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Exploration and mass propagation of nematodes entomopathogen *Steinernema* spp. wetlands on mustard (*Brassica juncea L.)*

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Key words: Entomopathogen, Mustard, *Steinernema* spp., *Tenebrio molitor*.

Abstract

The use of chemical pesticides in the context of pest control in mustard (*Brassica juncea L.)*plants is very dangerous. The used of biological agents of *Steinernema* spp. in the control of important pests is biological pesticide to overcome the problem of vegetable pest problems is e new hope foe us all so that farmers no longer us chemical pesticides that can interfere with human health. The utilization of biological agent in the form of election of entomopatogen nematode exploration on wetland from district of Barito Kuala, South Kalimantan. Test of biological agent formulation that is capable not only inhibits the development of plant pests (biopesticides). Experiment used a completely randomized design with each treatment ranging from control (without *Steinernema* spp.), 20, 40, 60, 80 and 100 tail population per unit, experiment with 3 repetitions, with 40 tail caterpillar as feed in mass propagation). The result of the study is that the population of entomopathogenic nematodes develops well in all treatment of *Steinernema* spp. DMRT test results showed that there were differences between treatments tested, only T1 and T2 treatment were not different in effect, because different initial population. Population with an initial population number of entomopathogen nematodes 80 and 100 tail tended to differ significantly and were stable in the number of late population development observations. In general that the use of caterpillar *T. molitor* is suitable in mass breeding entomopathogen nematodes.

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Introduction

Continuous planting to meet the growing vegetable needs resulted in pest problems also increased due to pest host always available at all times. To overcome these farmers are highly dependent pesticides so that the spraying of toxic materials is scheduled. While the cultivation of healthy plants can not rely solely on the use of certified healthy seeds and only the use of synthetic pesticides, because pests are always present in the land so that it always threatens the freedom of plants against pest problems (Bedding *et al.,* 1993)Increasing awareness of quality food, the requirements of pest control measures according to the Directorate General of Horticulture (2007), must meet the ecological aspects (not disturbing human health, natural enemy life and non-target organisms, environment and not cause harmful residues in the crops), social aspects (easy to implement, socially acceptable, and motivating community self-reliance) and technical aspects (combining harmonious, harmonious and balanced ways of controlling, prioritizing cultivation, physical / mechanical, biological, genetic, and pesticide use if necessary).

The role of horticultural crop commodities in the present and future in life and the national economy becomes very important. Behind its very important role, this commodity has constraints in cultivation and its management. The obstacle is the attack of plant pest organism (OPT), one of the pest is a pest insects are very detrimental to the farmers (Azharie, 2005). The disturbance will be able to decrease the quality and the crop production. Important plant pests such as *Plutella* spp *Lamprosema* spp *Spodoptera* spp and *Aphis* spp, *Lyriomysa* spp and as well as the presence of many diseases affecting vegetables especially the mustard greens is *Fusarium* sp, *Sclerotium* sp., and *Rhizoctonia solani* is a land-borne pathogens proven to always exist together in soil and often infect vegetable crops on young mustard and old crops on farmer's land.

The use of insect pathogenic nematodes has been developed for the biological control of several insect pests in various types of crops, whether food crops, plantations or horticulture (Grewal *et al*., 2005). Indeed, when compared with the use of chemical insecticides, the use of nematodes in general is more expensive (Arthurs *et al*., 2004), has a short life cycle, low stability, and for its application the user must have sufficient knowledge about nematodes ranging from knowledge to care until the storage of nematodes (Umamaheswari *et al*., 2006).

However, with the rules and restrictions of the Food Quality Protection Act of 1996 on the need for restricting the use of chemical insecticides in order not to poison humans (Anonymous, 1996) and the pressure and awareness of the public to reduce the use of chemical insecticides will encourage the development of safe biological controls, especially the use of the pathogenic nematodes *Steinernema* spp. (Koppenhöfer and Fuzy, 2009).

The study of the use of entomopathogenic nematodes on pests is important in view of the fact that there is a tendency for migration to organic farming, commercial market developments, attention to food safety, and the increasing number of pesticides withdrawn from circulation, leading to an increasingly important role of biological control. With this research is expected nanntinya can be an alternative in plant pests control, especially in mustard plants.

The challenge to meet the public demands for the availability of healthy and healthy quality foods leads to the use of synthetic pesticides to overcome pest diseases when planted or in storage needs to be reduced even avoided.

Therefore, an effective, efficient and environmentally friendly control alternative is needed. This research becomes important to be carried out considering the superior local biomedical exploitation as biopesticide as a biological agent in mustard plants has never been comprehensively studied to obtain appropriate technology.

Entomopathogenic nematodes are able to inhibit the development of important pests. But unfortunately the existence of that potential has not been explored comprehensively, especially in wetlands. Thus, this research tries to utilize the collaboration between entomopathogenic nematodes and is an alternative that has a good future and representative especially in wetlands as biopestisida for the main pest of mustard plant in order to fulfill requirement of mustard oragnik in the future.

The ultimate goal of this study is to obtain appropriate technology for the exploration and mass management of organic mustard plants using entomopatogen nematodes Steinerneme spp so as to affect the high productivity of vegetable crops, especially mustard greens.

It is expected that proper entomopathogenic nematodes then the management of plants, especially mustard greens can be profitable in a sustainable manner for the development of organic agriculture.

Research sites

The material used in this research is the sample soil taken from the horticultural cultivation land in the wetlands Balitra Garden of South Kalimantan experiment especially. Survey of the location and sampling is done on the land planting. Taking sampling of existing land vegetable crops. 10 Soil samples were taken around the root of the plant (*rhizozfer*) with a depth of 0-20 cm using a ground picker at a depth of 15 cm, a 45^0 angle of 500 grams. The soil is then put into a clear plastic size of 1 kg and then wrapped with aluminum foil so that the temperature and soil moisture can be maintained.

The equipment used in this research is microscope, glass slide, glass cover, petri dish, erlenmeyer flask, dropper dropper, tweezers, cetok, filter paper, plastic bag, jar, strimin cloth, gauze, glass jam jar, hand sprayer. This research was carried out in the field of experiment of Balittra and Laboratory of Agroteknologi.

Propagation Tenebrio mollitor

The sample soil that has been taken from the land is labeled according to the location of the pickup. *Tenebrio mollitor* feed larvae bought in the bird market, larvae are then wrapped with 10 gauze per pack. Sample Land Placement and Ground Feed larvae and *Tenebrio mollitor* feed larvae are placed inside polybags or glass jam jars labeled sample soil used. Volume of sample soil when using polybag as much as 500 gram and when using 200-300 gram of jams. Place *Tenebrio mollitor* feed larvae randomly. *Tenenbrio mollitor* baits are placed as many as 10 grams/500 grams of soil if in polybags and if in the bottle of jam as much as 4- 5 tails/300 grams of soil. Then given the aquadest as much as 100 ml so the ground remains moist and entomopatogen nematodes can live. Day two and so on also remain in add aquadest as much as 100 ml. *Tenebrio mollitor* feed duration in polybag or glass jelly bottle for 4-5 days.

The feed larvae are taken on the 4th or 5th day after the trapping is done. Pick up the larvae using a pair of tweezers first cleaned with 95% alcohol. Prepare the petri dish and then give the aqua glass cup as high as 5-6 mm. on top of aqua glass chunks placed in a petri dish with a strimine cloth and filter paper. The feed lever is placed on a filter paper, then a petri dish filled with a sterile aquadest until it touches the body of the feed larvae. The duration of the feed is in the 3-4 day petri dish.

Symptom observation

Dead bait larvae are observed for symptoms that appear on the morphology of the body. If there are symptoms of discoloration, the body becomes soft and if in the surgery the inner tissue becomes liquid but not foul then it can be concluded that the larvae have been attacked by entomopatogen nematodes (Azharie, 2005). According to Nurcholisah (2005), the infected feed larvae of *Steinernema* spp., will change color to beige, sometimes slightly greenish and the body softens, while the feed larvae turns reddish brown and the body remains hard then

infected with *Heterorhabditis* spp. If there are symptoms of feed larvae after 3 days of isolation, the liquid in the petri dish is taken as much as 0.5-1 ml and placed on a glass slide and covered with a glass cover. If the nematodes are visible, it is necessary to harvest as soon as possible. The liquid in the petri dish is put into the erlenmeyer flask and then diluted. After that the suspension is taken as much as 0.5-1 ml and seen under a microscope. If there are still many nematodes, it needs to be diluted again until they are seen in the microscope only slightly and can be counted. Counting is done 5 times from the same suspension.

Testing entomopatogen nematodes with Koch postulat test

This test is performed to test whether the symptoms that occur on the specimen are symptoms caused by entomopathogenic nematodes. This kochpostulat test uses only five *Tenebrio mollitor* feedlots suspected to be infected with entomopathogenic nematodes, to be reproduced in new and sterile feeds. If the infected symptom is the same as the previous one, then the infected feed larvae are entomopathogenic nematodes. Feed Testing in Mass Breeding:

The feed test is done by using hongkong caterpillar *Tenebrio molitor* with various population level. The initial populations tested were 20, 40, 60, 80, 100 and without population. The tested feed. Then the data is made report.

Experimental design

The experimental design used was a randomized block design (RBD) using entomopathogenic nematodes given 6 treatments and 3 replications, totaling 18 experimental units. Each of the 6 treatments used were: a) T1 100 tail, b) T2 80 tail, c) T3 60 tail, d) T4 40 Tail, e) T5 tail 20 and f) tail Control. Observations were made 2 times after 1 month 1 and second month. According Sulistyanto (2001) 24-48 hours of host insects will die from infection by entomopathogenic nematodes. The last observation was performed for 1 month after treatment using magnifying glass and surgery in host body.

The dead pest insects were reexamined with the Koch postulat method to ascertain the consequences of insect mortality. Variety analysis according to RBD design format used.

The experimental design used was a randomized block design with 5 replicates. The variance analysis was performed on the difference between treatments tested according to Duncan's multiple-range test (DMRT).

Results and Discussion

The results of the average analysis of the development of *Steinernema* spp. on *Tenebrio molitor* media is very suitable only that affect is the cloud population will affect the final population after 2 months of the pangamatan. Population with jumkah 100 tail and 80 tail that is 100 treatment of tail and 80 head per unit of experiment is ideal population for development of nematode at host body. 1media using its host insect larvae is hongkong caterpillar Tenebrio molitors as much as the development of *Steinernema* spp. the lowest is in population 0 or without treatment where the caterpillar Does not die until 0 days of observation (Table 1.).

Table 1. Results of propagation analysis Nematoda *Steinernema* spp on several treatments.

Source of variation	df	SS	MS	F-Calculate	F-Table	
					0,05	0,01
Treatment	4	60228362.252	15057090	18.56*	3.06	4.89
Error	15	12169999.772	81333.318			
Total	19	72398362.02				

*Influential very real.

DMRT results showed that there were differences between treatments tested, only T1 and T2 treatment were not different in effect, because different initial population (Table 2.).

The experimental results show the best nematode development is in the initial population of 00 and 80 heads per unit of experiment. Its own insects are hongkong worms (*Tenebrio molitor*). During the first 15 days no additional population of *Steinernema* spp. because the nematodes are still in the stage of infection to the hongkong caterpillar body. Nematodes *Steinernema* spp. is quick to find a suitable host *Steinernema* spp., entering the insect through natural holes (mouth, anus, or spiracles) feeding nematodes and developing in the host's body with the bacteria inside the gastrointestinal tract (Untung, 2001).

Table 2. DMRT results on the average rate of propagation rate of Nematodes *Steinernema spp*.

Treatment	The average propagation of *Steinernema* spp.
T_1 (Populasi 100 ekor per sat perconaan)	5424.80 a
T_2 populasi 80ekor per satuan per percobaan)	5370.00 a
T_3 (Populasi 60 ekor*Steinernema*)	2334.20 b
T_4 (*Piopulasi 40 dan 20 ekor*)	2324.920 c
T_5 20 ekor persatuan percobaan	12,00 d

Description: The number followed by the same letter behind the numbers shows a very real effect based on DMRT 5%.

After the 30th day of observation, the number of nematodes of *Steinernema* spp. greatly increased as much as 2443.13 because in the body of host insects in the digestive system of insects through hemokoel, solve the immune system of insects and immediately release the symbiont bacteria brought by the nematodes. Nematodes multiply in one cell of the insect's body along with the enzymes produced by bacterial cells located in the digestive tract of nematodes, breaking the body's tissue into nutrients suitable for nematodes. Nematodes can develop 2-3 generations and after depleted nutrients.

The nematodes will re-enter the infective cycle and migrate from the nutrient-depleted host by carrying bacterial cells in the gastrointestinal tract, out of the body of the insect larvae and can last for months (Untung, 2001).

On the 30th day the nematodes came out of the body of a host insect indicated by a change in the insect that is a soft or crushed insect. Nematodes *Steinernema* spp. *Tenebrio molitor* is thought to be quantitatively derived from insect larvae suitable for growth and development because *Tenebrio molitor* has nutrient content of 48% crude protein, ash content 3%, moisture content reaches 57%, crude fat 40%. The nutrient content found in *Tenebrio molitor* is good as a feed source for *Steinernema* spp. Some sources say that the content of fat present is higher than protein content (Untung, 2001).

According to (Harahap *et al*., 2004) the superiority of host larvae is easier to breed, cheaper propagation costs. The visually generated JI is more uniform in size and motility, retained in storage and lower contamination during the propagation process, resulting from extreme environmental survival. In the host's insect body there is an infective juvenile and all develop into females and males. JI on the nematodes *Steinernema* spp. can protect from unstable environmental factors and other microorganisms that can inhibit the growth of nematode (Poiner, 1979).

Effects on drought, high temperature and ultra violet rays even though the nematode is in water. Nematodes require high moisture that is over 80%. (Sulistyanto, 2001).

Nematodes *Steinernema* spp. also require oxygen, the higher the oxygen the faster nematode growth (Untung, 2001). The occurrence of population decline on days 60 to day 75 due to the available nutrients has been reduced suspected due to ingestion or evaporation in the media so that nematodes *Steinernema* spp. inactive and off.

Utilization can be used for nematode breeding, but artificial media containing only high protein from animals and is all the elements needed for growth of nematodes. However, this medium can cause a stinging smell during the breeding period. This will not be beneficial for the breeding that can take place (Wagiman *et al*.,2003).

On the 30th day of dog food treatment the development of nematodes *Steinernema* spp. not as fast as developing on the treatment of host insects. It is presumed that the nutrients available in the media are only for survival, but in the digestive tracts that the bacteria that already exist in the previous stock help nematode breeding, so only the resistant JI nematodes can develop so that high humidity can sustain the infective nematode life.

The result of mass nematode breeding experiments conducted in Balitsa Lembang shows that JI population production is significantly determined by various media. Media dog food significantly produces the highest JI production compared to goat and compost manure. Because dog food has the nutrients needed by the growth of nematodes. The population differences that occur from the five treatments are the nutrients present in the feed or the media vary so that the population of nematode development of *Steinernema* spp. are distinctly different.

Nematodes develop better in host insects than in extracts because nematodes are more susceptible to development within the body of the insect compared to processed media such as dog food, meat stew essence and heart-stew essence. Because the host insects reproduction more and more quickly. Of the five treatments for media using embroidered insect larvae or can be tested with *in vivo* the best is *Tenebrio molitor*.

Conclusion

The use of *Steinernema* spp. nematodes is the best in initial population of 100 and 80 tails in the effective jouvenil development of insect larvae on the *Tenebrio molitor* caterpillar host. Initial population growth below 60 tails per experiment unit. And *Tenebrio molitor* is particularly suitable for mass culture of entomopathogenic nematodes.

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