IOSR Journal Of Pharmacy *www.iosrphr.org*
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 6, Issue 6 Version. 3 (June 2016), PP. 76-81

The Efficacy Of Larvicides Of Leaves Of Yam Bean (*Pachyrhizuserosus)*As Botanical Insecticides Against Fly Larvae Myiasis *Chrysomyabezziana*

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Abstract:- The use of medicinal plants for the treatment of myasis cases in cattle have not been reported yet. One potential medicinal plants as a biopesticide is a yam bean plant (*Pachyrhizuserosus*). The leaf of yam bean has larvicidal efficacy against some insects. This study is expected to examine the effectiveness of yam bean leaves as a bothanical insecticides against fly larvae C*hrysomyabezziana*as a causative agent in vitro. This study is divided into 5 treatment groups. As many as 20 larvae 1, larvae 2 and larvae 3 of *Chrysomyabezziana* used for in vitro testing using plastic pots containing larvae media and ethanol extracts of leaves of yam bean with graded concentrations of 0.5, 1, and 2%. Coumaphos and sterile distilled water were used as positive and negative controls. The result showed that the ethanol extract of leaves of yam bean had an activity as larvicides against L1 and L2. The ethanol extract of the yam bean leaf showed the effectiveness as larvicides against L1 and L2 depending on the level of concentration of the extract which was mixed into the media. The higher the concentration of the extract was the higher the death rate of the larvae. At a concentration of 2%, it was capable of causing 100% pupae did not hatch. At L3 testing showed that the ethanol extract of the leaves was able to cause a decrease in hatchability at all concentrations. Testing L1 and L2 indicated the effectiveness of the extract as a stomach poison, while testing L3 showed an indication of a contact poison.

Keywords-*Chrysomyabezziana, leaf of yam bean, myiasis*

I. INTRODUCTION

The parasitic disease, which remains an issue in the Indonesian world of livestock is myiasis, the larvae infestations of flies into living tissue. The disease attacks all kinds of warm-blooded vertebrate animals including humans, so it is classified as zoonotic diseases. Based on the geographic distribution, the primary agent of causing myiasis is divided into three, namely Cochliomyahominivorax flies (*The New World Screwworm Fly*) spread across the Americas, *Wohlfahrtiamagnifica* flies spread across Europe to China and *Chrysomyabezziana* flies spread in the African tropics, subcontinent India, Southeast Asia including Indonesia and Papua New Guinea[3,4].

Myiasis causes huge economic losses, especially in areas which are the central of livestock farms. [7]noted the myiasis cases in endemic areas can reach 95%, which attack all kinds of animals and humans. Therefore, the body of the animal health world or *Office International des Epizooties (OIE)* classifies the disease into the list B, which is an infectious disease that has a socio-economic impact or has value health importance in a country in the international trade associated with products from animal origins.

During this time, controlling myiasis in general has used antibiotics and synthetic insecticides such as, coumaphos, diazinon, fenthion, ivermectin, amitraz, enrofloxacin, Spiramycin, through topical treatment and immersion (dipping) [8,9,20]. However, the use of synthetic insecticides are reported to have negative impacts, as developing race-resistant, killing natural enemies of pests, poisoning humans and domesticated livestock, cancer, residues in meat and milk, as well as causing environmental pollution [3]. Therefore, it is needed to have an alternative medicine that has several advantages, among others, easy, accessible, safe for animals and leaves no residue in products of animal origins. One alternative materials that can be used as myasis medicine is based on medicinal plants.

Previous research shows that families of plants that act as plant-based insecticide is *Leguminosae, Meliaceae, Annonaceae, Asteraceae, Piperaceae*, and *Rutaceae*. The plant of the *Leguminosae* family which has the potential to be developed into a bothanical insecticide for controlling myiasis is a yam bean plant. Rotenon compounds contained in yam bean supposedly efficacious as larvicidesand has been tested able to kill the larvae of *Aedesaegypti* mosquito and fly larvae *Musca domestica*. This compound is reported to have a mechanism to work by inhibiting the metabolism of insects [5].

Yam bean leaf extracts contain bioactive compounds that are useful as an insecticide plant. Information on the efficacy and safety of yam bean leaf against myasis case until now has not been found yet. This study aims to determine the potential of yam bean leaf extract as a biopesticide against Larvae 1 (L1), Larva 2 (L2) and Larvae 3 (L3) of the fly *C.bezziana* in vitro. The results of this study are expected to determine the consistency and effective dose in vitro and standardize the quality of plant materials.

II. MATERIAL AND METHODS

2.1 Plant materials and preparation

Yam bean leaf used in this study was obtained from the Center for the Study Biofarmaka of Bogor Agricultural University.

2.2 Extraction of plant materials

Simpliciayam bean leaves was extracted using maceration method. The maceration was conducted in 3 x 24 hours using 96% ethanol concentration. The comparison of the simplicia and the solvent is 1: 10. The viscous extract obtained from maceration was evaporated using a rotary evaporator at a temperature of 40-50 degree Celsius and 50 rpm.

2.3 Phytochemical screening

The ethanol extracts were used for the screening. The dried extracts were first reconstituted in the respective solvents used for their extraction and then tested by standard phytochemical method for the presence of alkaloid, flavonoid, tannin, saponin, andterpenoids[11].

Alkaloids test: Half gram of the extract was added to 5ml of 1% aqueous HCl on a steam bath andfiltered. 1 ml of the filtrate was treated with a few drops of Draggendorf's reagent and another 1 ml filtrate was treated with Wagner's reagent. The formation of precipitates was an indication of the presence of alkaloids.

Flavonoids test: Half gramof the extract was dissolved in 2ml of dilute NaOH solution. A few drops of concentrated H_2SO_4 were then added. Formation of yellow solution confirmed the presence of flavonoids.

Tannin test: Half gramof the extract was stirred with 10 ml of distilled water. The mixture was filtered and a few milliliters of 5% ferric chloride were added to the filtrate. A green coloration showed the presence of tannin.

Terpenoids test: Five ml of rhizome extract was mixed in 2 ml of chloroform and concentrated 3 ml of H_2SO_4. A reddish brown coloration at the interface showed the presence of terpenoids.

Saponins test: Fiveteen ml of distilled water was added to the extract and shaken vigorously until formation of a stable persistent form which indicated the presence of saponins.

2.4 The media manufacturing and testing in Vitro

An in vitro study conducted on two different media, i.e media Meat Blood Mixture (MBM) to test the first instar larvae (L1) and larval rearing Media (LRM) to test the second instar larvae (L2). The second test used to determine the larvicidal activity of the ethanol extract of leaves of yam bean as a stomach poison (venom digestibility). The MBM media was made by mixing 15 kg of ground beef with 30 mL of fresh cow's blood. The media LRM was made by mixing several materials include 450 g cow fresh frozen blood (*Marus*), 45 g skim milk, 45 g of egg powder, 50 g of CF 100 (powder paper), 1.0 mL of 10% formalin, and 980 mL of distilled water. At first, the cow fresh frozen blood was milled with a blender, after the liquid the other ingredients were incorporated into a blender and homogenized, then used for in vitro test medium [15]. Unlike the L1 and L2 test, an in vitro study aimed to determine the larvacides activity of the ethanol extract of the yam bean plant as a contact poison. The medium used was a mixture of ethanol extract of the yam bean plant with aquadest and DMSO 1% thus forming a liquid preparation in a certain concentration.

2.5 Extract test of natural ingredients in L1

Media MBM that had been mixed with extracts of natural ingredients with graded concentrations began from 0.5, 1 and 2 mg / mL, placed in plastic containers measuring 18.5 x 13.5 x 4.5 cm. A total of 20 (L1) each replicate was placed on the media and maintained in a room with a temperature of 30-32° C. The surviving larvae to second day was transferred to a new plastic containers and maintained on media LRM up to become pupae and hatched into imago [22].

2.6 Extracts test of natural ingredients L2

The medium used for this test was an LRM that had been mixed with extracts of natural ingredients with graded concentrations starting from 0.5, 1 and 2 mg / mL. A total of 215 LRM inserted into each plastic

container measuring 18.5 x 13.5 x 4.5 cm. Some 20 L2 per replicate were infested with the media and observed for its development up to become a pupa and imago[22].

2.7 Ethanol extract test of natural ingredients L3

This test was performed in a pot of drugs containing extracts of natural ingredients with graded concentrations of 0.5, 1 and 2 mg / mL. The test to the effects of contact poison conducted by immersing larvae into the test solution at one time, which is 10 seconds, 60 seconds and 180 seconds. A total of 20 larvae per replicate immersed in each treatment solution (10 mL) for 20 seconds, then drained using an aluminum sieve, then the larvae was placed on filter paper and transferred into plastic containers containing *vermicullite*. The larvae were incubated at a temperature of 30-32ºC to become pupae and hatch into imago [14].

The whole in vitro test for L1, L2 and L3 used five treatments, ie three treatments were challenged with ethanol extract (three graded concentrations), the positive treatment (using 1% caumaphos) and a negative control treatment (use distilled water). Each treatment consisted of five replications.

2.8 Variables and data analysis

Variables measured in vitro test was the number of deaths larva, pupa weight, hatchability pupa into the imago and physical condition of imago that hatch. The data were analyzed using the general linear model (GLM) with Minitab 16 program.

III. RESULTS

3.1 Extraction and Secondary Metabolites Content in Plants

The results of the ethanol extract of the plant showed the results of 1000 g of dried botanicals extracted yam bean leaves were 123, 3g, so that the resulting yield was 12.33%. Phytochemical screening results showed that yam bean leaves contain flavonoids, terpenoids, alkaloids and saponins. The active compounds in ethanol extract of yam bean leaves which act as larvicides was rotenon. Rotenon is a flavonoid compounds.

3.2 In Vitro Test Results

Table 1 shows that the ethanol extract of leaves of yam bean has activity as larvicides against L1. Ethanol extract of yam bean leaf showed the effectiveness of larvicides against L1 depended on the level of concentration of the extract, which was mixed into the media. The higher the concentration of the extract was, the higher the death rate of the larvae. The larvae that were given the leaf extract of yam bean was able to grow up into the pupa stage, but the weight was below normal (less than 26 mg), further the group of larvae that was fed 0.5% of yam bean leaf extract , which was about 85%, experienced disability on its wings. Treatment of 1% led all the hatched pupae experienced disability on its wings, while the treatment of 2% caused 100% pupa dried. The abovementioned results indicate that the active compound contains in the leaves of yam bean has activity as a stomach poison and *antifeedant*. Gastrointestinal toxins can be seen from the acute mortality in larvae 1 since treatment was performed, while antifeedant could be seen from the weight of pupae formed [19].

Table 1 The efficacy of larvicides of the ethanol extract of leaf of yam bean against larvae1 *C. bezziana*observed for 7 days post treatment

Dose Extract (%)	Larval mortalityof Larva 1 of*C.bezziana*	The weight ofpupa of the larvae 1*C.bezziana*	Hatched pupa of larvae 1*C.bezziana*
0	$0,00\pm0,00^{1}$	$36,03\pm0,38^{a}$	$95,00\pm5,00^{a}$
0.5	$22,00\pm4,47^{c}$	$11,40\pm0,58^{b}$	$9,00\pm7,42^{b}$
1	$43,00\pm10,37^{d}$	$10,23\pm1,71^{bc}$	$4,00\pm4,18^{bc}$
2	$66,00\pm4,18^{bc}$	$7,97\pm1,61^{cd}$	$0,00\pm0,00^{c}$
Drug control	$100,00\pm0,00^{a}$	$0,00\pm0,00^{e}$	$0,00\pm0,00^{c}$

Notes: Different superscript letters in the same column or row showed significantly different results $P < 0.05$. The death of larvae, pupae weights and pupae hatch were statistically analyzed separately.

Table 2 showed that the larvicidal activity of the ethanol extract of leaves of yam bean to the L2 tend to have the same effect as L1. The ethanol extract of yam bean leaf showed significant differences in the concentrations of 0.5% and 1%, but no significant different at a concentration of 2%. Based on the condition of larvae media, all media which was mixed with the extract of yam bean leaf inclined to dry and odorless. This situation was allegedly due to that the extract of yam bean leaf had strong antimicrobial activity so as to kill or inhibit the metabolism of spoilage bacteria. This was different from the positive control group. Although all the larvae died, but the positive control media tend to damp.

Table 2 The efficacy of larvicides of the ethanol extract of the yam bean leaf against larvae 2 *C.bezziana* observed for 7 days post treatment

Dose Extract (%)	Larval mortalityof Larva 2 of*C.bezziana*	The weight ofpupa of the larvae 2*C.bezziana*	Hatched pupa of larvae 2*C.bezziana*
0	0,00±0,00^{f}	36,05±0,13^{a}	96,00±4,18^{a}
0.5	39,00±8,94^{e}	11,79±1,42^{b}	44,00±11,94^{b}
1	59,00±6,52^{d}	10,32±1,06^{b}	15,00±7,91^{d}
2	70,00±6,12^{c}	8,89±2,81^{b}	0,00±0,00^{e}
Drug control	100,00±0,00^{a}	0,00±0,00^{c}	0,00±0,00^{e}

Notes: Different superscript letters in the same column or row showed significantly different results P <0.05. The death of larvae, pupae weights and pupae hatch were statistically analyzed separately.

Table 3 also showed that the extract group of yam bean leaf had larvicidal activity against L3. The efficacy of the larvicides was not effective as in test L1 and L2. In this test in regard to the obstacles the pupae to hatch, the smaller its hatchability, the more effective was the dosage form of the drug. In the administration of the drug with different immersing time, did not cause significant changes in the effect. The yam bean leaves group at the highest concentrations had the lowest hatchability when compared to the other treatment groups. The decline in hatchability due to the effectiveness of the active compound that was capable of acting as a contact poison, it called a contact poison because the tested larval stages 3 had already entered the final stages of larval 3 and was ready to become pupae. Activity that could affect the larvae was by direct contact with the cuticle.

Table 3 The efficacy of larvicidal solution of ethanol extract of yam bean leaves after immersion L3 with a long time as well as different concentrations

Dose Extract (%)	The weight of pupa of larva 3*C. bezziana*immersed			Pupa hatched of larva 3*C. bezziana*immersed		
	10 second	60 second	180 second	10 second	60 second	180 second
0	42,06±1,67^{a}	40,40±1,46^{ab}	39,39±1,36^{ab}	96,00±6,52^{a}	97,00±4,47^{a}	96,00±4,18^{a}
0.5	35,83±0,80^{b}	36,45±1,55^{bc}	35,98±2,02^{ab}	93,00±8,37^{a}	89,00±11,40^{ab}	86,00±9,62^{a}
1	34,99±1,59^{b}	33,55±1,88^{c}	32,19±2,28^{bcd}	71,00±12, 94^{ab}	59,00±9,62^{cd}	54,00±9,62^{b}
2	34,69±1,47^{b}	36,12±5,89^{bc}	33,01±3,22^{bcd}	42,00±5,70^{cd}	41,00±9,62^{def}	41,00±13, 87^{bcd}
Coumaphos	23,18±2,69^{c}	31,46±4,21^{c}	27,88±4,9 1^{cde}	26,00±13,87^{d}	18,00±6,71^{f}	19,00±9,62^{de}

Notes: Different superscript letters in the same column or row showed significantly different results P <0.05. Pupa weight and hatched pupa were statistically analyzed separately.

IV DISCUSSION

Based on in vitro test results proved that the yam bean leaves had activity against larvacidal*C.bezziana*. The active compounds are thought to play an important role in the activities of larvacidal is rotenon. Based on the dry weight, the content rotenon in the stem was of 0.03%, 0.11% in the leaves, 0.02% in the pods, and 0.66% in the seed. The content of pure rotenon in the ripe seeds ranged from 0.5 to 1.0% [1]. There are two hypothetical mechanisms of the active compound of yam bean plant in killing or inhibiting the growth of larvae of *C. bezziana*.

The first hypothesis is the active ingredient is toxic effect of the stomach.[19]stated that the absorption of insecticides which have the effect of gastrointestinal largely takes place in mesenteron (central part of the digestive tract). Mesenteron wall is composed of epithelial cells comprisetwo layers, i.e. lipid and protein which are scattered in certain parts of the lipid layer. The whole of these cells are lipophilic so as to facilitate the active compound diffuses into the cells. The presence of the active ingredients of yam bean plants that are ingested by the larvae will affect the metabolism of epithelial cells in the digestive tract. This hypothesis was supported by the results showing larva discoloration of the digestive tract that was blackish brown, pupa growth disorders or pupa changed into a small size, so it could not hatch into adult flies (imago).

The second hypothesis is the active ingredient of yam bean (rotenon) is the toxic effects of contact. In the immersion treatment of L3 allowed larvae *C.bezziana* contact with the active ingredient of the leaf yam bean. According to [19], the insecticides absorption which has contacts toxic's effect largely occurs in the cuticle. The active compound will penetrate the larvae through the body which are coated by a thin cuticle, such as inter-segment membranes, membrane joints and chemoreceptors at tarsus. Cuticular layer are lipophilic so that the active compound is able to diffuse from the outer cuticle layer through deeper layers towards hemolymph,

follow the flow of blood / hemolymph and distributed to all parts of the larval body of *C.bezziana*. As a result, the larvae aredying very fast.

Both of these hypotheses have the same basic working mechanisms. After rotenonget into the flow of hemolymph, the active compounds of the plant will work by blocking the action of enzymes cholinesterase, thus breaking acetylcholine into choline and acetic acid does not occur. As a result of the inhibited work of enzyme cholinesterase, the accumulation of acetylcholine compounds occur at the nerve ends, because most of acetylcholine cannot be hydrolyzed. This will result in excessive cholinergic activity, because the effector cells receive signals continuously. Clinical symptoms that can be seen in insects poisoned by rotenon is respiratory depression, convulsions and discharge from the anus (diarrhea) as a response to increased peristalsis [13].

IV. CONCLUSION

Ethanol extract of leaves of yam bean had larvacidal activity against larvae *C.bezziana* in vitro. The active compound (rotenon) contained in the seeds, stems and leaves of yam bean had properties as gastrointestinal poison, contact poison and antifeedant.

VI. ACKNOWLEDGEMENT

The authors are very grateful to the Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesian Research Center for Veterinary Science for the research facilities

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