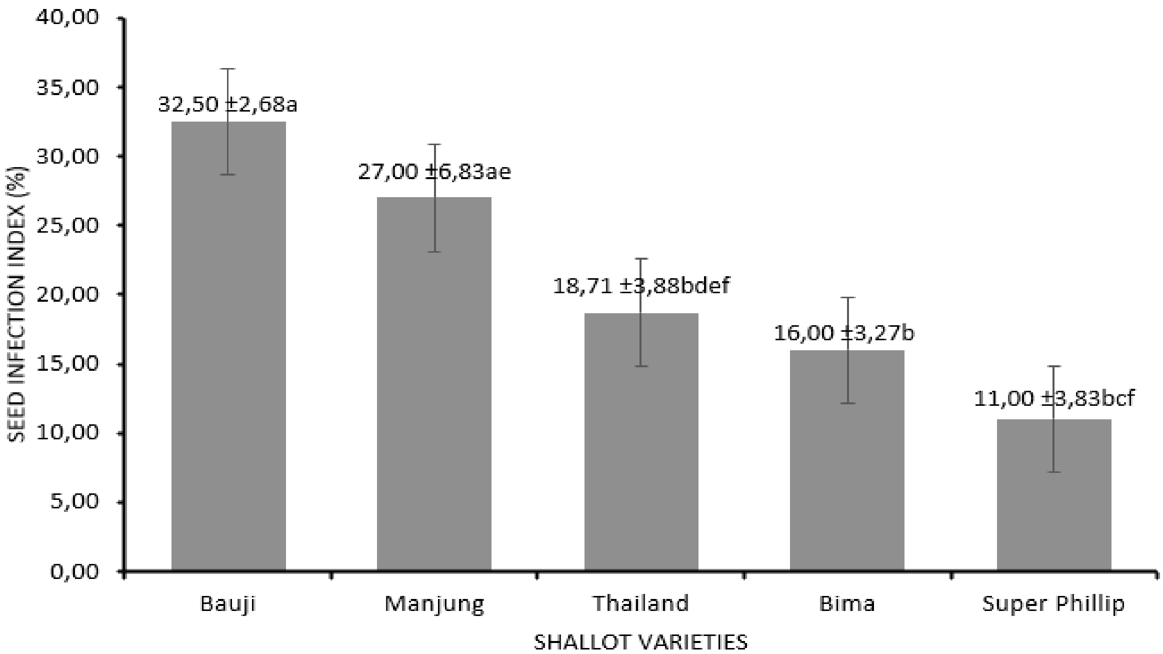

Figure 1: *Infection index of fusarium of bulb of five varieties of shallot.*

The results showed that the level of *Fusarium* infection in the Bima, Super Phillip, and Thailand varieties is still below 20%. In contrast, in the Manjung and Bauji varieties, the infection index value is high enough to reach more than 25%. This indicates that the Thaliland, Bima, and Super Phillip varieties are more resistant to the moler disease than the Bauji and Manjung varieties. The physical structure of the bulbs influences the resistance of shallots to *Fusarium* spp. The Thaliland, Bima, and Super Phillip varieties have stronger and thicker bulb layers and root tissues, making them more difficult to infect by the moler pathogen. In contrast, the Bauji and Manjung varieties have less intense epidermal cell walls, making the moler pathogen easily penetrate and infect shallot bulbs (Prakoso *et al*., 2017). The resistance of shallots to *Fusarium* spp. is also influenced by the genotype of the plant (Aprilia *et al*., 2020) and the accumulation of lignin in the shallot plate (Cahyaningrum *et al*., 2020). In addition, various inducers or elicitors can play a role in inducing plant resistance to pathogen attacks. The application of Bacillus spp. suppressed twisted disease on shallots and quantitatively increased the content of jasmonic and salicylic acids, serving as an induced resistance mechanism against pathogens (Wulan *et al*., 2022). The application of salicylic acid by immersing and spraying increases the resistance of shallot varieties to the twisted disease (Wijoyo *et al*., 2020).

The result also indicates that farmers contribute to the development of moler disease in Brebes by using shallot bulbs infected with moler pathogens. Infected bulbs can be a source of disease transmission in the field (Gupta and Kumar, 2020; Ozer and Koycu, 2004). Seed infection by pathogens can also lead to a decrease in seed vigor (Gebeyaw, 2020), making it crucial to control seed health in the agricultural sector (Klaedtke *et al*., 2022; Gupta and Kumar, 2020).

The level of pathogenic infection in seeds can be influenced by various factors, both related to the condition of the seed itself and the surrounding environment. Low-quality seeds or physical damage are more susceptible to pathogenic infections. The high moisture seed content is more susceptible to fungal and bacterial attacks because damp conditions support pathogen growth. Environmental conditions with high temperatures and high humidity can increase the risk of pathogenic infections in the seeds. High humidity, in particular, encourages the development of pathogenic fungi. Environments contaminated with pathogens, such as pathogenic infested soil or water, can increase the risk of infection of seeds. Likewise, incorrect plant rotation can cause the accumulation of specific pathogens in the soil, which can infect seeds. Poor treatment during harvest, drying, and storage can cause pathogens to infect seeds (Martín *et al*., 2022). *Fusarium* includes soil and seed pathogens, so this pathogen can spread far along with traded seeds.

Seed treatment test-2

The treatment of shallot bulbs with contact or protective fungicide was not able to increase the percentage of bulb growth in the field, nor did it reduce the intensity of moler disease in the shallot plantations tested for the Bima variety. On the other hand, seed treatment with systemic fungicide was able to suppress pathogen activity, resulting in a decrease in the intensity of moler disease and an increase in the percentage of bulbs growing in the field. The effect of seed treatment with fungicide on bulb emergence, moler disease, and yield on the Bima variety is presented in Table 1.

Table 1: *Effect of seed treatment using fungicides on the percentage of bulb emergence, disease intensity & control effectivity of moler, yield & the returned yield of Bima variety*

Fungicides	Mode of action	Dosage ($g.kg^{-1}$)[1)]	BE (%)	DI (%)	CE (%)	Yield ($kg.m^{-2}$)[2)]	Returned yield (%)
No treatment	-	-	95.33a	36,33 b	-	9,73 c	-
Chlorotalonil	Contact or protectant	1.875	95.67a	36,25 b	0,22	10,53 b	8,22
Mancozeb + carbendazim	Contact or protectant + Systemic	1.875	98.33c	10,83 a	70,19	12,77 a	31,24
Dithiocarbamate	Contact or protectant	1.875	96.67ab	38,50 b	-5,97	10,23 b	5,14

1) A gram active ingredient of fungicide per kg of bulb seed of shallot
2) The yield of the shallot sample plot with an area of one m^2
Means followed by the same letters in each column are significantly different based on DMRT at a level of 5%.
Abbreviations: BE = Bulb emergence, DI = Disease intensity, and CE = Control effectivity

Bauji and Manjung varieties have a fairly high level of *Fusarium* infection more than 25% higher than the seeds of Thailand, Bima, and Super Phillip varieties. This indicates that the two local varieties of Bauji and Manjung have lower resistance than the Bima varieties. In comparison, the two new varieties show a reasonably low infection rate, which is still below 20%. The resistance of plant varieties also affects the level of seed infection in types of plants that are resistant to producing seeds with lower levels of infection than vulnerable plants. By understanding these factors, farmers can take preventive steps to reduce the risk of pathogenic infections in seeds, such as the use of good cultivation techniques and healthy seed selection (Gullino and Munkvold, 2014), appropriate storage (Thivya, 2022), and treatment of seeds before planting (Askari-Khorasgani and Pessarakli, 2020; Baroroh *et al*., 2019; Kardava *et al*., 2023; Lamichhane *et al*., 2022; Munkvold *et al*., 2014).

The results showed that the treatment of shallot bulb seeds with contact or protective fungicide could not increase the percentage of seed growth in the field nor reduce the disease intensity of moler in the Bima variety tested. On the other hand, seed treatment with systemic fungicide was able to suppress pathogen activity, resulting in a decrease in the disease intensity of the moler and an increase in the percentage of the bulb seeds growing in the field. The treatment combination of contact or protective and systemic fungicides can increase the effectiveness of moler disease control by up to 70%, increase yield returned by 31%, and ensure the success of germination seeds by 98%. Systemic fungicides like Mancozeb + Carbendazim effectively reduced infection ($p < 0.05$) by 70%, likely due to their ability to penetrate and provide internal protection, whereas contact fungicides failed to reach the infection site inside the bulb. Mancozeb acts as a protectant on the surface of the bulb, inhibiting the attack of pathogens originating from the soil or those that have infested it. While Carbendazim, with its mode of action, inhibits beta-tubulin assembly in mitosis (Lewis *et al*., 2016), will enter the bulb tissue and affect the moler pathogen that has infected the bulb seeds. However, seed treatment with a systemic fungicide did not show any suppression of seed-borne pathogens in the early growth phase because *Fusarium* as a weak parasite rarely infects its host plant which still has a lot of energy.

Conclusions and Recommendations

The shallot bulb seeds have the potential to be an inoculum source of infection of the moler pathogens or the basal rot disease in the field. The level of *Fusarium* infection index varies from the highest to the lowest, respectively, in varieties of Bauji, Manjung, Thailand, Bima, and Super Phillip. Treating seed bulbs planted with contact and systemic fungicides can increase bulb emergence, reduce the intensity of moler or basal rot disease of shallot, and reduce the risk of yield loss in the field.

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Novelty Statement

This research provides empirical evidence that shallot seed bulbs used by Brebes farmers have the potential as an inoculum source of moler disease, and seed treatment with systemic fungicides is more effective in reducing the infection index than contacts.

Authors' Contribution

Salim Widono: Executed sampling, data collection, laboratory work, formal analysis, and formatted the manuscript.
S.H. Poromarto: conceptualization, writing review, and editing the manuscript.
Supyani: Formatted, reviewed, and edited the manuscript.
Hadiwiyono: supervision, data curation, methodology, writing review, and editing the manuscript.

Generative AI or AI assisted technology statement
The authorsdeclare that no generative AI was used in the creation of this manuscript

Conflict of interest
The authors have declared no conflict of interest.

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