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RESEARCH PAPER

Biosynthesis and applications of nanobas for increasing bacterial leaf blight resistance in shallot

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ABSTRACT

Nanobas is a nanosuspension derived from *Bacillus* sp. isolated from the rhizosphere of shallots, formulated as a biopesticide to mitigate the predominant diseases afflicting shallots. A significant pathogen of shallots is the bacterial leaf blight caused by *Xanthomonas axonopodis*, which can result in yield losses of up to 80%. This research aims to: 1) develop and characterize Nanobas as a nanosuspension formula, and 2) assess the efficacy of Nanobas in enhancing the resistance of shallots to bacterial leaf blight. The experimental methodology employed a completely randomized block design in field conditions. The study comprised five treatments: P0: control, P1: Nanosuspension *Bacillus* sp. Bm2-chitosan 10 mL/L, P2: *Bacillus* sp. Bm2 liquid formula 10 mL/L, P3: Chitosan 0.01%, P4: bactericide (copper hydroxide) 2 mL/L, with each treatment replicated five times. The treatments were applied by drenching 50 mL around the plant base. The variables observed included the visual characteristics of Nanobas, incubation period, disease intensity, effectiveness, and the structural and biochemical resistance of shallots. The findings revealed that Nanobas, as a *Bacillus* sp. Bm2-chitosan nanosuspension, exhibited colloidal stability, characterized by its ability to scatter light without sedimentation. The application of Nanobas resulted in a 30.28% reduction in disease intensity. Structural resistance was evidenced by the increased epidermal thickness in the cell walls of shallot leaves. The highest phenolic compound content was observed in the *Bacillus* sp. Bm2 liquid formula treatment, indicating that while the nanosuspension formula is efficacious as a biocontrol agent, it is less effective as a bioenhancer.

Keywords: *Bacillus* sp., biocontrol, bioenchancer, leaf blight, shallot

INTRODUCTION

One of the most significant diseases affecting shallots is bacterial leaf blight, caused by *Xanthomonas axonopodis*. This pathogen can lead to yield reductions of up to 80%, particularly under conditions conducive to disease development, such as susceptible plant genotypes and favorable environmental factors. The adoption of environmentally sustainable practices, including the utilization of beneficial rhizosphere bacteria isolated from healthy shallot plants, represents a promising alternative for managing bacterial leaf blight. Antagonistic rhizosphere bacteria are generally obtained from healthy plants. Shallot rhizosphere bacteria isolated using HiCrome selective medium and subjected to a pre-dilution heat treatment at 80 ºC for 20 min exhibited characteristics typical of *Bacillus* species, including Gram-positive staining and endospore formation. Previous isolations yielded 13 distinct isolates; however, following a series of antagonistic assays against *Fusarium oxysporum* f.sp. *cepae* and *Xanthomonas axonopodis*, four effective isolates were identified and designated as Bm1, Bm2, Bm3, and Bm4 (Djatmiko et al., 2023; Saputra et al., 2024). Shallot endophyte *Bacillus* sp. played the role in decreasing bacterial leaf blight by salicylic acid production (Resti et al., 2018). The twisted disease could be reduced by 70–90% with dipped and sprayed application of *B. velezensis* B-27 and *B. cereus* RC76 (Pratiwi et al., 2024).

Bacillus sp. has been formulated into a nanosuspension to enhance its efficacy, thereby enabling its use as an environmentally friendly biopesticide for disease management. Nanobas, a nanobiopesticide derived from *Bacillus* sp. isolated from the shallot rhizosphere, exemplifies this approach. Previous studies successfully formulated isolates Bm1 and Bm3 into nanosuspensions, with particle sizes of 224.1 nm and 244.3 nm, respectively, as determined using a particle size analyzer (PSA). These nanosuspensions inhibited *Fusarium oxysporum* f.sp. *cepae* by 10.72%

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and 11.41%, respectively, and effectively controlled moler disease (twisted disease) under screen-house conditions, achieving efficacies of 55.02% and 28.56%, respectively (Djatmiko et al., 2023).

In the present study, the Bm2 isolate was formulated into a nanosuspension using chitosan and sodium tripolyphosphate (TPP) to enhance its efficacy against moler and bacterial leaf blight diseases. Previous research demonstrated that *Bacillus* sp. isolate Bm2 effectively inhibited the growth of *Fusarium oxysporum* f.sp. *cepae in vitro* by 22.56% through an antibiosis mechanism, which induced hyphal swelling (Prihatiningsih et al., 2020). The ionic gelation technique employed in the biosynthesis of this nanosuspension involves cross-linking between polyelectrolytes mediated by multivalent ion pairs. The formation of these cross-linking bonds significantly enhances the mechanical stability of the resulting particles. Common polymer pairs used in ionic gelation include chitosan and tripolyphosphate. Chitosan is widely recognized for its mucoadhesive properties, ease of chemical modification, biocompatibility, low toxicity, and biodegradability, making it an excellent candidate for dosage-form development. The fundamental principle of nanoparticle formation in this method is based on ionic interactions between the positively charged amino groups (NH_2) of chitosan and the negatively charged polyanions of tripolyphosphate (TPP), resulting in the formation of a three-dimensional inter- and/or intramolecular network structure (Wibowo, 2022; Sreekumar et al., 2018).

MATERIALS AND METHODS

Research Site. This study was conducted in Linggasari Village, Kembaran District, Banyumas Regency, at an elevation of 117 m above sea level, geographically located at 7° 23' 48.6" S latitude and 109° 17' 07.9" E longitude, from May to July 2024.

Biosynthesis of *Bacillus* sp. Bm2-Chitosan Nanosuspension. The *Bacillus* sp. Bm2-Chitosan nanosuspension was synthesized by initially culturing *Bacillus* sp. Bm2 in a nutrient broth (NB) medium. The culture was incubated on a Daiki KBLee 3001 orbital shaker at 150 rpm and subsequently centrifugation centrifuged at 10,000 rpm for 15 min at room temperature. The resulting supernatant was purified through ethanol precipitation.

Nanoparticle preparation was initiated by dissolving 2 g of chitosan in 100 mL of acetic acid solution at pH 5. The *Bacillus* sp. Bm2 supernatant was then added dropwise to the chitosan solution under continuous stirring (Sulistyo et al., 2017). Various concentrations of *Bacillus* sp. supernatant and chitosan were prepared to determine the optimal conditions for nanoparticle formation. Initially, 500 µL of *Bacillus* sp. supernatant with a population density of 10^9 CFU/mL was transferred into an Eppendorf tube, followed by the addition of 490 µL of chitosan solution prepared in acetate buffer at pH 5.0. The mixture was homogenized by vortexing for 20 seconds. Subsequently, 10 µL of 0.03% tripolyphosphate (TPP) solution was added and immediately vortexed for an additional 20 seconds. Nanoparticle dispersion was evaluated based on the amount of insoluble *Bacillus* sp. supernatant to determine the optimal formulation, and particle size was measured using a Particle Size Analyzer (PSA).

Application of *Bacillus* sp. Bm2-Chitosan Nanosuspension. The application of the *Bacillus* sp. Bm2–chitosan nanosuspension to control bacterial leaf blight was carried out on shallots (*Allium cepa* var. *aggregatum*) of the Bima Brebes variety under field conditions. The experiment employed a randomized complete block design consisting of five treatments and five replications. The treatments were as follows: P0 (control), P1 (*Bacillus* sp. Bm2–chitosan nanosuspension at 10 mL/L), P2 (Bacillus sp. Bm2 liquid formulation at 10 mL/L), P3 (chitosan at 0.01%), and P4 (bactericide [copper hydroxide] at 2 mL/L).

All treatments were applied by pouring the solutions around the planting holes, considering that seedlings possess natural pathogen-suppressing capabilities. Because the study was conducted under natural field conditions, artificial inoculation was not performed, and the seedlings were naturally infected. Applications of the *Bacillus* sp. Bm2–chitosan nanosuspension and other treatments were carried out at seven-day intervals for a total of four applications, beginning when the plants were 11 days after planting.

Assessment of Bacterial Leaf Blight Disease Intensity. Disease intensity was evaluated using the formula proposed by Soedomo (2006), adapted for both screen-house and field research:

$$IP = \frac{\sum n_i \times v_i}{N \times Z} \times 100\%$$

IP = Disease severity;
n_i = Number of plants exhibiting blight symptoms per category;
v_i = Blight symptom category score;
Z = Highest blight symptom category score;

N = Total number of plants observed.

Blight symptom category scores were defined as follows: 0 (no symptoms), 1 (1–20%), 2 (21–40%), 3 (41–60%), 4 (61–80%), and 5 (81–100%).

The effectiveness of leaf blight disease control was determined by comparing disease intensity values between the control and treatment groups using the following parameters:

$$EF = \frac{(IPk - IPp)}{IPk} \times 100\%$$

EF = Control effectiveness (%);
IPk = Disease intensity in the control group;
IPp = Disease intensity in the treatment group.

Control effectiveness was classified as very effective (EF > 69%), effective (EF = 50–69%), less effective (EF = 30–49%), or ineffective (EF < 30%) (Mulyani et al., 2024).

The infection rate was calculated using the van der Plank (1963) formula:

$$r = \frac{2.3}{t}\left[\log\frac{1}{(1 - X_t)} - \frac{1}{(1 - X_0)}\right]$$

r = Infection rate;
X_t = Proportion of diseased tissue at time t;
X_0 = Proportion of diseased tissue at the initial observation (t = 0);
t = Observation time interval.

Plant Resistance Evaluation. Plant resistance was evaluated based on structural and biochemical parameters. Structurally, resistance was indicated by epidermal thickness, while biochemically it was assessed through total phenol content. For structural analysis, leaves were washed under running water and fixed in a formaldehyde–glacial acetic acid–alcohol (FAA) solution (5:5:90) for 24 hours. The samples were then rinsed with distilled water, and thin leaf sections were prepared using carrot tissue as a supporting medium. The sections were immersed in 5% sodium hypochlorite (NaOCl), stained with safranin, mounted on glass slides with 30% glycerin, covered with cover slips, and observed under a microscope.

For biochemical analysis, total phenol content was determined using gallic acid as a standard to generate a regression curve. Absorbance measurements were obtained using a UV–Vis spectrophotometer at a wavelength of 750 nm.

RESULTS AND DISCUSSION

Biosynthesis of *Bacillus* sp. Bm2-Chitosan Nanosuspension. The *Bacillus* sp. Bm2-chitosan nanosuspensions, referred to as Nanobas, are shown in Figure 1. These nanosuspensions exhibited clear, colorless appearances with no visible sedimentation and behaved as colloidal systems. The absence of color and high clarity indicate that the particles are extremely small, typically within the nanometer range, allowing light to pass through the solution with minimal scattering. Smaller particle sizes generally result in higher transparency due to reduced light scattering.

The particle size of the *Bacillus* sp. Bm2-chitosan nanosuspension was determined to be 334.6 nm using a Particle Size Analyzer (PSA). The stability of the nanosuspension can be initially assessed through visual observation of its clarity and color (Aghajani et al., 2015). Comprehensive characterization of nanosuspensions includes evaluation of parameters such as color, odor, presence of foreign particles, pH, particle size distribution, and laser light diffraction analysis (Jethara et al., 2014). The formulation of the nanosuspension enhances the solubility of the *Bacillus* sp. supernatant and improves its dispersibility when applied to soil.

Nanosuspensions significantly improve solubility and dissolution rate due to their fine particle size. This formulation increases wettability, particle surface area, and saturation solubility (Jahan et al., 2016). Nanoscale materials are capable of dissolving effectively in aqueous environments, thereby enhancing stability and facilitating improved absorption across plant cell walls. Consequently, their biological efficacy

Figure 1. Physical characteristics of the nanosuspension-based formulations. A. *Bacillus* sp. Bm2-chitosan nanosuspension; B. Nanobas.

is substantially increased (Shabani et al., 2022).

Application of Nanosuspension and Observation of Pathosystem Components. Observations of the pathosystem components indicated that the nanosuspension treatment reduced disease intensity by 30.28% compared with the control. However, no statistically significant differences were observed when this treatment was compared with Bacillus sp. Bm2 liquid formulation at 10 mL/L, chitosan at 0.01%, or the bactericide (copper hydroxide) at 2 mL/L, as shown in Table 1. The superior aqueous solubility of the nanosuspension enhances its stability, thereby contributing to the suppression of pathogen development. Previous studies have reported that nanosuspension application reduced disease progression in banana fruit by up to 68.9% (Vijayreddy et al., 2023).

Nanosuspensions are capable of targeting pathogens, functioning as protective agents or carriers for pesticides, fungicides, and herbicides, and enhancing host resistance (Devi et al., 2018; Singh et al., 2023). As such, nanosuspensions represent an advanced strategy for improving plant health against pathogenic threats (Elmer et al., 2018). Recent research has demonstrated that nano-fungicides synthesized using titanium dioxide in emulsion form effectively control plant diseases, including tobacco collar rot (Shabani et al., 2022).

Nanoscale suspensions possess distinct physicochemical properties, including enhanced thermal conductivity, catalytic activity, and biological effectiveness. In crop protection, nanomaterials act as protectants and carriers for biopesticides through mechanisms such as adsorption, encapsulation, or conjugation with active ingredients. These materials can be applied via foliar spraying, seed soaking, or root irrigation. Nanosuspensions also exhibit notable antifungal, antibacterial, and antiviral activities (Dutta et al., 2022).

The incubation period in the control group was shorter than that in the treated plants, as evidenced by the rapid progression of blight symptoms toward the basal regions of the leaves (Figure 2). Initial symptoms appeared as lesions or white spots with moist margins, which expanded rapidly in a water-soaked pattern. Disease progression was characterized by tissue indentation, shrinkage, chlorosis, shoot dieback, and ultimately leaf necrosis.

A significant difference in disease intensity was observed between all treatments and the control group. However, among the four treatments—*Bacillus* sp.-chitosan nanosuspension, *Bacillus* sp. Bm2 liquid formulation at 10 mL/L, chitosan at 0.01%, and the bactericide (copper hydroxide)—no statistically significant differences were detected. These results indicate that the nanosuspension treatment is comparable in effectiveness to the other treatments, excluding the control. The nanosuspension achieved a disease control effectiveness of 30.28%, which is classified as less

Table 1. Components of the shallot leaf blight pathosystem

Treatment	Incubation periode (dap)	Disease intensity (%)	Effectiveness (%)	Infection rate (unit/day)
P0	20	25.33 a	-	0.052
P1	25	17.66 b	30.28	0.034
P2	26	18.33 b	27.64	0.028
P3	25	18.00 b	28.94	0.034
P4	27	19.24 b	24.04	0.036

dap = days after planting; numbers followed by the same letter are not significantly different according to the LSD test at the 5% level. P0 = control; P1 = *Bacillus* sp. Bm2-chitosan nanosuspension (10 mL/L); P2 = *Bacillus* sp. Bm2 wettable formulation (10 mL/L); P3 = chitosan (0.01%); P4 = bactericide (copper hydroxide; 2mL/L).

Figure 2. Typical symptoms of bacterial leaf blight on shallot plants.

effective than conventional pesticides. Nevertheless, it offers a more environmentally friendly alternative due to its microbial-based nature and exhibited an infection rate of 0.034 units/day, comparable to that of the chitosan treatment. Nanosuspensions enhance not only solubility but also the safety and efficacy of biological control agents (Aher et al., 2017).

Nanosuspension Application and Plant Growth Components. Shallot plant growth showed an increasing trend compared with the control; however, statistical analysis revealed no significant differences among treatments, including in the number of leaves. Based on the vigor index, nanosuspension application improved plant vigor by 4–6%, as presented in Table 2. These findings indicate a positive effect on plant growth and resilience, warranting further evaluation of plant resistance mechanisms.

Nanotechnology is increasingly applied in agriculture as part of precision farming systems. This is largely due to the role of nanosuspension physicochemical properties in reducing the environmental impacts associated with conventional pesticide use (Sachan et al., 2023). Numerous studies have shown that nanotechnology-based formulations using silica carriers can enhance seed germination, promote plant growth, and improve overall plant development (Satya et al., 2024). In addition, cerium-based nanosuspensions have demonstrated significant potential in accelerating seed germination, improving plant growth, and influencing chlorophyll synthesis (Farhadihosseinabadi et al., 2019; Ramírez-Olvera et al., 2018).

Nanosuspension Application and Plant Resistance. Plant resistance was structurally indicated by increased epidermal cell wall thickness. Application of the nanosuspension resulted in a noticeable thickening of the epidermal cell wall, as shown in Figure 3. Biochemical resistance was assessed based on total phenolic content in shallot leaves, as presented in Table 3. Increased thickness of the epidermal and

Table 2. Plant growth components of shallot

Treatment	Plant height (cm)	Number of leaves	Plant vigor (%) at 51 dap
P0	32.34	12.66	86
P1	35.24	16.86	90
P2	32.06	12.95	92
P3	32.68	14.20	90
P4	34.02	14.64	92

dap = days after planting. P0 = P0 (control), P1 (*Bacillus* sp. Bm2–chitosan nanosuspension at 10 mL/L), P2 (*Bacillus* sp. Bm2 liquid formulation at 10 mL/L), P3 (chitosan at 0.01%), and P4 (bactericide [copper hydroxide] at 2 mL/L).

Table 3. Phenol test as an indicator of biochemical resistance

Treatment	Total phenol (mg GAE, 750 nm)
P0	1.030
P1	1.078
P2	1.166
P3	1.339
P4	1.478

P0 = P0 (control), P1 (*Bacillus* sp. Bm2–chitosan nanosuspension at 10 mL/L), P2 (*Bacillus* sp. Bm2 liquid formulation at 10 mL/L), P3 (chitosan at 0.01%), and P4 (bactericide [copper hydroxide] at 2 mL/L).

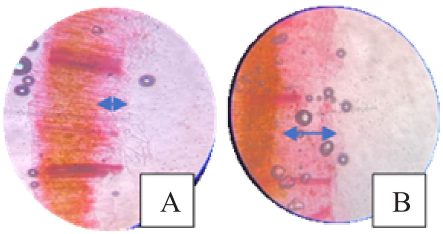

Figure 3. Thickening of the shallot leaf epidermal wall indicating structural resistance. A. Control; B. *Bacillus* sp.–chitosan nanosuspension treatment.

cuticular cell walls reflects enhanced resistance to pathogen invasion. This structural reinforcement is supported by the presence of biochemical compounds, including alkaloids, saponins, flavonoids, tannins, phenolics, steroids, and triterpenoids, which act as effective barriers against pathogen penetration (Heintz & Blaich, 1989; Rahmawati et al., 2019).

According to the data in Table 3, the bactericide treatment containing copper hydroxide exhibited the highest total phenol content. This finding indicates that although the *Bacillus* sp.–chitosan nanosuspension effectively suppresses disease and enhances structural resistance, it does not significantly induce biochemical resistance. Therefore, its primary utility lies in its role as an environmentally friendly biocontrol agent.

CONCLUSION

The biosynthesis of a nanosuspension-based biopesticide formulation utilizing *Bacillus* sp. isolated from the shallot rhizosphere exhibited distinct colloidal properties. Application of the nanosuspension biopesticide, termed Nanobas, resulted in a disease intensity reduction of 30.28%. In addition, Nanobas enhanced plant resistance, as indicated by increased epidermal thickness. The *Bacillus* sp.–chitosan nanosuspension proved effective as an environmentally friendly biocontrol agent for the management of bacterial leaf blight in shallots.

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AUTHORS' CONTRIBUTIONS

NP conceived and designed the study on the biosynthesis and application of Nanobas. DWK performed the nanosuspension formulation, analyzed and interpreted the data, and drafted the manuscript. NKW collected the data and contributed to manuscript preparation. NP, DWK, and NKW critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

COMPETING INTEREST

The authors declare no financial, general, or institutional competing interests.

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