Bioefficacy of entomopathogenic nematode, *Steinernema* sp. isolated from Timor Island as bioinsecticide on sweet potato weevil, *Cylas formicarius* (Fabr.) (Coleoptera: Brentidae)

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ABSTRACT

Bioefficacy of entomopathogenic nematode belongs to the genus *Steinernema* sp. were tested against mealworm *Tenebrio mollitor* with 50, 100, 150, 200 and 250 juveniles (IJs)/30 mealworm larvae. LC_{50} value was 105 IJs/ml for mealyworm. The invasion efficiency was lower as the density of nematodes increased. At highest density (250 IJs/ml), the efficiency of invasion of nematodes was 24.5 % whereas at lowest density (50 IJs/ml) the invasion efficiency reached 33%. For LT_{50} calculation, 30 larvae were put in contact with the nematode (density 250 IJs/ml) during 2, 4, 6, 8 and 10 hours. Mortality was recorded 48 hours after the contact periods, and LT_{50} value was obtained at 7.7 hours. After two weeks, nematode population could reach *ca*. 40,000 IJs/larva, 35,000 IJs/pupa and 29,000 IJs/imago. When applied using density 50 IJs/ml against *Cylas formicarius*, the nematode population grew up to *ca*. 4000 IJs/larva, 3600 IJs/pupa and 3400 IJs/imago with invasion efficiency of 10%, 8% and 7% respectively. A series of nematode density 1.5, 2.0, 2.5 and 3.0 x 10^4 IJs were poured on the soil surface in the plastic container where sweet potato tuber were buried. The tubers were previously and artificially infested by five pairs of weevil. Although in general the difference between treatments is not significant, the result showed that high mortality (>70%) of larva, pupa and adult of *C. formicarius* was observed in each tuber. It is concluded that the isolated nematode was not difficult to propagate *in vivo*, and their mobility to search and kill the weevil seemed promising as bioinsecticide.

Keywords: Pathogenicity, invasion efficiency, infective juvenile, nematode density, *Tenebrio mollitor*, mortality, LC_{50}, $LT_{50.}$

INTRODUCTION

Unlike the west region of Indonesia, where people use rice as main food-crop, corn and tuberous plant are the main food-crops for people of the province of Nusa Tenggara Timur, Indonesia. Sweet potato is the most cultivated tuberous plant in Timor Island. The most dangerous pest of sweet potato is weevil *Cylas formicarius* Fabr. (Coleoptera: Brentidae) (Kalshoven, 1981). Although sweet potato weevil is very important insect pest in reducing the yield, however, the control measure is very seldom practiced by farmers. The chemical control, although very effective, is not a wise technology to apply due the environmental hazard that could be entailed; moreover, the cost of chemical insecticide is high for the low price commodity like sweet potato. We are looking for the possibility in using local biocontrol agent, especially from the entomopathogenic group, to combat the insect pest.

Natural pathogen that have been reported as promising biocontrol agent against sweet potato weevil are fungi *Beauveria basiana* (Amalin and Vasquez, 1993) and nematode (Ekanayake, 2000). In line with the Integrated Pest Management (IPM) programme being developed, these two pathogen groups seem interesting to investigate further. For traditional farmers, mass production of nematode is believed more feasible than that of fungi that require more aseptic condition. The nematode *Heterorhabditis indicus* isolate INAH 17 has been attempted in green house and was judged effective (Chaerani and Waluyo, 1996). However, this nematode was isolated and tested in West Java, the region where the rainfall is usually high, so application to the dry region like Timor Island could face the problem of adaptation. To accelerate the adaptation of nematode to the field where they are applied, we decided to isolate the nematode from Timor Island and investigate their potency as biocontrol agent in the laboratory.

MATERIALS AND METHODS

Soil sampling and nematode extraction

Soil sample has been collected from rhizosphere of corn, dry land rice, peanut (village Manulai), bittergourd, corn,

sweet potato (village Noelbaki), spinach, eggplant, crucifers (village Oebufu). Three sample points have been determined randomly in 2 to 4 m^2 area. Using shovel, sub-sample of 100 cm^2 surface x 20 cm depth was taken to the laboratory. The shovel was cleaned and disinfected by ethanol 70% before use. About 150 g soil was placed in the plastic container, and 5 – 10 larvae of *T. mollitor* were introduced on the soil surface, and covered with lid. The container then inversely placed and incubated at room temperature. After 3 to 4 days, the cadavers were rinsed with sterile water and the nematode was extracted according White trap method (Kaya and Stock, 1997). The suspension of nematode obtained from this trap was kept in the refrigerator at 10^0C temperature for further study. To obtain the true entomopathogenic nematode, *ca* 100 nematodes consist of male and female were suspend in 10 ml water and poured into the Petri dish with sterile filter paper in the bottom. Five larvae of *T. mollitor* then released into the dish and allowed in contact with nematode for 3 – 4 days. The dead larvae were subject to White trap, and the nematodes freed from the host cadaver were then considered pathogenic. For identification, the nematode were put in the Ringer solution on the object glass and covered with ring glass. Nickle (1991), Mai and Mullin (1996), and Adams and Nguyen (2002) methodologies were used for nematode identification. For further study, mass propagation of the nematode was done using *T. mollitor* as insect host and followed by White trap method as described above.

Pathogenicity test against *Tenebrio mollitor*

Lethal Concentration (LC) was calculated using five densities of infective juvenile (IJs) nematodes *i.e.* 50, 100, 150, 200 and 250 IJs/ml. Suspension of each density was placed into the plastic container (diameter 5 cm) with filter paper in the bottom. To each container was introduced one larva of *T. mollitor*; thirty larvae were tested for each density. Mortality of larva was observed 48 hours after introduction of the larvae into the container. LC_{50} was calculated using Abbott's formula (Abbott, 1925). Observation was also done for the presence of nematodes in the cadaver by dissecting and counting the nematode that penetrated into the insect host. Invasion efficiency was determined by comparing the sum of nematodes successfully penetrated into the cadaver with the density applied to the filter paper (Epsky and Capinera, 1994). Lethal Time (LT) was calculated according to the method introduced by Glaser (1992), modified slightly by using only one nematode density, 250 IJs/ml. Larvae were kept in contact with nematodes during 2, 4, 6, 8 and 10 hours, as described above, then replaced in the new container free of nematodes. Mortality was observed 48 hrs after first contact between nematodes and insects.

Bioassay test against *Cylas formicarius*

Larvae, pupae and adult of *C. formicarius* were separated from the tuber that have been artificially infested. Filter paper was put in the bottom of Petri dish, and moistened by suspension of 250 IJs nematodes. Into each dish were introduced five larvae, pupae or adult. Experiment was replicated five times. After 48 hours contact period between insect and nematodes, the insect was dissected to observe the number of nematodes capable penetrated into the insect body. Other insect in the same batch was put in White trap dish to observe population growth of the nematode inside the *C.formicarius* cadaver. The effectiveness of entomopathogenic nematodes as biocontrol agent in searching the insect target has been conducted as well. One tuber was artificially infested by five pairs of weevil *C. formicarius* during two weeks in the container. Tuber were then deprived of the insect and buried in the soil placed individually in the plastic container. On the fourth weeks or two weeks after tuber have been buried, a series of nematode suspension: 15,000, 20,000, 25,000 and 30,000 IJs were poured to the soil as treatment for the larvae. This treatment was repeated four times. Similarly, such a treatment in other batch was conducted one week later to control pupae, and two weeks later to control adult. Four days post-application by nematodes, the tuber was dissected to count the living and dead larvae, pupae or adult by nematodes.

RESULTS AND DISCUSSION

Sampling of the nematodes

Nematodes have been successfully extracted only from village Manulai, while the samples from village Noelbaki and Oebufu were free from entomopathogenic nematodes (Table 1). The absence of entomopathogenic nematodes from these two villages is not fully understood, however, it is supposed to correlate with the fact that local farmers frequently applied insecticide carbofuran to control ants and soil cutworm.

Although the vegetation type was different between three villages surveyed, absence of nematodes was not considered under the influence of planted vegetation. We isolated nematodes from corn rhizosfer of village Manulai, but the same vegetation of village Noelbaki did not show similar result. Other survey conducted in corn vegetation revealed that entomopathogenic nematode could associate with corn stem borer. Using *T. mollitor* as insect trap, we expect that all nematodes collected are entomopathogenic. Identification study based on the

Tabel 1. Extraction of entomopathogenic nematode from soils of three villages in Kupang

Village	Vegetation	Nematodes
Noelbaki	Bittergourd	-
	Corn	-
	Sweet potato	-
Oebufu	Spinach	-
	Eggplant	-
	Crucifers	-
Manulai	Peanut	+
	Corn	+
	Dry land rice	+

- : absence + : presence

adult male and female morphological characters showed that all nematodes felt only into two families, Steinernematidae and Heterorhabditidae. Wouts (1991) stated that both groups do not posses stylet. The form and size of male spicula, the relative size of female vulva as compared to body were amongst principle guidance to identification of nematodes (Purnomo, 1998).

Quantitative population study of each nematode groups has not be undertaken, however, population density of steinernematid in general was higher than that of heterorhabditid in all vegetation surveyed. Steinernematid tend to distribute near the soil surface, unlike heterorhabditid that can move deeper in the soil Wouts, 1991). Both steinernematid and heterorha bditid from corn and peanut vegetation were surprisingly not able to develop further in the *T. mollitor* larvae. From rice vegetation, only steinernematid could show good growth under laboratory condition. This fact was not studied further, however, the different fitness exhibited by the nematodes was supposed to be affected by the growth of bacterial symbionts; except steinernematid from rice, this bacterial symbiont failed to compete and grow in the laboratory condition. The research was therefore focused on the pathogenicity study of *Steinernema* sp. which are issued from one parental source. Due to the certain limitations, *Steinernema* sp. identification until species level was not undertaken in this study.

Pathogenicity of *Steinernema* sp. on *T. mollitor*

Probit analysis indicated that the density of 105.0 IJs/ml (range 57.6 – 140.9) could provoke 50% mortality of host larvae (LC_{50} = 105. IJs/ml). Required time to kill 50% *T. mollitor* larvae population was obtained at 7.7 hours (range 6.4 – 9.7), if the density of 250 IJs/ml were used (LT_{50}). These values (LC_{50} and LT_{50}) obtained empirically, support the efficacious property of *Steinernema* against *T. mollitor.* The virulence of nematodes was not shown merely against insect larvae, but against pupae and adult weevil, nematodes seemed effective (Tabel 2). In this case suggestion that nematodes entered the host body via natural opening like mouth and anus seemed evident, since hard cuticle of host insect constitutes physical barrier against nematodes.

Life cycle of the nematode was so short that in two weeks, one host larva yielded *ca* 40,000 IJs nematodes. The yield decreased in the pupa and adult, which was not investigated in depth. However it was supposed probably due to the number of juveniles entering the insect body: more juveniles penetrated into the larva than into the pupa and adult. In fact, although a high number of nematode progeny was able to attain in a short period, the invasion efficiency was relatively low. This invasion efficiency was only 24.5 % off the 250 IJs applied to one larva (Tabel 3). Inversely, the efficiency was higher (33%) when only 50 IJs were applied to one host. Nematode reproduction in the host insect correlates with the number of JIs being capable to penetrate into the insect body (Leij,1995). At high density application, sum of JIs that invaded the host insect was also high, gave more progeny. In practical view, mass propagation of nematodes *in vivo* using *T. mollitor* as natural substrate seemed reasonable.

Bioassay of *Steinernema* sp. against *Cylas formicarius*

Larva, pupa and adult of *C. formicarius* have been proven to be vulnerable by *Steinernema* attack. The data supported the result obtained with *T. mollitor, ie* sum of IJs progeny produced in larval body was highest (4,020 IJs/larva) followed respectively by pupa (3,624 IJs/pupa) and adult (3,312IJs/young weevil). Both *T. mollitor* and *C. formicarius* are grouped in the same insect order, Coleoptera with undergo same type of development. This is the most plausible explanation on the different IJs produced in each developmental stadium. It is clearly understood that sum of IJs issued from *T. mollitor* was *ca* 10 fold than from *C. formicarius,* by considering the insect body size. Pouring of *Steinernema* suspension to the soil provokes mortality of *C. formicarius* at various degree.

Tabel 2. Infective juveniles produced by *Tenebrio mollitor* during two weeks observation

Week after infection	Number of IJs issued in each		
	Larva	Pupa	Adult
1st	26,016	23,376	21,024
2nd	14,624	11,536	8,320
Cumulative	40,640	34,912	29,344

Tabel 3. Invasion efficiency of *Steinernema* sp. into the *T. mollitor* larva

JI density	JIs penetrated ± S.E.	Invasion JIs efficiency (%)
50	16.4 ± 1.9[a]	33.0
100	21.6 ± 2.1[b]	21.6
150	25.6 ± 2.4[b]	17.1
200	32.2 ± 1.9[c]	16.1
250	61.2 ± 3.3[d]	24.5

Mortality was noted up to 11 larvae off 15.8 larvae in one tuber with application of 20,000 IJs. Total 9 pupae were observed in one tuber, 4.5 – 7 amongst them were dead. Likewise for young weevil, mortality > 50% was noted amongst 8.3 weevil present in one tuber (Tabel 4). Despite the high mortality (more than 70%) of each developmental instar inside the tuber, the nematodes progeny were not always found in each cadaver. In the soil, nematodes faced two barriers to find host insect: firstly they should pass soil before reaching the tuber, secondly once they found tuber, they should be able to detect the presence of insect inside the tuber. Increasing the number of IJs applied from 15,000 to 30,000 IJs would not parallel with the mortality of the host as a consequent. We observed a slight different between treatment in larval and pupal mortality, but not for young weevil. For young weevil, the mortality was relatively homogenous regardless the number of IJs applied. The dissection and count were performed on the fourth days post-application by nematodes. It is believed that four days period of infestation of nematodes into the soil was insufficient for all nematodes to reach the host insect. More numerous nematodes then could be expected if the period of observation were made longer. This experiment was not able to demonstrate the discriminate effect of different number of nematodes being applied. Nevertheless, the efficacious effect of *Steinernema* studied against *C. formicarious* was well demonstrated.

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Tabel 4. Presence and mortality of *C. formicarius* due to the treatment of *Steinernema* sp. in the laboratory

Stadia observed	Treatment (IJs/tuber)	Living	Dead	Total	Dead with nematode inside
Larvae (pupae and young weevils were not observed)	Control	14.8	1.0	15.8	0
	15,000	3.3	8.7	12.0	3.0
	20,000	3.3	11.0	14.3	3.5
	25,000	4.3	8.0	12.3	4.0
	30,000	4.0	9.0	13.0	4.0
Pupae (larvae and young weevils were not observed)	Control	8.5	0.5	9.5	0
	15,000	3.0	4.5	7.5	2.5
	20,000	2.7	5.7	8.4	2.3
	25,000	4.5	7.0	11.5	4.0
	30,000	2.0	7.5	9.5	2.8
Young weevils (larvae and pupae were not observed)	Control	7,7	0.7	8.4	0
	15,000	3.0	6.0	9.0	2.5
	20,000	2.8	6.0	8.8	2.0
	25,000	2.7	5.7	8.4	2.7
	30,000	1.7	6.7	8.4	3.0

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