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Exploration of Entomopathogenic Nematodes in Organic Rice Field in Sleman Regency

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Abstract

Entomopathogenic nematodes (EPNs), consisting of genera Steinernema and Heterorhabditis are biological agents proven effective in killing insect pests. Organic rice fields and sandy soil textures are potential areas for exploring the presence of EPNs. This research aims to determine the diversity of genera and populations of entomopathogenic nematodes in organic rice fields in Sleman Regency. The study was conducted on organic rice fields and the UPN "Veteran" Yogyakarta Plant Protection Laboratory from July – August 2023. EPNs were obtained from Prambanan Sleman and Ngemplak Sleman, with six plots as sample units in every location. EPNs were isolated from soil samples by using Tenebrio molitor larvae. The Whitehead tray method was used to isolate EPNs from the dead larvae, and the population of EPNs was counted. EPN population density at each location was analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test at the 5% level. EPNs were identified based on the symptoms of color changes on the cuticle and morphological characters. The result showed that entomopathogenic nematodes were found in organic rice fields in Prambanan and Sleman. The cuticles of T. molitor larvae that died were blackish brown. EPNs were found in Prambanan_6, Ngaliyan_1, and Ngaliyan_2. The highest population of EPNs was on Ngaliyan_1 (38,00 EPNs/ml), and the lowest was on Prambanan_6 (2,13 EPNs/ml). Based on the color changes of dead larvae and morphological characters of EPNs, EPNs found in organic rice fields in Prambanan and Sleman were identified as Steinernema spp.

Keywords: Steinernema, biological agents, Tenebrio molitor, identification, morphological

1. Introduction

Entomopathogenic nematodes (EPNs) are one of the biological agents proven effective in killing insect pests [12]. EPNs are deadly insect parasites that live in soil, are classified under the Nematode Phylum, and are members of the Steinernematidae and Heterorhabditidae. They also have demonstrated remarkable efficacy in managing both sol and above-ground pests as highly effective biological control agents [12]. EPNs are considered eco-friendly for controlling certain pests instead of synthetic chemical insecticides [3]. EPNs are specific to their target pests and do not harm non-target organisms [8]. EPNs are self-replicating and can persist in the soil for several years, providing long-term control of pests [16].

EPNs from the genera *Steinernema* and *Heterorhabditis* are biological agents capable of reaching insects in difficult-to-reach habitats, high reproductive capacity, and safety for humans, vertebrates, and the environment [11]. EPNs can act as a control agent for pests on agricultural plants, such as rice stem borers, green planthoppers, sugar cane worms, oil palm tree borer caterpillars, armyworms on cabbage and mustard greens, subterranean termites on sugar cane, and so on [6] [15]. EPNs can infect and kill the host quickly by forming a symbiotic relationship with EPNs symbiont bacteria, which produce toxins [11]. Both *Heterorhabditis* and *Steinernema* are mutualistically associated with the genera *Photorhabdus* and *Xenorhabdus* bacteria, respectively [9].

Entomopathogenic nematode species that have been found in Indonesia are *Steinernema hermaphroditum* in Maluku [19], *Steinernema feltiae* in East Java [1], *Steinernema huense*, *Steinernema pakistanesse* [6], and *Heterorhabditis indica* in West Java, East Java, Bali and Maluku [13]. EPNs are found in soil with a sandy soil texture and high soil moisture and in vegetation soil with high organic matter [14]. EPN in East Java was found in crumbly textured soil (not clay and not too sandy) [20]. EPN is found in vegetated soil of horticultural vegetables, such as eggplant, chili, and tomato plants [22].

Sleman Regency has a loose soil texture in clay with a high sand content. On the other hand, the development of organic rice in Sleman Regency is considered massive. Organic rice fields and sandy soil textures are potential areas for exploring the presence of EPNs. The EPN population is higher on land with livestock manure than land without vegetation [14]. Exploration of various types of EPNs is important as a first step in developing biological agents. EPNs play a role in controlling insect pests and indirectly increase farmers' income. This research aims to determine the diversity of genera and populations of entomopathogenic nematodes in organic rice fields in Sleman Regency.

2. Material and Methods

The research was conducted on organic rice fields and the UPN "Veteran" Yogyakarta Plant Protection Laboratory from June to August 2023. The organic rice farming that will be the object of study is rice fields located in Prambanan - Sleman and Ngemplak - Sleman.

2.1 Soil sampling

Soil sampling was carried out on organic rice planting land. The sampling technique was carried out randomly [20]. On each field, six (6) plots of land were taken as sample units. In one plot of land, five soil samples were taken diagonally (one center point and four diagonal points). The soil in each sample was taken as much as 200 grams at 5 to 10 cm from the soil surface using a shovel. Then each soil sample was put into the same plastic container (homogenized).

2.2Trapping entomopathogenic nematodes

The method used for EPN trapping is a modified Bedding and Akhurst (1975) isolation method [4]. The soil sample for each sample unit (200 grams) was transferred into a plastic container with a volume of 1000 ml. Ten (10) *Tenebrio molitor* larvae were wrapped in gauze and put into each container. The container was covered with gauze and left in a dark room for 3-5 days. To maintain soil moisture, spray it with water every day. The gauze containing *T. molitor* larvae in the plastic container was opened after 7-10 days. Dead larvae (infected by entomopathogenic nematodes) will show several symptoms, including swelling, the body becoming flabby, the host's body tissue becoming watery soft, and changing color.

2.3 Extraction-isolation and population calculation

Dead insect larvae were transferred to filter paper Ø 17 cm to be placed on a support Ø 13 cm (whitehead tray method [24]). The buffer, filter paper, and dead insect larvae are placed in a closed jar. The jar is filled with distilled water until it touches the edge of the filter paper and left for 14-21 days. The purified water is then put in a beaker. The nematodes obtained were observed or placed in a storage bottle. The suspension volume was made to 100 ml. The suspension was stirred, and 5 ml was taken and then poured into a counting dish. The nematode suspension was observed with a stereoscopic microscope. After observation, the suspension was put back in the beaker. Nematode suspension collection was repeated three times. EPN population density at each location was analyzed using analysis of variance (ANOVA). If there were significant differences, it was continued with Duncan's Multiple Range Test (DMRT) at the 5% level.

2.4 Identification of entomopathogenic nematodes

Identification of EPNs could be done by observing the symptoms that appear on the body of *T. molitor* larvae, including the condition of the larval body and the Occurrence of color changes on the larval body. If the larval body color changes to blackish brown, then the larvae are infected by *Steinernema* spp., but if the caterpillar is red or red old, then the larvae are infected with *Heterorhabditis* spp. [2][21].

Morphological identification of EPN is carried out by looking at the morphological characters and morphometric measurements of nematodes. The morphological characteristics observed were head shape, stoma, cuticle, esophagus, tail shape, and body length [18]. Before identification, preparations are made. Nematodes are fished out and placed on a glass object dripped with glycerol. Then, quickly cover with a glass cover and smear nail polish on the edges. The preparations were observed using a binocular microscope.

3. Results and Discussion

3.1 Mortality of *Tenebrio molitor* larvae

The results of trapping EPNs using Tenebrio molitor larvae could be seen from the death of the larvae. Based on observations of *Tenebrio molitor* larvae, larvae died in all treatments except Ngaliyan_5 (Figure 1). Isolates that showed more than 50% mortality were Prambanan_6, Ngaliyan_1, Ngaliyan_2, Ngaliyan_3, Ngaliyan_4, and Ngaliyan_6. The results of the isolation of entomopathogenic nematodes (EPNs) using *T. molitor* larvae as bait from organic rice fields showed that *T. molitor* larvae that died were blackish brown, both in Prambanan and Ngaliyan isolates (Table 1, Figure 2). These symptoms followed the results of previous research that *T. molitor* larvae infected with EPNs showed changes in the color of the larval cuticle, which was initially light brown to caramel brown, and the body tissue became soft and did not smell bad [2]. Color changes and changes in body texture that occur in insects are caused by the presence of the symbiont bacteria *Xenorhabdus* sp., which is in the nematode's body. These bacteria release exotoxins and extracellular enzymes, which ultimately damage the insect's hemolymph, then the nematode will cause paralysis in the insect, followed by the insect's death [26].

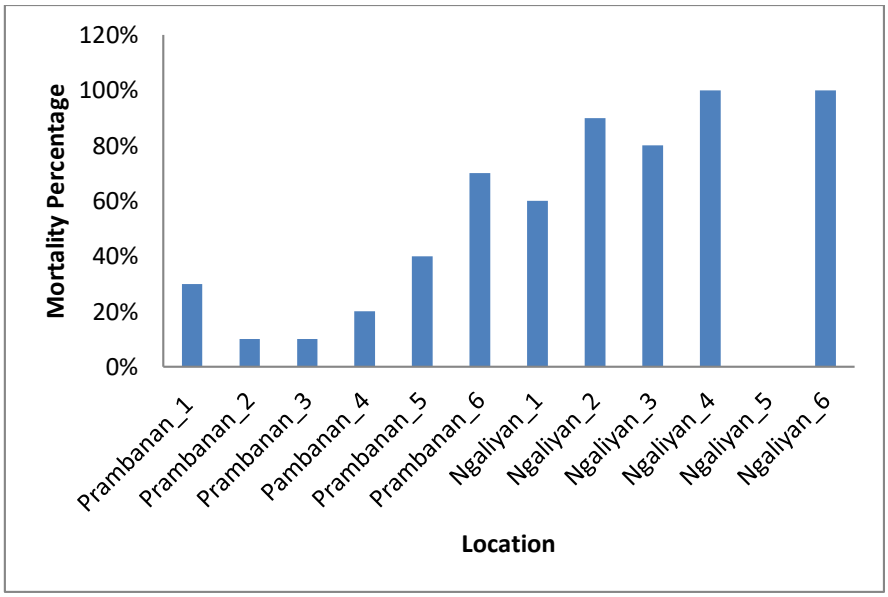


Figure 1
Mortality percentage of *Tenebrio molitor* larvae resulting from trapping Entomopathogenic Nematodes

Table 1
Tenebrio molitor larvae appearance resulting from trapping Entomopathogenic Nematodes

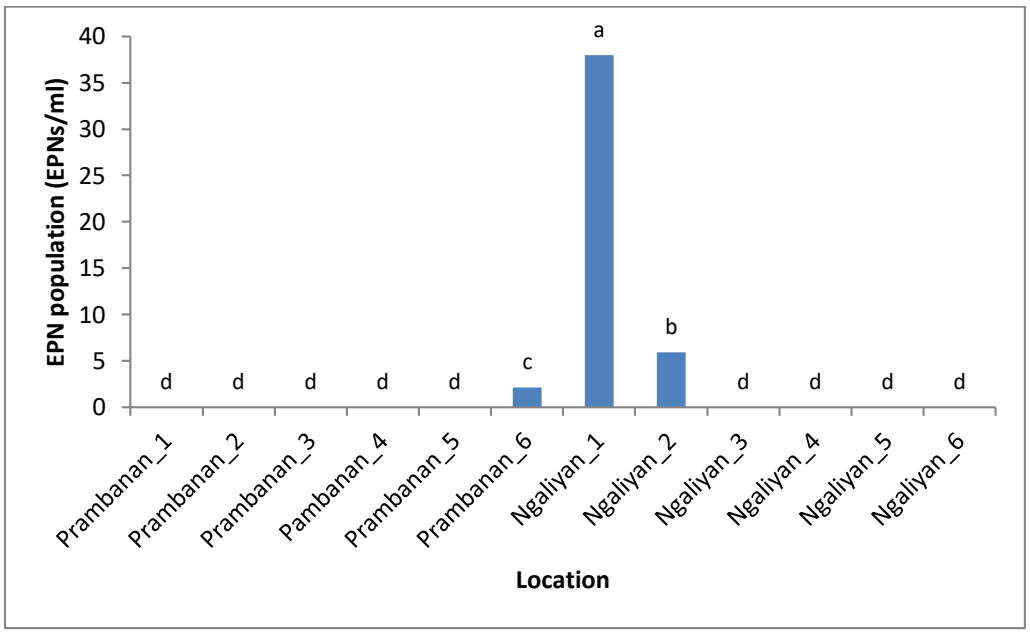
Location	Larvae appearance
Prambanan_1	Blackish brown
Prambanan_2	Blackish brown at the top
Prambanan_3	Blackish brown at the top
Pambanan_4	Blackish brown color
Prambanan_5	Blackish brown
Prambanan_6	Blackish brown
Ngaliyan_1	Blackish brown
Ngaliyan_2	Blackish brown
Ngaliyan_3	Blackish brown
Ngaliyan_4	Blackish brown
Ngaliyan_5	-
Ngaliyan_6	Blackish brown

Figure 2
Color changes in *Tenebrio molitor* larvae attacked by entomopathogenic nematodes

3.2 Population of Entomopathogenic Nematodes

The extraction-isolation results showed that entomopathogenic nematodes were found in soil samples Prambanan_6, Ngaliyan_1, and Ngaliyan_2 (Table 2). Not all *T. molitor* died due to attacks by entomopathogenic nematodes. The highest population of EPNs was on Ngaliyan_1 (38,00 EPNs/ml), and the lowest was on Prambanan_6 (2,13 EPNs/ml). The higher the nematode population density, the higher the larval mortality [23]. Differences in EPN abundance in various locations are caused by several factors, including the source of organic material, biotic environmental conditions, and abiotic environmental conditions [14]. Environmental factors such as soil type, soil function, climate, soil water content, and soil temperature can affect the occurrence, persistence, and species variation of EPNs [17].

The rice planted in the Prambanan_5 and Prambanan_6 locations has been cultivated organically since 2008, while the rice in other Prambanan locations in 2015. The organic rice system in Prambanan uses manure and rabbit urine fertilizer. They also used vegetable pesticides such as mindi leaves and biological agents such as *Paenibacillus* sp. to control pests and diseases. Organic rice cultivation at the Ngaliyan location began in 2019. The fertilizer used is manure made by farmer groups and additional humic acid, while to control pests and diseases, farmers used biological agents (*Bacillus* sp., *Beauveria bassiana*, and *Trichoderma* sp.) and botanical pesticides with amethyst plants to control bird pests. Irrigation for Prambanan organic rice is through wells, while for Ngaliyan organic rice is through wells and ponds with filters. The high number of EPNs at the Ngaliyan location could be due to the intense use of organic fertilizer and humus and the application of beneficial microorganisms.



Note : Common letter in the same column are not significantly different according to Duncan's Multiple Range Test at P = 0.05

Figure 2
Entomopathogenic nematodes (EPNs) population in organic rice field.

3.3 Identification of Entomopathogenic Nematodes

EPNs were identified based on the symptoms of color changes on the cuticle and morphological characters. The cuticles of *T. molitor* larvae that died were blackish brown in Prambanan and Ngaliyan isolates (Table 1, Figure 1). This characteristic indicated that the genus of EPNs that attacked was *Steinernema*. Larvae infected with *Steinernema* change color from light brown to cream, while larvae infected with *Heterorhabditis* change color to brick red or brown [7]. The color of the larvae turned brown when they died due to *Steinernema* infection [10].

The nematodes found in three locations (Prambanan_6, Ngaliyan_1, and Ngaliyan_3) were in the juvenile phase and had the same morphological characteristics. Nematodes have a characteristic slender body, straight habitus, anterior end slightly rounded and fused with the body, no stylet, cylindrical stoma, no second cuticular sheath, long, narrow esophagus, slightly widened pro corpus, not annulated, and conoid tail (Figure 2, 3, 4). According to its morphology, this nematode species refers to the *Steinernema* species (Rhabditida: Steirnematidae) [1][2]. *Steinernema* is a genus of entomopathogenic nematodes that has the characteristics of a long and cylindrical stoma, with or without a second cuticular sheath, reduced esophagus and intestine, and tail conoid or filiform [18]. The third-stage infective juvenile has a slender body, habitus straight, cuticle with fine

transverse striae, head continuous with body contour, slightly truncate, not annulated, long esophagus, narrow, procorpus slightly expanded, narrowing in the isthmus and base bulb pyriform, tail conoid tapering gradually [10].

Figure 2
Morphology of the Prambanan_6 entomopathogenic nematode as a whole (A), anterior part (B) and posterior part (C)

Figure 3
Morphology of the Ngaliyan_1 entomopathogenic nematode as a whole (A), anterior part (B) and posterior part (C)

Figure 4
Morphology of the Ngaliyan_2 entomopathogenic nematode as a whole (A), anterior part (B) and posterior part (C)

Based on body size, the nematodes Prambanan_6, Ngaliyan_1, and Ngaliyan_3 respectively have body lengths of 310 µm, 539 µm, and 563 µm (Table 4). *Steinernema* nematodes in the infective juvenile stage have varying body lengths of 350 – 1200 µm [19]. *Steinernema* spp. in the infective juvenile stage, which have a length of between 500-600 µm are *S. carpocapsae*, *S. kushidai*, *S. rarum*, *S. ritteri*, *S. asiaticum*, *S. tami*, etc. [19]. Different body lengths of nematodes in the same species can be caused by various factors, including bacterial food, sex, growth temperature, and developmental mechanisms [27]. The body width of the nematodes found ranged from 27-28 µm. *S. carpocapsae* has a body width of 24 – 27 µm at the infective juvenile stage [28], *S. pakistanense* has a body width of 24-29 µm [25], *S. abbasi* has a body width of 27-30 µm, and *S. kushidai* has a body width of 22-31 µm [19]. EPN diversity with other organisms can be caused by management practices

such as irrigation, planting density, variety selection, soil processing methods, fertility inputs, use of pesticides and various other factors [5].

Table 4
Measurement of body length and body width of entomopathogenic nematodes

Sample units	Body length (µm)	Body width (µm)
Prambanan_6	310,34	28,88
Ngaliyan_1	538,73	27,26
Ngaliyan_2	563,45	28,37

4. Conclusion

Entomopathogenic nematodes were found in organic rice fields in Prambanan and Sleman. Based on the color changes of dead larvae and morphological characters of EPNs, EPNs found in organic rice fields in Prambanan and Sleman were identified as *Steinernema* spp.

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