Mixed Formula of Neem Leaves Extract and Curcumin as Botanical Insecticides for Sustainable Agriculture

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Abstract. Neem extract posses important bioactivities such as insect repellent, antifeedant, growth inhibitor, and other insecticidal properties, while curcumin has many bioactivities as antioxidant, antiinflamation, antibacterial, as well as anticancer. Both natural substances were mixed as botanical insecticides formula. Its efficacy against bean pod sucking bugs in vitro were evaluated. Several stages were done in this research, i.e. neem leave extraction, curcumin extraction, mixed formula production, and efficacy evaluation of mixed formula against bean pod sucking bugs in laboratory. The result of research showed that the mixed formula of the botanical insecticides containing neem leaves extract and curcumin caused mortality of bean pod sucking bugs in vitro significantly. The mortality percentage of bugs was up to 96,25%. Therefore, it is emphasized that mixed formula of botanical insecticides containing natural substances is an ecofriendly product for agriculture.

1 Introduction

Indonesia as a country with the second largest biodiversity in the world, has various flora which is as source of environment-friendly active substances. This condition is a big potency for developing a research and pesticide based on local plants.

One of the plants used as botanical insecticides is neem trees (*Azadirachta indica*). This plants contain active substance azadirachtin, which is mostly found in its leaves and seeds. The neem botanical insecticides is popular for having wide spectrum and is proven to control 300 kinds of pests. However, the effectiveness of this botanical insecticides is hindered by its character of being broken down by sun shine easily so that its efficacy decrease and must be applied repeatedly. This problem causes unpractical use of botanical insecticides and the effect, the farmers are not interesting in using it. Therefore, the protection toward active substance is needed so that its efficacy is high and durable.

Chemical substance addition functioning as botanical insecticides stabilizer such as tertiary butyl-p-cresol, 8-hydroxy quinoline, and tertiary butyl hydroquinon, has been done and even patented in India. Those three chemical subtances are proven to extend half life and improve azadirachtin stability to sun shine. Unfortunately, the chemical stabilizer added to the botanical insecticides give bad effect to human health. The rec idue carried away in various agriculture product and is consumed by human being can irritate skin, eyes, throat and causes liver disorder and fetus as well as very poisonous to aquatic organism. Based on those facts, the availability of safe and natural stabilizer to agricultural product and human healthy as well as being capable of protecting botanical inseticides from photodegradation is needed. One of the natural stabilizers fullfilled those criteria is curcumin compound.

Curcumin is a secondary metabolite, natural felonic from *Curcuma domestica*. Many researches had been done on curcuma potency as antioxidant, antiinflamation, anti-bacterial, and anticancer. In this research, the addition of curcumin in the neem botanical insecticides was expected can improve its efficacy and so far, the role of curcumin as antibacterial in improving botanical insecticides efficacy has not been done yet.

One of pests controlled by using the neem botanical insecticides is bean pod sucking bugs, *Riptortus linearis*. This bugs were considered as one of major pests in soybean crops which can decrease the production until 80%. Many attemps were done to control this bugs by using chemical insecticides which clearly give bad effect to human beings and environment. Therefore, a safe and environment-friendly control is necessary by using the neem botanical insecticides. As an agricultural country, Indonesia has not been successful in achieving self-supporting soybeans. Every year, Indonesia has to import soybeans for 60-65% from all national soybeans needs which is 220 tons.

This research was undertaken to in vitro evaluate the efficacy of the botanical insecticides containing mixed neem leaves extract and curcumin extract towards the mortality of bean pod sucking bugs, *R. linearis.*

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2 Method

The research was conducted in Plant Protection Laboratory, Faculty of Agriculture - Wijaya Kusuma Surabaya University, and Organic Chemical Laboratory, Faculty of Science and Technology – University of Airlangga. The neem leaves were collected from two districts in Gresik, East Java, Indonesia.

2.1. Procedures

a. Preparation of Neem Leaves Extract

A weighed quantity of dried neem leaves were washed with tap water, wind dried, and then blended into powder to be used for the extraction. The neem leaves powder was then soaked in ethanol, and kept for a night. The mixture was then filtered off and separated between the residue and filtrate. The filtrate was collected and were subjected to rotary evaporator and subsequently concentrated under pressure and stored at 4°C in air tight bottle.

b. Isolation of Curcumin from the Rhizome of Curcuma domestica

Rhizome of *C. domestica* was washed with flowing water, sliced and wind dried for three days. The sliced *C. domestica* was then grinded and soaked with redistilled etanol in maceration flask for two days. The mixture was filtered off, and the filtrate separated from the residue. The filtrate was collected, and concentrated by vacuum evaporator and gave a viscous brown yellowish extract. The curcumin extract was filtered off, washed with cold *n*-hexane and dried.

c. Rearing of Bean Pod Sucking Bugs in Laboratory

Bugs were collected from the soybean field at Randubangu village - Mojokerto, East Java. Tested insects used in this research were obtained as rearing result in laboratory. Nymphs and adults of the bug were held in a cage filled with fresh long beans as bugs feed. Nymphs moult four times and develop into adults within 10- 21days. Adults were brown and active for 30-35 days. The bugs used as test insect were adults stadium.

d. Efficacy Test of the Mixed Formula of Neem Leaves Extracts 20%-curcumin Against Bean Pod Sucking Bugs Mortality in vitro

This step was done by using Complete Random Design (CRD) with four replications for each of treatment. Different treaments done were mixed formula of neem leaves 20% extract concentrate with various curcumin concentrate addition which were 0 (control), 8, 12, 16, and 20 ppm. Twenty adults of bean pod sucking bugs were infested to each cage. The observed parameter is the precentage of bugs mortality stated in the following formula:

$$\% \text{ mortality} = (X/Y) \times 100\%$$

where X was amount of all the dead bean pod sucking bugs and Y was amount of bean pod sucking bugs totally.

2.2. Data Analysis

The collected data were statistically analysed by using ANOVA (Analysis of Variance). Comparison of treatmen means was performed using LSD (Least Significant Difference) test at $p<0,05$ probability level.

3 Results and Discussion

3.1 Rearing of bean pod sucking bugs in laboratory

The research step could be done well and all the steps with its ages are presented in Table 1.

Table 1. Life span of Bean Pod Sucking Bugs in Laboratory

Life stages	Age (days)
Eggs	3-8
Nymphs stage 1	3-4
Nymphs stage 2	2-4
Nymphs stage 3	1-4
Nymphs stage 4	3-6
Nymphs stage 5	1-3
Adults	30-35
Total	43-64

Based on the table, it is known that a life cycle of bean pod sucking bugs lasted for 43-64 days.

3.2. Bean Pod Sucking Bugs Mortality in Laboratory

The Analysis of Variance (ANOVA) showed that the mixed formula of neem leaves extract 20% with various curcumin concentration influenced the bean pod sucking bugs mortality significantly. It is presented in Table 2.

Table 2. Analysis of Variance the influenced of mixed formula of neem leaves extract 20% - curcumin towards the bean pod sucking bugs mortality in laboratory, seven days after treatment

Source of variance	Df	Sum of squares	Mean squares	F-test	Sig.
Curcumin concentration	4	128.500	32.125	6.763*	.003
Error	15	71.250	4.750		
Total	19	199.750			

Note: Remarks * = influenced significantly at 5% level

Eventhough, the result of ANOVA indicated that the mixed formula neem leaves extract 20% - curcumin influenced towards the bean pod sucking bugs mortality significantly, there was no best concentration of treatment produced the highest mortality of bugs mortality in laboratory. It is presented at Table 3.

Tabel 3. The number of bugs mortality in the mixed formula of neem leaves 20% extract with curcumin extract various concentrate

Curcumin concentration (ppm)	The total number of adults mortality	Percentage (%)
$K_2 = 12$	77^{a}	96,25
$K_3 = 16$	74^{ab}	92,50
$K_1 = 8$	73^{bc}	91,25
$K_4 = 20$	72^{cd}	90,00
$K_0 = 0$ control	49^{e}	81,67

Note: *The numbers followed by the same letters on the same columns were not significantly different at LSD 5%*

Based on data, it is identified that the highest mortality of bugs were obtained at mixed formula of neem leaves extract 20% - curcumin 12 ppm (K_2). This treatment was different with others (K_0, K_1, and K_4) but it was not different signicantly with K_3. Reaching certain concentration, the higher the curcumin addition in neem leaves extract, the number of bugs mortality is increasing. The mortality of bean pod sucking bugs in the treatment of mixed formula of neem leaves extract 20% with various curcumin concentration can be seen in Figure 1.

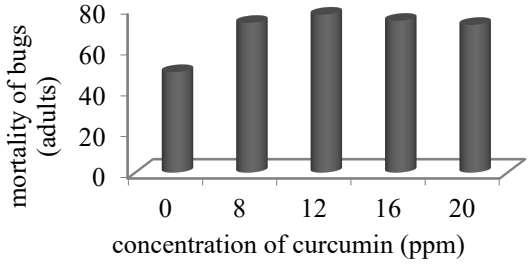

Figure 1. Mortality of bean pod sucking bugs in mixed formula of neem leaves extract 20% with various curcumin concentration

4 Conclusion

The life cycle of bean pod sucking bugs consists of six stadia which every stadium is marked with moulting. In one life cycle, 200-300 nymphs are produced. All these bugs life span need 43-64 days. The result of bean pod sucing bugs rearing that done by [1] showed that one life cycle of this bug needs 49-94 days. Meanwhile, [2] got one life cycle of bean pod sucking bug in 47 days. It is assumed that some factors influence the growth and development of these bugs such as nutrient, temperature, humidity, as well as types of feed. [3] mentioned that the optimum temperature needed for hatching the eggs is 20-35°C. [4] evaluated the influence of feeding soybean pods and seeds to *Riptortus pedestris* and *Halyomorpha halys* in laboratory. They reported, bugs which fed on pods had longer development times and 2.2 - 5.0 times higher mortality rates than bugs which fed on seeds. Furthermore, adult longevity of *R. pedestris* and *H. halys* fed on pods was 8.4 and 7.5 days shorter, respectively, than that of bugs fed on seeds. They concluded that soybean pods were found to be a suitable food source for both bugs despite some negative effects.

Based on Table 1, it is known that the longest stage in a life cycle of bugs is adults. [5] stated that adults constitute the most significant stadia of bugs attacking soybeans. According to [4] the bugs feed soybean through punctures made in the pod by inserting piercing-sucking mouthparts and forming a stylet sheath to convey downward saliva containing digestive enzymes for tissue breakdown and upward for the extraction of plant liquids. The other function of the stylet sheath is to deliver substances that influence wound healing, defense signaling pathways, and the emission of volatile substances by the plant [5][6]. On seeds, feeding punctures appear as minute dark spots, and feeding on seeds reduces yield, quality, and germination. Furthermore, the bugs preferentially feed on young, tender growth and developing seeds (growth stages R4-R6 in soybean). The peak infestation of stink bugs in soybean fields generally occurs at the mid to late pod filling stage (stages R5-R7). At soybean fields, [7] found *R. pedestris* and *H. halys* present in at the mature pod (stage R8) in fall, suggesting that this stage of soybean is also suitable for these bugs feeding.

The bugs that attacking at the forming pods stage will cause pods dry and fall. While the attack at growth and developing seeds will produce deflated pods and seed, dry up, then fall. The attack of bugs on the seeds filling stage causing the beans to become black and the attack at the ripening stage of pods cause brownish black spots on seeds and become wrinkles. Meanwhile, the bugs that attack the mature pods or before harvest can cause hollow seeds. The damage sign of bugs attacks are characterized by the presence of brown spots on the seed or inner pod skin [5].

The result of research indicated that mixed formula of neem leaves extract-curcumin has high efficacy towards bean pod sucking bugs. The mortality precentage of these bugs in this treatment up to 96,25%. The efficacy

of neem botanical insectiside in influencing pests has been proven by Sonyaratri [9] used neem leaves extract 1,5% to totally inhibit the development of *Sitophilus zeamais* Motsch. The neem leaves extract 1,5% was used by [10] to control grasshopper and larvae in *Solanum melongena*. The result of research conducted by [11] showed that neem leaves extract 1,5% able to decrease the attacks intensity of *Nezara viridula* and *R. linearis* up to 53,74% at 12 weeks after plantation. Meanwhile, the use of neem leaves extract 10% is capable of surpressing *Aphis gossypi* at soybean [12].

According to [13], neem leaves consists of four natural chemical compound which have function as pesticide active subtances, i.e. azadirachtin, salanin, meliatriol, and nimbin. Azadirachtin ($C_{35}H_{44}O_{16}$) is beneficial as antifeedant, antioviposition, repellent, as well as growth-regulating that disturb the reproduction and growth of insects [14]. Moreover, [15] and [16] explain that azadirachtin is acting as ecdyson blocker which can inhibit the system of ecdyson, a hormone whose functions in the process of insects metamorphosis. Insect will be disturbed during the process of moulting or eggs hatcing or the developtment of nymphs becomes adults. Usually the failure in this process affects the insect mortality. [17] added that a group of major compounds of neem will disturb sexual communication process which can inhibit mating and eggs-lying, causing infertility, poisons the nymphs and adults, as well as inhibit cithin production. Azadirachtin is considered nontoxic to mammals, fish and pollinators, having low mammalian toxicity with LD_{50} of >5000 mg/kg for rat. It is classified by Environment Protection Agency (EPA) as class IV. It is felt that none of the synthetic pesticides developed so far has the excellent virtues of neem in pest management.

Neem is being used to manufacture natural or bioinsecticides, which are environmentaly friendly and do not have any toxic effects on plants and soil. Neem insecticides are used to protect both food as well as cash crops [18]. The insecticidal effect of neem has been proved on several insect groups, including Lepidoptera, Diptera, Coleoptera, Homoptera and Hemiptera. Nevertheless, there are advantages associated with neem as botanicals insecticides i.e. it is degraded rapidly from sunlight, air, and proper moisture, which generally makes them less toxic to the environment, but may also require them to be applied more often, applied correctly, and with more precise timing. Botanicals act quickly to stop feeding of insect pests and often cause immediate paralysis or cessation of feeding, but they may not cause the insect's mortality for hours or days. Furthermore, most botanicals have low to moderate toxicity to mammals, yet they are still poisons and pose a hazard to humans or to the environment. Botanicals cost more than synthetic insecticides and may not be readily available.

[19] (*unpublished report*) got 50,8% bean pod sucking bugs mortality by using neem leaves extract 20% without curcumin addition. In fact, the addition of curcumin on neem extract has primary purpose to protect the solution from photodegradation. This analysis had been conducted on other research. On the other hand, the addition of curcumin was also intended to improve neem extract efficacy against bean pod sucking bugs. Based on the results of this study, it is understood that the curcumin addition able to increase the mortality of bean pod sucking bugs. It is assumed, that curcumin degradation resulted ferulic acid compounds that can increase the activities of neem botanical insectisides synergically. According [20], at pH above neutral, i.e. when dissociation takes place, curcumin undergoes a rapid hydrolytic degradation. The main decomposition products have previously been identified as feruloyl methane, ferulic acid. [21] reported that ferulic acid derivates have potential bioactivities towards *Aphis fabae Scopoli, Tetranychus cinnabarinus,* dan *Culex pipiens pallens*. Further mentioned, ferulic acid A is a natural phenolic compound that can be isolated from many staple foods, including fruits, vegetables, cereals, and coffee. This compound and its derivatives exhibit a wide range of therapeutic effects [22], with applications including anticancer [23][24], antidiabetic [25], cardio protective [26], neuroprotective [27], and anti-inflammatory activities [28][29]. Octopamine is present in high concentrations in various insect tissues. The octopaminergic system in insects performs insecticidal action with minimum non-target effects [30]. Many octopamine derivatives exhibit moderate insecticidal activity [30][31][32][33]. It is reported that the substituted ferulic acid amide derivatives 7 and the corresponding hydrogenated ferulic acid amide derivatives 13 possess excellent levels of antiviral activity together with good levels of insecticidal activity. Furthermore, these compounds displayed good insecticidal activities against insects with piercing-sucking mouthparts, which can spread plant viruses between and within crops.

The utilization of ecofriendly-botanical insecticides play a big role in order to implement Sustainable Agriculture in Indonesia. It can be recommended as an ecofriendly and sustainable strategy in the management of agricultural pests. The Sustainable Agriculture aims at reducing the incidence of pests and diseases to such a degree that they do not seriously damage crops without upsetting nature balance. One of the aims of The Sustainable Agriculture is to rediscover and develop strategies whose cost and ecological side-effects are minimal. [34] said, botanical pesticides are active principles derived from plants for the management of human and animal pest organisms. It can be said to be biologically active ingredients, principally derived from plants, for the management of human and animal pest organisms. The botanicals are generally highly biodegradable, relatively harmless to non-target organisms and the environment, as well as their use in crop protection is a practically sustainable alternative [35]. Related to its highly biodegradable, the botanicals become inactive within hours or a few days. This reduces the negative effect on beneficial organisms and they are relatively environmentally safe. According to [36], the botanicals are based on a single active ingredient, plant derived insecticides comprise an array of chemical compounds which act concertedly on both behavioural and physiological processes. Thus the chances of pests developing resistance to such

substances are less likely. Furthermore, the botanicals generally act in one of two ways, either as a contact poison when sprayed on the insect or as a stomach poison when applied to the plant and eaten by the insect. In other words, most the botanicals are contact, respiratory, or stomach poisons.

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