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THE EFFECT OF *Bacillus thuringiensis* TOXIN Cry1A.105 AND Cry2Ab2 ON THE SURVIVAL OF THE NON-TARGET PEST, *Spodoptera litura*

PENGARUH TOKSIN Bacillus thuringiensis *Cry1A.105 DAN Cry2Ab2 TERHADAP KELANGSUNGAN HIDUP HAMA BUKAN SASARAN,* Spodoptera litura

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ABSTRACT

Spodoptera litura is one of the important insect pest of maize besides the notoriously damaging corn borer, *Ostrinia furnacalis*. *S. litura* has been the target of various controls including the use of *Bacillus thuringiensis* (Bt) toxin Cry1A.105 and Cry2Ab2. This study was conducted to evaluate the acute effect of Bt toxin Cry1A.105 and Cry2Ab2 on the growth and development of *S. litura* from larval to adult stages. Two sublethal concentrations were used; 0.1875 and 0.0469 ppm for Cry1A.105, and 0.0008 and 0.0003 ppm for Cry2Ab2. The bioassay using diet dipping was carried out on a CRD with three experiments and five repetitions. The observation was carried out on the mortality and survival rates of *S. litura*. The mortality reached 28% when the larvae were treated with 0.1875 ppm and 20% with 0.0469 ppm of Cry 1A.105. The exposed larvae and pupae were smaller than control. Larval and pupal weight were 117.0 and 165.6 g with 0.1875 ppm, while control were 212.9 and 211.2 g. Cry1A.105 also longer the larval stage, larval stage with higher and lower concentration were 24.5 and 22.3 day, while control was 20.5 day. The resulted pupae from larve which exposed by Cry1A.105 were less than control; there were 40% at concentration 0.1875 ppm and control 61%. The two concentration of Cry2Ab2 produced similar mortality of 20%. Similarly, Cry2Ab2 affected pupal to adult stages development. The longevity of pupal stage with concentration 0.0003 ppm was 9.5 days, followed by 0.0008 ppm (9.1 days) and control (10.1 days). The adult emerge on the highest concentration was 47.4% while control only 34.6%. There results showed that both Cry1A.105 and Cry2Ab2 were detrimental to the survival of *S. litura* which is the non-target insect of transgenic Bt maize.

Keywords: Cry1A.105, Cry2Ab2, maize, non-target pest, *Spodoptera litura,* survival

INTISARI

Spodoptera litura *merupakan salah satu hama penting yang menyerang tanaman jagung, selain* Ostrinia furnacalis. *Belakangan ini* O. furnacalis *diketahui telah menjadi target dari berbagai macam cara pengendalian termasuk penggunaan toksin* Bacillus thuringiensis *(Bt) Cry1A.105 dan Cry2Ab2. Penelitian ini dilakukan untuk mengetahui efek akut toksin Bt Cry1A.105 dan Cry2Ab2 terhadap pertumbuhan dan perkembangan* S. litura *dari larva sampai imago. Dua konsentrasi subletal yang akan digunakan adalah; 0,1875 dan 0,0469 ppm untuk Cry1A.105, dan 0,0008 dan 0,0003 ppm untuk Cry2Ab2. Pengujian dilakukan dengan menggunakan metode celup pakan dan Rancangan Acak Legkap dengan tiga perlakuan dan lima ulangan. Pengamatan dilakukan terhadap mortalitas dan kelangsungan hidup* S. litura*. Mortalitas mencapai 28% pada larva yang dipaparkan dengan 0,1875 ppm dan 20% dengan 0,0469 ppm Cry 1A.105. Larva dan pupa yang terkena toksin berukuran lebih kecil. Berat larva dan pupa yang terpapar toksin dengan konsentrasi 0,1875 ppm, masing – masing 117,0 dan 165,6 g, sedangkan kontrol masing – masing 212,9 dan 211,2 g. Cry1A.105 juga dapat memperpanjang stadia larva. Lama stadia larva dengan konsentrasi tertinggi dan terendah adalah 24,5 dan 22,3 hari, sedangkan kontrol 20,5 hari. Jumlah pupa yang berhasil terbentuk dari larva yang terpapar toxin Cry1A.105 lebih sedikit dibandingkan dengan kontrol; pada konsentrasi 0,1875 ppm sebesar 40%, sedangkan kontrol sebesar 61%. Kedua konsentrasi dari toksin Bt Cry2Ab2 menyebabkan mortalitas yang sama yaitu 20%. Cry2Ab2 juga berpengaruh terhadap lama stadia pupa dan tingkat keberhasilan pembentukan imago. Lama stadia pupa dengan konsentrasi 0,0003 ppm adalah 9,5 hari, diikuti dengan konsentrasi 0,0008 ppm (9,1 hari) dan kontrol (10,1 hari). Jumlah imago terbanyak terdapat pada perlakuan dengan konsentrasi 0,0008 ppm sebesar 47,4% sedangkan pada kontrol hanya 34,6%. Hal ini menunjukkan bahwa toksin Bt Cry1A. 105 dan Cry2Ab2 juga berpengaruh terhadap kelangsungan hidup* S. litura *yang merupakan serangga bukan sasaran dari tanaman jagung transgenik Bt.*

Kata kunci: Cry1A.105, Cry2Ab2, hama bukan sasaran, jagung, kelangsungan hidup, Spodoptera litura

INTRODUCTION

Maize is one of the most important crop in Indonesia. It is used as foods, feeds, and raw materials for many industries. Two regions in Indonesia, the islands of Madura and Nusa Tenggara, are especially known to grow maize for livestock feeds (Respati *et al*., 2013). The demand of maize in Indonesia is expected to increase along with the growth of the country's population, however, maize production is often insufficient that this commodity is continued to be imported every year. The production of dry grain in 2013 amounted of 18.51 million tons decreased to 4.5% from 2012 (BPS, 2014a). The reduction of national maize production is also raised the import rate. Indonesia imported maize for 2.9 million tons in 2013, whereas in 2014 the number was expected to achieve 3.6 million tons (BPS, 2014b). The dependence on maize import will create the negative impact to the domestic maize production. If the problem continues, the country's food security and the livestock businesses sustainability would be threatened.

The reduction of maize production in Indonesia is influenced by several factors, one of them is pests and diseases. There are a number of pests recorded on maize such as stem borer (*Ostrinia furnacalis*), ear borer (*Helicoverpa armigera*), seed flies (*Atherigona* sp.), *Aphids* sp., grasshopper (*Valanga nigricornis*), army worm (*Spodoptera litura*), and grubs (*Lachnosterna* sp. and *Holotrichia* sp.) (Kalshoven, 1981). *S. litura* is the main pest on a number of agricultural crops in Southeast Asia and spread out in the entire Africa and Europe, however it is considered less importance than *O. furnacalis* on maize. The percentage of maize lost in yield by *O. furnacalis* ranged between 20–80% (Nonci, 2004), while the attack of *S. litura* amounted to 0–57% (Halim *et al.,* 2014). *S. litura* is a polyphagous insect, which infest a number of crops such as tomato, cotton, tobacco, rice, cacao, orange, sweet potato, peanut, soybean, potato, cabbage, and maize (Kalshoven, 1981).

Pest control on maize is mainly targeting *O. furnacalis* as the main source of yield loss. Many control methods have been employed to keep this pest on check including biological control and synthetic chemical pestisides (Subiadi *et al*., 2014). One of the currently developed biological control method is the use of *Bacilus thuringiensis* (Bt), either conventionally by direct spraying or inserted within the transgenic maize crops throught genetically modification procedure (George & Crickmore, 2012). There are many toxins that have been used to create transgenic maize such as Cry1A.105, Cry1Ab, Cry2Ab2, Cry34Ab1, Cry35Ab1, Cry3Bb, Cry3Bb1, and Cry1F (Roh *et al.,* 2007). Two of them, the Cry1A.105 and Cry2Ab2 are expressed in the transgenic maize MON 89034, which has been developed in America since 2009. The target insect pests of MON 89034 are *H. zea*, *O. nubilalis*, *O. furnacalis, S. frugiperda*, *Diatraea saccharalis,* and *D. grandiosella* (PSA, 2015). Bt toxins can also be employed to control *S. litura*. Cry1C is expressed in tobacco and is proven to increase the mortality of *S. litura* larvae from 76.9% to 100% within 72 hours after treatment (Lin *et al*., 2003). Furthermore, Cry1C, Cry1Ac, and Cry2Ab which are expressed in cotton are also demonstrated to influence the survival of *S. litura* by deterring the growth of the late instar larvae (Naik *et al*., 2013).

Bt toxin is known to have high specificity to their target pest up to the species level (Ruut *et.al.*, 2001). However, maize productivity may decline because of non-target pests which increase in number after the reduction of the main pest population. On the other hand, there is also report which stated the impact of Bt toxin on the non-target pest (BRAD, 2009). As *S. litura* and *O. furnacalis* belong to the same order, the Lepidoptera, therefore, the use of Cry1A.105 and Cry2Ab2 targeting *O. furnacalis* may also affect *S.litura*. The impact of both toxins on this army worm has yet to be determined by the release of transgenic maize in Indonesia. This study was arranged to determine the acute effects of Bt toxin Cry1A.105 and Cry2Ab2 on the development of *S. litura* larvae to its adult stage.

MATERIALS AND METHODS

The Collection and Rearing of Spodoptera litura

The starting population of *S. litura* was collected from cabbage plantation in Muntilan, Central Java. Thirty larvae were collected and reared in plastic jars (diameter 14 cm, height 6 cm), fed with organic pakcoy (*Brassica rapa*) leaves. The leaves were obtained from the nearby supermarket, and were cleaned before given to the insects. The larval containing jars were then sealed with a gauze. The diet was replaced every two days until the larvae pupating. The pupae were left in the jar until they molted to adults. The adults were paired and kept in the separate jar to mate. The jar was layered with a paper for eggs laying and were given the 10% honey solution. The resulted egss were collected and transferred to plastic vial (diameter 3.5 cm, height 4.5 cm). The jars were labelled with the eggs laying date. The third generation of first instar (neonate) were used in the treatment.

The Artificial Diet

Bioassays were carried out with the artificial diet. The diet recipe has been used for mass rearing of *O. furnacalis* in the Toxicology Laboratory of Universitas Gadjah Mada since 2009, and can also be used for *S. litura* larvae. The diet was consisted of red beans, wheat bran, yeast powder (fermipan), ascorbic acid, sorbic acid, vitamin mix, casein, tetracycline, agar, and distilled water. The beans were soaked in water for ± 24 hours, and then finely blended. Others ingredients (except agar) were added into the blender and mixed thoroughly. The agar was dissolved in distilled water. The blended ingredients were poured onto the agar and mixed completely. The mixture was then poured into the plastic trays and allowed to cool. The diet were finally stored in the refrigerator (5°C) until used.

The Bioassays

Determination of sublethal concentrations. The bioassay was preceeded by preparing the toxin solutions. Two Bt toxins namely Cry1A.105 and Cry2Ab2 (Monsanto, St Louis, Missouri, USA) were employed in this study. The original concentration of the stock toxins of Cry1A.105 and Cry2Ab2 were 96 and 31 ppm respectively. Half of these concentrations or 48 and 15.5 ppm were used in this bioassay, based on the previous assay conducted for *O. furnacalis* (Trisyono, 2015, unpublished). One ml of each toxins was mixed with 9 ml of buffer. Cry1A.105 was dissolved in the buffer containing 25 mM CAPS, pH 10.3, 1 mM benzamidine-HCl, 0.1 mM EDTA, and 0.2 mM DDT, while Cry2Ab2 was dissolved in the buffer containg 50 mM CAPS, pH 11, and 2 mM DDT. Ten ml of the two toxins were then diluted in 10 series of concentration to obtain the working solutions for bioassay. The working solutions for Cry1A.105 ranged from 48 to 0.0938 ppm, while for Cry2Ab2 their range was from 15.5 to 0.0008 ppm. However, when these solutions were used on the first instar of *S. litura*, only Cry2Ab2 that resulted in more than 50% mortality, while Cry1A.105 only gave less than 50% mortality (Attachment 1). Therefore, we doubled the highest concentration of Cry1A.105 from 48 to 96 ppm. Thus, the series of concentrations for Cry1A.105 ranged from 96 to 0.1875 ppm (96, 48, 24, 12, 6, 3, 1.5, 0.75, 0.375, and 0.1875 ppm), while the same range of concentrations of 15.5 to 0.0008 ppm (15.5, 5.1667, 1.7222, 0.5741, 0.1914, 0.0638, 0.0213, 0.0071, 0.0024, 0.0008 ppm) of Cry2Ab2 previously used for *O. furnacalis* were again utilized in this study. Increasing concentrations did not result a significant increase in mortality, therefore four out of the ten series of concentrations were employed to determine the sublethal concentratios (Table 1). These four concentrations were; the highest, two of the middle, and the lowest concentrations. The series concentration of Cry1A.105 used were 96, 6, 3, and 0.1875 ppm, while for Cry2Ab2 the series were 15.5, 0.1914, 0.0638, and 0.0008 ppm.

The toxins were administered through feed dipping (FD) method. The artificial diet was cut, weighed ± 1 g, and then dipped for 10 sec in toxin solution. The larvae on the control treatment were fed with the same amount of artificial diet previously dipped in distilled water. The diets were left to dry on a piece of filter paper for ± 15 minutes, before each of them was placed inside the test vials (diameter = 3.5 cm, hight = 4.5 cm). Ten first instar (neonate) were added to each vials. The vials were then properly labelled.

The larval mortality were observed on the third day and seventh day after treatment (DAT). Larvae which survived their third day were removed individually to the new vial equipped with a cube of toxin free diet. Each experiments was repeated five times. The mortality rates observed on 3 and 7 DAT were then used as the basis for the sublethal effect bioassay to the growth and development of *S. litura* larvae.

Table 1. The mortality of early molted larvae of *Spodoptera litura* treated with *Bacillus thuringiensis* toxins Cry1A.105 and Cry2Ab2 on 7 day after treatment

Toxin	Concentration (ppm)	No. of neonate larvae	Mortality (%)
Cry1A.105	96	50	72
	6	50	34
	3	50	36
	0.1875	50	40
	0	50	8
Cry2Ab2	15.5	50	52
	0.191	50	28
	0.064	50	36
	0.0008	50	46
	0	50	4

Effects of Cry1A.105 and Cry2Ab2. The lowest concentration and one fourth of the lowest concentration were used in this bioassay. Therefore, the series of toxin solutions used were 0.1875 and 0.0469 ppm for Cry1A.105 and 0.0008 and 0.0003 ppm for Cry2Ab2. In this bioassay, FD method was again employed under CRD. Each experiment was consisted of five replicates, while each replicate employed ten first instar. The larva was placed individually in each vial. A cube of toxin treated diet (1 g) was given to the larva and replaced with the toxin-free cube whenever it was finished.

The observation was conducted every day until the larvae reached the adult stage. The observed parameters were covering the mortality rate on 3 and 7 DAT, the weight of larvae and pupae, and the longevity of larvae and pupae. The larval weight was measured on 7 and 14 DAT, while the pupae were weighted on the second day after pupation. The weights were measured using electronic analitical scale (SHIMADZU AW 120 max 120 g d = 0.1 mg). Larval stage was counted from the first DAT until they pupated, while pupal stage was counted as the time needed from pupation to adult eclosion. The resulted data were analysed using ANOVA. Significant difference among the experiment were analyzed with Duncan's Multiple Range Test at 5% level using SAS 9.1.3 Portable software.

RESULTS AND DISCCUSSION

Determination of Sublethal Concentration

The mortality rate of *S. litura* larvae on 7 DAT treated with both Cry1A.105 and Cry2Ab2 were not very different from each other (Table 1). The mortality due to different concentration was not prominent although the difference between the lowest and the highest concentration reached 512× for Cry1A.105 and 19.683× for Cry2Ab2. Therefore, the lowest concentration (0.1875 ppm) and one fourth of the lowest concentration (0.0469) were chosen as the working concentrations for the rest of the studies. On Cry1A.105 the testing concentration was 0.1875 ppm and 0.0469 ppm, while on Cry2ab2 the concentrations of 0.0008 ppm and 0.0003 ppm were selected. These concentrations were then employed to test the effect of both Cry1A.105 and Cry2Ab2 on the survival of *S. litura.*

The Effect of Bt toxin Cry1A.105 on Spodoptera litura

Bt Cry1A.105 caused the mortality, reduxed weight gain, and prolong the length of larval stadium (Table 2). Larval mortality between the toxins treated and the control treatments was significantly different on both 3 and 7 DAT, and slightly different from one concentration of the treatment to another. On 3 DAT no mortality was observed on control treatment, while 12 and 16% of mortality were found on the concentration of 0.0469 and 0.1875 ppm respectively. Similarly, on 7 DAT only 2% mortality was observed in control treatment, while 20 and 28% of mortality was seen on the concentration of 0.0469 and 0.1875 ppm, respectively. Larval weight on 7 DAT was not significantly different among all concentration including control (ranging from 3.2 to 5.1 g), however on the 14 DAT the highest concentration of 0.1875 ppm was resulted in the significantly lighter larvae (117.0 g) than control (212.9 g), but not significantly different to the larvae treated with lower concentration of 0.0469 ppm (141.6 g). The length of the larval stadium was slightly longer on the Bt Cry1A.105 treated larvae with 22.3 and 24.5 days on the lower and higher concentration respectively, compared to the control with 20.5 days. The percentage of pupation was higher on the control treatment (72%), compared to the toxins treated larvae with 36 and 30% for 0.0469 and 0.1875 ppm respectively.

The weight of pupae and the percentage of pupae that reached the adult stage was slightly different between the toxins treated and the control, but similar among the toxins treated treatments (Table 2). The weight of male pupae on control treatment (211.2 g) was significantly higher than the pupae resulted from the treated larvae with the concentration of 0.1875 ppm (165.6 g), however they were similar to the pupae resulted from the larvae treated with the lower concentration of 0.0469 ppm (198.1 g). On the contrary, the weight of female pupae on control treatment (246.6 g) was similar to the pupae resulted from the larvae treated with the concentration of 0.1875 ppm (227.9 g), but significantly heavier to the pupae resulted from the larvae treated with lower concentration of 0.0469 ppm (205.4 g). The percentage of pupae that reached the adult stage was higher (61%) on control treatment compared to both the toxins treated treatments, with the percentage of these two were almost similar with 33.3 to 40% on the lower and higher concentration respectively. The length of pupal stadium was not different from each treatment, which was ranged from 9.3 to 10.2 days.

The Effect of Bt toxin Cry2Ab2 on Spodoptera litura

Similar to Cry1A.105, Cry2Ab2 was also caused larval mortality (Table 3). The mortality of the toxins' treated larvae was higher on both 3 and 7 DAT to the control, but not to each other. On 3 DAT no mortality was observed on control treatment, but 8 to 10% mortality were found on the concentration of 0.0003

Table 2. Mortality, growth, and development of newly hatched larvae of *Spodoptera litura* treated with *Bacillus thuringiensis* toxins Cry1A.105

Stage	Parameters of observation	Concentration (ppm)		
		0	0.0469	0.1875
First instar	No. larvae	50	50	50
	Mortality 3 DAT	0	12	16
	Mortality 7 DAT	2	20	28
	Weight (mg) 7 DAT	4.3 a	5.1 a	3.2 a
	Weight (mg) 14 DAT	212.9 a	141.6 ab	117.0 b
	Larval stage (day)	20.5 b	22.3 ab	24.5 a
	Larvae → pupae (%)	72	36	30
Pupae	Weight ♂ (mg)	211.2 a	198.1 ab	165.6 b
	Weight ♀ (mg)	246.6 a	205.4 b	227.9 ab
	Pupal stage (day)	10.2 a	9.3 a	9.5 a
	Pupae→ adult (%)	61.1	33.3	40

DAT (day after treatment); numbers followed by the same letter for each parameters in the same rows are not significantly different on 5% level according to Duncan's Multiple Range Test.

Table 3. Mortality, growth, and development of newly hatched larvae *Spodoptera litura* treated with *Bacillus thuringiensis* toxin Cry2Ab2

Stage	Parameters of observation	Concentration (ppm)		
		0	0.0003	0.0008
First instar	No. larvae	50	50	50
	Mortality 3 DAT	0	8	10
	Mortality 7 DAT	4	20	20
	Weight (mg) 7 DAT	3.0 a	3.3 a	3.4 a
	Weight (mg) 14 DAT	158.5 a	179.5 a	121.0 b
	Larval stage (day)	23.6 a	22.2 a	21.8 a
	Larvae → pupae (%)	52	32	38
Pupae	Weight ♂ (mg)	198.5 a	188.0 a	181.7 a
	Weight ♀ (mg)	237.1 a	199.9 a	197.1 a
	Pupal stage (day)	10.1 a	9.5 ab	9.1 b
	Pupae→ adult (%)	34.6	43.8	47.4

DAT (day after treatment); numbers followed by the same letter for each parameters in the same rows are not significantly different on 5% level according to Duncan's Multiple Range Test.

and 0.0008 ppm respectively. Similarly, on 7 DAT only 4% mortality was observed in control treatment, while 20% mortality was seen on both toxins concentrations. Larval weight on 7 DAT was not significantly different among all concentration and control (ranging from 3.0 to 4.3 g). However, on the 14 DAT while on the higher concentration of 0.0008 ppm was significantly lesser in weight (121.0 mg) than both the 0.0003 ppm (179.5 ppm) concentration and to the control (158.5 ppm). The length of the larval stadium was not different from each other treatments. The percentage of pupation was also higher on the control treatment (52%), compared to the toxins treated larvae with 32 and 38% for 0.0003 and 0.0008 ppm respectively.

The weight of pupae and the percentage of pupae that reached the adult stage not different between the toxins treated and the control (Table 2). The weight of male pupae on control treatment (198.5 mg) was not different from the pupae resulted from the treated larvae with the concentration of 0.0008 ppm (188.0 mg) and to those resulted from the larvae treated with the higher concentration of 0.0008 ppm (181.7 mg). The weight of female pupae in overal was higher than that of male, but also not significantly different in all treatments. On control treatment it was 237.1 mg, while on the lower and higher concentrations was 199.9 and 197.1 mg respectively. The percentage of pupae that reached the adult stage on control was surprisingly a little bit lower (34.6%) compared to both the toxins treated treatments with 43.8 and 47.4% on the lower and higher concentration respectively. The length of pupal stadium was not different from each treatment, which was ranged from 9.1 to 10.1 days.

Bt toxin Cry1A.105 and Cry2Ab2 caused variable rate of mortality on *S. litura* larvae (Table 2 and 3). Cry1A.105 was able to kill 12 to 16% of larvae on 3 DAT which rose around 1.7× on 7 DAT or 20 to 28%. The higher concentration of 0.1875 ppm gave a slight but not significantly higher mortality than the 0.0469 ppm. Cry2Ab2 performed much better than Cry1A.105, where with the concentrations that were 512× lower, both concentrations had killed 8−10% of larvae on 3 DAT, which was multiplied to 2−2.5× on 7 DAT to 20%. These results showed that Cry2Ab2 was better than Cry1A.105. This result was different from those on *O. furnacalis* and *S. frugiperda* where both toxins produced similar mortality rate of 100% and more than 95% respectively, or on *D. grandiosella* where Cry2Ab2 resulted in 95% mortality while Cry1A.105 caused 90−95% mortality (BRAD, 2009).

The similarity of the results between the two toxins might be caused by various factors. One of them might be the non-target nature of *S. litura*. Ruut *et al.* (2001) and George & Crickmore (2012) found that the host-specific nature of Bt toxins, might induce the production of different toxins crystal, which might not be suitable for the non-target pests. The selectivity of Bt toxin is determined by toxin structure and other factors on the body of the target insects. The binding process of the toxin onto the receptor in the midgut can only be successful whenever they are compatible to each other (Gill, 1995).

The administration of Bt toxin Cry1A.105 and Cry2Ab2 generated different effects on *S. litura* larval and pupal weights (Table 2, 3). Cry1A.105 reduced *S. litura* larval and pupal weight, while Cry2Ab2 did not. The larval weight on 3 DAT on the two toxin treatments were not different to control and to each other. However, on 14 DAT the higher concentration of Cry1A.105 (0.1875 ppm) significantly reduced larval weight compared to the control, but there was no significant different between the lower concentration of 0.0469 ppm to the control. Similarly, Cry1A.105 reduced pupal weight significantly, but no notable reduction was observed on the pupae resulted from the larvae treated with Cry2Ab2. These differences might be caused by the different amount of toxins being given, where Cry1A.105 concentrations were much higher than Cry2Ab2. According to Tampubulon *et al.* (2013), the appetite of *S. litura* larvae infected by Bt are reduced and they even ceased to eat, which further reduces larval weight and slowing down their movement.

The longevity of larvae and pupae of *S. litura* were influenced by different Bt toxins (Table 2, 3). Cry1A.105 prolonged the longevity of *S. litura* larval stage but did not affect the longevity of pupal stage, while Cry2Ab2 did not prolong the longevity of larval stage but that of pupal stage. Furthermore, both Cry1A.105 and Cry2Ab2 decreased the number of larvae that successfully molted to pupae (Table 2, 3). On both concentrations of Cry1A.105 the number of pupae were reduced to 0.4 to 0.48 of the control, while on Cry2Ab2 it was reduced to 0.7 to 0.6 of the control. Similarly, the number of successfully molted adults on Cry1A.105 were 0.54 to 0.65× of the control. However, this number on the control treatment of Cry2Ab2 was less than that on the toxins treated insects by 0.21 to 0.27. In general, these results were consistent with the findings of Bortolotto *et al*. (2015) with toxin Cry1F and Cry1F + Cry1A.105 + Cry2Ab2 can reduce larval growth of *S. eridania*, although this larva was not their insect target. According to Puspita (2015) Cry1A.105 sublethal concentration can decrease the rate of growth and delay the development of *O. furnacalis* larvae into the pupal and adult stages. Similarly, sublethal concentration of Cry2Ab2 also reduce the development of *O. furnacalis* larvae, cause larval mortality, decrease pupal weight, adults' fertility, and eggs viability. Moreover, larvae which survive to adult stage will produce male adult (Triyani, 2015).

The affect of Bt toxins were different. Even for target species from different countries or regions, sensitivities to expressed toxins vary widely. It cannot be expected that the same species-specific and even population-specific sensitivity to Bt toxins will apply between different environments and across continents. Local non target species like butterflies of conservation concern and heritage value may therefore be at risk (Bøhn *et al*., 2010). Monsato conducted extensive studies testing the Cry1A.105 and Cry1A.105 proteins for activity against a range of both target and non target insect species, the results also show that both the Cry1A.105 and Cry2Ab2 proteins are highly specific in insecticidal activity against lepidopteran insects and have little activity against non-lepidopteran insects (PSA, 2015).

CONCLUSION

Bt toxin Cry1A.105 and Cry2Ab2 targeting *O. furnacalis* could affect the non target *S. litura* survival. The administering Bt toxin Cry1A.105 and Cry2Ab2 on sublethal concentration produced different negative effect on the survival of *S. litura* in laboratory condition. Higher concentration of Bt toxin Cry1A.105 administered to *S. litura* larval diet was given higher mortality, lowered larval and pupal weight, prolonged larval stage and reduced the number of the resulted pupae.

However, higher concentration of Bt toxin Cry2Ab2 given to *S. litura* increased their larval mortality and prolonged pupal stage.

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Attachment 1. The mortality test of *Spodoptera litura* larvae on 3 and 7 DAT with *Bacillus thuringiensis* toxin Cry1A.105 and Cry2Ab2

Toxin	Concentration (ppm)	No. larvae	Mortality (%) ± SD	
			3 DAT	7 DAT
Cry1A.105	48	30	16 ± 0.89	36 ± 0.71
	0.593	30	18 ± 0.44	28 ± 1.15
	0.198	30	12 ± 0.44	13 ± 0.50
	0.002	30	8 ± 0.58	14 ± 0
	0	30	4 ± 0.55	4 ± 0
Cry2Ab2	15.5	30	18 ± 1.10	52 ± 0.84
	0.191	30	2 ± 0.45	28 ± 0.84
	0.064	30	14 ± 1.14	36 ± 0.89
	0.0008	30	18 ± 0.45	46 ± 0.89
	0	30	0 ± 0	4 ± 0.55

DAT (Day after treatment)