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INTEGRATION OF BOTANICAL PESTICIDE AND ENTOMOPATHOGENIC FUNGI TO CONTROL THE BROWN STINK BUG *RIPTORTUS LINEARIS* F. (HEMIPTERA: ALYDIDAE) IN SOYBEAN

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ABSTRACT

Integration of botanical pesticide and entomopathogenic fungi to control the brown stink bug* Riptortus linearis *F. (Hemiptera: Alydidae) in soybean. The efficacy of botanical pesticides i.e. *Annona squamosa* seed powder (ASP) or *Jatropha curcas* seed powder (JSP) integrated with entomopathogenic fungi, *Lecanicillium lecanii* to control brown stink bug, *Riptortus linearis* F. was studied at Muneng Research Station, the Indonesian Legume and Tuber Crops Research Institute (ILETRI) in June up to September 2011. The purpose of the research was to determine the efficacy of integration the natural pesticides i.e, *A. squamosa* seed powder (ASP) and *Jatropha curcas* seed powder (JSP) and entomopathogenic fungi *L. lecanii* to control brown stink bug. The treatments were application of (1) ASP 50 g/l, (2) JSP 50 g/l, (3) ASP 50 g/l + *L. lecanii* 10^7/ml, (4) JSP 50 g/l + *L. lecanii* 10^7/ml, (5) *L. lecanii* 10^7/ml, (6) deltametrin and (7) control (untreated). Application of ASP and JSP combined with *L. lecanii* decreased the hatched eggs by 84% and 82%, respectively. However, reduction of hactched egg from a single application of ASP and JSP were 56-61% . ASP and JSP combined with *L. lecanii* reduced both nymphs and adult stink bug population built up and damage (empty pod). Application of ASP and JSP in combination with *L. lecanii* did not affects the survival of generalist predators such as *Paederus* sp., *Oxyopes* sp. and *Coccinella* sp. as opposed to chemical insecticide that did. The integration of ASP or JSP with entomopthogenic fungi *L. lecanii* was able to increase the efficacy of brown stink bug control.

Key words: botanical pesticides, egg mortality, *L. lecanii*, pod sucking bug, soybean

ABSTRAK

Perpaduan antara pestisida nabati dan cendawan entomopatogen untuk pengendalian hama kepik coklat* Riptortus linearis *F. pada tanaman kedelai. Uji efikasi pestisida nabati yakni serbuk biji *Annona squamosa* (SBS) dan serbuk biji jarak (SBJ) yang dikombinasikan dengan cendawan entomopatogen *Lecanicillium lecanii* untuk mengendalikan hama kepik coklat (*Riptortus linearis* F.) telah dilakukan di kebun percobaan (KP) Balai Penelitian Tanaman Kacang-kacangan dan Umbi-umbian di Muneng (Probolinggo) dimulai pada bulan Juni sampai dengan September 2011. Tujuan penelitian ini untuk mengakaji efektifitas perpaduan antara pestisida nabati tepung biji sirsak, *Annona squamosa* dan tepung biji jarak, *Jathropha curcas* dengan cendawan entomopatogen *L.lecanii* untuk pengendalian hama pengisap polong kedelai *R. linearis* F. Perlakuan adalah: (1) SBS 50 g/l, (2) SBJ 50 g/l, (3) SBS 50 g/l + *L. lecanii* 10^7/ml, (4) SBJ 50 g/l + *L. lecanii* 10^7/ml, (5) *L. lecanii* 10^7/ml, (6) deltametrin dan (7) tanpa pengendalian. Aplikasi kombinasi SBS, SBJ dengan cendawan *L. lecanii* mampu menggagalkan penetasan telur kepik coklat masing-masing 84% dan 82%. Sementara itu, efikasi SBS dan SBJ yang diaplikasikan secara tunggal hanya mampu menggagalkan penetasan telur berkisar 56-61%. Aplikasi SBS dan SBJ yang dikombinasikan dengan cendawan *L. lecanii* mampu menekan jumlah populasi nimfa dan imago pengisap polong maupun kerusakan polong (polong hampa). Aplikasi SBS dan SBJ yang dikombinasikan dengan cendawan *L. lecanii* tidak berdampak buruk terhadap kelangsungan hidup serangga predator seperti *Paederus* sp., *Oxyopes* sp., dan *Coccinella* sp. dibandingkan dengan insektisida kimia. Integrasi kombinasi pestisida nabati SBS, SBJ dengan cendawan entomopatogen *L. lecanii* mampu meningkatkan efikasi pengendalian hama kepik coklat.

Kata kunci: kedelai, *L. lecanii*, mortalitas telur, pengisap polong, pestisida nabati

INTRODUCTION

The green stink bug, *Nezara viridula* F., the lesser green stink bug *Piezodorus hybneri* Genn. and the brown stink bug *Riptortus linearis* F. are the most important species of pod sucking pests of soybean in Indonesia. Based on the population and their distribution in Indonesia Tengkano *et al.* (2005; 2006) indicated that brown stink bug *R. linearis* is the most dominant species among the three species. Direct feeding of the insect reduce 50% of seed vigor and 80% yield loss, respectively.

At the moment the pest control of soybean pest, including the brown stink bug mainly depends upon chemical insecticides. However, the chemical only affects the nymphs and adults, and does not affects the eggs. Therefore, insecticide application to high population of brown stink bug is uneffective. Prayogo (2009) investigated the use of spore suspension of entomopthogenic fungi, *Lecanicillium lecanii* to control the brown stink bug. He found that *L. lecanii* fungus infected or colonized eggs of stink bug and the infection decreased significantly on hatched of infected eggs. This fact suggest that the fungus acts as an ovicidal to the brown stink bug.

The advantages of *L. lecaniii*, instead infect stink bug eggs, the fungus also infects both the nymphs and the adults (Prayogo, 2004; Prayogo *et al.*, 2004). In field application, however, the efficacy of fungal conidia will reduce, due to the decrease of their viability as the affected by a biotic factor, especially UV rays. Therefore, to maintain their conidial viability in field condition, integrated application with other control agents that may synergistic are recommended (Hirose *et al.*, 2001; Depieri *et al.*, 2005; Kim *et al.*, 2010). The compatibility of *L. lecanii* and botanical insecticide is measured on vegetative, generative development (*in-vitro*) that affect on insect mortality (*in-vivo*) (Castiglioni *et al.*, 2003; Depieri *et al.*, 2005; Prayogo, 2011b). They found a number woody and herbacious plants, such as *Anonna* seed powder (ASP), *Jatropha* seed powder (JSP) and *Aglaia odorata* leaf extract contain insecticidal actions. These plants cause high mortality of pod stink bug eggs (Prayogo, 2010, 2011a, 2011b). In addition, these three plants enhanced the fungus development as expressed in larger colony and number of conidia produced by *L. lecanii* in growth substrat contain plant extracts *in-vitro*. From previous experiments it was shown that integrated application of entomopthogeniic fungi *L. lecanii* and neem seed powder increased the effectiveness control by reduction the number of stink bug nymphs. 50 g/l of *Annona squamosa* seed powder (ASP) and *Jatropha curcas* seed powder (JSP) were the optimum concentration when they were applied with *L. lecanii* (Prayogo 2010 & 2011a). Therefore, the efficacy biological control of brown stink bug using natural and entomopthogenic fungi *L. lecanii* in field condition was studied.

MATERIALS AND METHODS

Study Site. The experiment was conducted at Muneng Research Station (Probolinggo), the Indonesian Legume and Tuber Crops Research Institute (ILETRI) in June to September 2011. The experiment was arranged in randomized complete block design (RCBD) with three repetitions. Spraying the stink bugs with (1) ASP 50 g/l, (2) JSP 50 g/l, (3) ASP 50 g/l + *L. lecanii* 10^7/ml, (4) JSP 50 g/l + *L. lecanii* 10^7/ml, (5) *L. lecanii* 10^7/ml, (6) deltametrin and (7) control (untreated) were applied.

Insect Preparation. Both nymphs and adults of *R. linearis* were collected using sweeping net from soybean field at Kendalpayak Research Station, Malang in November 2010 and reared in the screen cage and fed with stringbean. Feed was replaced in every two days using fresh beans. To serve an oviposition site, a bundle of fine threads were hanged in the screen. To avoid variation in development stage of insects all new eggs laid were collected and grouped based on egg oviposition time or periods. All nymphs produced from laboratory mass rearing were used in the treatments.

Culture of Entomopthogenic Fungi *L. lecanii*. The higher virulent isolate Ll-JTM1 of *L. lecanii* (Prayogo, 2009) was cultured using *potato dextrose agar* (PDA) in Petridish (dia. 9 cm). At 21 days after inoculation (DAI), the conidia were harvested using wetted fine brush and suspended in erlenmeyer flask containing strile water. The conidia suspension was homogenized with 2 ml/l Tween 80 and shaked in 30 minutes. The haemocytometer was used to calculate conidia density of 10^7/ml under microscope.

Preparation of Natural Pesticide. Laboratory experiment in 2009 was identified that *Annona* seed powder (ASP) and *Jatropha* seed powder (JSP) were compatible with *L. lecanii* to control brown stink bug eggs (Prayogo, 2011a). The dry seeds of *Annona* and *Jatropha* collected from Probolinggo were finely ground and stored in a refrigerator. The botanical pesticides were studied under field condition. A 50 g of each seed powder that was taken either alone or in combine with entomopathogenic fungi, *L. lecanii* were dissolved with 1 l a strile water in erlenmeyer flasks.

Field Experiment. Soybean seeds of Wilis variety were grown in 7 m x 5 m rise bed/ plot, 40 cm x 10 cm plant spacing, and maintained two plants/hole. A standard cultivation was used, and fertilized using Urea 50 kg/ ha, SP 36 100 kg/ha and KCl 100 kg/ha. At 35 days after planting (DAP), 25 freshly eggs were inoculated on uppersite leaves and sticked using Arabic gum, then all plants were caged with metal-framed tile screen (Figure 1).

Natural pesticide suspensions either alone or in combination with 10^7/ml conidia of entomopatogenic fungi, *L. lecanii* were applied on the caged soybean once in the afternoon reduce the effect of UV rays. The dose of 2 ml/plant was applied.

Data Collection. The data collected includes (1) egg mortality, (2) number of normal pods, (3) empty pods (4) seed weight/plant, (5) seed weight/plot, (6) number of stylet punctures/seed, and (7) predator.

Data Analysis. The data was analyzed using Minitab 14. The effect among the treatments were analyzed using *Duncan's multiple range test* (DMRT 5%).

RESULTS AND DISCUSSION

The Egg Mortality. The *Annona* seed powder (ASP) and *Jatropha* seed powder (JSP) mixed with conidia of *L. lecanii* significantly affected on egg mortality. The high egg mortality (fail to develop to nymphs) were 84%, 82.7% and 80%, respectively for ASP, JSP combined with *L. lecanii*, and the fungus in single application (Figure 2). However, the combination of natural pesticides with entomopthogenic fungus was more effective to reduce stink bug egg development than single pesticide which suppressing by 56% and 61.33%, respectively for ASP and JSP. These natural pesticides may contain different active chemicals that act as ovicidal to stink bug eggs.

The evident of synergism between natural pesticides and entomopthogenic fungi *L. lecanii* was indicated as the increase in efficacy. It was found that the fungi and ASP, JSP acted as ovicidal on different insects species and nematodes (Shinya *et al.* 2008a & 2008b). Seed oil from both natural pesticides block natural micropyle that interfere on physiolocal process on egg development. *Jatropha* contains 63.16% oil higher than that of another plants (Akbar *et al.,* 2009). This exeperiment also proved that jatropha seed contain more oil than sesame, beans and tobacco (Gunstone, 2004; Marti'nez-Herrera *et al.,* 2006; Banapurmath *et al.,* 2008; Kumar & Sharma, 2008).

Figure 1. Inoculated and caged soybean in field experiment

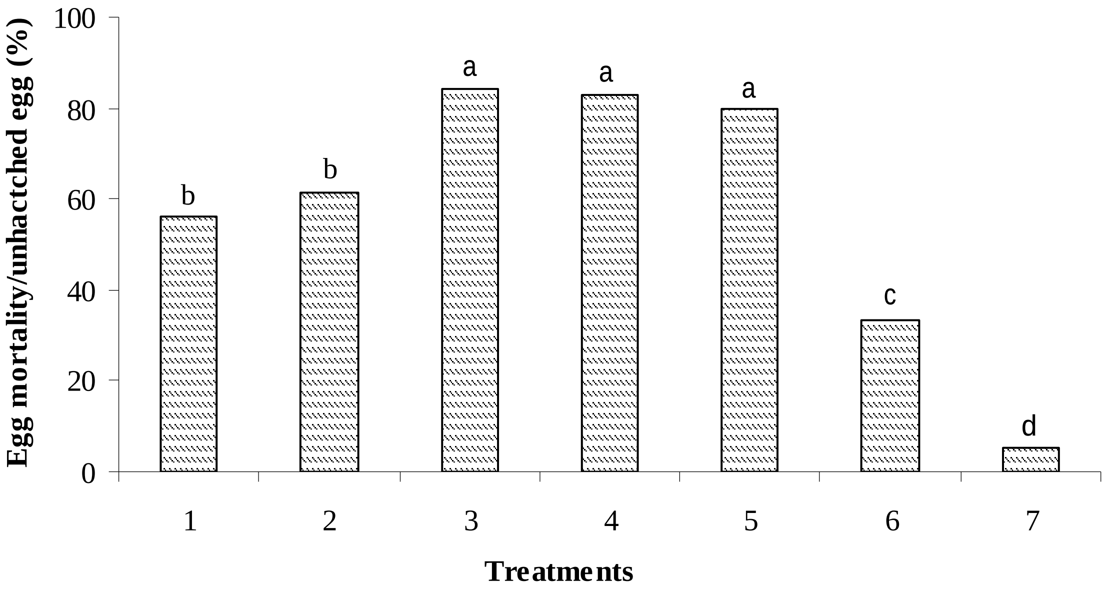

Figure 2. The mortality of brown stink bug eggs applied using natural pesticides and entomopthogenic fungi *L. lecanii*.(1)= ASP 50 g/l, (2)= JSP 50 g/l, (3)= ASP 50 g/l + *L. lecanii* 10^7/ml, (4)= JSP 50 g/l + *L. lecanii* 10^7/ml, (5)= *L. lecanii* 10^7/ml, (6)= deltametrin and (7)= check (no application).

The synergistic in pest control through integration of number of pest control agents was recommended thus pest was control more effective (Purwar & Sachan 2006). Feng *et al.* (2004) reported that the synergism of entomopathogenic fungus *Beauveria bassiana, Paecilomyces fumosoroseus* and imidacloprid pesticide increased control of green house whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyurodidae) than in a single application of agents.

A single application of ASP and JSP suppressed the number of egg development, and deltamethrin cause egg mortality 66.70%. The active chemicals of deltamethrin did not act as ovicidal. It was suggested that the active chemicals of deltamethrin drop blocked the microphyle disturbing the natural apperture where the embryo of stink bug exclosion and the embryo died inside (Islam *et al.,* 2009).

Number of Empty Pods. Damaged pod and seed by pod sucking bug, and pod borer insects directly affects on deformation seed and reduction on number of pods, aside of another physiological aspects of plant it self. Feeding of pod sucking bug by stylet punctures causes pod empty, and seed abortion.

Feeding of brown stink bug cause empty pod. The symptom was clearly shown on black spots on seed as a wound of stylet punctures. In this experiment, all sample plants were caged with fine tile screen and inoculated with fresh eggs, thus uninffected eggs will normally developed into adult. Therefore, the occurence of pod and seed damage causes by both nymphs and adults were still observed.

A higher number of 12.7 pods (45.78%) empty pod was observed on untreated or checked plot, however, the treatment did not significantly differ that from ASP with 10.67 (27.59%) of empty pod in a single aplication (Figure 3). The least empty pod 2.34 (4.87%) was on plot treated with ASP in combination with *L. lecanii* fungus, however, this treatment did not significantly differ that from JSP combined with *L. lecanii* (3.67%). The effective treatments (*L. lecanii* + natural pesticides) increased mortality and reduced of eggs development. The least developed nymphs and adult were the least damage occured. The high number of nymphs and adults feeding both on pod and developed seed increases the number of empty pod.

Number of Healthy Pods. The more healthy pods (48 pods) were recorded from ASP combined with *L. lecanii* (Figure 4). And the least healthy pods (27.67 pods) were recorded on untreated plot. Deltamethrin sprays did not effectively control brown stink bug damage as indicated in the number of healthy pods similar to that of un-treated plot. Mode of action deltamethrin as contact and gut poison, thus did not kill the stink bug eggs. Therefore, the population of stink bug caused severe damage on soybean. Apllication of ASP and JSP both in single application or combined with

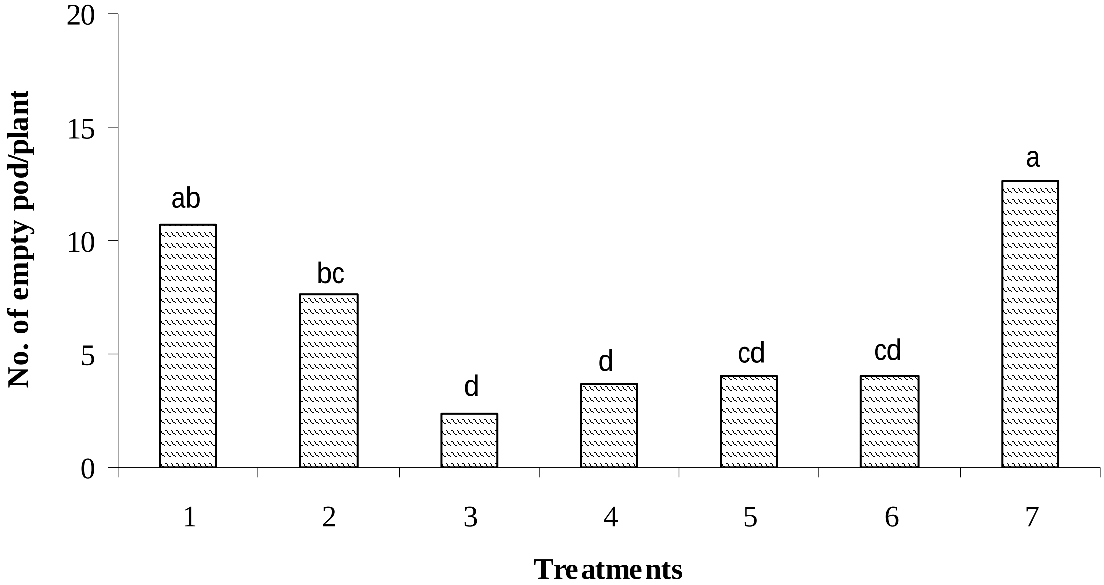

Figure 3. Number of empty pod as affected by combined application of ASP, JSP and *L. lecanii* fungus. (1)= ASP 50 g/l, (2) = JSP 50 g/l, (3)= ASP 50 g/l + *L. lecanii* 10^7/ml, (4)= JSP 50 g/l + *L. lecanii* 10^7/ml, (5)= *L. lecanii* 10^7/ml, (6) = deltamethrin, and (7) = control (no treatment).

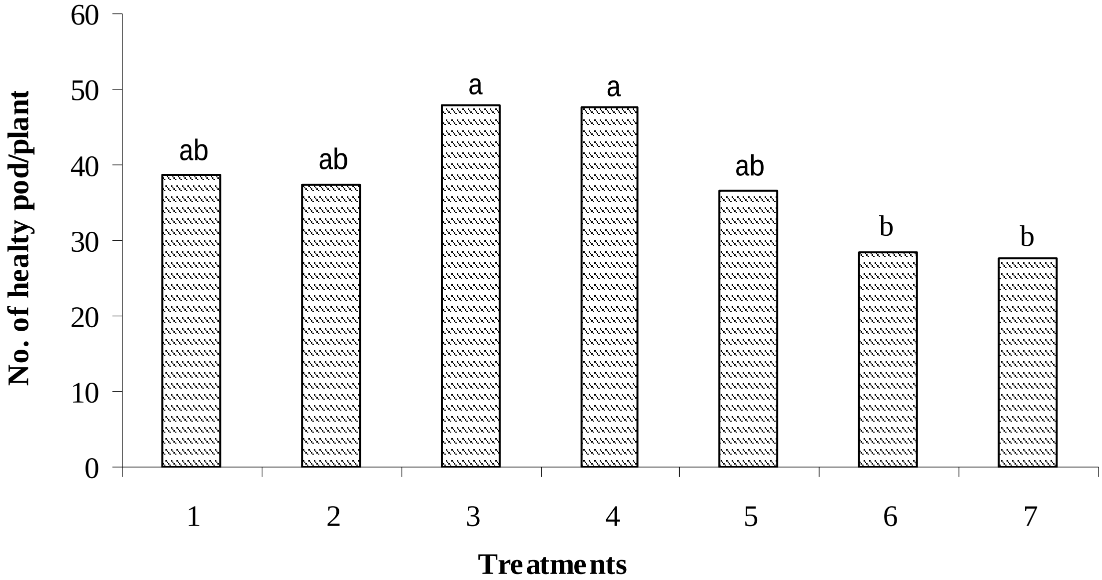

Figure 4. Number of healthy pod as affected by combined application of ASP, JSP and *L. lecanii* fungus. (1)= ASP 50 g/l, (2)= JSP 50 g/l, (3)= ASP 50 g/l + *L. lecanii* 10^7/ml, (4)= JSP 50 g/l + *L. lecanii* 10^7/ml, (5)= *L. lecanii* 10^7/ml, (6) = deltamethrin, and (7) = control (no treatment).

L. lecanii more effective to control the stink bug eggs as the fungus act as ovicidal.

Number pods on plot treated with natural pesticide of JSP applied in combination with *L. lecanii* fungus were more (47 pods), which did not different from ASP and *L. lecanii*. Number of pod from single apllication of *L. lecanii* fungus, ASP and JSP was equal to application of *L. lecanii* combined with ASP and JSP. Although number of pod from ASP, JSP and *L. lecanii* were similar, however, did not clearly expessed in their seed weight reduction.

Number of Stylet Punctures on Soybean Seed. Number of stylet puncture on seeds was indicated as

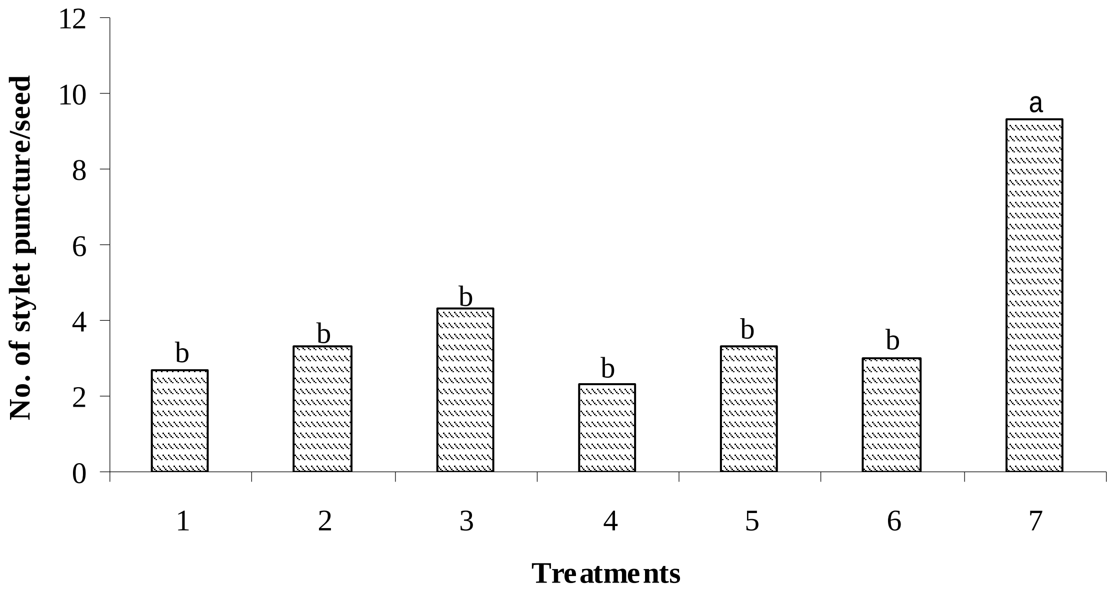

Figure 5. The application of natural pesticide and entomopathogenic fungus on stylet punctures on seed. (1)= ASP 50 g/l, (2)= JSP 50 g/l, (3)= ASP 50 g/l + *L. lecanii* 10^7/ml, (4)= JSP 50 g/l + *L. lecanii* 10^7/ml, 5= *L. lecanii* 10^7/ml, 6= deltamethrin, and 7= control (un-treated).

damage caused by stink bug feeding. In addition, the seed damage was also affected by the depth of stylet punctures on seed. The more stylet puntures of 9.34/ seed were observed on un-treated plot (Figure 5), and the least stylet punctures of 2.34/seed were found on JSP combined with *L. lecanii*, and did not differ from other treatments with exception on un-treated plot.

Prayogo (2009) revealed that only 2 stylet punctures/seed were found on soybean seed from plot treated with *L. lecanii* in combination with natural oil, however, application of the fungus without natural oil was 12 stylet punctures/seed. It was suggested that addition of natural oil from *Annona* and *Jathropha* seed protected the conidia from dry condition, thus maintaining their viability and survival rate of the conidia (Nyam *et al.*, 2009; Kim *et al.*, 2010; Kim & Je, 2010). Natural oil contains lecitin and casein reduced the polarity, thus increase the mixtures between oil and water. Addition of natural oil maintained the conidia viability of the fungus up to 10 days when exposing into soybean (Prayogo, 2009). However, without natural oil the conidia viability only one day after application. Ganga-Visalakshy *et al*. (2005) revealed that addition of conidia protectant avoiding lecitin from UV effect was suggested (Ibrahim *et al.*,1999). Protectant increased the air humidity as a requrement for conidia germination before infection (Inyang *et al.*, 2000; Verhaar *et al.*, 2004; Lazzarini *et al.*, 2006; Silva *et al.*, 2006).

Dry Seed Weight. Control of pod stink bug through application of ASP, JSP and *L. lecanii* fungus on the bug eggs sigificantly affect on seed weight/plant. The higher dry seed weight/plant 7.03 g was obtained from JSP and *L. lecanii* treatment (Figure 6), and the lowest dry seed weight 3.70 g was from un-treated plot. The seed weight of ASP + *L. lecanii* treatment was 6.50 g/ plant, which statically did not differ from JSP + *L. lecanii* and ASP + *L. lecanii* and a single apllication of *L. lecanii* fungus with 5,84 g seed weight. However, the seed weight in a single application of the fungus was lower than that application i.e. natural pesticide and entomopathogenic fungus *L. lecanii*.

The dry seed weight of ASP and JSP treatments in a single application was 4,13-4,83 g respectively. The dry seed weight of plot treated with deltamethrin 2 ml/l was 5.44 g/plant. It seemed that dry seed weight was lower than ASP, JSP and *L. lecanii* either in single or combined application. Based on this data, it was clearly shown that application of JSP + *L. lecanii* fungus was more effective than that other treatment. It means that with plant population of 250.000-300.000 plants/ha we expected soybean yield was aproximately 1.7-2.1 t/ha.

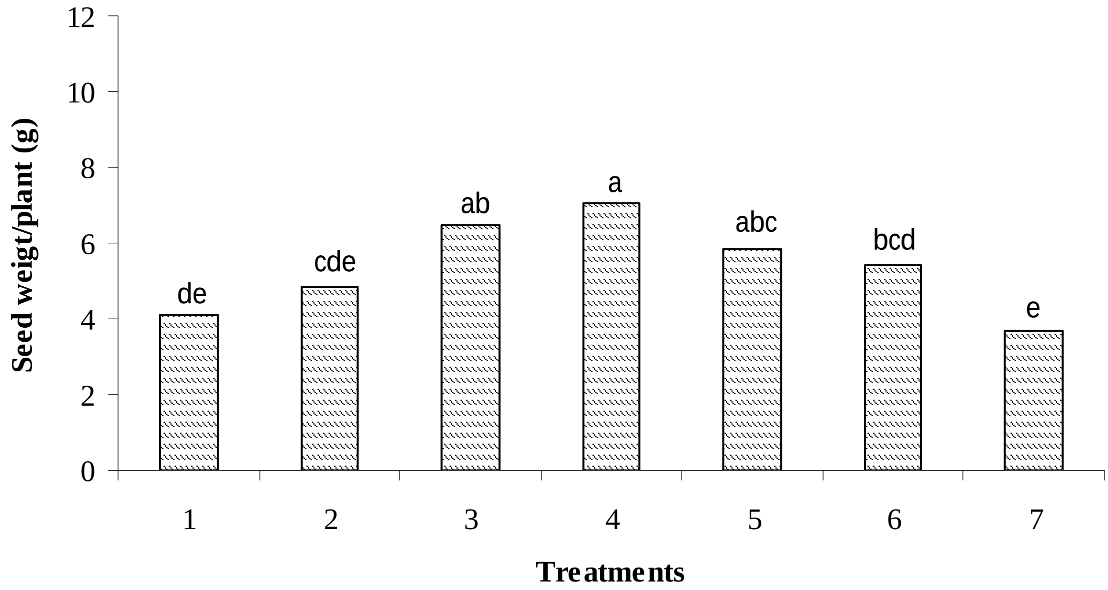

Figure 6. Dry seed weight of soybean as affected either single application or combination of ASP,JSP and *L. Lecanii* fungus. (1)= ASP 50 g/l, (2)= JSP 50 g/l, (3)= ASP 50 g/l + *L. lecanii* 10^7/ml, (4)= JSP 50 g/l + *L. lecanii* 10^7/ml, (5)= *L. lecanii* 10^7/ml, (6)= deltamethrin, and (7)= control (un-treated).

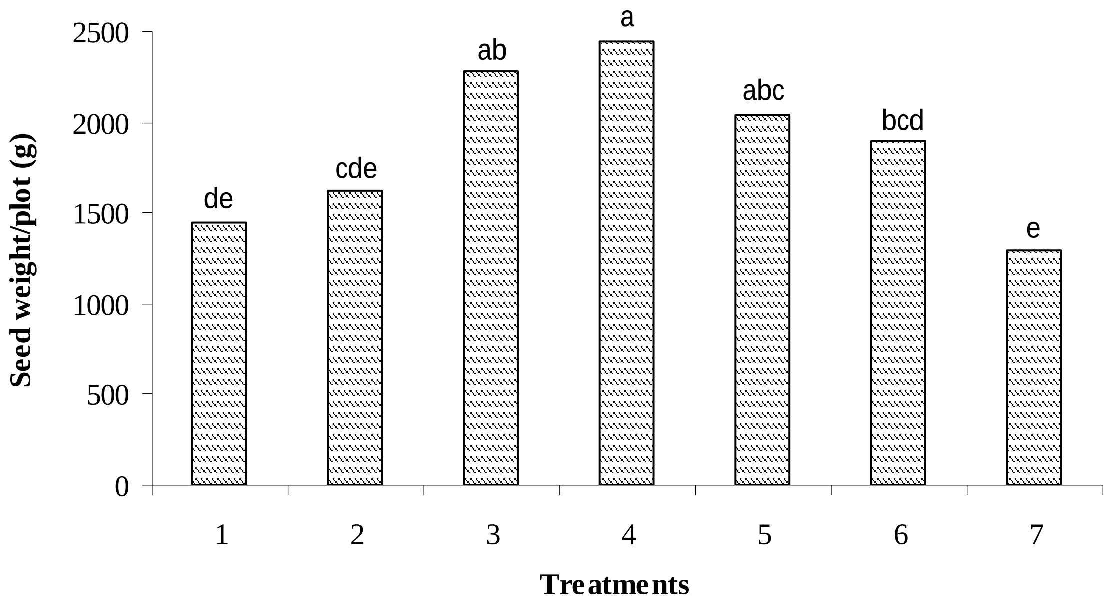

Figure 7. Seed weight/plot treated with natural pesticide and entomopthogenic fungi *L. lecanii*.(1)= ASP 50 g/l, (2)= JSP 50 g/l, (3)= ASP 50 g/l + *L. lecanii* 10^7/ml, (4)= JSP 50 g/l + *L. lecanii* 10^7/ml, (5)= *L. lecanii* 10^7/ml, (6)= deltamethrin, and (7)= control (check).

Its mean that the integration of natural pesticide and entomopathogenic fungi *L. lecanii* would maintain soybean yield up to 1.5 t/ha supporting program to increase soybean yield in Indonesia. Most of soybean growers (95%) mainly use insecticide in pest control. However, chemical insecticides maintain soybean yield by 1.3-1.6 t/ha lower than that of integrated control system.

Seed Weight/plot. It was found there was a positive correlation between seed weight/plant and seed weight of 7 m x 5 m plot. The high yield/plot of soybean from plot treated was integration of JSP + *L. lecanii,* ASP + *L. lecanii,*and single application of *L.lecanii* respectively were 2447 g, 2276 g, and 2042 g (Figure 7), and application of deltamethrin was lower i.e 1892 g. However, the seed obtained from the application of ASP and JSP in a single application was lower than plot treated with *L. lecanii* and deltamethrin. The least seed of 1299 g was recorded in the check (un-treated plot).

Regarding on seed yield it was revealed that integration of natural pesticide and entomopthogenic fungi of *L. lecanii* increased the control of stink bug by 36% and 33% respectively for ASP + *L. lecanii,* and JSP + *L. lecanii*. The integration of these agents on stink bug control could safe or reduce yield loss up to 46% and 39 % respectively. Therefore, integration of natural pesticide and entomopthogenic fungi *L. lecanii* was recommended.

Natural Enemies. It was found that natural enemies were not affected by natural pesticide either in a single or int combination with *L. lecanii* fungi application than deltamethrin, as indicated with high population of *Paederus* sp. and *Coccinella* sp. (generalist predator). Number of 28 *Coccinella* sp. and 20 *Paederus* sp. were found, however, the population of *Oxyopes* sp. were lower. It was observed that the high distribution population of *Oxyopes* sp. mainly on rice field and peanut. These three generalist predators preys were Homoptera, Hemiptera and Lepidopterans insect, both eggs nymphs and as well as the larvae.

O. Javanus (Oxyopidae: Araneae) was effective predator for the common cutworm *Spodoptera litura,* brown stink bug *R. linearis,* green stink bug *N. viridula* and *E. zinckenalla* both eggs, larvae or nymph and prey 12 insects/day (Tengkano & Bedjo, 2002) similar to *Paederus* sp. (Taulu, 2001). This evident suggested that the population of the predators in soybean should be maintained to control soybean pests under economic threshold.

Chemicals pesticides totally affected generalist predators population such as *Paederus* sp., *Oxyopes* sp., and *Coccinella* sp. No more predators were found, exept one *Coccinella* sp. These species were known as an agressive predator in soybean canopy. Integration of ASP, JSP and entomopathogenic fungi *L. lecanii* did not affect the survival of natural enemies.

CONCLUSIONS

Integration of natural pesticides of ASP, JSP and entomopthogenic fungi, *L. lecanii* increased the efectiveness to control stink bug by 46%. Natural pesticides ASP, JSP and entomopthogenic fungi *L. lecanii* did not affect the survival of generalist predators such as *Paederus* sp., *Oxyopes* sp., and *Coccinella* sp. also predator was more sensitive to chemical insecticide. ASP, JSP and *L. lecanii* were integral part of integrated pest management of pod stink bug in soybean.

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