

Field Efficacy of *Steinernema carpocapsae* Against Lima Bean Pod Borer, *Etiella zinckenella* Treitschke, Attacking Groundnut

Dwinardi Apriyanto^{#1}, Nadrawati^{#2}, Tri Sunardi^{#3}, Dotti Suryati^{*}

[#] Department of Plant Protection, University of Bengkulu, Bengkulu, 38371, Indonesia
E-mail: ¹kangdwi2010@gmail.com, ²nadrahpt@ymail.com, ³trisunardi@yahoo.co.id

^{*} Department of Plant Culture, University of Bengkulu, Bengkulu, 38371, Indonesia
E-mail: dottisuryati@yahoo.com

Abstract— Lima bean pod borer (LBPB), *Etiella zinckenella* Trietschke, is major insect pest of soybean in Indonesia. Currently it was also reported attacking groundnut in some parts of Sumatera causing serious yield loss. Insecticide treatment is not only ecologically unsafe, but also is not practical due to concealed behavior of the larvae within pods below soil surface. Systemic insecticide might be so but unsafe as it might leave unaccepted residue in harvested pods. It is clear that safer more effective technology such as biological control is badly needed. A Field study was conducted at the University of Bengkulu Research Station in 2012-2013. The objectives was to determine the effectiveness of non local strain of entomopathogen nematode (EPN), *Sterinenema carpocapsae*, to control LBPB attacking groundnut. Groundnut seeds were sowed in plots which were later treated with *S. carpocapsae* produce by Biological Control Laboratory University of Jember at the dose of 500.000 JI m⁻². EPN was sprayed upon plants and soil surface twice at plant age of 45 and 65 days after sewing (DAS). There were insecticide treatment and control plots as comparison. Observations of damaged pods and the presence of LBPB larvae were done at plant age of 63, 77, 90 DAS and at the haesting date. Data were subjected to analysis of variance and the means of damaged pods were separated with least significant different (LSD). The result revealed that in two years study, damaged pods consistently tended to be less in plots treated with *S. carpocapsae* or insecticide than those in controls, indicating that it might be effective against PLPB. The lack of significant different between EPN treated and control plots might be as a result of low percentages of EPN larval survival due shipment problem (i.e. inappropriate packaging and long transportation that toke several days) or it might be that the strain was less adapted yet to local conditions. I speculate that local strain of EPN might be more effective to control LBPB attacking groundnut.

Keywords— Pod borer; Entomopathogen; groundnut

I. INTRODUCTION

Lima bean pod borer (LBPB), *Etiella zinckenella* Treitschke, is one of the major insect pests of soybean in many parts of the world [1]. The importance of this species in Indonesia is noted in some publications [2], [3], [4]. It was reported recently that this species was also considered as serious pest of groundnut.

LBPB was first reported as causing severe pod damage on groundnut in Indonesia in 2008 [4], [6]. The damage incurred by LBPB was as high as 78% or more, and some groundnut fields even were abandoned because farmers would loss more due to harvesting cost.

From field experiment it was observed that high incidence of LBPB was occurred in different altitude from 0 - > 800 m asl., though, it was more serious at lower altitude [7]. Other report showing LBPB as a serious pest of groundnut was

from field survey in West Sumatera [8] which indicated similar results.

In soybean, farmers relay much on chemical insecticide uses to control LBPB, with consequences of any deleterious impacts to the environment that follows if it is not wisely applied. Insecticide treatments to control LBPB on groundnut are not only ecologically unsafe, but also not practical due to concealed behavior of the larvae inside pods below soil surface. Systemic insecticide might be so but unsafe as it might leave unaccepted residue in harvested pods. Reference [9] stated that injudicious use of insecticide in soybean is not effective in controlling LBPB populations; instead it destroys natural enemies, especially parasitoids.

In groundnut, many farmers just ignore the incidence of this insect pest in field, with risk of losing yield. High incidence of pod damage has discouraged farmers to grow groundnut. It is clear that safer and more effective technology such as biological control is badly needed.

There are many documented studies on the potency of using natural enemies, including entomopathogen to control insect pest populations [10], [11], [12]. However, there were only view intensive studies that examined the potency of natural enemy to combat *E. zinckenella* in soybean [13], let alone in ground nut [see 8]. Entomopathogen nematode (EPN) has been used to control many insect pest species with good results and has been produced in commercial scale [14], [15]. Infective juvenile (IJ) of EPN was reported as good biological control agent for soil dwelling or concealed insect pests [14]. Infective juvenile is 3rd larval instar of EPN. We have examined local (i.e. native) isolates of EPN and was able to induce mortality on 3rd LBPB larvae up to 80 % or more (unpublished data), which is not much different from ones reported reference [16] and [17]. Though, we have not successfully mass produced them. There are several advantages of using EPN for insect pest control. EPN is more easily incorporated into integrated pest management program, due to its resemblance in behavior to insecticide or other chemicals use for plant protection. Technology for mass production is available. Field application of EPN may be done using similar defined standard methods and equipment to that of applying insecticides. Furthermore, it is compatible with many chemical insecticides [17].

In this paper we reported results of field trials to evaluate the effectiveness of introduced EPN, *Steinernema carpocapsae*, for controlling LBPB attacking groundnut. We compared EPN with chemical insecticide and control blank (no control measure).

II. MATERIAL AND METHOD

A. Experimental Design and Treatment Applications

Field trials were conducted in Faculty of Agriculture's Experiment Station, University of Bengkulu. The soil type is ultisol with pH of 4.5 as measured in dry season. This study was conducted in four trials (planting seasons). Seeds were sowed singly in 30 cm within and 20 cm between rows. The plants were fertilized with Urea, SP-36 and KCl at dosis of 70, 125 and 70 ha⁻¹, respectively. Weeding was done as necessary.

The experiment plots were originally two trials of split-plot design with main plots of trap crop for LBPB and subplot of control applications, in the first year (2012) and two trials of control applications (Chemical insecticide, Crude extract of neem leaf) in complete block randomized design (RCBD), in the second year (2013). The trap crops incorporated in the first year trials were four rows of *Crotalaria juncea* or soybean, which was grown in a peripheral pattern (i.e. surrounding corresponding plots). The main plots, including control (without trap crop), were arranged in randomized block. Subplot treatments were EPN, EPN + crude extract of neem leaf, Deltametrin (Decis® 2.5 EC), and control (blank). The treatments were replicated three times.

The main plots were of ca. 50 m x 20 m, each was split into 4 subplots of 25 m x 10 m. EPN (*S. carpocapsae*) obtained from Biological Control Laboratory of Agriculture Faculty, University of Jember was applied twice at plant age of 45 and 65 days after sewing (DAS), at dose of 500.000 IJ

m⁻². Infective juvenile of *S. carpocapsae* was diluted into 12.5 l and applied for 250 m⁻² subplot and the applications were done with low pressure knapsack sprayer. EPN application using sprayer with static pressure until 14 bars did not injure the nematode. Furthermore, the passage through flat nozzle did not affect the viability of nematode infective juvenile [18]. Whereas, insecticide and neem extract were applied in 7 days interval at plant age of 35-70 DAS.

B. Data Collection and Analysis

LBPB incidence was observed every 2 weeks, at 63, 77, and 90 DAS, with 10 sample plants taken randomly. Observation was also done at harvest with 30 sample plants. Within each sample plant, all pods were inspected for the presence of damage sign (holes) and LBPB larvae. Undamaged pods taken from 30 plants that were sampled randomly at harvest were weighed per plant basis. Data were analysed with ANOVA and means were separated with least significant different (LSD).

In this report, the main plots (trap crops) were ignored due to their lack of effect on LBPB incidence. Neem leaf extract also was omitted because gave no addition effects as compared to EPN only. Therefore the data were pooled for statistical analyses comparing EPN, insecticide and control in randomized block design, which considered replications and control measures (EPN, Insecticide, and control) as variation sources.

III. RESULTS AND DISCUSSION

In general, the incidences of LBPB (damaged pods on groundnut) were very low, except for the last trial. We found only few larvae from sample plants, and therefore it is not included in this discussion. This low incidence does not support the notion that LBPB is one of the most important insect pests of groundnut as has been noted in several publications, e.g. [1], [2], [5]. Low LBPB incidence in our study was probably due to isolated location where trials were conducted that has never been use for legumes. The location was surrounded by oil palm plantation and secondary forest growth, and as such, population buildup of LBPB had never occurred in that area. In the fourth trial the population might have grown much higher after previous three consecutive trials and therefore caused more damage to pods (Fig 1).

Mean numbers of damage pods consistently tended to be less in plots treated with insecticides or EPN than those in controls. However the different was not statistically significant.

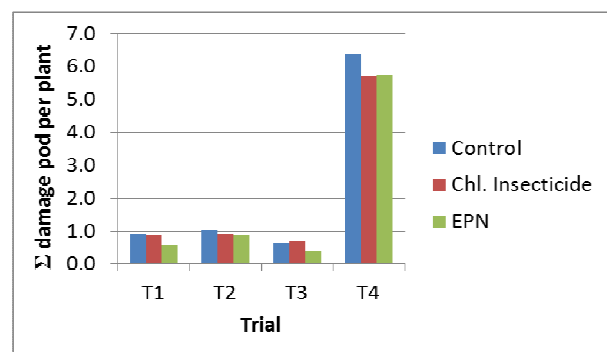


Fig 1. Number of damage pods by *Etiella zinckenella* in four filed trials.

There are variations in undamaged pod yield, but not consistent between EPN or insecticide treated plots and control (Fig 2). In a field efficacy trial for diamondback moth control, reference [17] found larvae mortality up to 60% at day 7th, with EPN formulated in Water Dispersible Granules (WDG), suggesting that, to some extent, EPN is effective to control insect pest. Pod weight did not differ significantly between EPN or insecticide treated plots with those of controls. Pod production is much lower in the fourth trial. This was probably as a result of continuous use of the same location (plots) for groundnut growing.

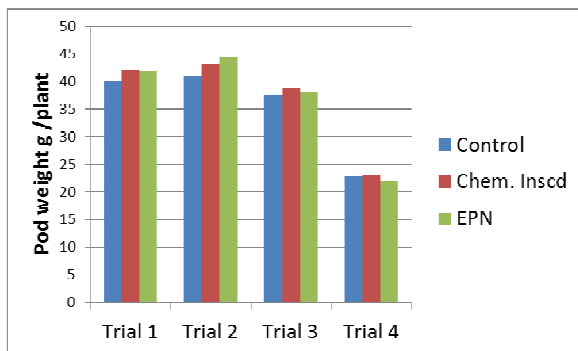


Fig 2. Per plant bases of pod weight in four trials

There are some explanations why EPN had not been able to reduce the incidence of LBPB significantly in our study. Reference [15] stated that nematode, host, and the environment as important factors that might influence field efficacy. In our trials, EPN resource was far away from the location of field trial, so that they suffered from shipment (e.g. packaging and long transportation). They were sent via carriages which took 5 days to get our office. In laboratory observations, we found only $\pm 60\%$ of IJ of EPN that were alive and further EPN mortality might occur in field after being applied. We did not bioassay to determine their viability. In addition, the EPN isolate might not be quite adapted to local conditions. The soil pH where field trials were conducted was 4.5 (acid), which may be different from the origin of the strain of EPN used. The possible effect of soil pH has been suggested as an important factor affecting natural occurrence of EPN [18], [20]. In addition, it might also be that the EPN is not quite adapted to often disturbed soil due to frequent tillage as has been mentioned in reference [16]. The effect of temperature was reported in [21] indicating that EPN infectivity increases with temperature with the highest larval mortality of *G. mellonella* by *S. feltiae* at 26°C. We did not measure soil temperature in our study. Working with *Heterorhabditis bacteriophora* against the fruit fly, *Anastrepa ludens*, Reference [22] in a laboratory study reported that soil moisture also affected the efficacy of EPN in infecting the third instar of the host. Mortality of *A. ludens* increased when the soil moisture was increased from 9% (w/w) until 18%, but then decreased at further higher moisture. We did not measure the soil moisture, but monthly rainfall was high within the planting season both in 2012 and 2013, ranging from 288 – 632 mm.

Efficacy of EPN is also affected by the method of application [23] who reported a field test comparing between

two methods, irrigation versus gel formulated spray. Effective pest suppression was obtained in Gel spray, not in irrigation method.

Reference [14] cited that EPN efficacy for soil-dwelling insects may be ineffective if behavior in host finding, attachment, or penetration by infective juveniles is reduced because of host behavior change. Host behaviors may evolve in the form of increasing defecation rate which may reduce EPN infection via anus. It may also be in the form of CO₂ output reduction or releasing CO₂ in a burst such that reducing chemical cues, or forming a physical barrier in the form of impenetrable cocoons or soil cells before pupation.

Inasmuch that the difference was not significant, the facts that the LBPB tended to be lower in EPN than in control plots might indicate that EPN is effective against this insect species. Efforts to use a local isolate (strain) of EPN have been done in Bengkulu. We have explored and did a bioassay of a local (native) isolate in our laboratory using 3rd LBPB larvae that resulted in 80% mortality (unpublished data). However, mass production has not been successful due to failure in isolating and culturing of the bacterial symbiont, *Xenorhabdus*. This bacterium is one that is responsible for causing the larval death of the host. Infective juveniles of EPN release the bacterial symbiont when they infect larvae and consume the devoured tissue of infected larvae [16]. Larval death is a result of physiological changes of some immunity enzymes (e.g. phenoloxidase, acetylcholinesterase and carboxylesterase), digestive enzymes (e.g. protease), and hydrolyzing enzymes (e.g. amylase, trehalase, invertase) within the infected insect [24].

It is not practical to use the natural host (insects) for EPN mass-production (i.e. *in vivo*), as it will be very laborious and costly. *In vitro* mass-production (i.e. in artificial diet + *Xenorhabdus*) of EPN has been successfully done in many laboratories, using different artificial diets [14]. However, we have not been successful in isolating and culturing the bacterial symbiont. Without the bacterial symbiont, the productivity of infective juveniles is very low and therefore impractical. With the aforementioned conditions, it is necessary that a local isolate of EPN and the bacterial symbiont be explored and studied, so that they can be used for LBPB biological control in any locality in this country. I speculate that a local strain of EPN might be more effective for controlling LBPB attacking groundnut.

IV. CONCLUSIONS

The incidence of LBPB in groundnut is alarming, especially in Sumatera. In new planting areas the incidence might increase in several growing seasons. *Steinernema carpocapsae* has the potential as a biological control agent for combating LBPB attacking groundnut as has been seen by more or less consistent lower damage to pods in EPN treated plots than in control plots. However, it needs further study to find and mass-produce an effective local isolate(s).

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