Jurnal Perlindungan Tanaman Indonesia, Vol. 24, No. 2, 2020: 182–187
DOI: 10.22146/jpti.44032
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Feeding Inhibition by Chitosan on Larvae of *Spodoptera litura* (Lepidoptera: Noctuidae)

Sulistia Ningsih[1)]*, Nugroho Susetya Putra[1)], & Y. Andi Trisyono[1)]

[1)]Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada
Jln. Flora No. 1, Bulaksumur, Sleman, Yogyakarta 55281 Indonesia

**Corresponding author. E-mail: ningsihsulistia92@gmail.com*

Received March 12, 2019; revised March 29, 2019; accepted December 19, 2020

ABSTRACT

The high usage of synthetic insecticides for controlling *Spodoptera litura* could be detrimental to the environment, especially on non-target organisms. Therefore, more environmentally friendly pest management techniques should be used, for example, using a natural product such as chitosan. The objective of this study was to understand the effect of feeding inhibition by chitosan on the feeding activity of the third instar larvae of *S. litura*. The feeding inhibition test was carried out using the choice methods in a factorial completely randomized design (CRD) with seven treatments: i.e. chitosan 5×10^3, 15×10^3, 25×10^3, 35×10^3, 45×10^3 ppm and profenofos 0.18×10^3, 0.34×10^3 ppm, and one control treatment. The test of the choice methods was carried out by three larval laying positions: (1) between the control and treatment feeds (Position A), (2) above the treated feed (Position B), and (3) above the control feed (Position C), and were replicated three times. The non-choice test was done in a completely randomized design (CRD) with seven treatments plus control and were replicated four times. The results showed that the chitosan in the concentration range of 5×10^3–45×10^3 ppm reduced feeding by *S. litura* larvae by 2.587 to 34.974% in the choice method, and 11.610 to 50,712% in the non-choice method. This feeding inhibition increased significantly with the increment of chitosan concentration. However, the inhibition effects by chitosan was weaker than the inhibition by profenofos LC_{50} in both tests at a concentration of 0.34×10^3 ppm: 44.331% and 62.491% respectively. In conclusion, chitosan with a concentration of 45×10^3 ppm at all larval laying positions showed the highest value of feeding inhibition activity on the third instar larvae of *S. litura* compared to other chitosan concentrations in both methods.

Keywords: chitosan; feeding inhibition; *Spodoptera litura*

INTRODUCTION

The army worm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), is an important horticulture pest on approximately 120 types of plants (CABI, 2017). To this date, syntetic insecticides are still the main option to manage *S. litura* in the field. However, continuous and overuse of insecticides have detrimental effects on the environment (Untung, 1984). As reported by Irawan (2017) and Setiawati, *et al.* (2015), the application of *λ-sihalotrin* 25 EC (25 g/L) and carbofuran (Furadan 3 G) on long beans had negative effects on the predatory spider, *Pardosa pseudoannulata*, and reduced soil arthropod species richness by 30.67–34.32%. Thus, alternative management techniques that are environmentally friendly are required.

Chitosan is a natural material that has good potential to be developed as an insect pest control agent. It is one of the biopolymers resulted from chitin distilation that is contained in most arthropods, Crustacean shell, such as shrimps, crabs, and oysters (Badawy & El-Aswad, 2012). Chitosan is used in agriculture as substances to increase plant growth (Ianca, 2010; Suptijah *et al.,* 2010; Boornlertnirun *et al.*, 2008), inhibits fungi growth (Pamekas *et al*., 2009), elicitors of plant resistances (Damayanti *et al.,* 2013), and as an insecticide (Zhang *et al.,* 2003; Badaway *et al.*, 2005; Rabea *et al*., 2005; Badawy & El-Aswad, 2012).

Beside having insecticidal properties, it is confirmed that at concentration of 0.9%, chitosan affects feeding preference of *Aphis craccivora* on long bean (Megasari *et al.*, 2014). In addition, if it is modified with metals, such as nickel (Ni) and mercury (Hg), it has feeding inhibition activity on *S. littoralis* larvae (Badawy & El-Aswad, 2012).

Current research on the use of chitosan have heavily focused on its effects on plant pathogens and sucking-

type insects, while chewing-type insects have been less studied. Therefore, the goal of this study was to test the feeding inhibiton effects of chitosan against 3rd *S. litura* larvae, the most voracious stage.

MATERIALS AND METHODS

Spodoptera litura Rearing

S. litura were collected from sunflower plants located in Sinduharjo Village, Ngaglik District, Sleman Region, Special Region of Yogyakarta and also obtained from the Indonesia Sweetener and Fiber Crops Research Institute (Balittas), Malang. Both colonies were reared at the Laboratory of Entomology, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta since 2017.

Larvae of *S. litura* were kept in plastic containers covered with mess clothes (18×11×10 cm) and fed with artificial diets (Singh & Moore, 1985; with slightly modification; Table 1). Containers were cleaned daily and diets were change every 2 days or based on larvae needs. Pupae were collected and placed in a petri-dish (diameter = 9 cm; height = 2 cm) with a wetted cotton to maintain humidity of the container. Petridish was placed in a transparent plastic container (diameter = 17 cm; height = 19.5 cm) that was closed using mess clothes, and enabled pupae to hatch into adults. Adults were fed with 10% honey solution until they laid eggs.

Table 1. Diet composition used for *Spodoptera litura* rearing based on Singh & Moore (1985) with modifications

Ingredient	Amount	Units
red beans	75	Gram
wheat bran	75	Gram
yeast	25	Gram
gelatine	14	Gram
benzoate	2	Gram
ascorbate acid	3	Gram
distilled water	1	Liter

Chitosan Preparation

Pure chitosan were obtained from Black tiger shrimp shells (90% of purification) provided by CV. Bio Chitosan Indonesia (Cirebon, Indonesia). Chitosan were disolved into 1% acetate acid before used in tests.

Choice Test of Feeding Inhibition Activity

Choice-test of feeding inhibition was set as a Factorial Complete Randomized Design (CRD) to determine feeding activity of *S. litura* in similar condition to field condition. Diets were made into cubicle shapes (1× 1× 1 cm; weight ±3 g). The diets were than immersed for 10 seconds into chitosan solutions at 5 different concentration, including 5×10^3, 15×10^3, 25×10^3, 35×10^3, and 45×10^3 ppm, and profenofos solutions at concentrations of 0.1810^3 ppm (LC_{20}) and 0.34×10^3 ppm (LC_{50}) as a comparison, while diets used as controls were immersed into 1% acetate acid solutions. Diets were then air dried on filter paper, and noted as weight before larval feeding. Treated and control diets weres placed respectively on the left and right of plastic containers (diameter = 10.5 cm; height = 7.5 cm) and labelled based on treatments.

Tests were done by placing ten 3rd instar larvae in 10 plastic containers (diameter = 10.5 cm; height = 7.5 cm) that were separated by treatment. Larvae were placed at 3 positions, which were between control and treated diet (Figure 1A), on top of treated diets (Figure 1B), and on top on control diets (Figure 1C). Each treatment were replicated 3 times and as much as 630 3rd *S. litura* larvae were used in this choice test. Feeding inhibition activity was calculated using the formula from Hassanali & Bentley (1987). Feeding inhibition activity using the choice test:

$$\text{Feeding inhibition activity (FI) (\%)} = \frac{C-T}{C+T} \times 100$$

C = Diet weight before larvae feeding- diet weight after larvae feeding on the control diet

T = Diet weight before larvae feeding- diet weight after larvae feeding on the treated diet

No-Choice Test of Feeding Inhibition Activity

This tests was done to confirm results from the choice test by forcing larvae to one type of diet. Procedure used were similar to the choice test and set as a Completely Randomized Design (CRD).

Diets treated with chitosan, profenofos, and control diets were placed in separated plastic containers (diameter = 6.5 cm; height = 4.8 cm). Each experimental unit consisted of 10 3rd instar *S. litura* larvae placed into separated containers. Larvae placement only used one treatment, which was on the designated diet (Figure 2). Each treatment was replicated 4 times resulting in

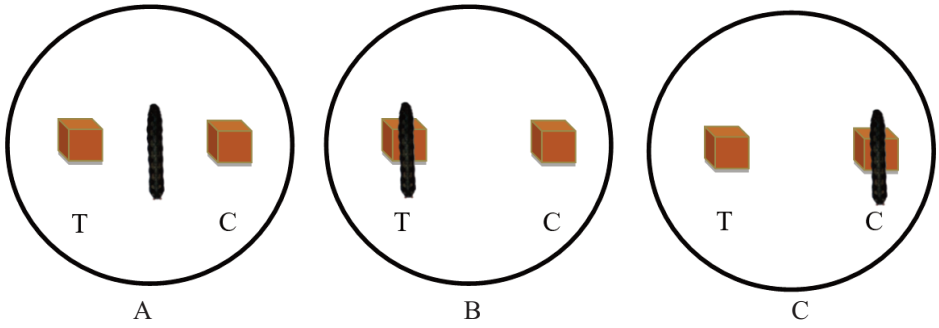

Figure 1. Choice test method with larva placement A (larva placed between control and treated diet), B (larva placed on treated diet), and C (larva placed on control diet)
T = Treated diet
C = Control diet
= Larva

320 larvae used in this experiment. Remaining diets were then weighted. Diets consumed by larvae were determined by calculating the differences of weights before and after larval feeding. Observations were done several times over 48 hours.

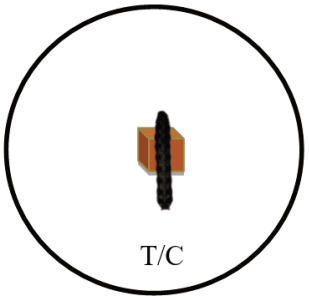

Figure 2. No-choice test method
T = Treated diet
C = Control diet
= Larva

To calculate water moist loss from diets due to evaporation was done by using a parallel diet that was not fed to larvae. The diet was then weighted and differences were calculated. Feeding inhibition activity was calculated using the formula from Hassanali & Bentley (1987). Feeding inhibition activity using the choice test:

$$\text{Feeding inhibition activity (FI) (\%)} = \frac{C\text{-}T}{C} \times 100$$

C = Diet weight before larvae feeding- diet weight after larvae feeding on the control diet
T = Diet weight before larvae feeding- diet weight after larvae feeding on the treated diet

Statistical Analysis

Inhibition percentages of the choice tests were tested using an ANOVA as a Factorial CRD with two factors including solution type concentration and larvae placement position. Significant differences between treatments were then tested used a post-hoc Tukey HSD test at $\alpha = 5\%$. All statistical tests were done using R-3.4.4, 2018.

RESULTS AND DISCUSSION

Choice-Test of Feeding Inhibition Activity

Results showed that feeding inhibiton was significantly affected by compound types and concentration, but not by the larva placement position and there were no interactions between these 2 factors (Table 2). Feeding inhition increased respectively to the concentration of chitosan and profenofos regardless of the position of the larva.

Chitosan concentration of 45×10^3 ppm showed feeding inhibition of 34.974, 36.557 and 34.556%. However, this inhibition was not larger than profenofos LC_{50} of 0.34×10^3 ppm on all larval position of 44.331, 45.090, and 46.152%.

No Choice-Test of Feeding Inhibition Activity

Feeding inhibition using the no-choice test had similar results with the choice test. Higher chitosan concentrations had significantly higher feeding inhibition effects. Feeding inhibition of chitosan and profenofos showed similar patterns (Table 3).

Higher chitosan concentration created thicker film coatings that covered the diets and disrupted larval feeding. Decreased eating can occur due to

Table 2. Feeding inhibiton of 3rd instar *Spodoptera litura* larva at various concentration of chitosan and profenofos at 3 different larval placement position for the choice test

Compound[c]	Concentration (ppm)	Position A $\overline{X}$(%) ± SD [ab]	Position B $\overline{X}$(%) ± SD [ab]	Position C $\overline{X}$(%) ± SD [ab]
Chitosan	5×10^3	2.587 ± 0.3 f A	3.291 ± 1.4 f A	2.488 ± 0.7 f A
	15×10^3	8.934 ± 0.4 e A	8.651 ± 0.92 e A	7.302 ± 0.6 e A
	25×10^3	16.859 ± 2.0 d A	16.305 ± 0.4 d A	16.843 ± 1.4 d A
	35×10^3	25.787 ± 1.5 c A	23.884 ± 0.9 c A	25.002 ± 0.9 c A
	45×10^3	34.974 ± 1.1 b A	36.557 ± 1.4 b A	34.556 ± 1.9 b A
Profenofos	0.18×10^3	14.385 ± 0.9 d A	14.869 ± 1.5 d A	17.608 ± 3.4 d A
	0.34×10^3	44.331 ± 2.6 a A	45.090 ± 0.6 a A	46.152 ± 1.6 a A

[a] Feeding inhibition mean
[b] Standard deviation
[c] No interactions between concentration and larva placement position
Position A= larva placed between treated and control diet
Position B= larva placed on treated diet
Position C= larva placed on control diet
Mean at each concentration followed by the same letters in the same column were not significantly different based on Tukey post-hoc test (α= 5%). Lowercase letters shows difference between concentrations, while uppercase letters indicate difference between larval placements.

Table 3. Feeding inhibiton of 3rd instar *Spodoptera litura* larvae with the no-choice test

Compound	Concentration (ppm)	$\overline{X}$ (%) ± SD [ab]
Chitosan	5×10^3	11.610 ± 5.9 d
	15×10^3	30.760 ± 8.8 c
	25×10^3	42.142 ± 12.0 bc
	35×10^3	43.769 ± 9.2 abc
	45×10^3	50.712 ± 5.7 ab
Profenofos	0.18×10^3	38.828 ± 5.0 bc
	0.34×10^3	62.491 ± 5.0 a

[a] Feeding inhibition mean
[b] Standard deviation
Means at each concentration followed by the same letter were not significantly different based on the Tukey post-hoc test (α= 5%).

the deterrent effect and sublethal concentrations that reduce feeding rates. Chitosan is a natural biopolymer with semipermeable characteristics and creates coating films that have been reported to be used as seed coating that effectively repel *Agrotis ipsilon*, soybean pod borer, and soybean aphid feeding activity by 82.89, 87.24, and 80.21% respectively at concentrations of 5% (Zeng *et al.*, 2012). However, this study was only able to decrease feeding rates by 35.0% at the choice test and 50.7% at the no-choice test when using chitosan concentrations of 45×10^3 ppm. These values were not higher than the profenofos LC_{50} at 0.34×10^3 ppm treatment regardless with choice or no-choice tests. This implies that chitosan did not effectively inhibit feeding activity of 3rd instar *S. litura* larvae.

Antifeedant is a compound applicated at low concentration, but is able to highly inhibit feeding rates (Purrington, 2017). Polyphagous insects, including *S. litura,* have the ability to adapt to antifeedant compounds. In addition, this compound does not have high enough toxicity so that the adaptation by larvae occurs more quickly. Since chitosan is not able to reduce the feeding rate of *S. litura*, this also means that chitosan has no effect on its development and growth. In this phase *S. litura* will adjust to chitosan, so that its growth and development are not disturbed.

Results form this study were different from previous studies using pure chitosan (molecule weight 2.27×10^5 g/mol with concentration of 4 g chitosan/kg of artificial diet) and chitosan with nickel (Ni) and mercury (Hg) addition, which were able to inhibit *S. littoralis* feeding by 76, 90.2, and 86.8%. This feeding inhibition of pure chitosan was not significantly higher than chitosan with Ni and Hg addition (Badawy & El-Aswad, 2012).

According to Zhang *et al.* (2003), chitosan have insecticidal properties against lepidopteran and homopteran insects. Chitosan activity was higher in *Plutella xylostella*, about 72% compared to *Helicoverpa armigera* and *S. exigua* which were only 40% at a concentration of 3g/L, and *Rhopalosiphum padi*, *Metopolophium dirhodum*, and *A. gossypii*, i.e. 60–80% at a concentration of 600–6000 mg/L. In addition, application of chitosan at concentrations of 0.9% on long beans affected *A. craccivora* feeding preferences based on low populations and no lethal effects on this species is recorded (Megasari *et al.*, 2014). This may be caused by chitosan's ability to induce resistant mechanisms of plants, such as the accumulation of phytoalexin, PR (pathogenesis related), proteinase inhibitors, and various enzymes including peroxidase. Peroxidase can induce lignification of plant's cell walls that act as a physical barrier that reduce herbivor's preferences (Hadrami *et al*., 2010).

CONLUSION

Feeding inhibition of 3rd instar *S. litura* larvae due to chitosan increased respectively with the increase of concentration (5×10^3–45×10^3 ppm). Chitosan concentration of 45×10^3 ppm inhibited feeding activity the most at all larval posistion placement compared to other concentrations regardless the choice or no-choice test.

ACKNOWLEDGEMENT

We would like to thank *Lembaga Pengelola Dana Pendidikan* (LPDP) for funding this research and program of the first author.

LITERATURE CITED

Badawy, M.E.I. & A.F. El-Aswad. 2012. Insecticidal Activity of Chitosans of Different Molecular Weights and Chitosan-Metal Complexes against Cotton Leafworm *Spodoptera littoralis* and Oleander Aphid *Aphis nerii*. *Journal of Plant Protection Science* 48: 131–141.

Badawy, M.E.I., E.I. Rabea, T.M. Rogge, C.V. Stevens, W. Steurbaut, M. Höfte, & G. Smagghe. 2005. Fungicidal and Insecticidal Activity of *O*-acylchitosan Derivatives. *Polymer Bulletin* 54: 279–289.

Boornlertnirun, S., C. Boonraung, & R. Suvanasara. 2008. Application of Chitosan in Rice Production. *Journal of Metals, Materials and Minerals* 18: 47–52.

CABI (Centre for Agriculture and Bioscience International). 2017. *Spodoptera litura* (Taro Caterpillar). http://www.cabi.org/isc/datasheet/44520, modified 20/05/2017.

Damayanti, T.A., S. Haryanto, & S. Wiyono. 2013. Pemanfaatan Kitosan untuk Pengendalian *Bean common mosaic virus* (BCMV) pada Kacang Panjang. *Jurnal Hama dan Penyakit Tumbuhan Tropika* 13: 110–116.

Hadrami, A.E., L.R. Adam, E.I. Hadrami, & F. Daayf. 2010. Chitosan in Plant Protection. *Journal of Marine Drugs* 8: 968–987.

Hassanali, I. & M. Bentley. 1987. Comparison of The Insect *Antifeedant* Activities of Some Limonoids. Proc. 3rd. *International Neem Conference* 1986: 683–689.

Ianca, B.F. 2010. *Pengaruh Perlakuan Kitosan terhadap Pertumbuhan Tanaman Kedelai (Glycine max) Selama Fase Vegetatif dan Awal Fase Generatif*. Skripsi. Institut Pertanian Bogor, Bogor (ID). 50 p.

Irawan, D. 2017. *Pengaruh Aplikasi Insektisida terhadap Serangga Hama dan Musuh Alami pada Pertanaman Kacang Panjang (Vigna sinensis L.)*. Tesis. Institut Pertanian Bogor, Bogor (ID). 44 p.

Megasari, D., T.A. Damayanti, & S. Santoso. 2014. Pengendalian *Aphis craccivora* Koch. dengan Kitosan dan Pengaruhnya Terhadap Penularan *Bean Common Mosaic Virusstrain Black Eye Cowpea* (BCMV-BlC) pada Kacang Panjang. *Jurnal Entomologi Indonesia* 11: 72–80.

Pamekas, T., C. Sumardiyono, & N. Pusposendjojo. 2009. Ekstraksi, Karakterisasi dan Daya Penghambatan Kitosan Alami terhadap Jamur *Colletotrichum musae* secara *In Vitro*. *Jurnal Perlindungan Tanaman Indonesia* 15: 39–44.

Purrington, C.B. 2017. Antifeedant Substances in Plants, p. 1140–1145. *In* B. Thomas, B.G. Murray, D.J. Murphy (eds.), *Encyclopedia of Applied Plant Sciences*. Academic Press, Amsterdam.

Rabea, T.M., M.E.I. Badawy, T.M. Rogge, C.V. Stevens, M. Höfte, W. Steurbaut, & G. Smagghe. 2005. Insecticidal and Fungicidal Activity of New Synthesized Chitosan Derivatives. *Pest Management Science* 61: 951–960.

Setiawati, W., H. Jayanti., A. Hudayya, & A. Hasyim. 2015. Pengaruh Insektisida Karbofuran terhadap Kerusakan dan Kehilangan Hasil Kentang Akibat Serangan *Gryllotalpa hirsuta* Burmeister (Ortoptera: Gryllotalpidae) serta Dampaknya terhadap Keanekaragaman Artropoda Tanah. *Jurnal Hortikultura* 25: 54–62.

Singh, P. & R.F. Moore. 1985. *Handbook of Insect Rearing* 2nd ed. Elsevier. Amsterdam (NL). 522 p.

Suptijah, P., A.M. Jacob, & S. Mursid. 2010. Teknik Peranan Kitosan dalam Peningkatan Pertumbuhan Tomat (*Lycopersicum esculentum*) Selama Fase Vegetatif. *Akuatik-Jurnal Sumberdaya Perairan* 4: 9–14.

Untung, K. 1984. *Pengantar Analisis Ekonomi Pengendalian Hama Terpadu*. Andi Offset, Yogyakarta (ID). 92 p.

Zeng, D., X. Luo & R. Tu. 2012. Application of Bioactive Coatings Based on Chitosan for Soybean Seed Protection. *International Journal of Carbohydrate Chemistry* 2012: 1–5.

Zhang, M.I, T. Tan, H. Yuan, & C. Rui. 2003. Insecticidal and Fungicidal Activities of Chitosan and Oligo-Chitosan. *Journal of Bioactive and Compatible Polymers* 18: 391–400.