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Full Length Research Paper

Bioactivity of methanolic seed extract of *Barringtonia asiatica* L. (Kurz) (Lecythidaceae) on biological characters of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

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ABSTRACT

Plants are known to have various chemical compounds that have potential to be developed as insecticides. One of the potential plants to be developed as a source of insecticides is *Barringtonia asiatica* (Lecythidaceae). This research was conducted to determine toxicity of methanolic seed extract of *Barringtonia asiatica* to mortality and biological character of *Spodoptera litura*. The evaluation of toxicity was carried out using feeding method. Result of this research indicated that methanolic seed extract of *B. asiatica* had insecticidal activity with LC_{50} at concentration of 0.30% and LC_{90} at concentration of 0.80% in 13 days after treatment with LT_{50} at 4.8 days. In addition, methanolic seed extract of *B. asiatica* caused decrease of larval weight, tend to increase duration time of development, reduced leaf consumption and decrease of egg amount oviposited by female of *S. litura*.

Keywords: *Barringtonia asiatica*, seed, extract, mortality, biological character, *Spodoptera litura.*

INTRODUCTION

Various active ingredients originally from plants have been known and tested against insects. At least 2000 plant species have been reported toxic to various plant pests, and more than 850 active compounds from plants have been tested against insects (Grainge and Ahmed, 1988; Prakash and Rao, 1997). During the last decade there is improvement of big enthusiasm in seeking of insecticide compounds from plant (Schmutterer, 1995). Syahputra (2001) reported from various districts in Indonesia, there were more than 40 potential plant species that could be used as botanical insecticides.

Botanical insecticides have long been applied by the farmers. One of the plants having a potency to be developed as insecticide is *Barringtonia asiatica* Kurz (Lecythidaceae) with common name sea poison tree or in Indonesia known as bitung (Ecology and Evolutinonary Biology Greenhouse (EEBG), 2006).

B. asiatica is known to have active compounds which cause mortality to insect pests. Methanolic seed extract of *B. asiatica* was toxic to *Crocidolomia pavonana* with value of LC_{50} equal to 0.66% at 7 days after treatment (dat). The application of seed extract of *B. asiatica* also has influenced oviposition with effective concentration equal to 0.96% which causes *C. pavonana* female not to lay the eggs at the crop. Larval response indicated that the extract of *B. asiatica*, besides had toxic character also had antifeedant activity (Dono and Sujana, 2007). Therefore, methanolic seed extract of *B. asiatica* had a potency as insecticide. One of the active compounds in seed of *B. asiatica* is saponin (Herltz *et al*., 2002; Burton *et al*. 2003, Cannon *et al.* 2004). At some places *B.*

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asiatica is used as ancient medicine and fish poison (The Cook Island Nature Heritage Trust, 2005). Active compound in seed of *B. asiatica* which poisoned fish is group of saponin compounds (Tan, 2002; EEBG, 2006). One of the most toxic compounds against fish from *B. asiatica* seed extract is ranunkosida VIII (Burton *et al,* 2003). Research about seed extract of *B. asiatica* has been conducted but its toxicity to *Spodoptera litura*(Fabricius) (Lepidoptera: Noctuidae) has not been known. This research was conducted to know the influence of *B. asiatica* seed extract on mortality and biological characters of *S. litura.*

S. litura is widespread insect, polyphagous, and can eat all parts of crop, especially at dry season (Department of Indonesian Agriculture, 2005). Controlling of *S. litura* in general is still by using synthetic insecticide. Unfortunately, usage of synthetic insecticides has reported able to generate resistance of insect target, resurgence, and secondary pest out break. Other side impact of usage of synthetic insecticide is environmental contamination and killing of non-target organism (Profit, 1993). Therefore, an alternative insecticide, that was relatively safe for environment and has minimal or no side effect to non target organism, was required. An alternative method for controlling of the pest was botanical insecticide from seed extract of *B. asiatica*.

MATERIALS AND METHODS

Rearing of Test Insect *Spodoptera litura*

Test insect larvae, *S. litura,* was collected from Brassicaceae plantation in Lembang. Larvae obtained from the field were placed in plastic box and feed with taro leaf, and pupation took place in plastic box with sterile soil. Pupae were put in screen cage and adults emerged from pupae was fed with dilution of honey 10% which have been permeated in cotton. In the cage, some leaves and pieces of taro were placed for adult oviposition. Egg mass at taro leaf was removed into separate plastic box. At the time of egg hatching, larvae ware moved to bigger plastic box and fed with taro leaf.

Extraction of Seed of *Barringtonia asiatica*

Seeds of *B. asiatica* was obtained from the field (altitude: 750 m above the sea level) of Campus Universitas Padjadjaran, campus Jatinangor, Sumedang, West Java, Indonesia. Seed was taken away from the fruit, then sliced thin and wind-dried. The dried seeds were refined by using blender to form flour. Flour of the seeds at weight of 2969.34 g was soaked in 10.68 litres of methanol. Methanol was used as solvent because of active compound contain in seed of *B. asiatica* was polar compound. The solution was filtered using filter paper and evaporated using rotary evaporator at temperature of 40ºC with low pressure until all solvent evaporated and yielded of 781.48 g crude extract. The crude extract was stored in refrigerator at temperature ± 4ºC until being used.

Experiment 1. Insecticidal Activity of *B. asiatica* Against *Spodoptera litura*

The extract was tested against second instar larvae of *S. litura* using a leaf residue feeding method. *Extract* was tested at six concentrations that were expected to cause between 0 – 100% insect mortality. The range of concentrations was determined by preliminary test. The treatment with each concentration was replicated six times.

Examination was done with taro leaf dipping method. The extract diluted with mixture of aquadestilata with Agristic (0.05%). Two treated or control leaf disks were placed in glass petri dishes (9 cm in diameter) lined with towel paper, and 10 first instar *S. litura* were placed in each dish. The test larvae were fed with treated leaves for 72 hours, then fed untreated leaves until fourth instar. Parameter observed was mortality of insect.

Regression relationship between extract concentration and percentage of insect mortality were determined by probit analysis (Finney, 1971). Calculations of sublethal concentrations of extract were used for other experiments.

Experiment 2. The Effect of *B. asiatica* Seed Extract on Fecundity and Biological Character of *Spodoptera litura*

The treatment procedures were similar with the same as Experiment 1. Concentration of extract tested at this research equivalent with LC_{30}, LC_{50}, and LC_{70} determined at experiment 1. In each level concentration of test and control larvae was observed until becoming adult. Imagos that were successfully emerged from pupae were paired and then put into different plastic cage (diameter 6.5 cm, height 30 cm). The imago fed with diluted honey (30%) and taro leaf put in plastic container as egg trap.

Observation was done every day for larval weight using digital weighting-machine, feed wide eaten, pupa weight, emerge of adult, number of eggs oviposited during the female of adult lived. Larval weight was measured by electrical weighing-machine. While width of food leaf consumed was measured with paper millimeter block. Data obtained were analysed with analysis of variance and continued with multiple range test at significance level of 5%.

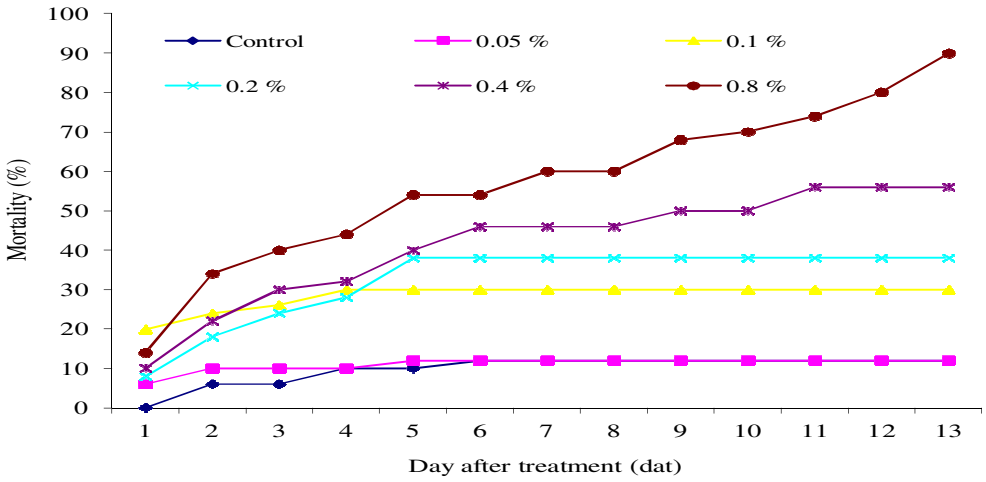

Figure 1. Mortality of *S. litura* larvae caused by treatment of methanolic seed extract of *B. asiatica*

Table 1. Parameter regression probit relationship between concentration of methanolic seed extract of *B. asiatica* and mortality of *S. litura*

Day analisys (DA)	a ± SE	b ± SE	Value of LC_{50} (%)	Confidential limits (95%)	Value of LC_{90} (%)	Confidential limits (95%)	g
3	-0.55 ± 0.26	0.71 ± 0.38	5.89	-	363. 53	-	1.01
5	-0.82 ± 0.24	1.08 ± 0.38	1.18	0.56 – 27.33	17.99	3.22 – 47.42	0.29
8	0.32 ± 0.23	1.27 ± 0.35	0.55	0.33 – 1.57	5.57	1.82 – 196.3	0.29
10	0.51 ± 0.23	1.46 ± 0.36	0.44	0.29 – 0.88	3.31	1.39 – 34.35	0.23
12	1.18 ± 0.28	2.37 ± 0.49	0.31	0.22 – 0.43	1.09	0.71 – 2.72	0.16
13	1.12 ± 0.26	2.15 ± 0.46	0.30	0.21 – 0.41	1.17	0.74 – 3.05	0.15

a = Intercept
b = Coefisien of regression
SE = Standard error
g = Potential index of significance

RESULTS AND DISCUSSION

Toxicity of Methanolic Seed Extract of *Barringtonia asiatica* to *Spodoptera litura* Larvae

Mortality of test insect increased equivalently with increasing concentration of methanolic seed extract of *B. asiatica* applied. In all treatment, larval mortality was seen since 1 day after treatment (dat). Improvement of mortality at concentration of 0.05%, 0.1%, and 0.2% was seen stable at 6 dat, while at concentration of 0.4% and 0.8%, improvement of mortality always happened until 11 dat (Figure 1).

Increase of test larval mortality was seen in the first day until 6 DAT. Increasing of test larval mortality after seventh DAT was not significant and at low concentration the larval mortality tend to be stable. The result of research conducted by Dono and Sujana (2007) indicated that methanolic seed extract of *B. asiatica* tested to *Crocidolomia pavonana* cause death and inhibit the development of larvae.

Bioactivity of active compound in methanolic seed extract of *B. asiatica* worked slowly with mortality response equal to 90% at concentration of 0.8% at 13 DAT. Saponin is principal active compound in extract of *B. asiatica* acted as stomach poison so that it doesn't cause death of larva immediately (Agrell *et al*. 2004).

Methanolic seed extract of *B. asiatica* has insecticidal activity to *S. litura* larvae. Based on result of lethal concentration (LC_{50}) analysis, the value of LC_{50} since 3 DATuntil 5 DATwere decreasing and stable at 12 DAT. Value of LC_{50} obtained at 13 DATwas equivalent with concentration of 0.30% (Table 1).

Amount of larval mortality generally occurred at initial instar compared late instar. Difference of level of defence in detoxyfication of poison compound in body causing

Table 2. Analysis LT_{50} methanolic seed extract of *B. asiatica* to S. *litura* larvae

Extract concentration (%)	a ± SE	b ± SE	Value of LT_{50} (days)	Confidential limits (95%)	g
0.2	-1.25 ± 0.31	1.01 ± 0.34	16.9	10.02 – 27.07	0.42
0.4	-1.09 ± 0.29	1.11 ± 0.33	9.5	6.22 – 20.64	0.33
0.8	-1.02 ± 0.29	1.74 ± 0.34	4.8	3.36 – 4.46	0.14

a = Intercept
b = Coefficient of regression
SE = Standard error
g = Potential index of significance
LT = Lethal time

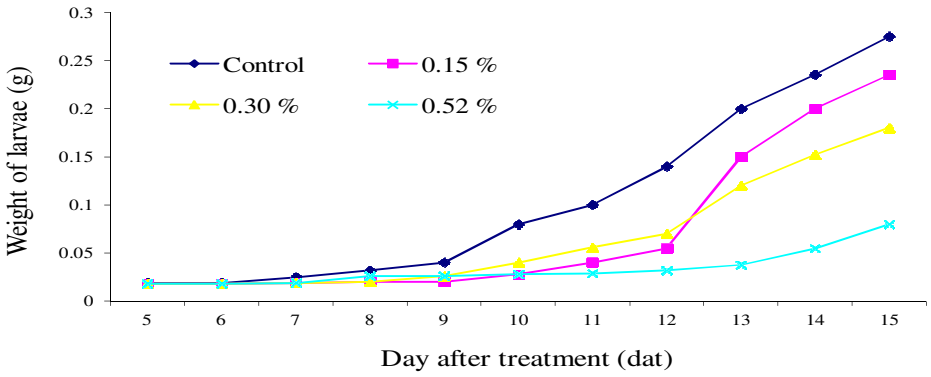

Figure 2. Average of weight of *S. litura* larvae on each methanolic seed extract of *B. asiatica* treatment at concentration equivalent with LC30 (0.15%), LC50 (0.30%), and LC70 (0.52%)

death response will differ in. According to Syahputra *et al.* (2006), if larva consumes active compoundm initial instar of larva more sensitive to toxic compound and would easily to die compared to late instar of larva because of the late instar has stronger defence reaction compared to initial instar.

Death symptom of larvae showed that initially the larvae were not active,the body looked smaller, seen wrinkled, and then dry. This was indicated that active compound in the form of saponin contain in methanolic seed extract of *B. asiatica* wasthe main cause of death of larva at all of treatment. According to Chaieb *et al.* (2001) saponins in the insect body can disturb cell permeability and cause decreased size of the cell because of losing of cell fluid. The breakdown this cell permeability is caused by the reaction between saponins with cholesterol and phospolipid of the cell membrane (Menger and Keiper, 1998).

B. asiatica seed extract at lower concentration of extract required more time to cause mortality of test insect compared with height concentration. *B. asiatica* seed extract at concentration of 0.8% was killing 50% of insect test slowly (LT_{50} equivalent with 4.8 days), while at concentration of 0.2%, value of LT_{50} far longer that was 16.9 days (Table 2). Saponin extracted from plant cause death of test insect sufficiently long (Agrell *et al.* 2004).

Influence of Methanolic Seed Extract of *B. asiatica* to Biological Character of *Spodoptera litura* Larvae

Influence of Methanolic Seed Extract of *B. asiatica* on Weight of Larvae

Larval weight was depended on quantum of food consumed. Decrease in feeding activity caused as a result of toxic compound eaten by larval test can influence growth rate and larval weight (Ambarningrum *et al.,* 2009). This caused difference in improvement of larval weight for every concentration of *B. asiatica* seed extract tested. Improvement of average of larva weight at control and concentration of 0.15% increasing so closing 0.3 g while at concentration of 0.30% and 0.52%, improvement of average of larva weight still below (under 0.2 g) (Figure 2).

Difference of average of larva weight in each concentration showed that at height concentration of methanolic seedextractof *B. asiatica* can reduce feeding

Table 3. Influence of *B. asiatica* seed extracts to leaf area consumed by *S. litura* larvae

Extract concentration (%)	Leaf area consumed at 2 dat (%) (x ± SE) (N)	Leaf area consumed at 4 dat (%) (x ± SE)(N)
Control	2.40 ± 0.86 (60) b	20.75 ± 3.07(60) c
0.15 (LC_{30})	1.88 ± 0.36 (60) b	14.30 ± 6.79 (58) b
0.30 (LC_{50})	1.87 ± 0.48 (60) b	12.26 ± 5.51 (40) b
0.52 (LC_{70})	0.93 ± 0.23 (60) a	5.05 ± 2.05 (38) a

The mean followed by different letter was significance different (α = 0.05)
x = average of leaf consumed (mm^2)
SE = Standard error
N = Number of alive larvae
dat = day after treatment

Table 4. The effect of methanolic seed extract of *B. asiatica* to developmental time of *S. litura* larvae (day)

Extract Concentration	Developmental Time Instar I–II (day) (x ± SE) (N)	Developmental Time Instar II–III (day) (x ± SE) (N)	Developmental Time Instar III-IV (day) (x ± SE) (N)
Control	2.0 ± 0.00 (60)	1.7 ± 1.64 (60)	3.0 ± 0.74 (58)
0.15 %	2.0 ± 0.00 (60)	1.9 ± 0.40 (46)	3.7 ± 0.48 (44)
0.30 %	2.0 ± 0.00 (60)	2.0 ± 0.22 (33)	4.0 ± 0.25 (29)
0.52 %	2.0 ± 0.00 (60)	2.2 ± 0.44 (26)	4.0 ± 0.24 (21)

x = Mean of duration of larval development (days)
SE = Standard error
N = Amount of larvae alive

activity of *S. litura,* causing decreasing of average of larval weight. Toxic compounds contained in seed extract of *B. asiatica* can influence response of insect physiology (Dadang and Prijono, 2008).

Influence of Methanolic Seed Extract of *B. asiatica* to Leaf Area Consumed by *Spodoptera litura* Larvae

Leaf area consumed by the test larvae indicated smaller with increase of the extract concentration (Table 3). The extract (saponin as main active compound contained in the methanolic seed extract of *B. asiatica*) indicated to act as antifeedant. Result of Herlt *et al*. (2002) research indicated that two main saponins isolated from *B. asiatica* shown anifeedant activity against *Epilachna*. The same results were indicated by some researchers (Adel *et al.,* 2000; Brown (2006); Szczepanik *et al*., 2001; Shinoda *et al*., 2002; Agerbirk *et al*., 2003; Agrell *et al*., 2003; Szczepanik *et al*., 2004).

Insect is a heterotroph which must consume other organisms to obtain molecules rich in energy which is needed to its growth and development (Meyer 2005). When the nutrition is not enough, it showed disturbance to metabolic process of the insect which would cause death or abnormal growth and development process.

Influence of Methanolic Seed Extract of *B. asiatica* to Duration of Larval Development

Methanolic seed extract of *B. asiatica* influenced development time of larvae from instar II until instar IV. At higher concentration of *B. asiatica* seed extract lengthen duration of development time of the test larvae (Table 4). When larvae consumes toxic active compound, some of larvae will death, while another larvae will maximize energy source in the body to eliminate the toxic compound so that the growth and development retarded (Syahputra *et al*., 2006; Tomia, 2008).

Influence of *B. asiatica* Seed Extract to Pupal Weight and Emergence of *S. litura* adult

B. asiatica seed extract influenced pupal formation and weight, and adult emergence (Table 5). Pupal formation at treatment was smaller than control pupae. This indicated that nutrition supplay annoyed at the larval stage. The growth of larvae becomes pursued and finally will influence development of *S. litura* larvae when forming pupa. Forming of imperfect pupa can reduce emergence of insect adult.

There was failure in pupal formation at treatment with

Table 5. Effect of *B. asiatica* seed extract on pupal forming and weight, adult emergence of *S. litura*

Treatment	Pupal forming (%)	Pupal Weight ± (SE) (g) (N)	Adult emergence (%)
Control	39.65	0.28 ± 0.34 (20)	0.69
0.15 % (LC_{30})	39.53	0.28 ± 0.23 (17)	0.53
0.30 % (LC_{50})	35.71	0.26 ± 0.21 (15)	0.52
0.52 % (LC_{70})	33.33	0.25 ± 0.17 (11)	0.45

SE = Standard Error
N = Number of insect test
g = gram

Table 6. Amount of egg laid by adult of *S. litura* emerged from test insect treated with methanolic seed extract of *B. asiatica* in larval period

Extract concentration (%)	Amount of eggs (x ± SE)
Control	1343± 0.22 a
0.15 (LC_{30})	1236± 0.12 a
0.30 (LC_{50})	1172 ± 0.42 a
0.52 (LC_{70})	1138 ± 0.15 a

x = Number of eggs reproduced
SE = Standard error

higher concentration of extract. It indicated the lack of nutrition supply to *S. litura* larvae as resulted inhibition of insect feeding activity by active compound of *B. asiatica* seed extract. In addition, failure in pupal formation could be due to decreasing of food consumption by the insect as a result of toxic compound (Adel *et al*., 2000).

The treatment with concentration of 0.15%, the pupa that was formed successfully, equal to 39.53% with emergence of adult equal to 0.53%, while at concentration of 0.52%, pupal formed only equal to 33.33% with emergence of adult only 0.45%. Pupa that was not formed perfectly at concentration of0.52% caused decreasing of adult emergence. The data indicated that there was decreasing activity of methanolic seed extract of *B. asiatica* which in line with decreasing of extract concentration.

Influence of Methanolic Seed Extract of *B. asiatica* to Fecundity of *Spodoptera litura* Female

Number of eggs yielded at all of treatments did not different (Table 6). This indicated that active compound in methanolic seed extract of *B. asiatica* did not caused decrease of reproduction level of *S. litura* with indicated that most of toxic compound that successfully enters into larval body was successfully neutralized when caterpillar grows from initial instar towards final instar in parallel with moulting process of insect.

The value followed by the same letter not significantly different (Duncan test at α= 0.05)

The findings above increasingly strengthens estimation that active compound activity coming into larval body of *S.litura* as a result of treatment of methanolic seed extract of *B. asiatica* will decline and disappear in line with growth and development from larval stadium, pupa and then adult. Defence mechanism in insect body will try to eliminate toxic compound coming into the insect body and cause imago to successfully emerge from pupa will return to normal growth and can lay eggs again normally.

CONCLUSION

Result of this research indicated that methanolic seed extract of *B. asiatica* had insecticidal activity with LC_{50} at concentration of 0.30% and LC_{90} at concentration of 0.80% in 13 days after treatment with LT_{50} at 4.8 days. In addition, Methanolic seed extract of *B. asiatica* coused decrease of larva's weight, time of development, reduced leaf consumption and decrease of egg amount oviposited by female of *S. litura*. Therefore, methanolic seed extract of *B. asiatica* has potential to be developed as an insecticide to control *Spodoptera.*

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REFERENCES

Adel MM, Sehnal F, Jurzysta M (2000). Effects of alfalfa saponins on the moth *Spodoptera littoralis*. Journal of Chemical Ecology 26, 1065-1078

Agerbirk N, Olsen CE, Bibby BO, Frandsen HO, Brown LD, Nielsen JK, Renwick AA (2003). A saponin correlated with variable resistance of *Barbarea vulgaris* to the diamondback moth *Plutella xylostella*. *Journal of Chemical Ecology* 29, 1417-1433

Agrell J, Anderson P, Oleszek W, Stochmal A, Agrell C (2004). Combined effects of elevated CO2 and herbivore damage on alfalfa and cotton. *J. Chem. Ecol.* 30, 2309-2324

Agrell J, Oleszek W, Stochmal A, Olsen M, Anderson P (2003). Herbivore induced responses in alfalfa (*Medicago sativa*). *J. Chem. Ecol.* 29, 303-320

Ambarningrum TB, Pratiknyo H, Priyanto S (2009). Indeks Nutrisi dan Kesintasan Larva *Spodoptera litura* F. yang Diberi Pakan Mengandung Ekstrak Kulit Jengkol (*Pithecellobium lobatum* Benth.). Jurnal HPT Tropika. Volume 9, No. 2: 109-114. Tahun 2009.

Burton RA, Wood SG, Owen NL (2003). Elucidation of a new oleanane glycoside from *Barringtonia asiatica*. ARKIVOC 2003(xiii). Department of Chemistry and Biochemistry, Brigham Young University, Provo. *Utah 84602 page*. 137-146. Available online at http://www.arkat-usa.org/get-file/19025/ (accesed on December 2010).

Brown AE (2006). Mode of action of landscape insecticides and miticides. Department of Entomology, Maryland Cooperative Extension, university of Maryland.

Chaieb I, Halima-Kmel MB, Hamouda MHB (2001). Effect of diet addition with *Cestrum parquii* (solanaceae) extract on some Lepidoptera pests *Pieris brassicae* (Pieridae) and *Spodoptera littoralis* Boisduval (Noctuidae). Med. Fac. Lanbouww. Unigent, 66/2a: 479-480.

Dadang Prijono D (2008). Insektisida Nabati; prinsip, pemanfaatan dan pengembangan. Departemen Proteksi Tanaman, Fakultas Pertanian, Institut Pertanian Bogor, Bogor. pp. 1- 65.

Departement of Indonesian Agriculture (DEPTAN) (2005). Ulat Grayak (online). Tersedia: http://www.detan.go.id/ditlinhorti/opt/bw_merah/ulat_grayak.htm (accesed on December 2010)

Dono D, Sujana N (2007). Aktivitas insektisida ekstrak daun, kulit batang, dan biji *Barringtonia asiatica* (Lecythidaceae) terhadap larva *Crocidolomia pavonana* (Lepidoptera : Pyralidae). Disampaikan pada Simposium Nasional PEI. Revitalisasi Penerapan PHT dalam Praktek Pertanian yang Baik. Menuju Sistem Pertanian Berkelanjutan, Sukamandi 10 – 11 april 2007.

Dono D, Wirahadian I (2007). Ekstrak Metanol Biji *Barringtonia Asiatica* (Lecythidaceae): Pengaruh Letal dan Antioviposisi terhadap *Crocidolomia Pavonana* (Lepidoptera: Pyralidae). Agrikultura 18 (3), 217-224.

Ecology and Evolutionary Biology Green House (2006). *Barringtonia asiatica* kurz. Universit of Connecticut. Available online at: http//www.eeb.unconn.edu/acc_num/200201850 html. Accesed on April 2010.

Herlt, AJ, Mander LN, Pongoh, Rumapuk RJ, Tarigan P. 2002. Two major saponins froem seeds of *Barringtonia asiatica*: putative antifeedants toward *Epilachna* sp. Larvae. (Abstract). J. Nat Prod. 65 (2):115-120. Available online at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieveanddb=PubMedandlist_uids=11858740anddopt=Abstract (Acessed on January 23, 2011)

Kardinan A (2000). Pestisida Nabati. Seminar Nasional PHT Promo 2000. Bandung. 18 hlm.

Kardinan A (2005). Pestisida Nabati dan Teknik Aplikasi. Penebar Swadaya Jakarta. Hlm 87.

Maryam Purbadi (1997). Uji Kemangkusan Beberapa bahan Insektisida Botanik terhadap Hama Perusak Daun Melati *Palpita unionalis* Hubn. Jurnal Holtikultura. Vol. 7 No.1. 550-556.

Menger FM, Keiper JS (1998). Digtonin as a chemical trigger for the selective transformation of giant vesicle. Angew. Chem. Int. Ed.

Meyer JR (2005). Insect nutrition NC State University. Available online at http://www.cals.ncsu.edu/course/ent425/tutorial/nutrition.html 9 accesed on January 12, 2011)

Shinoda T, Nagao T, Nakayama M, Serizawa H, Koshioka M, Okabe H, Kawai A (2002). Identification of a triterpenoid saponin from a crucifer, *Barbarea vulgaris*, as a feeding deterrent to the diamondback moth, *Plutella xylostella*. *J. Chem. Ecol.* 28: 587-599.

Syahputra E (2001). *Hutan Kalbar sumber pestisida Botanic :Dulu, Kini, dan Kelak* (Online) Tersedia : http://rudyct.tripod (Accesed on Oktober 2010).

Syahputra E, Prijono D, Dadang, Manuwoto S, Darusman LK (2006). Respon Fisiologi *Crocidolomia pavonana* terhadap Fraksi Aktif *Calophyllum soulattri*. Jurnal Hayati. Volume 13, No.1, Maret 2006. hlm. 7 – 12.

Szczepanik M, Bialy Z, Jurzysta M (2004). The insecticidal activity of saponins from various *Medicago* spp. against Colorado potato beetle, *Leptinotarsa decemlineata* Say. *Allelopathy J.* 14, 177-185.

Szczepanik M, Krystkowiak K, Jurzysta M, Bialy Z (2001). Biological activity of saponins from alfalfa tops and roots against Colorado potato beetle. *Acta Agrobotanica* 54 (2), 35-45.

Tomia A (2008). Penggunaan ekstrak bawang hutan sebagai insektisida botani untuk menekan pertumbuhan *Spodoptera litura* F. secara *in-vitro*. Jurnal Ilmiah Agribisnis dan Perikanan (Agrikan UMMU-Ternate). Volume 1 Oktober 2008.