



Enhancing *Capsicum annuum* L. Disease Resistance with Carrier and BSF Larval Gut Microbes

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Abstract

Red chili (*Capsicum annuum* L.) is one of the essential vegetable commodities in Indonesia. Nevertheless, the production of red chili is affected by disease due to fungal infection. Previous research showed that the consortial formulation of bacterial and *Trichoderma* without a carrier from Black Soldier Fly (*Hermetia illucens*) larva gut enhanced the disease resistance of red chili. Regardless, the research has not overcome several diseases caused by fungal infection. This study aims to analyze the effect of adding kaolin, talc, and zeolite as carriers with bacterial and *Trichoderma* consortia from BSF larval gut on the disease resistance of red chili caused by fungal infection. This experimental study was carried out in a randomized block design. The treatments were consortial of bacterial and *Trichoderma* + carrier kaolin (BTrK), talc (BTrT), zeolite (BTrZ), positive control of consortial without a carrier (K+(1)), positive control of inorganic treatment (K+(2)), and negative control of plants without treatment (K(-)). The results were analyzed by calculating disease incidence (DI) and severity intensity (SI) scores to determine disease resistance. New findings prove that BTrK enhanced resistance to fungal infection, namely cercospora leaf spot, leaf rust, and powdery mildew. BTrT formulation enhanced resistance to symptom severity. The study concluded that carrier formulation enhanced the resistance of red chili.

Keywords: Agriculture, carrier, biofungicide, disease resistance, red chili

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Introduction

Indonesian cuisine is known internationally due to its abundance of spices and herbs in various cuisines. Spicy cuisine is often found in all regions of Indonesia and uses chili as the main spice. There are various types of chilies in Indonesia, one of which is red chili (*Capsicum annuum* L.), which provides color and flavor to dishes. Almost all regional cuisines in Indonesia use red chili as a spice. Based on the National Statistical Bureau, the average consumption of red chili per capita in a week is 3,4 grams (Badan Pusat Statistik, 2024). The data suggest that red chili is an important agricultural commodity.

The production of red chili is affected by disease. Diseases due to fungal infection are the most common and cause more damage than any other pathogen (Nazarov et al., 2020). Those fungal infections can be prevented and treated

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by adding a biocontrol agent, for instance, *Micrococcus* (Patel dkk., 2021). Previous research has found beneficial microbes, namely *Micrococcus*, *Bacillus* sp., and *Trichoderma* fungi, inside the Black Soldier Fly (*Hermetia illucens*) larva gut (Kristi dkk., 2024; Sanjaya dkk., 2023). Following research also proved that the formulation of the consortium of bacterial and *Trichoderma* without a carrier from those BSF larva gut enhanced the disease resistance of red chili. However, the research has not resolved several diseases caused by fungal infection. The formula used in the study was only a consortia suspension of bacteria and *Trichoderma viride* diluted with sterile distilled water (Kristi dkk., 2024).

Formulation without a carrier causes the material to have a limited storage time (Myo et al., 2019). Supplementing probiotics to plants without a carrier also increases the risk of microbial dispersal in agricultural areas, and the short life span of microbes. Hence, the microbes

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are unable to function optimally. Adding a carrier to probiotic formulations escalates inoculation success in crops since the carrier supports microbial growth and provides protection during application to the rhizosphere. Carriers are also shown to carry multi-strain inoculations and support inoculum survival, consequently, microbes can function optimally in providing benefits to plants (Sohaib et al., 2020). The combination of various carrier and microbial formulas resulted in different effects on plant growth. Application of *Rhizobium* sp. + *Azotobacter* sp. biofertilizer formula using peat + kaolin or peat + kaolin + phosphate rock or biochar + zeolite carrier managed to enhance pod filling and soybean weight compared to other formulas (Aksani et al., 2021).

Thus, the study aims to explore an alternative solution through the development of a bioformulation with a solid carrier. The carrier serves as a microbial storage medium that can also enhance the disease resistance of red chili to avoid chemical fungicide usage. The carrier material must be easy to find, low-cost, and environmentally friendly to encourage local farmers in its application (Aksani et al., 2021). Therefore, in this study, three types of carriers were selected, namely kaolin, talc, and zeolite, to analyze how the effect of adding carriers with a consortium of bacteria and *Trichoderma* fungal from BSF larval gut isolates as formulations on disease resistance of red chili caused by fungal infection.

Methods

Study area

The research was conducted at the Botanical Garden of the Universitas Pendidikan Indonesia, located in Sukasari District, Bandung City, at an altitude of 923 m asl with coordinates 6°51'44.2"S 107°35'41.8"E. This research was conducted for four months, from December 2023 to April 2024.

Sample preparation

The research was experimental quantitative with a Randomized Block Design (RBD) with six treatments and four repetitions. The number of repetitions was determined based on Federer's repetition formula (Rozi et al., 2018). The population in this study was the

red chili cultivar Lembang-1. The samples in this study were red chili treated with organic treatment, namely, consortia of bacterial and *Trichoderma* + carrier with kaolin carrier (BTrK), talc (BTrT), zeolite (BTrZ), positive control of bacterial and *Trichoderma* without carrier (K+(1)), positive control of inorganic treatment with chemical pesticides + fungicides (K+(2)), and negative control of untreated plants (K(-)). The plants were grown outside the greenhouse.

The carrier combination treatment (BTrK, BTrT, and BTrZ) for each plant was carried out by applying 20 g per plant. The combination was given to the soil around the roots of red chili plants every two weeks (Sutarman & Prahasti, 2022). The positive control, specifically organic treatment without a carrier (K+(1)), received 10 mL of each liquid bacteria and the liquid *Trichoderma* consortium every two weeks (Kristi et al., 2024). The positive control, specifically inorganic treatments (K+(2)), received insecticides (Curacron brand) and fungicides (Heksa brand). The application was referred to the usage recommendations listed on the label, respectively, insecticides 1.5 mL/L and fungicides 1.5 mL/L.

Data Collection

Data collection started from one week after treatment until the beginning of the generative phase. Data collection was carried out every three days to see the time of appearance of disease symptoms on plants. Disease symptoms that appear on plants are documented using a phone camera. Furthermore, the diseases are identified through literature studies. Abiotic factor data taken are soil pH and moisture, light intensity, temperature, and air humidity. Rainfall data was retrieved from the online database of the National Geophysical Meteorology and Climatology Agency (BMKG, 2024).

Data Analysis

Plant disease resistance is determined through disease incidence and disease severity (Aditya et al., 2015). Disease incidence was determined using the Disease Incidence (DI)

formula (Hafsah et al., 2023). The severity of disease or health conditions shown in treatment can be determined using the formula of Severity Intensity (SI) Non-Absolute by the Directorate of Horticultural Protection, Ministry of Agriculture of the Republic of Indonesia. The Non-Absolute Severity Intensity (SI) formula was chosen because plants under fungal infection have the possibility of not experiencing damage to all organs (Warduna et al., 2011).

The value or score in each attack category is determined by calculating the damage that occurs on the plant according to previous research (Azwin et al., 2022). Data of Disease Incident (DI) and Severity Intensity (SI) were statistically analyzed using IBM SPSS Statistics 23 software to interpret the data. Data were presented as mean rank and hypothesis testing. The normality test and homogeneity of variances were verified using the Kolmogorov-Smirnov and one-way ANOVA. Significant differences among treatments were evaluated using the Kruskal-Wallis test, followed by Dunn's post hoc test in R software (version 4.3.2). Differences were considered statistically significant at $p_{adj} < 0.05$.

Results and Discussion

The research revealed three types of diseases caused by fungi. The three diseases were *Cercospora* leaf spot caused by *Cercospora capsici*, rust caused by *Phoma* sp., and powdery mildew caused by *Leveillula taurica* (Figure 1). *Cercospora* leaf spot or frog-eye forms a circular lesion on the leaf, typically has a light grey center and noticeable

dark brown margins, resembling a frog's eyes (Figure 1. A). Rust symptoms seem similar to *Cercospora* leaf spot, but the lesions are irregular with an uneven margin and typically light brown color (Figure 1. B). Powdery mildew symptoms in this study reveal an irregular brownish-yellow spot in the adaxial part of the leaf rather than covered in white "powder" (Figure 1. C). All these morphological symptoms are in line with a prior study (Aglave, 2019).

Abiotic factors, environmental conditions, and virulence of fungal pathogens affect plant health and resistance conditions at various levels of plant growth, starting from the seedling (Beka & Pichiah, 2021). Abiotic factors, such as air temperature, soil moisture, soil pH, and biotic factors such as vegetation around the host plant and the distance between the pathogen and the host plant, affect the fungal infection in plants (Merges et al., 2018). The average temperature at the study site was 27°C and the average air humidity was 75%, and the average rainfall was light (BMKG, 2024).

Cercospora capsici fungus lives at an optimum temperature of 22.5-23.5°C, relative humidity of 77-85%, and can be spread through splashing water, wind, and garden tools that have been exposed to contact with the fungus (Aglave, 2019). *Phoma* sp. fungus thrives in the rainy season and high humidity. *Phoma destructiva* fungus causes rust spots on chili fruits and leaves (Deb et al., 2020). *Leveillula taurica* fungus spores can be dispersed by wind, splashing water, contact through insects, and thrive in warm temperatures (20-30°C) and relative humidity of 40-90% (Aglave, 2019)

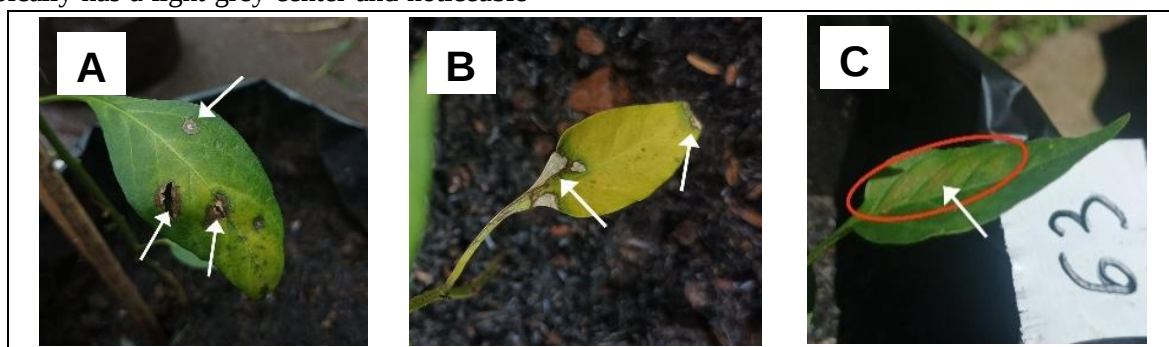


Figure 1. Fungal disease: A) cercospora leafspot; B) leaf rust; C) powdery mildew

Table 1. Disease Incidence (DI) and Severity Intensity (SI)

Treatment	Disease Incidence (%)	Category	Severity Intensity (%)	Category
K-	96,15	Highly susceptible	28,37	Severe
K+(1)	96,15	Highly susceptible	25,48	Moderate
K+(2)	69,23	susceptible	17,31	Moderate
BTrK	80,76	Highly susceptible	21,63	Moderate
BTrT	78,84	Highly susceptible	19,71	Moderate
BTrZ	80,76	Highly susceptible	21,15	Moderate

Table 2. Kruskal-Wallis Test

	Disease Incidence	Severity Intensity
df.	5	5
Asymp. Sig.	0.003	0.003

Table 3. Mean Rank of Kruskal-Wallis Test

Treatment	Disease Incidence	Severity Intensity
K-	53.92	55.35
K+(1)	51.35	49.27
K+(2)	27.58	25.81
BTrK	33.56	37.00
BTrT	34.69	32.31
BTrZ	35.81	37.27

According to the value of disease incidence (DI) and severity intensity (SI) plants with carrier treatments are slightly higher resistant to disease attacks compared to plants that are only received a liquid consortium of bacterial and *Trichoderma* from BSF larval gut isolates without carriers (K+(1)) and plants without treatment (K-) (Table 1.). The resistance of *C. annuum* can be categorized based on disease incidence (DI) and severity intensity (SI) values (Aditya et al., 2015). The DI category was determined by disease intensity scale (%): ≤ 10 = highly resistant, ≤ 20 = resistant, ≤ 40 = moderate, ≤ 70 = susceptible, ≥ 70 = highly susceptible (Hafsah & Amelia, 2022). Meanwhile, the SI Category was determined based on the severity intensity scale (%): 0 = healthy (H), <11 = mild (M), 11–25 = moderate (Mo), 25–75 = severe (S), >75 = total loss (T) (Warduna dkk, 2011). In this study, DI represented the spread of the disease and the increase in infected plants per treatment, whereas SI indicated the severity and progression of the disease. On that account, the disease resistance level in each treatment could be determined.

In addition to categorization, the data were also statistically tested to determine significant differences. Tests of normality and homogeneity of variance indicated that the DI and SI data were not normally distributed. Therefore, the Kruskal-Wallis non-parametric statistical test was carried out to evaluate treatment effects. The result showed a significant difference between treatment data, as the Asymp. Sig is less than 0,05 (Table 2). Furthermore, the comparison among treatments was conducted using mean rank value, where the lower mean rank indicates the least disease incidence or severity, reflecting an enhanced effect on chili plant resistance. In contrast, a high mean rank indicates more disease incidence or severity compared with other treatments.

The lowest mean rank of DI in the carrier treatment was found in the kaolin carrier treatment (BTrK) at 33.56, followed by talc (BTrT) at 34.69, and zeolite (BTrZ) at 35.81 (Table 3). Although the differences were not substantial, the higher value of mean rank suggests that plants treated with kaolin carrier with bacteria and *Trichoderma* consortium from BSF gut isolates were more resistant to disease

infection compared to the talc or zeolite carrier treatments.

The lowest mean rank SI among the carrier treatments was found in the talc carrier treatment (BTrT) at 32.31, followed by the kaolin (BTrK) at 37.00 and the zeolite (BTrZ) at 37.27 (Table 3). The mean rank values of kaolin (BTrK) and zeolite (BTrZ) carriers were almost identical, differing only by 0.27. The lower mean rank value indicates that the talc carrier treatment with bacteria and *Trichoderma* consortium from BSF gut isolates was more effective in preventing disease severity due to fungal infection. The lowest mean rank across all treatments in both DI and SI data was found in the positive control of inorganic treatment (K+(2)), with a mean rank of 27.58. However, both ID and SI mean rank values of carrier treatments were close to the inorganic treatment mean rank, suggesting that carrier treatment can serve as a potential substitute for chemical fungicides.

Analyzed statistics suggest carrier application with bacterial and *Trichoderma* consortium from Black BSF larval gut isolates (Treatments BTrK, BTrT, and BTrZ) was associated with lower disease incidence and severity, compared to liquid application of bacteria and *Trichoderma* consortium without carrier from BSF larval gut isolates (K+(1)). Carriers provide a place for microbes to survive and improve microbial effectiveness when biopesticides or biofertilizers are applied to plants (Aksani et al., 2021). Bacteria and *Trichoderma* from BSF larval gut isolates are beneficial in increasing the resistance of red chili caused by fungal infection, as a result of *Trichoderma viride* contained in BSF larval gut isolates functions as an antifungal (Kristi et al., 2024).

The gut of Black Soldier Fly (BSF) larvae is home to microbiota that play a role in BSF physiological processes, including polysaccharide biosynthesis, metabolism, extracellular structure formation, and other processes. Among the 11,000 types of bacteria that exist in the BSF larvae gut, the species with the highest abundance come from the genera *Enterococcus*, *Acinetobacter*, *Providencia*, *Enterobacter*, and *Myroides* (Zhineng et al.,

2021). The gut of BSF larvae also contains bacteria of the *Bacillus* and *Micrococcus* genus and fungi of the *Trichoderma* genus (Kristi et al., 2024; Sanjaya et al., 2023).

In this study, microbes from the gut isolates of BSF larvae were utilized as biofungicides to enhance the disease resistance of red chili against pathogenic fungi. The microbes used were the fungi *Trichoderma viride* and *Trichoderma harzianum*, as well as probiotic bacteria; *Bacillus subtilis*, *Micrococcus* sp., and *Enterobacter* sp., which were then combined with a carrier material to maintain microbial viability. The treatment materials in this study were obtained from the Vegetable Plant Research Institute, Lembang, Indonesia.

Throughout this study, microbes from BSF gut isolates were combined with three different types of carriers to determine the best carrier that suits the probiotics and can increase disease resistance in red chili due to fungal infection. Kaolin, Talc, and zeolite carriers were chosen as the study materials as they fulfill the requirements of carriers; able to retain water, are non-toxic, easily available, economical, and commonly used as a carrier material for biopesticides or biofertilizers (Karaca et al., 2023).

Trichoderma is a genus of fungi that has been widely used as a biocontrol agent against plant diseases. *Trichoderma* spp. can be inoculated into a carrier to increase their viability and efficiency in plant protection. Dry formulations of *Trichoderma* can use solid materials, for example, talc, to maintain the survival rate of conidia, dormancy, or viability of conidia during storage, without harming the *Trichoderma* conidia (Martinez et al., 2023). *Trichoderma* is commonly applied to the agricultural rhizosphere or plant root zones to induce plant systemic resistance (ISR) without physical contact between the plant and the biocontrol agent (Ownley et al., 2010). *Trichoderma viride* and *Trichoderma harzianum* can protect plants from pathogenic fungi as they have the ability of mycoparasitism by inhibiting the growth of mycelium of pathogenic fungi through adhesion, mycelial

entanglement, penetration, and lysis of mycelial cell walls (Yassin et al., 2021).

The physical properties of the carrier particles affect the efficacy of *Trichoderma harzianum* since they affect the stability, solubility, and bioavailability of *Trichoderma*. The size and shape of the carrier particles enable the effective action of *Trichoderma* on the target tissue. The results of using talc nanoparticles as a *Trichoderma harzianum* carrier to test chickpea (*Cicer arietinum*) resistance to the fungal pathogen *Fusarium oxysporum* f. sp. *ciceris* showed mycelium inhibition of 2.56% at a concentration of 50 ppm, compared to the use of lignite and fly ash carriers with 100% inhibition effectiveness. Talc carrier has the largest particle size, specifically 73.46 nm, compared to lignite (41.55 nm) and fly ash (53.92 nm) (Supriya et al., 2024).

Based on the results of statistical tests on the value of disease incidence (DI), plants treated with kaolin carrier (BTrK) have the smallest mean rank value, indicating a lower level of disease incidence compared to other carrier treatments. On account of kaolin particles being medium in size, they are more effective in responding to and resisting infection due to pathogenic fungi. Based on the results of statistical tests on the value of severity intensity (SI), red chili plants treated with carrier talc (BTrT) show resistance to severity. The mean rank value of severity intensity (SI) of BTrT plants is the lowest, presumably since the talc carrier used in this study has a finer particle size among kaolin and zeolite carriers. Consequently, talc carriers are more efficient in preventing the worsening of pathogen damage symptoms.

The kaolin and talc carriers, apart from the particle size that supports probiotics in optimizing their work, also have several advantages. Kaolin can release fungicides slowly and has a good ability to absorb and retain water, therefore, it provides a favorable niche for probiotics. The ability to absorb and retain good water, optimum PH, and not be toxic is also possessed by talc. Thus, talc can support microbial growth and survival (Rui-Hong et al., 2023; Singh et al., 2020). The

carrier properties of kaolin and talc support the activity of microbes from BSF larval gut isolates, enabling them to enhance disease resistance.

The DI and SI values of the zeolite treatment (BTrZ) were slightly higher, presumably because the zeolite size was the largest among the other two carriers in this study. However, the mean rank values of IP and IS of zeolite were still much lower than the organic control without carrier (K+(1)) and the negative control without treatment (K-), thus, it can be interpreted that the zeolite treatment (BTrZ) was still able to withstand the infection of pathogenic fungi and was sufficient to prevent the damage caused by the symptoms of attack to being too severe. Similar to kaolin and talc, zeolite has a water-holding capacity and cation exchange capacity, therefore, it can release fungicides slowly (Cataldo et al., 2021). Zeolites added to agricultural soil infected with *Sclerotinia sclerotiorum* reduced disease severity and increased the wet weight of lettuce plants (Poulaki et al., 2020).

According to statistical analysis, *C. annuum* L. treated with kaolin carrier with probiotics from BSF (BTrK) showed lower DI and SI values than probiotic treatment without carrier (BTr). Additionally, to determine the significant differences between treatments, a post hoc test was performed.

Post hoc Dunn's test showed significant differences between groups when $P_{adj} < 0.05$. As shown in Table 4, the DI differed significantly between inorganic (K+(2)) and liquid treatment (K+(1)), as well as between inorganic (K+(2)) and control (K(-)). For the SI, a significant difference was found only between inorganic (K+(2)) and control ((K(-)). These post hoc results indicate that the addition of a carrier with bacterial consortium and *Trichoderma* from the BSF gut isolates, namely BTrK, BTrT, and BTrZ, did not differ significantly from the inorganic fungicides treatment. On that account, this also supports the suggestion that carrier-based treatment could serve as an alternative to inorganic fungicides.

Table 4. Dunn Post Hoc Test

Comparison	Disease Incident		Severity Intensity	
	Z	P.adj	Z	P.adj
K+(2) – K+(1)	-3.00	0.04	-2.85	0.06
K+(2) – BTrK	-0.76	1.00	-1.36	1.00
K+(1) – BTrK	2.23	0.38	1.49	1.00
K+(2) – BTrT	-0.89	1.00	-0.79	1.00
K+(1) – BTrT	2.10	0.53	2.06	0.58
BTrK – BTrT	-0.13	1.00	0.57	1.00
K+(2) – BTrZ	-1.03	1.00	-1.39	1.00
K+(1) – BTrZ	1.96	0.74	1.46	1.00
BTrK – BTrZ	-0.27	1.00	-0.03	1.00
BTrT – BTrZ	-0.14	1.00	-0.60	1.00
K+(2) – K(-)	-3.32	0.01	-3.59	0.00
K+(1) – K(-)	-0.32	1.00	-0.73	1.00
BTrK – K(-)	-2.55	0.15	-2.23	0.38
BTrT – K(-)	-2.42	0.22	-2.80	0.07
BTrZ – K(-)	-2.28	0.33	-2.19	0.41

Conclusions

The carrier (kaolin, talc, zeolite) potential with bacterial consortium and *Trichoderma* formulation from BSF larvae isolated has been reported in this study. The experimental results indicate that plants with carrier treatments significantly enhanced disease resistance compared to plants without carrier treatments and untreated controls. Plants treated with kaolin carrier (BTrK) with probiotics from BSF larval gut isolates provided resistance to fungal infection as evidenced by the lower mean rank value of disease incidence (DI) compared to other treatments. Plants treated with talc carrier (BTrT) provided resistance to the severity of disease symptoms caused by fungi, as evidenced by the mean rank value of severity intensity (SI), which was lower than the other treatments.

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