Sublethal effect of physic nut wangi variety oil (Jatropha curcas L.) on Helicoverpa armigera Hubner

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Abstract. The use of chemical pesticides has a variety of negative impacts that are harmful to the environment and humans. Therefore, it is necessary to develop environmentally friendly pest control techniques. To support the development of environmentally friendly pest control techniques, research related to the sublethal of physic nut wangi variety oil on H.armigera. is needed. The experimental design used Completely Randomized Design (CRD). The test used 6 level concentrations (0.1gr/L, 5ml/L, 10ml/L, 20ml/L, and 40 ml/L). Analysis of variance (ANAVA) at α 95% followed by Duncan multiple test at α 5% was carried out. The result of sublethal effect of physic nut test showed that concentration of physic nut wangi variety and IP2A provenance oil did not cause mortality of H. armigera larvae, but inhibited the growth such as indicated by lower weight of pupae and prepupae in treated larvae than in control. The highest percentage of inhibition of prepupae and pupae weight was found in physic nut wangi variety oil with concentration 40 ml/L that show respective results of 57,41% and 65,38%. The highest percentage of inhibition of the number of eggs on next generation, number of succeeded eggs transform into larvae and percentage of succeeded eggs transform into larvae were found in physic nut IP2A provenance oil with concentration 40 ml/L that show respective results of 96,70% and 98,42%. The increasing concentration of physic nut wangi variety and IP2A provenance oil caused a significant increase in the sub lethal effect when the larvae were exposed to these oils. Physic nut wangi variety and IP2A provenance oil can be developed into botanical pesticides because it has the advantage of leaving residues that are easily biodegradable, resulting in secondary metabolic that can be used as a poison, safe for natural enemies and can be used as component in integrated pest management

1. Introductions

Helicoverpa armigera is a significant pest on several agricultural commodities in Indonesia. Severe plant damage due to this pest could decrease the crop production significantly. The control of this pest is mainly achieved by the use of chemical insecticide such as endosulfan, methomyl, monocrotophos, quinalphos, chlorpyriphos, fenvalerate and cypermethrin [1]. Contrarily, the use of the insecticide has lead to serious environmental pollution (water, air, and soil), pest resistance, pest resurgence, mortality of natural enemy, and toxicity to human and non target organism [2,3]. Therefore, the need of the future is to develop an eco-friendly approach to controlling insect pests that should be able to regulate pest populations and safe to the environment. Lately, the use of botanical pesticide offered new possibilities of promising control techniques against insect pests.
The use of botanical pesticides has developed since the Persian era but since the discovery of chemical pesticide, the use of chemical pesticide increasing every year and leads to out of control [4]. There are several conditions to be able to develop botanical pesticides, such as plant material easily found, supply in large quantities is not constrained, safe for the environment, safe for non-target organisms, safe for natural enemies, safe for humans, and has lethal effect to insect pest. Botanical pesticide has many advantages over chemical pesticide, such as minimal health hazards and environmental pollution, safe to non-target organism, no adverse effect on plant growth, and safe for humans [5,6].

The physic nut wangi variety those that have oil ingredients have not been widely studied in Indonesia. On the other hands, physic nut has been investigated for its usefulness in several countries including: China, the Philippines, United States, Thailand, India, Singapore, and several other developing countries. In addition to functioning as biofuels or biofuel, it also can be used as an antimicrobial and molluscicidal, and insecticidal. The oil extract from physic nut wangi variety are known to contain chemicals that affect insect life, namely the cusrin, phorbol esters and triglycerides. This chemical contains proved to have high effectiveness on *H. armigera*, *Pectinophora gossypiella*, *Anrasca bigattula*, *Ostrinia nubilalis*, *Sesamia nonagrioides*, *Busiole fusca*, *Callobruchus maculates*, *Sitophilus zeamaic*, *Bombyx mori*, and *Coptotermes vastator* [7,8,9,10]. The chemical content of physic nut oil could lead to death in *Achaea janata*, *Spodoptera litura*, and *Crocidolomia binotalis* [11].

The physic nut wangi variety oil contains insecticide chemical content and could be developed as botanical pesticides to control pest especially *H. armigera*. The use of physic nut wangi variety oil is still limited, and so far there are no document on their potentials for a wider adoption for insect control. Therefore, further development of botanical pesticide based on physic nut wangi variety oil is needed. The quantification of the lethal effect of physic nut oil against insect pest has been traditionally used to determine appropriate lethal doses for pest control [12]. Nevertheless, the estimated lethal doses may only be a one part measure of the deleterious effects. If we only look on the lethal effect of botanical pesticide, it difficult to compete with a chemical pesticide, because botanical pesticide has a slow activity to kill insect pest in hours. Sublethal effect usually influence insect pest besides lethal effect in the field condition [13].

Information on the sublethal of physic nut wangi variety oil has not been available. Therefore, it is necessary to do research on this topic to provide information about the use of physic nut wangi variety oil as an insecticide that can be used by farmers to control larvae of *H.armigera* in an eco-friendly way. The objective of this study is to determine the sublethal of physic nut wangi variety oil on *H.armigera*.

2. Materials and Methods

2.1. Experimental Insect

The research was conducted at the Laboratory of Pest and Disease of Indonesian Sweetener and Fiber Crops Research Institute from May to November 2011. The research used bollworm (*H.armigera*). This larvae were collected from Corn and Cotton plantation in Karangploso Experimental Station, Malang, East Java. Larvae from the field were allowed to feed on corn (*Zea mays*) in a plastic jar (1.5 kg) that was covered with a gauze. The larvae were mass rearing in laboratory condition. They were kept until developed into pupae. When larvae was transform into pupae stadium, this pupae were transfered to another plastic jar up to adult emergence. Male and female moths were allowed to mating and produce egg masses. The egg masses were kept for hatching. The newly hatching larvae were collected and reared on artificial diet to produce 2nd instar. The second instar of F2 generation were used for bioassays.

2.2. Physic nut wangi variety oil extraction

The physic nut wangi variety and IP2A provenance seeds were collected from physic nut fields in Karangploso Experimental Station and surrounding area of Malang, East Java. Phyic nut that used in this research were wangi variety and IP2A provenance. Two kilograms of physic nut seeds were dried to obtain a moisture content of approximately 15 %, and then the seeds were cut into small pieces.
approximately 2 - 4 cm in length. This material was put into manual oil press machine and pressed. From this machine we obtain the oils and it was used for bioassay.

2.3. The sub lethal effect of physic nut wangi variety on H. armigera
In order to ascertain the sub lethal effect of physic nut wangi variety, a test was carried out with concentrations of 5 ml/L, 10 ml/L, 20 ml/L, 40 ml/L, 1 gr detergent/L and water as control. The experimental design Completely Randomized Design (CRD). On this test we used physic nut wangi variety and as toxicity level comparison we used IP2A provenance. Each of concentration rates including control used 25 second instar larvae. The corn fruit was replaced with new one daily. The larvae were cultured until developed into next generation to known the sub lethal effect of physic nut oils on this pest. The sub lethal effect parameter were weight of prepupae and weight of pupae, number of eggs on next generation, number of eggs, number of succeeded eggs transform into larvae and percentage of succeeded eggs transform into larvae.

2.4. Data analysis
The results were analyzed by ANOVA and if the results were significantly different (α 5%), continued with Duncan Multiple Range Test.

3. Result and Discussions
In addition to the lethal effect, physic nut wangi variety oil has a sub lethal effect. This sub lethal effect is needed to support the development of physic nut wangi variety oil as a botanical pesticide. Because the presence of sub lethal effects provides added value to the function of botanical pesticides to compete with chemical pesticides.

Table 1. Effect of physic nut wangi variety on prepupae and pupa weight of H. armigera

| Concentration            | weight of prepupae (gr) | Percentage inhibition of prepupae (%) | weight of pupae (gr) | Percentage inhibition of pupae (%) |
|--------------------------|-------------------------|--------------------------------------|---------------------|----------------------------------|
| Control (water)          | 0.54 *a                 |                                      | 0.52 a              |                                  |
| Control (1 g detergent/L)| 0.53 a                  |                                      | 0.51 a              |                                  |
| 5 ml/L physic nut oil    | 0.40 b                  | 25.93 a                              | 0.31 b              | 40.38 a                          |
| 10 ml/L physic nut oil   | 0.35 c                  | 35.19 b                              | 0.28 b              | 46.15 a                          |
| 20 ml/L physic nut oil   | 0.32 c                  | 40.74 c                              | 0.24 c              | 53.85 b                          |
| 40 ml/L physic nut oil   | 0.23 d                  | 57.41 d                              | 0.18 d              | 65.38 c                          |

* The average in colloum followed by the same letter is not significantly different by DMRT (α = 5%).

Table 2. Effect of physic nut wangi variety on number of eggs, number of succeeded eggs transform into larvae, and percentage of succeeded eggs transform into larvae of H. armigera

| Concentration            | Number of eggs | Percentage inhibition of number of eggs (%) | Number of succeed eggs transform into larvae | Percentage inhibition of succeed eggs transform into larvae (%) | Percentage of succeed eggs transform into larvae (%) |
|--------------------------|----------------|--------------------------------------------|--------------------------------------------|---------------------------------------------------------------|--------------------------------------------------|
| Control (water)          | 661.0* a       |                                            |                                            | 645.75 a                                                      | 98.03 c                                         |
| Control (1 g detergent/L)| 645.5 a        |                                            |                                            | 634.25 a                                                      | 98.26 c                                         |
| 5 ml/L physic nut oil    | 274.75 c       | 58.85 b                                    | 86.0 b                                    | 86.68 a                                                      | 36.68 b                                         |
| 10 ml/L physic nut oil   | 342.25 b       | 48.22 a                                    | 80.5 b                                    | 87.52 a                                                      | 24.34 a                                         |
| 20 ml/L physic nut oil   | 372.0 b        | 43.72 a                                    | 76.0 c                                    | 88.22 a                                                      | 20.48 a                                         |
| 40 ml/L physic nut oil   | 286.5 c        | 56.66 a                                    | 55.87 d                                   | 91.35 b                                                      | 19.50 a                                         |

* The average in colloum followed by the same letter is not significantly different by DMRT (α = 5%).
In order to ascertain the sub-lethal effect of physic nut wangi variety oil on *H. armigera*, an observation was conducted on the development of *H. armigera* which had been applied with physic nut wangi variety oil by using the parameter of weight of pre pupae and weight of pupae, number of eggs on next generation, number of succeeded eggs transform into larvae and percentage of succeeded eggs transform into larvae.

After application of physic nut oil, some larvae escape from death and could neutralize the toxin which enter to the haemocoel and then larvae continue growth to the next stage. The concentration of physic nut wangi variety and IP2A provenance oil did not cause mortality of *H. armigera* larvae, but inhibited the growth such as indicated by lower weight of pupae and prepupae in treated larvae than in control. The application of physic nut wangi variety and IP2A provenance oil were able to inhibit the weight of pre pupae respective results of 57,41% and 55,55% (Table 1 and 3). The highest percentage of inhibition of prepupae and pupae weight was found in physic nut wangi variety oil with concentration 40 ml/L that show respective results of 57,41% and 65,38%.

Besides effect to the weight of prepupae and pupae, the application of physic nut wangi variety and IP2A provenance also inhibited the number of eggs on next generation, number of succeeded eggs transform into larvae and percentage of succeeded eggs transform into larvae such as indicated by lower the number of eggs on next generation, number of succeeded eggs transform into larvae in treated larvae than in control. The application of physic nut wangi variety and IP2A provenance oil were able to inhibit the number of eggs on next generation and number of succeeded eggs transform into larvae respective results of 56,66%, 91,35%, 96,70%, and 98,42% (Table 2 and 4). The highest percentage of inhibition of the number of eggs on next generation, number of succeeded eggs transform into larvae and percentage of succeeded eggs transform into larvae were found in physic nut IP2A provenance oil with concentration 40 ml/L that show respective results of 96,70% and 98,42%. The increasing concentration of physic nut wangi variety and IP2A provenance oil caused a significant increase in the sub lethal effect when the larvae were exposed to these oils.

### Table 3. Effect of physic nut IP2A provenance on prepupae and pupa weight of *H. armigera*

| Concentration          | weight of prepupae (gr) | Percentage inhibition of prepupae (%) | weight of pupae (gr) | Percentage inhibition of pupae (%) |
|------------------------|-------------------------|---------------------------------------|----------------------|-----------------------------------|
| Control (water)        | 0,54* a                 | 0,43 a                                |                      | 95,48 d                           |
| Control (1 g detergent/L) | 0,53 a                | 0,41 a                                |                      | 95,48 d                           |
| 5 ml/L physic nut oil  | 0,41 b                  | 24,07 a                               | 0,35 b               | 96,70 a                           |
| 10 ml/L physic nut oil | 0,35 c                  | 35,19 b                               | 0,28 c               | 98,42 a                           |
| 20 ml/L physic nut oil | 0,30 c                  | 44,44 c                               | 0,21 c               | 98,42 a                           |
| 40 ml/L physic nut oil | 0,24 d                  | 55,55 d                               | 0,14 d               | 98,42 a                           |

*The average in column followed by the same letter is not significantly different by DMRT (α = 5%).

### Table 4. Effect of physic nut IP2A provenance on number of eggs, number of succeeded eggs transform into larvae, and percentage of succeeded eggs transform into larvae of *H. armigera*

| Concentration          | Number of eggs | Percentage inhibition of number of eggs (%) | Number of succeeded eggs transform into larvae | Percentage inhibition of succeeded eggs transform into larvae (%) | Percentage of succeeded eggs transform into larvae (%) |
|------------------------|----------------|---------------------------------------------|-----------------------------------------------|-----------------------------------------------------------------|---------------------------------------------------|
| Control (water)        | 698,00* a      | 666,50 a                                    | 95,48 d                                      | 95,48 d                                                        |
| Control (1 g detergent/L) | 628,50 a      | 666,50 a                                    | 95,48 d                                      | 95,48 d                                                        |
| 5 ml/L physic nut oil  | 115,75 b       | 83,52 a                                     | 93,61 a                                      | 93,61 a                                                        |
| 10 ml/L physic nut oil | 163,00 b       | 76,65 a                                     | 93,69 a                                      | 93,69 a                                                        |
These oils contain toxin such as phorbol ester and curcin. The chemical content of phorbol ester in provenance IP2A oil = 7.8 μg / ml and curcin = 22.6 μg / ml. Whereas the chemical content of phorbol ester in physic nut wangi variety = 3.76 μg / ml and curcin = 46.03 μg / ml. This phorbol ester content is toxic to *Achaea janata*, Thrips, and *Bombyx mori* silkworm [14,15,16].

The survival larvae had the ability to neutralize or detoxify the poison that enter the body so that the poison did not cause death. However, the consequence was that the detoxification process needs energy from the body, which should have been used for growth and development of the insect. This naturally resulted in an inhibition of the insect growth, vis-à-vis a healthy insect [17]. This was the reason why the application of the physic nut wangi variety and IP2A provenance oils caused the lower weight of prepupae and pupa compared with the control. The number of eggs produced from an imago from each concentration is strongly influenced by the content of food / nutrients consumed by insects. Food ingredients that are consumed are ingredients that have contained toxins from physic nut oil. Phorbol ester from physic nut oil has enter to the body and accumulated in the haemocoel which results in nerve damage and decreased number of eggs. Some eggs do not hatch and eggs become sterile. Phorbol ester content which is toxic can affect the ovipositor, as anti-oviposition and ovicidal in insects namely glycerol fatty acids and triterpene pentacyclic acids [18,19].

Physic nut wangi variety oil has the chemical phorbol ester which can function as a contact poison and stomach poison. Physic nut wangi variety oil can cause abnormal growth of *H. armigera* larvae, reduced and fertile egg hatching percentage. There is a type of hormone that affects when skin changes [20]. Besides causing a decrease in egg hatchability, phorbol ester compounds when accumulated in insects will affect nerve and digestive tract cells, disrupting the regulation of metamorphosis, especially the analysis and treatment. These compounds also affect the nervous function of insects [21]. The effect of the phorbol esters content in physic nut wangi variety oil has a function as an effective poison against *H. armigera*. Physic nut wangi variety oil can be used as a botanical pesticide and effectively kill larvae from three corn caterpillars *O. nubilalis*, *S. monogrioides*, *B. fuscescens* [22,23,24]. It is even toxic to *Bombyx mori* caterpillars, *Coptotermes vestator*, white mice, anti-bacterial, anti-virus, and molluscs [25,26].

The quality of eggs produced by a female imago can also be affected by nutrition in the needs of insect life cycle. Another concentration treatment in provenance IP 2 A shows the effect of decreasing egg production, because it is influenced by the chemical content in physic nut oil. Phorbol ester content which is toxic can affect the ovipositor, as an anti-oviposition and ovicidal in insects, namely glycerol and pentacyclic acid triterpene [17].

Physic nut wangi variety and IP2A provenance oil can be developed into botanical pesticides because it has the advantage of leaving residues that are easily biodegradable, resulting in secondary metabolic that can be used as a poison, safe for natural enemies and can be used as component in integrated pest management [25]. Phorbol esters that activate cellular targets important C Chinese protein (CCP) are important toxic components, which can cause poisoning. Phorbol esters not only affect the workings of metabolism but also affect the morphology of insects into infertility, and maturity of sexual hormones, and result in a decrease in egg and egg production to become fertile.

### 4. Conclusions

The concentration of physic nut wangi variety and IP2A provenance oil did not cause mortality of *H. armigera* larvae, but inhibited the growth such as indicated by lower weight of pupae and prepupae in treated larvae than in control. The highest percentage of inhibition of prepupae and pupae weight was found in physic nut wangi variety oil with concentration 40 ml/L that show respective results of 57.41% and 65.38%. The highest percentage of inhibition of the number of eggs on next generation, number of succeeded eggs transform into larvae and percentage of succeeded eggs transform into larvae were found

| Concentration      | Mean Weight Prepupae | Mean Weight Pupa | Mean Weight Egg Hatching Percentage |
|--------------------|-----------------------|------------------|--------------------------------------|
| 20 ml/L physic nut oil | 28.50 c | 95.84 b | 14.25 d | 97.86 a | 3.93 a |
| 40 ml/L physic nut oil | 23.75 d | 96.70 b | 10.50 d | 98.42 a | 21.19 b |

*The average in colloun followed by the same letter is not significantly different by DMRT (α = 5%).

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