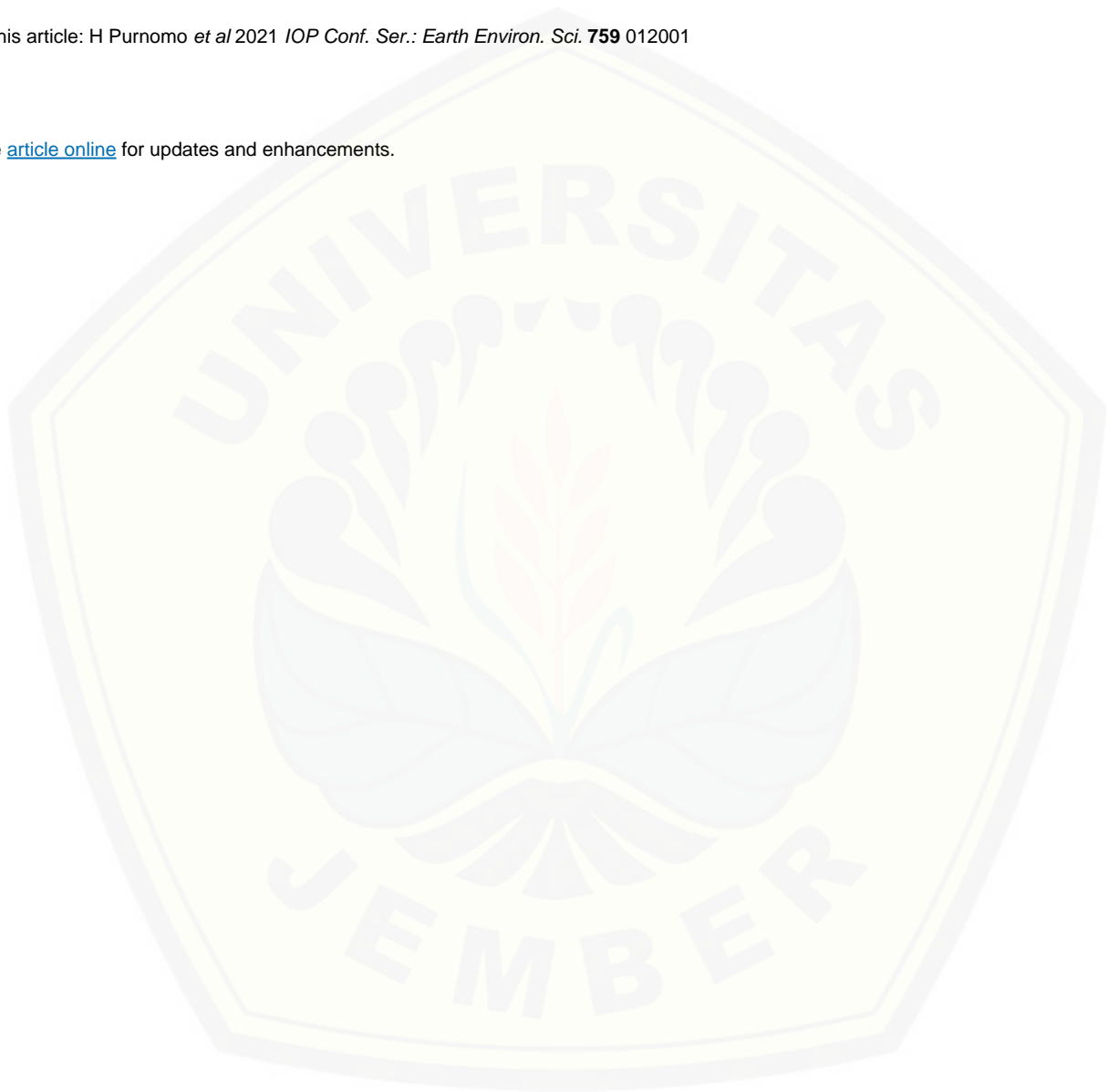


PAPER • OPEN ACCESS

## Virulence of *Steinernema* spp. an entomopathogenic nematodes Indonesia isolates against larvae of white grub *Lepidiota stigma* F (Coleoptera: Scarabaediae) in the laboratory condition

To cite this article: H Purnomo *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **759** 012001

View the [article online](#) for updates and enhancements.



### The Electrochemical Society

Advancing solid state & electrochemical science & technology

The ECS is seeking candidates to serve as the

#### Founding Editor-in-Chief (EIC) of ECS Sensors Plus,

a journal in the process of being launched in 2021

The goal of ECS Sensors Plus, as a one-stop shop journal for sensors, is to advance the fundamental science and understanding of sensors and detection technologies for efficient monitoring and control of industrial processes and the environment, and improving quality of life and human health.

Nomination submission begins: May 18, 2021



# Virulence of *Steinernema* spp. an entomopathogenic nematodes Indonesia isolates against larvae of white grub *Lepidiota stigma* F (Coleoptera: Scarabaeidae) in the laboratory condition

H Purnomo<sup>1\*</sup>, N T Haryadi<sup>1</sup>, M Hoesain<sup>1</sup>, E Zahro'in<sup>2</sup> and Nuryatiningsih<sup>2</sup>

<sup>1</sup>Departement of Agrotechnology, Faculty of Agriculture, University of Jember, Kalimantan Street 37, Jember, 68111, Indonesia

<sup>2</sup>Balai Besar Perbenihan dan Proteksi Tanaman Perkebunan Surabaya, Mojoagung Street No. 52, Jombang, 61482, Indonesia

\*E-mail : haripurnomo.faperta@unej.ac.id

**Abstract.** *Lepidiota stigma* (F) (Coleoptera; Scarabaeidae) is the key pest in sugarcane plantation caused devastated damaged on roots. The objectives of this research are to determine virulence of *Steinernema* sp, an entomopathogenic nematodes to larvae of *L. stigma* in laboratory. The Experiment was conducted in the laboratory, Agrotechnology Department University of Jember. Filter paper and sand method was used to determined the effective concentration of entomopathogenic nematodes against larva of white grub. The result showed that all entomopathogenic nematodes able to kill larvae of white grub *L. stigma* at all level concentration and soil depth. The result also convinced that *Steinernema* sp. origin from Kediri was more virulence compared with the other isolates.

## 1. Introduction

*Lepidiota stigma* (F) (Coleoptera: Scarabaeidae) is becoming a key pest in Sugarcane plantation as long as the farmers have moved sugarcane cultivation from paddy field to a marginal field land which is typically sandy soil type. Their larva is soil dwelling insect and the feeding activity causing significant damage of both the roots of the sugarcane plant and the regenerative portion of its underground stem. Sugarcane attacked by larva seemed to wilt as the transport of nutrient substances and the water stopped. This is due to its roots as a means of absorbing substances truncated nutrient and water damage. Symptoms of plants marked with the withering of sugarcane shoots, then dry the leaves and eventually collapsed and died [1]. In addition to attacking sugarcane, larvae of *L. stigma* also attacks maize, cassava, coffee, rubber, taro, yam, coconut, watermelon, beans, young pineapple, and pumpkin [1].

Recently report of *L. stigma* infestation on sugarcane caused total loss more than 40 ha sugarcane plantation field at East Java and 500 ha was affected in Central Java and Yogyakarta and caused up to 61 % loss of production and reducing of sugar production more than 22.5 ton per harvest time [3].



*L. stigma* has about one year of life cycle [4]. The adult is light brown colour with white spot at elytra and the larvae is scarabaeidae form with characteristic C-shape body, three thoracal legs and chewing type mouth. They have three larval stages and the late instar larva is about 75 mm in 11 in diameter. The female of *L. stigma* lay eggs on December and will hatch becoming first larvae instar early January and develop to second and third instar larvae till July and started pupae on August. The Adults will emerge and fly as terrestrial insect start on November [1].

All Farmers and National Sugar Mill Manufacture have controlled this pest with two tactics i.e. mechanic and systemic insecticides. The mechanic control was done by collecting larva during ploughing as land preparation. Diazinon, an insecticide has applied to control of larvae; unfortunately, both tactics has not given satisfied result whereas population of the larvae still high over the year (personal communication).

Consider with negative impact of insecticides application to soil and environment, we need an alternative control of population of *L. stigma*. Using biological control agents such as entomopathogenic nematodes to control soil dwelling insect pest has been reported against white grub [5; 6;7;8;9;10], but there is little known using entomopathogenic nematodes against *L. stigma*.

Entomopathogenic nematodes (EPNs) belonging genus *Steinernematidae* and *Heterorhabditidae* has long history to use for killing soil dwelling insect [2]. They can enter to the body of the larvae through natural opening such as mouth, anus, and spiracles [3], since they entered the body they will release a symbiotic bacterium that very specific relationship with the species of nematodes, *Xenorhabdus* bacteria has mutualistic relationships with the genus of *Steinernematidae* and *Photorhabdus* bacteria with *Heterorhabditidae* [4]. The bacteria kill larvae and start multiply inside the haemocoel and make environmental suit for growth and developing of nematodes inside the haemocoel. After several generations inside the larvae body, the juvenile infective will exit from the paralysis body of the dead larvae and search a new host [5].

The objective of this research was to determine virulence of *Steinernema* spp, an entomopathogenic nematodes to larvae of *L. stigma* in the laboratory condition. So the result of our study will be help farmers to control these white grub and promoting biological control tactic in the white grubs control management.

## 2. Material and Methods

### 2.1. Collecting of Entomopathogenic Nematodes

The research was conducted from January to Mei 2013 at Biological Control Laboratory, Plant Pests and Diseases Department, Faculty of Agriculture, The University of Jember.

All EPNs used in this experiment were collected from three different area which is known as Sugarcane growing area and the most population endemic area of *L. stigma* at East Java. i.e. Banyuwangi, Jember and Kediri. The diagonal system was used to collect 5 soil subsample in the sugarcane plantation. Each subsample soil was taken at least 30 cm of soil depth and each we mixed all subsample soil as 2 kg soil sample and transported to the laboratory.

In the laboratory, a plastic glass (200 cc) was filled one third of volume with moist soil sample then we added larvae of *Tenebrio molitor* (meal worm) instead of larvae of wax moth *Galleria mellonella* due to very cheap price from local bird market as a bait [6], then we filled with soil sample until total amount of the volume. All plastics glass of soil samples were incubated at dark condition for 7 days. After 7 days, the dead larva of *T. molitor* was collected, rinsed with sterile deionized water (SDW) and put in White traps as described by Woodring and Kaya [7] and incubated for next 7 days. Nematodes was harvested and identified as *Steinernema* sp according the symptom colour of dead meal worm larvae becoming brown or dark brown and then were given name as Kacangan Isolates (orogin from Banyuwangi region) , Kalisat Isolates (Origin from Jember region) and Kediri Isolates (Origin from Kediri region) and used for experiment.

### 2.2. Virulence of Entomopathogenic nematodes isolates to the larvae of *L. stigma*

A Randomized Complete Design was used to set up the experiment with Concentration of infective juvenile of nematodes as a treatment i.e. 100, 500, 1000, 1500 and 2000 infective juvenile/ml. Sand assays method was used to determine virulence of entomopathogenic nematodes to second instar larva of *L. stigma*. Plastic cup (5 cm in diameter) was introduced second larva of grub (1/cup) then filled with 4 g sterilized moist sandy soil. 5 ml of nematodes suspension/cup were pipetted on the top of surface arena (cup) each concentration. Control treatment was sprayed with sterile deionized water (SDW). The experiment was replicated thirty times for each concentration of the treatments. All cups were incubated for 24h. The mortality of the larvae of white grubs was recorded each day for 14 days observation.

### 2.3 Infectivity of entomopathogenic nematodes to the larvae of *L. stigma*

To deal with the nematodes whether they can infect the larvae of *L. stigma* as long as they live beneath the soil, we conducted Experiment 2 which was to determine infectivity of entomopathogenic nematodes to the larvae of *L. stigma* at different depth in soil. The experiment used Randomized Complete Design with the length of the depth of soil as treatments. The experiment arena was a polyvinyl column with 5 cm in diameters and different height from 10, 15, 20, 25 and 30 cm (as treatments). We layered the bottom of the column with nylon mesh to avoid the larvae of *L. stigma* run out the experimental arena and to keep the larvae at fix position in column. We put one second larvae instar of *L. stigma* each column, then we filled it with sterile moist sandy soil to total volume. 500 infective juvenile per/ml was sprayed on the top of the column and after that all column were incubated at the dark condition. The experiment was replicated thirty times. Mortality of the larvae in each column was recorded at 7, 14, 21 and 28 days after application. To observe of the mortality, we checked the larvae each time observation whether they dead or not by the symptom of the larvae body and mobility, if they change the colour of the body and no given responses when we touch it, we stated dead.

### 2.4 Analysis of Data

All mortality data dealing with Dose-response at Experiment 1 was analysed using probit analysis to calculate  $LC_{50}$ . All mortality data of experiment 2 was analysed using ANOVA and mean comparison using Tukey at 5 %. All statistics calculation used StatView software 5.01 version [8]

## 3. Results and Discussion

In the experiment 1, the result showed that two *Steinernema* sp. from Kalisat and Kediri killed larvae of *L. stigma* starting from 3 days after treatment (DAT), while Kacangan Isolat killed start at 4 DAT. Mortality increases with the length of observation time and reached 100% mortality at day 14. (Figure 1). Kediri isolates showed greater killing ability compared with Kalisat isolates and Kacangan isolates in term of time of killing. In 10 DAT Kediri isolate at all concentration had range mortality of grub larvae 86.7-100 %, however Kalisat and Kacangan Isolate had range mortality of grub larvae 56.7-100 % and 56.7-96.7 at the same times respectively.

Probit analysis also indicated that  $LC_{50}$  of Kediri isolate (95 % fiducial limits) was 76.87 (37.74 - 121.55) IJs/ml smaller than others isolates.  $LC_{50}$  Kalisat Isolates (95 % fiducial limit) was 197.96 ij/ml (119.1-278),  $LC_{50}$  Kacangan Isolates (95 % fiducial limits) was 346.11 ij/ml (218 – 481.85) respectively. This result revealed that *Steinernema* sp. Kediri Isolates more virulence compared with Kalisat and Kacangan Isolates.

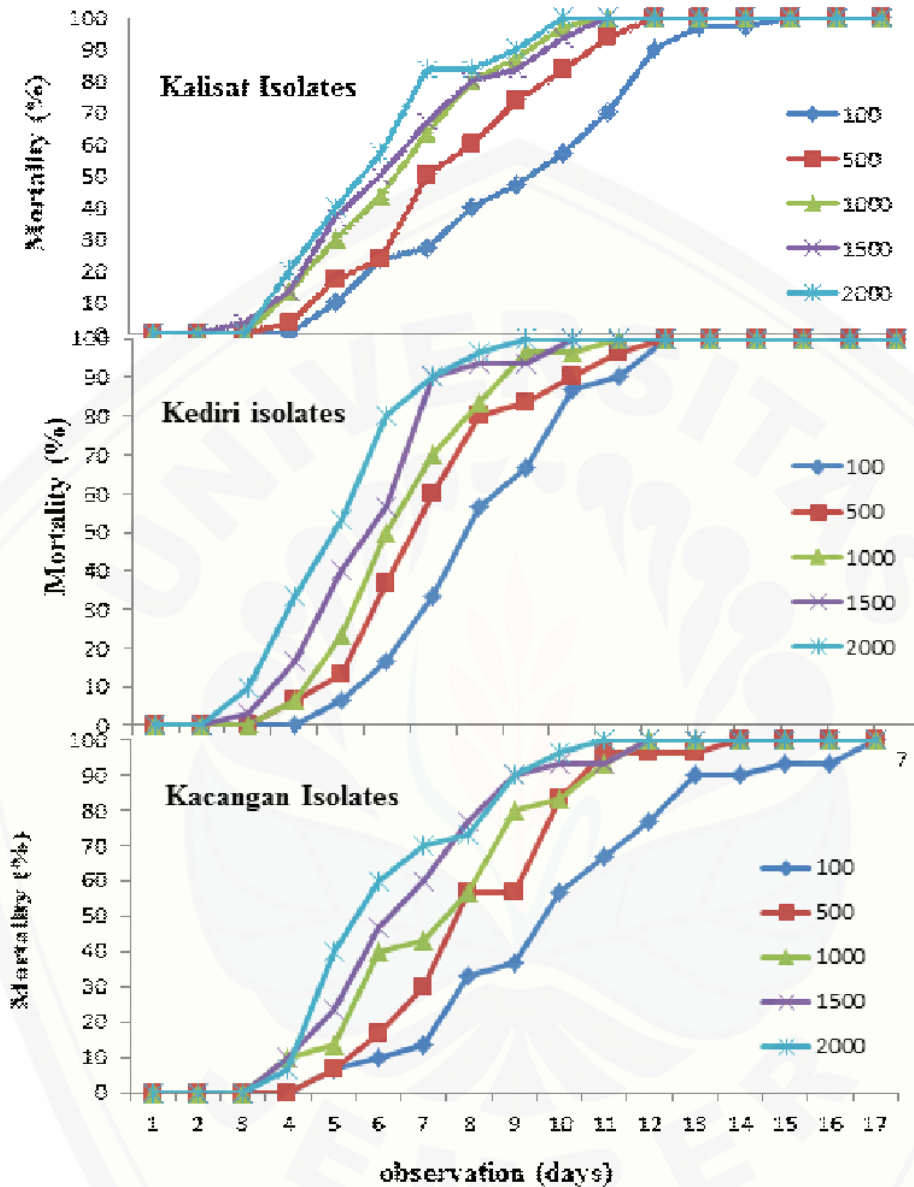


Figure 1. Dose-response *Steinernema* sp. against second larva *L. stigma*

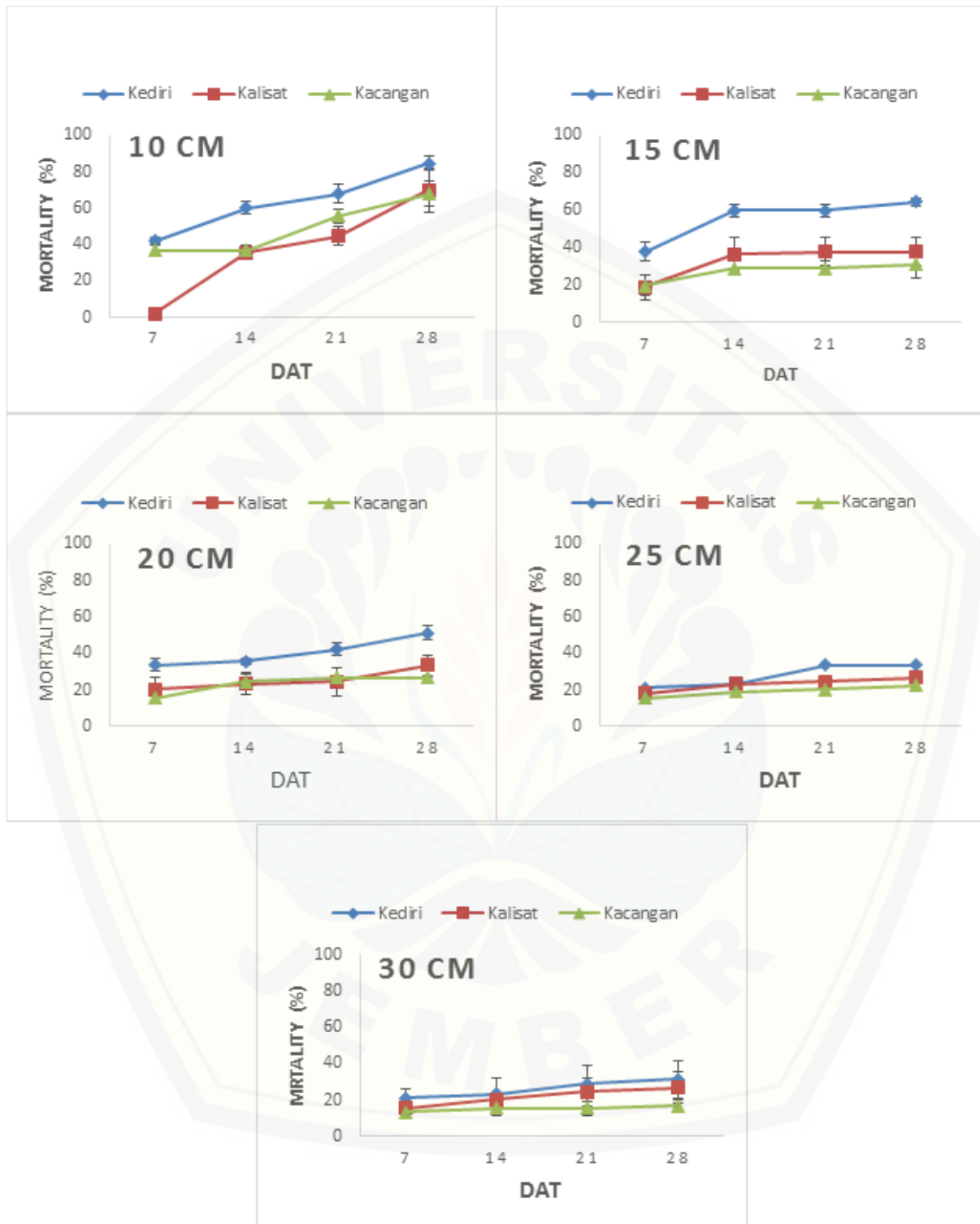
In experiment 2, the results showed that all *Steinernema* sp. isolates (Kediri, Kalisat, and Kacangan ) able to kill second larva instar of *L. stigma* from 10 to 30 cm of soil depth from first week of observation with varied mortality respectively (Figure 2). There was significant difference in interaction between EPNs isolate and Depth of Soil ( $F_{(2, 174)} = 10.53, P < 0.0001 \alpha = 5 \%$ ) Tukey-Kramer Mean comparison indicated that Kediri isolates caused significant greater mortality to larvae of *L. stigma* than others isolates at all the length of soil depth, but there was no significant different between Kalisat and Kacangan Isolat (Table 1)

The research is based on concern for the problems larvae of *L. stigma* attack on sugarcane plantations, where larva of *L. stigma* to be a major problem, though not the only type white grub in sugarcane, but *L. stigma* has been known that the most damaging in sugarcane. Larvae of *L. stigma* is not only found in sugarcane but have also damaged other crops such as coffee, sengon and golf turfgrass (Purnomo, unpublished data). The research seems to be the first report on the use of EPN to control larvae of *L. stigma* in Indonesia.

As nematode species/strain plays a key role in the success of insect control, our present study indicate that all EPNs isolates able to kill *L. stigma* larvae either in experiment 1 or 2 and also show that EPNs from Kediri more virulent than the others EPNs isolates. Koppenhofer and Fuzy [9] reported that EPNs species have different attraction to white grub species, although the degree of virulence was varied depend on EPNs species. Grewal *et al.* [10] also revealed that genera *Heteroranditis* more consistence to kill white grubs larvae than genera *Steinernema* in the field trial and equal with pesticides application.

Our present study, we used sandy soil for the test of virulence of *Steinernema* sp. as similar with the type of soil whereas nematodes was collected, so the difference of treatment we need just length of depth of soil. The results showed that the virulence of all nematodes isolates were decreases as long as increasing soil depth, so we suggest that for controlling the larva of *L. stigma* when the larva still near at the surface of soil its mean on first larva stages or two larval stages, because they are always at 10-20 cm soil depth, so *Steinernema* sp. will difficult to reach third larva stages that live more than 30 cm soil depth.

This study shows that the biological control of larvae *L. stigma* using entomopathogenic nematodes isolated from sugarcane growing areas can be an alternative to the control of mechanical control and pesticides which not only require huge costs and time but also negative impacts of pesticide application on agricultural land.



**Figure 2.** Effect length soil depth on virulence *Steinernema* sp. against second larva *L. stigma*

Our result showed that all isolates of EPNs were able to kill the larvae *L. stigma* and entomopathogenic nematodes from Kediri are recommended to be a potential biological control agent when viewed from the ability to kill in a variety of soil.

The results also show that EPNS can effectively kill the larvae *L. stigma* as shown other research reports on the use of EPN in other larval species in the same family Scarabaeidae [6; 8; 10].

**Table 1.** Mean comparison of effect isolate on mortality of *L. stigma* larvae

Isolates	Mortality Mean	Notation
Kediri	14.722	a
Kalisat	17.272	b
KAcangan	2.550	b

Means followed by same letters was not significant different based on Tukey-Kramer mean comparison at 95 %

Integrated Pest Management always the ideal strategies use to maximise effect of control through combination of tactics control. Combination of nematodes with entomopathogenic fungi such as *Metarizhium anisopliae* and *Beauveria brongniartii* are also a good choice considering fungi production is very easy and can be done by the farmers themselves. Choo *et al.* [11] reported that combination of *S. carpocapsae* and *B. brongniartii* given significant result to kill white grubs' larvae at golf Course in Korea. Combination EPNs and BT also had sinergitic and additive effect for controlling Scarab Grubs. Ansari *et al.* [12] also reported the similar result that combination between *M. anisopliae* and EPNs had synergetic effect to control *Hoplia philanthus*. Koppenhofer and Fuzy [9] also reported that combination of anthranilic diamide, chlorantraniliprole, and the entomopathogenic nematode *Heterorhabditis bacteriophora* had a synergistic or additive Effect on the oriental beetle, *Anomala orientalis* mortality. The next question to be answered is how to apply the EPNs in the field in terms of how to provide a large role in the amount of the nematode to be applied given that most sugar cane plantation is belonging ordinary farmer not sugarcane mill manufacture which has limited financial resources. EPN production technology on a large scale through in vitro techniques for farmers is not easy in terms of the technology required and the cost. So that the in vivo production techniques may be the best option for farmers producing nematode, but its cost at labour and times. So if the EPNs will use as an alternative control of *L. stigma* on sugarcane we suggest that training how to mass production of EPNs is essential need for farmer.

#### 4. Conclusion

*Lepidiota stigma* (F) (Coleoptera; Scarabaeidae) is the key pest in sugarcane plantation caused devastated damaged on roots. The objectives of this research are to determine virulence of *Steinernema* sp, an entomopathogenic nematodes to larvae of *L. stigma* in laboratory. The result showed that all entomopathogenic nematodes able to kill larvae of white grub *L. stigma* at all level concentration and soil depth. The result also convinced that *Steinernema* sp. origin from Kediri was more virulence compared with the other isolates.

#### 5. References

- [1] Kalshoven LGE and Van der Laan PA 1981 *Pests Crop Indonesa* (Jakarta : Ichtar Baru)
- [2] Klein M 1990 *Efficacy against soil-inhabiting insect pests* In: Gaugler, R & Kaya, HK Entomopathog nema-Iodes Biol Control
- [3] Koppenhöfer AM, Grewal PS and Fuzy EM 2007 *J Invertebr Pathol.* **94**(3)184–95



- [4] Griffin CT, Boemare NE and Lewis EE 2005 *Biology and Behaviour* (Oxfordshire:CABI Publishing)
- [5] Poinar GO, Gaugler R and Kaya HK 1990 *Entomopathogenic nematodes in biological control* (Florida : CRC Press Boca Raton)
- [6] Bedding RA and Akhurst RJ 1975 *Nematologica*. **21**(1)109–10
- [7] Woodring JL, Kaya HK 1988 *Steinernematid and heterorhabditid nematodes: a handbook of biology and techniques* South Coop Ser Bull
- [8] SAS 1999 *Statistical Analysis System* (North Carolina: Institute Cary)
- [9] Choo HY, Kaya HK, Huh J, Lee DW, Kim HH and Lee SM 2002 *Biocontrol* **47**(2)177–92
- [10] Grewal PS, Power KT, Grewal SK, Suggars A and Haupricht S 2004 *Biol Control* **30** (1)73–82
- [11] Koppenhöfer AM, Choo HY, Kaya HK, Lee DW and Gelernter WD 1999 *Biol Control* **14** (1):37–44
- [12] Ansari MA, Tirry L and Moens M. 2004 *Biol Control*. **31**(2)172–80

