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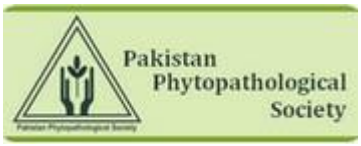

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THE EFFICACY OF COST-EFFECTIVE BIONEMATICIDE AGAINST POTATO CYST NEMATODE *GLOBODERA ROSTOCHIENSIS*

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ABSTRACT

Potato cyst nematode (*Globodera rostochiensis*) infection causes yield loss of up to 80%. Various attempts have been made to suppress their infection on potato. However, *G. rostochiensis* infection remains a problem that has not been fully resolved. One of the potential techniques to control their population is the use of biological control agents. In previous studies, we have succeeded to isolate 3 rhizobacteria (*Bacillus* sp.) and 1 endophytic bacterium (*Pseudomonas dimunita*) from the rhizosphere and root of the coffee plant. In this study, the bionematicide was formulated using four bacterial isolates and inexpensive materials. Bionematicides were tested for their effectiveness on land infected with *G. rostochiensis* (227 per 100 mL) of soil. We compared the effectiveness of the bionematicides at various doses. As a control, a common nematicide was employed. The bionematicide also reduced the number of cysts and the number of female nematodes in the field. Moreover, it also showed that the bionematicide was able to increase the height of potato plants. Bionematicide application also improved various potato growth parameters. The tested formula also increased the number of tubers per plant. The study demonstrated that the most effective and recommended bionematicide concentration was 4% for every 100 mL in each plant.

Keywords: *Bacillus*; endophyte; formulation; *Pseudomonas*; rhizobacteria.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the selected commodities to support the food diversity program to achieve sustainable food security. Potatoes are deemed a staple food because they contain calories, carbohydrates, minerals, and vitamins (Rose and Vasanthakaalam, 2011). In addition, potatoes have a high economic value (Moussa and Solieman, 2016). However, every year potato production in Indonesia is suboptimal due to lower production compared to market demand, even though potatoes are still imported to meet market demand. Commercially, potatoes are highly valuable because potato tubers can be processed into various processed products and used as substitute food ingredients that are healthy and safe for the community (Hussain, 2016).

The need for potatoes in Indonesia is increasing every year, which increasingly out-numbers national

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production. Java is the largest potato producing area with a production of 745,817 tons or about 55.34% of the total national potato production. Potato crop productivity in Indonesia is still relatively low at 17.67 tons ha^{-1} compared to that in subtropical countries such as the USA and Netherlands at 37.40 tons ha^{-1} and 45.10 tons ha^{-1}, respectively. This low productivity is influenced by many factors, one of which is the *Globodera* spp. infection (Mustika, 2005).

Globodera spp. is known as the potato cyst nematode. This nematode is distributed in tropical cold areas and subtropical temperate areas (Dandurand *et al.,* 2019; Shahid *et al.,* 2019). This nematode grows very well in cold soil temperatures, whereas prolonged exposure to high soil temperatures limits their development and reproduction. (Kaczmarek *et al.,* 2019). Soil moisture at field capacity makes it easier for J2 of the nematodes to move around. However, the nutrient content of the soil has little effect on nematodes, except for those made by plant activity (Devine and Jones, 2001). In addition, the level of soil pH tolerance that is suitable for the growth of potatoes is also suitable for the growth and development of *Globodera* spp. (Mulder and Van Der Wal, 1997).

The average losses due to *Globodera* spp. in many countries ranging from 50% to 80% (Kaczmarek *et al.,* 2019). Even in Australia, the costs incurred due to this nematode infection in the last 20 years are substantial, reaching an average of $18.7 billion ranging from $11.9 to 27.0 billion per year (Hodda and Cook, 2009; Mburu *et al.,* 2020; Rehman, 2021). Hadisoeganda (2006) reported that in Indonesia, especially in Batu City – East Java, the average potato production of 1.5 ha can reach 24 tons. The production dropped drastically to 7 tons per 1.5 ha after being infected with *Globodera rostochiensis*. Potato cyst nematode reduces leaf area for photosynthesis and disturbs the root system, gradually declining potato production. The disturbances in the root system result in stunted plants and cause leaves to wither and turn very bright yellow (De Ruijter and Haverkort, 1999; Dandurand *et al.,* 2019). Short, filthy roots and tiny, dark-brown granules that resemble copper can be seen when the soil's rhizosphere is removed. The granules adhere to the roots, but some of them fall and distribute themselves about the roots. Plants are more vulnerable to infections from other pathogens like *Phytophthora* sp. and *Ralstonia solanacearum* as a result of the disruption of water and nutrient uptake by roots (Fiers *et al.,* 2012).

Despite various controlling measures performed, *Globodera rostochiensis* infection in Indonesia remains a serious problem that has not been fully resolved. The potato cyst nematode is quite difficult to control conventionally because of its unique niche (Price *et al*., 2021). These nematodes are endoparasites and can form cysts which are defense structures that may survive for decades (Dandurand *et al.,* 2019). An effective, efficient, sustainable, and environmentally friendly control technique is needed to control *Globodera rostochiensis* infection in potato plants. One solution that can be applied is the application of biological control agents such as rhizobacteria and endophytic bacteria (Widianto *et al.,* 2021). In some previous studies, the population of *Pratylenchus coffeae* has been successfully suppressed by rhizobacteria and endophytic bacteria (Asyiah *et al.,* 2015; Asyiah *et al*., 2018). These bacteria have also been proven effective in producing protease and chitinase enzymes, which are essential for solubilizing phosphate (P) and fixing nitrogen (N).

Biological agents can directly produce extracellular enzymes and anti-nematode compounds. Istifadah *et al.* (2018) reported that endophytic bacteria isolated from potato plant roots had the potential to control potato cyst nematodes. These bacteria control nematodes by producing extracellular enzymes such as chitinase and protease. Both enzymes can degrade nematode cell walls and nematode eggs which are mostly composed of protein and chitin (Subedi *et al.,* 2020). Furthermore, biological agents can produce volatile compounds in the form of Hydrogen Cyanide (HCN), which are toxic to nematodes (Yousif *et al*., 2017).

Indirectly, biological agents can control nematodes through resistance induction mechanisms (Molinari and Leonetti, 2019; Poveda *et al.,* 2020). Plants treated with biological agents generally produce higher levels of defense compounds, such as tannins, saponins, and glycosides than those without biological agents. These three compounds are plant defense compounds that play a role in warding off various types of pathogens (Rosyidah *et al.,* 2014). The application of biological agents can also increase the production of Pathogen-Related Protein (PR-Protein). PR-Protein is a compound that increases plant resistance, even to plant-parasitic nematodes (Forghani and Hajihassani, 2020).

In a previous study, we succeeded in developing a bionematicide to control root-knot nematodes in

tomatoes. The formula consisted of rhizobacteria, endophytic bacteria, and carriers in the form of organic materials. The purpose of this study is to evaluate the bionematicide and its effectiveness in controlling *Globodera rostochiensis*.

METHODS

Time and research site: The study was conducted in Sumber Brantas village, Bumiaji sub-district, Batu city, East Java, Indonesia. The village is located at an altitude of about 1,200 masl. It is one of the largest potato production centers in Indonesia and one of the areas where the potato fields are heavily infected with *Globodera rostochiensis*. The study was carried out from November 2020 to April 2021.

Source of Bacteria Isolates: We employed three rhizobacteria isolates and one endophytic bacteria isolate that had previously been isolated, identified, and described (Table 1). The isolates consisted of two genera, namely Pseudomonas and Bacillus. Each isolate has been proven compatible to be combined into a consortium.

Table 1. Isolates used in this study

Isolates code	Species	Status	References
SK.07	Bacillus sp.	Endophyte	(Asyiah *et al*., 2015; Asyiah *et al*., 2018)
SK.14	Bacillus sp.	Endophyte	
KB.14	Bacillus sp.	Endophyte	
PD.01	*Pseudomonas dimunita*	Rhizobacteria	

Formula of cost-effective bacteria-based bionematicide: Bacterial isolates were cultured into a consortium on Bean Sprout Extract Broth (BSEB) media. A total of 200 g of bean sprouts in 1,000 mL of distilled water are slowly boiled. The extract suspension was then filtered using a 100-mesh sieve. Each 1,000 mL of bean sprout extract was then mixed with sugar (20 g L^{-1}) and sterilized using an autoclave. This suspension was then referred to as BSEB.

One stroke of ose needle of each bacterial isolate was cultured in 250 mL BSEB for 48 hours and shaken at 300 rpm. This process was done 30 times simultaneously, eventually producing 7,500 mL of bacterial consortium suspension.

Fresh cow dung, amino acids, vitamins, and molasses were used as a carrier, with details of the composition being the confidential trading condition of *Tiga Kreasi Bersama* Limited Partnership, Indonesia. All carriers were mixed in 1,000 L of water. The bionematicide mixing plant was equipped with an air pump to avoid anaerobic conditions. After all the carriers were mixed, then 7,500 mL of the bacterial consortium suspension was mixed and then incubated for 30 days. Suspensions formed after 30 days of incubation were hereinafter referred to as bionematicides. To ensure the quality of the bionematicide formula, an analysis was carried out to determine the bacterial density, total auxin, and heavy metal content of the formula (Asyiah *et al.,* 2021).

Condition of Experimental fields: Our initial analysis showed that the average number of *Globodera rostochiensis* cysts in the research site was 227 per 100 mL of soil. The texture of 10 soil fractions was observed using the pipetting method and calculating the percentage of fractions (Table 2). The physical characteristics of the soil must be observed since they have a large impact on the life of nematodes. Nematodes like soil holes with greater pore diameters.

Table 2. Soil physical properties in the experimental field

Fractions	Diameter (µm)	Percentage (%)	Total Percentage (%)
Sand	>1,000	11.97	43.1
	500 – 1,000	8.99	
	200 – 500	11.43	
	100 – 200	8.52	
	50 – 100	2.19	
Dust	20 – 50	18.55	50.27
	10 – 20	7.90	
	2 – 10	23.82	
Clay	0.05 – 2	1.92	6.63
	0 – 0.05	4.71	

In addition to the physical properties, we also observed the soil's chemical properties in the experimental field. The chemical properties observed included organic-C, nitrogen, C/N ratio, P_2O_5, Morgan K_2O, and pH H_2O (Table 3).

Table 3. Soil chemical properties in the experimental field

Characteristics	Value
Organic-C	4.72 g 100 g^{-1}
Nitrogen	0.55 g 100 g^{-1}
C/N Ratio	9
Available P	787 mg 1000 g^{-1}
Available K	972 ppm
pH H_2O	6.9

Field experiment: *Granola Kembang* potato variety was planted in the research site following a complete randomized block design. The study involved 7 treatments, with 5 replications, and each replication consisted of 16 experimental plants. The treatments dealt with the concentration of bionematicide. As a positive control, pesticides with carbofuran as an active ingredient were used, and as a negative control, water without the mixture of other ingredients was used. We also compared our bionematicide with commercial bionematicides commonly used by farmers around the research site (Table 4).

Table 4. The treatments used in the study

Treatment codes	Description
K-	Water only
K+	5 g carbofuran per plant on the initial planting
P1	1% bionematicide
P2	2% bionematicide
P3	3% bionematicide
P4	4% bionematicide
P5	1% commercial bionematicide

The K+ treatment was only applied once at the beginning of planting. By contrast, P1, P2, P3, P4, and P5 treatments were applied once a week for 4 months. Treatments P1 to P5 applied 100 mL of bionematicide per plant. Furthermore, all treatments applied chemical fertilizers to all treatments, following the recommended dosages. In addition, during potato cultivation, pathogenic fungi were controlled using the recommended dosage of synthetic chemical fungicides (Asyiah *et al*., 2021).

The observation variables consisted of agronomic and pathological variables. The agronomic variables included plant height, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, the number of tubers per plant, average weight per tuber, and tuber weight per plant. The pathological variables included the average cyst per plant and the average female potato cyst nematode per plant.

DATA ANALYSIS

Data were analyzed using a Two-Way Analysis of Variance. When differences were identified, a follow-up Duncan Multiple Range Test (DMRT) with a 95% confidence interval would be performed. The analysis was carried out using DSAASTAT version 1.101 (Asyiah et al., 2021).

RESULTS

Biological and Chemical Quality of Bionematicides

Preliminary analysis showed that the developed bionematicide formula supported microbial growth. Bacteria of the genus *Bacillus* sp. were evident in the bionematicide formula with the highest density compared with other bacteria, reaching 3.9 × 10^9 CFU mL^{-1}. Furthermore, the density of *Pseduomonas* sp. was 2.6 × 10^9 CFU mL^{-1}, which was slightly smaller than that of *Bacillus* sp. The observations on *Azotobacter* sp. showed a density of 1.7 × 10^8 CFU mL^{-1}.

Based on the bacterial activity, we analyzed the population of N-fixing bacteria and P-solubilizing bacteria. The analysis results showed that there were 1.1 × 10^9 CFU mL^{-1} of N-fixing bacteria and 1.4 × 10^9 CFU mL^{-1} of P-solubilizing bacteria in the bionematicide formula used in this study. Furthermore, the test of *Escherichia coli* and *Salmonella* sp. contents showed that these two bacteria were present in very low and harmless populations, namely < 3 MPN mL^{-1}.

The auxin content test suggested that the applied bionematicide formula contained auxin of 0.528 mg L^{-1}. This bionematicide formula was also tested for metal content, and the results showed that this formula did not contain As, Hg, and Pb. The test also identified 0.31 ppm of Cd, 0.44 ppm of Cr, and 2.4 ppm of Ni. The data on density, total auxin, and heavy metal content in the applied bionematicide formula are presented in Table 5.

Table 5. Bacterial density, total auxin, and heavy metal content in bionematicide formulas

Parameters	Concentrations
Bacillus sp.	3.9×10^9 CFU mL^{-1}
Pseudomonas sp.	2.6×10^9 CFU mL^{-1}
Azotobacter sp.	1.7×10^8 CFU mL^{-1}
N-fixing bacteria	1.1×10^9 CFU mL^{-1}
P-solubilizing bacteria	1.4×10^9 CFU mL^{-1}
Escherichia coli	< 3 MPN mL^{-1}
Salmonella sp.	< 3 MPN mL^{-1}
Auxin	0.528 mg L^{-1}
As	0.00 ppm
Hg	0.00 ppm
Pb	0.00 ppm
Cd	0.31 ppm
Cr	0.44 ppm
Ni	2.4 ppm

The effect of Bionematicide Formula on the Growth of Potatoes Infected with *Globodera rostochiensis:* Potato height was measured from the first week to the sixth week of treatment involving bionematicide. The results showed that one week after application, potato height varied, with P4 (22.56 cm) identified as the highest, followed by P3 (22.12 cm), P2 (20.12 cm), P5 (18.52 cm), P1 (16.04 cm), K+ (18.48 cm), and K- (18.04 cm). In the 2nd to 6th observations, the pattern of plant height in each treatment showed a similar pattern to that in the first week of observation. The last observation (6th week) demonstrated that the treatment with the highest plant was P4 (54.82 cm), followed by P3 (47.00 cm), K+ (43.66 cm), P2 (43.36 cm), P5 (40.46 cm), P1 (39.82 cm), and K- (39.54 cm). In the 6th week of observation, that plant in P4 was found 35.49% higher than that in P5. In addition, the very plant was 25.56% higher than that in K+ and 38.64% higher than that in K-. The data on potato height from each observation are presented in Table 6.

Table 6. Plant heights under various treatments

Treatments	Plant Heights (cm) On Week					
	I	II	III	IV	V	VI
K+	18.48 b ± 0.29	22.18 b ± 0.42	30.04 b ± 0.57	34.02 c ± 0.19	38.76 c ± 0.93	43.66 b ± 0.60
K-	18.04 b ± 0.59	21.74 ab ± 0.26	26.98 a ± 0.87	31.36 a ± 0.29	35.80 a ± 0.69	39.54 a ± 1.27
P1	16.04 a ± 0.48	20.88 a ± 1.34	26.70 a ± 1.85	31.92 ab ± 1.29	34.86 a ± 0.87	39.82 a ± 0.71
P2	20.12 c ± 0.69	23.64 c ± 1.09	31.54 c ± 1.42	35.78 d ± 1.41	38.02 bc ± 1.44	43.36 b ± 1.61
P3	22.12 d ± 0.29	25.04 d ± 1.39	33.20 d ± 0.84	38.54 e ± 0.73	44.16 d ± 2.79	47.00 c ± 3.24
P4	22.56 d ± 0.47	27.58 e ± 0.48	34.52 d ± 0.90	40.58 f ± 1.07	47.62 e ± 1.33	54.82 d ± 3.01
P5	18.52 b ± 1.76	25.04 cd ± 0.93	29.06 b ± 0.55	32.74 b ± 1.71	36.40 ab ± 1.83	40.46 a ± 2.06

Note: Numbers in the column followed by the same letter were insignificantly different at the *p*-value of 0.05 (Duncan Multiple Range Test).

The analysis results of shoot fresh weight showed that P4 (52.65 g) obtained the highest fresh weight, followed by P3 (45.20 g), P2 (37.73 g), P1 (35.33 g), P5 (33.98 g), K+ (32.98 g), and K- (31.33 g). This finding suggested that P4 was the best treatment with a significant difference compared with the other treatments. Although P1, P5, and K+ had different shoot fresh weights compared with K-, these were not statistically different. When compared with the control, the plant fresh weight in P4 was 59.64% and 68.04% higher than those in K+ and K- treatments, respectively.

The observation of root fresh weight (without tubers) marked the highest root fresh weight in P4 (35.18 g), followed by P3 (30.45 g), P2 (26.38 g), P1 (20.81 g), K+ (22.68 g), P5 (22.40 g), and K- (20.81 g). Like the fresh plant weight, P4 was significantly different compared to other treatments. The P1, P5, and K+ treatments had different shoot fresh weights from K-, but these were not statistically different. When compared with the control, the plant fresh weight in P4 was 55.11% and 69.05% higher than those in K+ and K-, respectively.

The observation of shoot dry weight showed that the

highest shoot dry weight was evident in P4 (5.15 g), followed by P3 (3.80 g), P1 (3.25 g), P2 (3.10 g), P5 (2.95 g), K+ (2.90 g), and K- (2.60 g). Although there were some differences between K+, K-, P1, and P2, these were not significantly different. P4 showed the highest dry weight, which was significantly different from the other treatments. P4 was 77.58% and 98.07% higher compared with K+ and K-, respectively. Furthermore, the observations of root dry weight showed that the highest dry weight was found in plants in P4 (4.35 g), followed by P3 (2.55 g), P2 (2.40 g), P5 (1.85 g), K- (1.85 g), K+ (1.70 g), and P1 (1.65 g). The plant root weight in P4 was found the highest, and this was significantly different from the other treatments. The root dry weight in P4 was 155.88% and 135.13% higher than those in K+ and K-, respectively. The data on shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight are presented in Table 7.

Table 7. The fresh and dry weight of potato shoots and roots under various treatments

Treatments	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
K+	32.98 ab ± 4.31	22.68 ab ± 3.80	2.90 ab ± 3.15	1.70 a ± 0.51
K-	31.33 a ± 4.76	20.81 a ± 3.55	2.60 a ± 2.18	1.85 a ± 0.45
P1	35.33 ab ± 4.89	25.28 ab ± 8.19	3.25 ab ± 3.51	1.65 a ± 0.63
P2	37.73 b ± 5.18	26.38 bc ± 8.60	3.10 ab ± 3.18	2.40 ab ± 1.82
P3	45.20 c ± 8.06	30.45 cd ± 8.25	3.80 b ± 3.93	2.55 ab ± 1.85
P4	52.65 d ± 13.40	35.18 d ± 11.05	5.15 c ± 3.62	4.35 b ± 4.79
P5	33.98 ab ± 6.68	22.40 ab ± 7.03	2.95 ab ± 2.44	1.85 a ± 0.42

Note: Numbers in the column followed by the same letter were insignificantly different at the p-value of 0.05 (Duncan Multiple Range Test).

The effect of Bionematicide Formula on Potato Yield Infected with *Globodera rostochiensis:* Potatoes with the highest average number of tubers were found in P4 (5.75), followed by P2 (4.65), K- (4.45), P3 (4.45), P1 (4.00), K+ (3.95), and P5 (3.75). Concerning the number of potato tubers, only P4 was significantly different from the control treatment. However, P4 showed insignificant results compared with P2. Compared with the number of tubers in the control treatment, the number of tubers in P4 was 45.56% and 29.21% higher than K+ and K-, respectively.

The analysis showed that P3 (183.82 g) generated the highest tuber weight compared with other treatments, followed by P4 (163.02 g), K- (158.81 g), K+ (156.85 g), P2, (150.82), P5 (142.15 g), and P1 (129.49 g). Despite the significant difference in tuber weight per plant, K+, K-, P2, P3, P4, and P5 were not significantly different. This indicated that the application of the bionematicide formula did not give significant effects on the tuber weight in each plant.

In addition to observing the average tuber weight per plant, we observed the average weight per tuber. This observation showed that P3 (52.6 g) had the highest average weight per tuber, followed by K+ (48.49 g), P5 (59.66 g), K- (41.65 g), P4 (39.76 g), P2 (37.3 g), and P1 (35.16 g). The weight per tuber in P3 proved the highest value, but this was not significantly different from K+, K-, P4, and P5. P1 and P2 were not significantly different from K+, K-, P4, and P5. The data on tubers in each treatment are presented in Figure 1.

The Effect of Bionematicide Formula on *Globodera rostochiensis* Population: Our observation demonstrated that the lowest number of nematode cysts was found in P4 (112.33), followed by P3 (140.43), K+ (145.33), P5 (157.67), P2 (166), P1 (174.67), and K- (195). The number of cysts in P4 was significantly different from that in the other treatments. When compared with K+ and K-, the number of cysts in P4 was lower by 22.70% and 42.39%, respectively. Based on these data, P4 treatment was the most effective treatment to reduce the number of *G. rostochiensis* cysts in the field.

The observation results on the number of female nematodes showed that P4 (11.30) generated the lowest yield, followed by P3 (14.45), P2 (20.10), P1 (20.80), P5 (21.80), K+ (22.45), and K- (25.10). P4 produced significantly different results from the other treatments. Furthermore, when compared with K+ and K-, the number of female nematodes in P4 was lower by 49.66% and 54.98%, respectively. The data on the number of female cysts and nematodes in each treatment are presented in Figure 2.

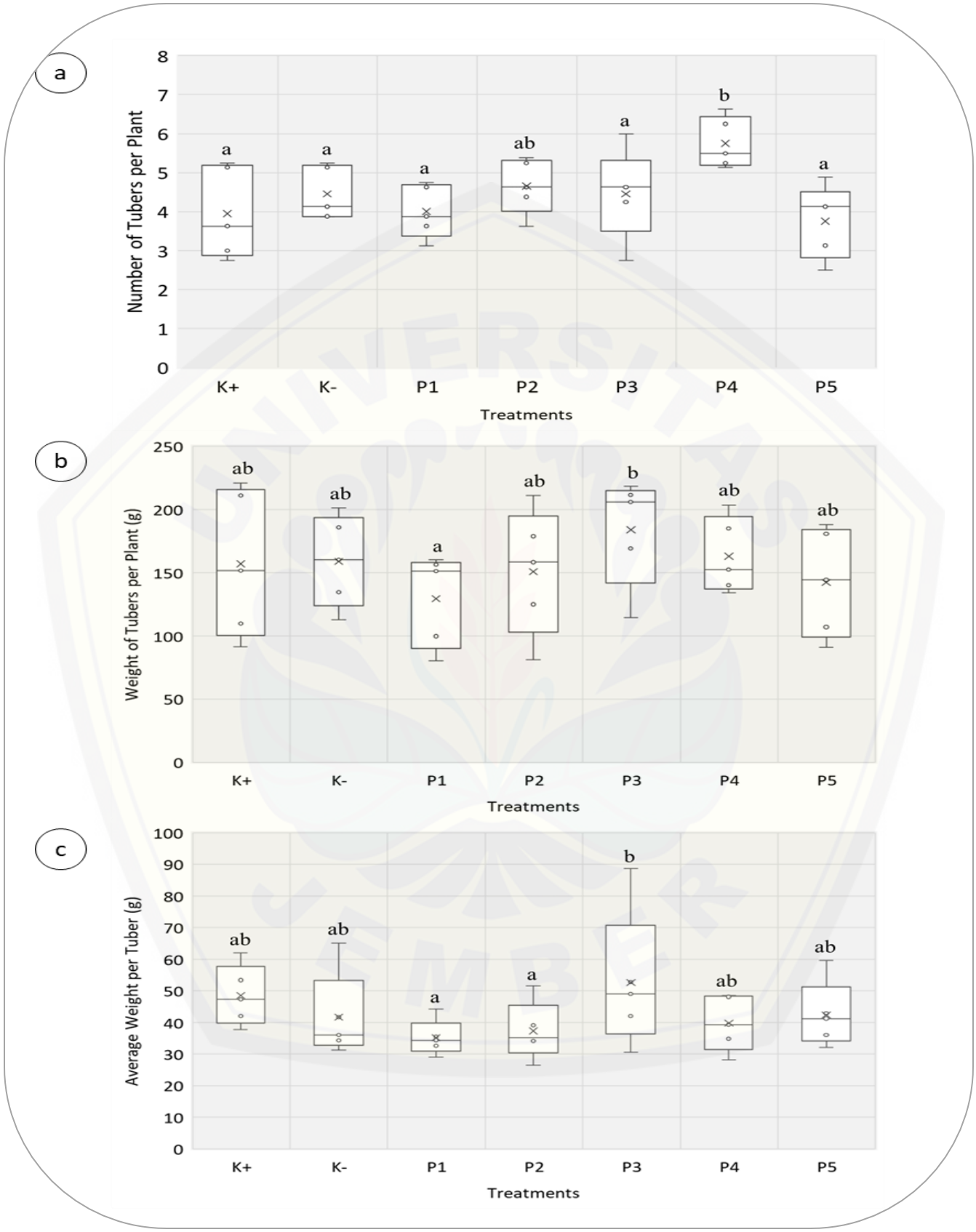

Figure 1. Potato yields under various treatments

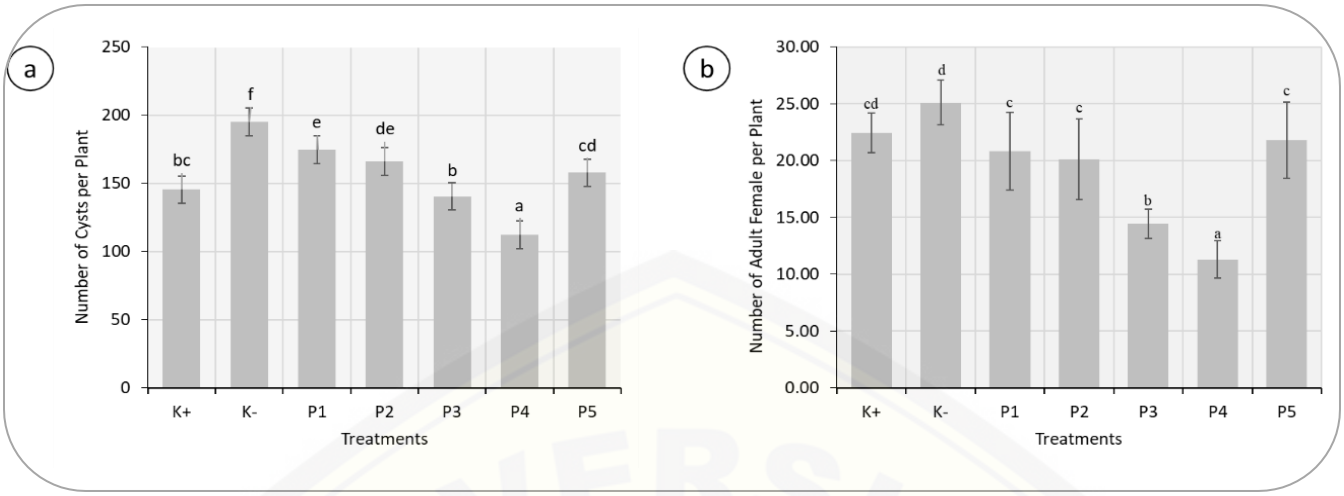

Figure 2. The number of cysts and female nematodes per plant

DISCUSSION

Microbial formulations as biological agents require proper nutrition. Good formulas can sustain microbial viability during storage (Soumare *et al.,* 2020). In this study, the population of Pseduomonas sp., Bacillus sp., and Azotobacter sp. was fairly dense. This is in coherence with a previous study by Patil *et al.* (2013) who formulated biofertilizers using enriched organic matter with a fairly dense microbial population. In this study, 0.528 mg L^{-1} of auxin was also detected in the applied bionematicide formula. This finding supports Merzaeva and Shirokikh (2010) who state that rhizobacteria and endophytic bacteria can produce auxin hormones that are beneficial for plant growth.

Rhizobacteria and endophytic bacteria can produce compounds to suppress the number of parasitic nematodes and improve plant growth (Tian *et al.,* 2007; Sidhu, 2018). The potato cyst nematode causes symptoms in 2 phases. The first phase occurs at the beginning of the growing season, with such symptoms as reduced photosynthetic rate (due to disturbances in nutrient uptake, hormone signaling, and water-plant relationships), increased allocation of photosynthetic products to roots, fewer and smaller stem production, and smaller leaves (Sukhomlin *et al*., 2019). The second phase occurs in the middle of the end of the growing season. The symptoms in this phase include leaves dying more quickly, fewer new leaves, reduced water and nutrient absorption (causing wilting in hot and dry weather), and reduced number and weight of tubers (Djebroune *et al.,* 2020).

Increased plant height and other growth parameters occur due to the presence of auxin in plants, especially Indole Acetic Acid (IAA). This is supported by Marathe *et al.* (2017) who argue that the inoculation of IAA-producing Pseudomonas spp. in *Glycine max* can stimulate plant growth. Bacteria that live in the Rhizosphere belonging to the Plant Growth Promoting Rhizobacteria (PGPR) can produce IAA hormones (Mehmood *et al.,* 2018). Auxin (IAA) affects the elongation of plant cells is by stimulating certain proteins in the cell plasma membrane to pump H+ ions to the cell wall (Hager, 2003; Muraro *et al.,* 2013). This H+ ion activates certain enzymes, thereby breaking some of the hydrogen cross-links of the cellulose molecular chains that make up the cell wall (Rayle and Cleland, 1977; Morsomme and Boutry, 2000). Plant cells then elongate due to the entrance of water through osmosis. After the elongation, the cell continues to grow by resynthesizing the cell wall and cytoplasm (Taiz, 1984).

In general, rhizobacteria and endophytic bacteria can increase plant growth through various mechanisms. Sivasakthi *et al.* (2014) reported that rhizobacteria of the genus Pseudomonas sp. and Bacillus sp. can fix nitrogen from the environment. N is an element needed in the greatest amount, so it is called a primary macro nutrient. Generally, nitrogen makes up 1-5% of the body weight of plants. Plants uptake N in the form of ammonium (NH_4^+) or nitrate (NO_3^-). N can be obtained from organic matter, soil minerals, or the addition of organic fertilizers (Leghari *et al*., 2016). The presence of N-fixing bacteria in the root area of soil will certainly support N availability for plants (Igiehon and Babalola, 2018).

In addition to fixing N, rhizobacteria and endophytic

bacteria were also acknowledged to dissolve P (Satyaprakash *et al.,* 2017). P is also one of the primary macronutrients required by plants in large amounts to support growth and yield. Plants take up P from the soil in the form of $H_2PO_4^-$ ions. The concentration of P in plants ranges from 0.1 to 0.5%, lower than that of N and K. P serves as a storage and transfers energy for all plant metabolic activities (Abel *et al*., 2002; Rafi *et al.,* 2019). Postma *et al.* (2010) reported that bacteria from the genera Pseudomonas, Bacillus, and Serratia were able to solubilize P, so that P which was previously bound by other elements, such as Al and Fe, could be made readily available to plants.

In this study, one treatment, P4 (4% bionematicide formula), successfully increased plant height, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight. In the other treatments, the effect of bionematicides on plant growth was not significantly evident. This may be because the need for nutrients to support plant growth has been met by the application of inorganic fertilizers according to the recommended dose in potato cultivation.

The observation of potato tubers in this study showed that a significant difference was only identified in the number of tubers per plant compared with the control. The parameters of tuber weight per plant and weight per tuber documented varied results but, in general, these were not significantly different from the control. This phenomenon can occur because bacteria can stimulate plants to produce more tubers. The increase in the number of potato tubers resulting from the application of biological agents has also been reported by Ekin (2019). Although the number of tubers in all treatments was significantly different from that in the control, the bionematicide treatment did not lead to any significant difference in tuber weight. This finding is related to the uptake of potassium by plants. According to Koch *et al.* (2020), the formation of carbohydrates in tubers is influenced by potassium nutrients. In the same vein, Hasanuzzaman *et al.* (2018) argue that potassium helps plants to synthesize protein and carbohydrates and ensures smoother carbohydrate translocation. In photosynthesis, potassium plays an important role in opening stomata, which eventually results in effective photosynthesis and optimal formation of organic compounds (Oosterhuis *et al.,* 2014). The accumulation of carbohydrates in the tuber affects the weight per tuber. The more carbohydrates are translocated in the tuber, the more the tuber weight increases. Produced through photosynthesis, the dry matters accumulated in the tubers include carbohydrates, proteins, and vitamins (Sawicka *et al*., 2015). In this study, the effect of the bionematicide formula on tuber weight was not significant, presumably because the potato plants had been treated with synthetic fertilizers based on recommended dose.

The research results demonstrated that the different doses of bionematicides affected the number of *G. rostochiensis* cysts and the number of female nematodes. The analysis results showed that the six treatments had a significant effect on the number of cysts. The treatment that could reduce the high number of cysts was P4. The decrease in the number of cysts was presumably because the bacteria in the bionematicide produced the chitinase enzyme. The chitinase enzyme is known to control Potato Cyst Nematodes from the egg stage (Cronin *et al.,* 1997). Inhibited cyst development will reduce the number of nematodes, as documented in the treatments with bionematicides compared with the control.

In this study, P4 treatment was able to reduce the number of female nematodes attached to the roots. The decrease in the number of potato cyst nematodes *Globodera rostochiensis* may be due to the bacteria in the bionematicides, including *Pseudomonas diminuta* and *Bacillus subtilis*, both of which are genera capable of producing chitinase enzymes. The chitinase enzyme degrades pathogenic cell walls which are composed of chitin compounds, such as in potato cyst nematode cell walls (Veliz *et al.,* 2017; Banerjee and Mandal, 2019). Another form of symbiosis carried out by endophytic bacteria on plants is by colonizing plant tissues, especially the roots. The bacteria will enter the plant tissue and occupy the intracellular space, leaving no room for pathogens (Anjum *et al.,* 2019; Firdous *et al.,* 2019; Khan *et al.,* 2020). The colonization also decreases the nutrients for pathogens because nutrients in the form of exudate or substrate will be available for endophytic bacteria (Morales-Cedeño *et al.,* 2020).

According to De Gonzalo *et al.* (2016), endophytic bacteria also produce lignin which strengthens plant cell walls, thus preventing pathogens from infecting plants. When the development of potato cyst nematode was inhibited, the number of nematodes in the roots would be reduced, compared with the control. Chitinase enzyme is produced by bacteria to control nematodes by

degrading the middle layer of nematode eggs and inhibiting the hatching of *Globodera rostochiensis* eggs by 70%. In addition to chitinase enzymes, biological agents from the bacterial class are also able to control nematodes through the production of protease enzymes and the volatile HCN compound (Abd El-Rahman *et al.,* 2019). Indirectly, the presence of microbes in the bionematicide formulas can also increase plant resistance through resistance induction mechanisms, which has been reported by Choudhary and Johri (2009). Induced plants generally have a higher content of anti-nematode compounds (Mhatre *et al.,* 2019). Enzymes such as protease and chitinase have direct mechanisms for nematode control. In this study, bionematicides were effective at reducing the number of cysts but less effective at reducing the number of adult female nematodes due to the disparity between their life niches. Generally, cysts are located in the rhizosphere, whereas adult female nematodes inhabit protected root tissue. This explains why the efficacy of bionematicides varies between cyst control and adult female nematode control.

CONCLUSION

This study has corroborated that the bionematicide formula with active microbial rhizobacteria and formulated endophytic bacteria with an inexpensive carrier can effectively control the potato cyst nematode *G. rostochiensis*. The recommended concentration is 4% in every 100 mL for each plant.

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AUTHORS' CONTRIBUTIONS

INA, DHT, and APP performed the experiment and wrote the manuscript; JP, DN prepared the research designs, administered bacterial isolates, and supervised research activities; SW, LW, and KF co-authored the manuscript and performed statistical analyses.

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