Innovation of Standardized Extract of Yam Bean Seed and Red Betel Leaf as Bioinsecticide for Myiasis Treatment

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ABSTRAK

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Miasis pada ternak menyebabkan kerugian yang signifikan secara ekonomi maupun produksi. Penggunaan bahan kimia untuk mencegah atau mengendalikan miasis pada ternak sering digunakan, namun penggunaan insektisida berbahan dasar kimia dalam jangka waktu tertentu dapat menimbulkan dampak negatif. Penelitian ini bertujuan untuk mengetahui khasiat kombinasi ekstrak etanol biji bengkuang dan daun sirih merah secara in vitro sebagai sediaan yang mampu membunuh semua stadium larva Chrysomya bezziana agen penyebab miasis. Penelitian ini terbagi menjadi 11 kelompok perlakuan. Masing-masing sebanyak 20 Larva instar 1 (L1), Larva instar 2 (L2), dan Larva instar 3 (L3) C.bezziana digunakan untuk pengujian in vitro menggunakan pot plastik yang berisi media larva dan ekstrak etanol biji bengkuang, daun sirih merah, serta kombinasi ekstrak etanol biji bengkuang dan daun sirih merah dengan konsentrasi bertingkat 0,5%, 1%, dan 2%. Asuntol dan akuades steril digunakan sebagai kontrol positif dan negatif. Hasil penelitian menunjukkan bahwa kombinasi ekstrak etanol biji bengkuang dan daun sirih merah pada konsentrasi 2% mampu menyebabkan 100% kematian larva dan 100% pupa tidak menetas. Pengujian L3 menunjukkan bahwa kombinasi ekstrak etanol biji bengkuang dan daun sirih merah mampu menyebabkan penurunan daya tetas pada semua konsentrasi. Pengujiaan L1 dan L2 untuk mengindikasikan efektivitas ekstrak sebagai racun perut, sedangkan pengujian pada L3 sebagai indikasi racun kontak. Biji bengkuang dan daun sirih merah memiliki daya larvasida terhadap beberapa jenis larva serangga Chrysomya bezziana.

Kata Kunci: Bioinsektisida, *Chrysomya bezziana*, Miasis, Biji Bengkuang, Daun Sirih Merah

ABSTRACT

Mustika AA, Sutardi LN, Purwanto ES, Nefo F, Andriyanto, Wientarsih I, Sawitri DH, Wardhana AH. 2024. Innovation of standardized extract of yam bean seed and red betel leaf as bioinsecticide for myiasis treatment. JITV 29(1):16-28. DOI:http://dx.doi.org/10.14334/jitv.v29.i1.3158.

Myiasis causes severe economic and production losses in animals. Chemicals are frequently employed to prevent or control myiasis in animals, however long-term exposure to chemical-based pesticides can have severe effects. This study aims to assess the effectiveness combination of ethanol extract of yam bean seeds and red betel leaf in vitro as a preparation capable of killing all larval stages of Chrysomya bezziana, the primary agent responsible for myiasis. This study consisted of eleven treatment groups. Twenty instar 1 (L1), 2 (L2), and 3 (L3) C. bezziana larvae were used for in vitro testing utilizing plastic pots containing larval media and ethanol extract of yam bean seeds, red betel leaf, and their combination as well as a combination of 0.5%, 1%, and 2% concentrations of ethanol extract of yam bean seeds and red betel leaf. Asuntol and sterile distilled water were utilized as positive and negative controls, rerspectively. The results demonstrated that the combination of 2% ethanol extract of yam bean seeds and red betel leaf was able to induce 100% larval death and 100% pupal failure. The L3 test demonstrated that the combination of yam bean seed ethanol extract and red betel leaf reduced hatchability at all doses. The L1 and L2 tests suggested that the extract was efficient as a stomach poison, whilst the L3 test indicated that it was also a contact poison. Yam bean seeds and red betel leaf show larvicidal efficacy against multiple species of Chrysomya bezziana insect larvae.

Key Words: Bioinsecticide, *Chrysomya bezziana*, Myiasis, Red Betel Leaf ,Yam Bean Seeds

INTRODUCTION

Myiasis is an infestation of fly larvae in living vertebrate tissues and organs, as well as in necrotic tissues. In endemic regions, the prevalence of myiasis reaches 95 %, affecting all animal species, including humans. Therefore, the World Organization for Animal Health (WOAH) categorizes this disease as list B, which is an infectious disease that has a socio-economic impact or a health importance value on a country in international trade associated to products of origin (Mustika et al. 2016a). The primary agents that cause myiasis are divided into three groups based on their distribution: the *Cochliomya hominivorax* fly (The New World Screwworm Fly) that is prevalent in the Americas, the *Wohlfahrtia magnifica* fly that is prevalent from Europe to China, and the *Chrysomya bezziana* fly that is prevalent in the tropics and parts of Africa (Wardhana and Diana 2014). This disease is caused by *Chrysomya bezziana,* an obligate parasite (Wulandari and Pemayun 2019).

Myiasis typically affects animals, including sheep, goats, cows, and other vertebrates (Zuleika 2015). Myiasis is prevalent in tropical regions, particularly among those from poor socioeconomic classes (Singh and Kaur 2019). The high incidence of myiasis in the tropics is caused by inadequate hygienic conditions and the increased aggressivity of myiasis-causing flies (Kristinawati et al. 2019). Myiasis has become a severe global issue due to the international spread of flies, particularly in tropical and subtropical settings. Cases of myiasis in ruminants in Iraq (Zhou et al. 2019), small animals in Australia (Welch et al. 2014), and both small animals and ruminants in South Africa (Mukandiwa et al. 2012) are examples of the rapid expansion of myiasis around the globe.

Myiasis causes significant economic losses, especially in areas where livestock is prevalent. Myiasis can be dangerous if not treated immediately; if left untreated for an extended period of time, it will target important organs and cause secondary infection. Due to myiasis, the condition of cattle will become weakened, with decreased hunger, fever, a drop in milk output and body weight, and even anemia (Susari et al. 2020).

Myiasis control is not yet excellent, despite the use of antibiotics and synthetic insecticides such as coumaphos, diazinon, fenthion, ivermectin, amitraz, enrofloxacin, and spiramycin by topical therapy (spraying) and dipping (Mustika et al. 2016a). The use of synthetic pesticides can result in adverse effects such as the emergence of resistant strains, threats to food safety, and environmental degradation (Ballweber and Baeten 2012). Therefore, alternate treatments, such as medicinal plant-based preparations, are required for the treatment of myiasis in the field.

Traditional and modern therapeutic compounds have been derived from medicinal plants (Khater 2012). Historically, plant-derived bioactive chemicals have been utilized as an effective parasiticide against resistant populations (Molento et al. 2020). In situations of myiasis, nutrient-rich vegetables might be utilized as an alternative treatment. Previous research has demonstrated that plants from the Meliaceae, Annonaceae, Asteraceae, Piperaceae, and Rutaceae families can be utilized as bioinsecticides, including for the treatment of myiasis (Wientarsih et al. 2017). Yam bean seeds (*Pachyrhizus erosus*) and red betel leaf (*Piper crocatum*) have the potential to be used as myiasis bioinsecticides.

Rotenon is the insecticide-potent active component of the yam plant. The rotenone chemicals in yam have been shown to be effective larvicides against Aedes aegypti and Musca domestica mosquito larvae. This chemical is said to impede insect metabolism as its mode of action (Oguh et al. 2019). The ethanol extract of yam bean seeds has been proven to be effective as a larvicide against the parasite *Chrysomya bezziana*, which causes myiasis in livestock (Mustika et al. 2016a). Essential oils, carvcrol, eugenol, chavicol, flavonoids, arecolin, and tannins are present in red betel leaf phytochemicals. Carvacrol is an antiseptic and disinfectant, but eugenol is a pain reliever. Red betel leaf essential oil contains chavicol and eugenol, which have antibacterial properties. When used to cure infections caused by harmful bacteria in the body, red betel leaf's antimicrobial qualities are quite effective (Kurniawati et al. 2014). Red betel leaf contains flavonoids, arecolin, and tannins, which promote wound healing and blood flow (Widhyari et al. 2018). In the treatment of myiasis, it is anticipated that the interaction between these two medicinal herbs will be synergistic. This study intends to establish *in vitro* whether a combination of ethanol extract of yam and red betel seeds is effective against all stages of the myiasis-causing parasite *Chrysomya bezziana*.

MATERIALS AND METHODS

This study was conducted in multiple phases. Using the Liquid Chromatography Mass Spectrometry (LCMS-MS) instrument, the active chemical is identified in the first step. In the second phase, L1, L2, and L3 *C. bezziana* larvae were examined *in vitro* for larvicidal activity.

Extraction of yam bean seeds and red betel leaf

The maceration procedure was used to extract dried simplicia yam bean seeds and red betel leaf. Three 24-hour macerations were performed using 96% ethanol as the solvent. The proportion of simplicia to solvent was 10 :1. The extract produced from maceration was then evaporated using a rotary evaporator at 40-50 °C and 50 rpm in a temperature range of 40-50 °C (Mustika et al. 2016a).

Identification of active compound

Using a Liquid Chromatography Mass Spectrometry (LCMS-MS) apparatus, the active components of yam bean seeds and red betel leaf will be identified. UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS ThermoScientific with Accucore C18 column, 100 x 2.1 mm, 1.5 m was utilized as the LCMS (ThermoScientific).

In vitro test of the combination of yam extract and red betel leaf extract at L1 and L2

In vitro studies were conducted on two distinct media: meat-blood mixture (MBM) media for the first instar larval test (L1) and larval rearing media (LRM) media for the second instar larvae test (L2) (L2). MBM medium that has been combined with yam bean seed extract and red betel leaf at tiered concentrations of 0.5%, 1%, and 2% are placed in a plastic container measuring 18.5 x 4.5 x 4.5 cm. Twenty (L1) of each replication were placed on the media and maintained at a room temperature of 30–32°C. The larvae that survived until day 2 were transferred to new plastic containers and raised on LRM media until they developed into pupae and hatched into imago. L2's examination process is identical to L1's, but LRM media are employed (Wardhana et al. 2014).

In vitro test of the combination of yam extract and red betel leaf extract at L3

The test was conducted in a medicinal pot containing 0.5%, 1%, and 2% concentrations of yam bean extract and red betel leaf in stratified proportions. The larvae were immersed in the test solution for 10 seconds, 60 seconds, and 180 seconds as part of the contact toxic effect test. Twenty larvae per replication were submerged in 10 ml of each treatment solution for 10, 60, and 180 seconds. The larvae were incubated at 30-32 °C until they developed into pupae and hatched into imago (Spradbery 2002).

RESULTS AND DISCUSSION

Analysis of the active compound in yam bean seed

The LC-MS results for the ethanol extract of yam bean seeds revealed that the extract contains 168 active compounds. Figure 1 presents the chromatogram from the LC-MS analysis of the ethanol extract of red betel leaf

Rotenone, curcumin, and coumarin are the active compounds in the ethanol extract of yam bean seeds that have potential as insecticides (Figure 2). In addition, yam contains the active substances rotenone, coumarin, coumestan, isoflavone, isoflavanone, and other isoflavonoid groups, according to a review of the scientific literature (Basukriadi and Wilkins 2014; Mustika et al. 2016b).

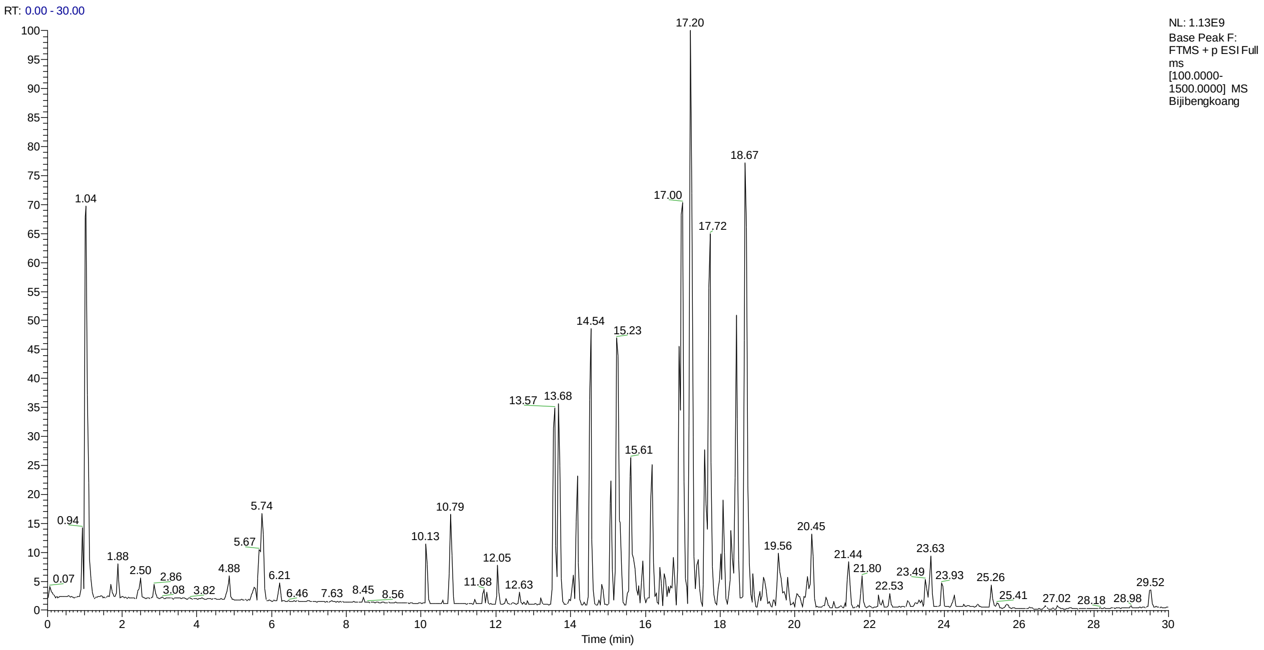
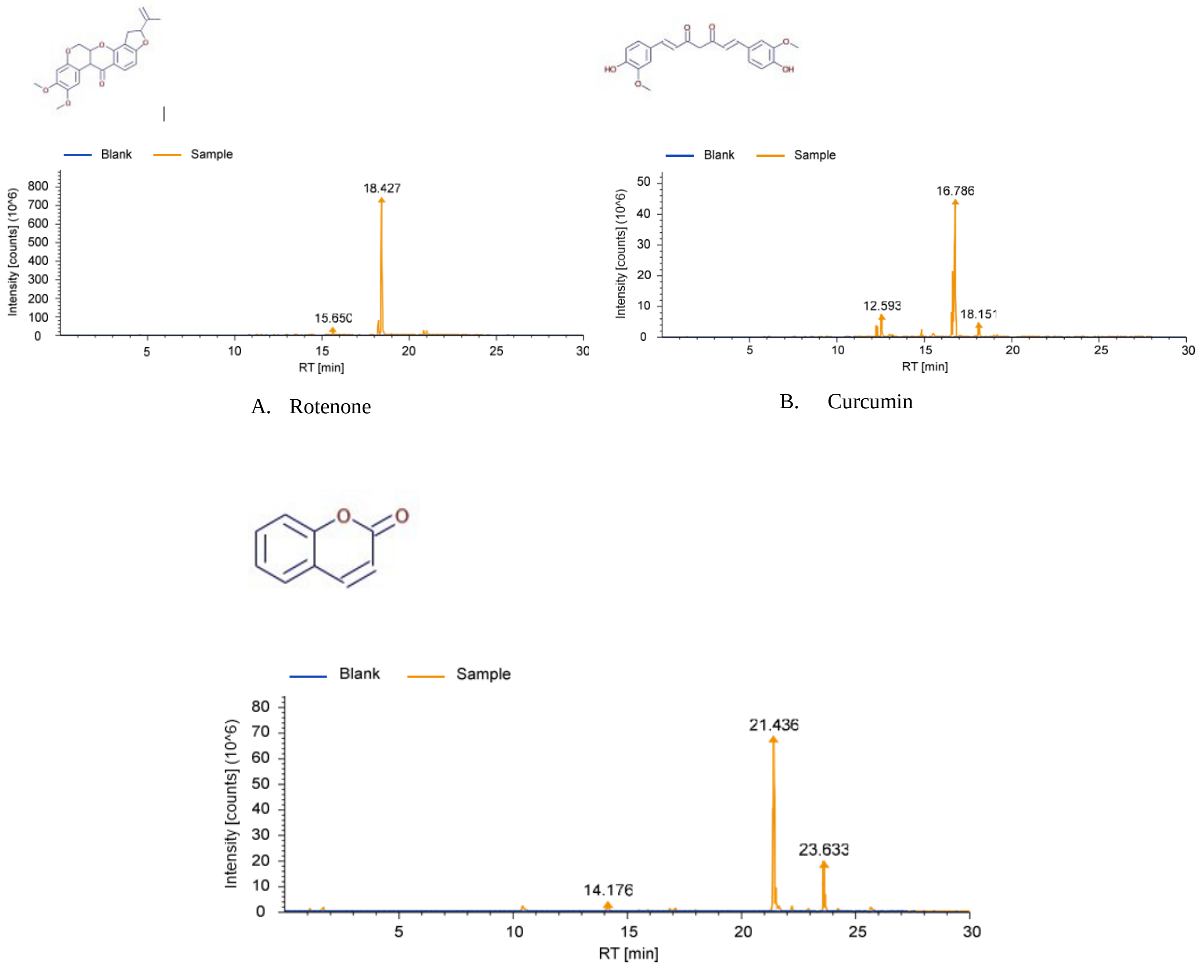


Figure 1. LC-MS chromatogram graph of yam bean seed extract



A. Rotenone

B. Curcumin

C. Coumarin

Figure 2. The results of the LC-MS analysis of yam bean seeds active compound (A: rotenone¸ B: curcumin, C: coumarin)

Rotenone ($C_{23}H_{22}O_6$) functions as a larvicide by inhibiting nicotinamide adenine dinucleotide CoQ reductase (complex 1) in the initial phase of mitochondrial electron transport (Barceloux 2008). Blocking mitochondrial electron transport prevents the generation of energy that will be sent to the cell and disrupts the physiological function of the cell, leading to the larvae's death (Ahdiyah and Purwani 2015). Curcumin ($C_{21}H_{20}O_6$) is an insoluble flavonoid molecule that is soluble in acetone, ethanol, and dimethylsulfoxide. Curcumin has a molecular weight of 368.37 g/mol and a boiling point of 183°C, according to (Sethi et al. 2009). Organophosphate and carbamate insecticides target the enzyme acetylcholinesterase, which plays a key role in synaptic transmission and is a primary molecular target (Matiadis et al. 2021). Curcumin is recognized to inhibit acetylcholinesterase enzyme activity similarly to pyridostigmine bromide and malathion (Rao et al. 2021). Coumarin ($C_9H_6O_2$) is a phenylpropanoid having a six-ring lactone structure and a 2H-1-benzopyran-2-one core (Isnawati et al. 2008). A number of the pharmacological effects of coumarine have been thoroughly examined. Coumarin can stimulate the generation of reactive oxygen species (ROS) and reduce the selectivity of cell membranes (Barbosa et al. 2018).

Analysis of the active compound in red betel leaf

The LC-MS study of red betel leaf ethanol extract revealed that the extract included 208 chemicals as active components. Figure 3 illustrates the chromatogram of the ethanolic extract of red betel leaf as determined by LC-MS.

Quassin and coumarin are the possible insecticides found in the ethanol extract of red betel leaf, according to a review of the relevant literature (Figure 4). Quassin ($C_{22}H_{28}O_6$) is the first member of the class C Tetracyclic C20-quassinoids to be isolated from *Picrasma*.

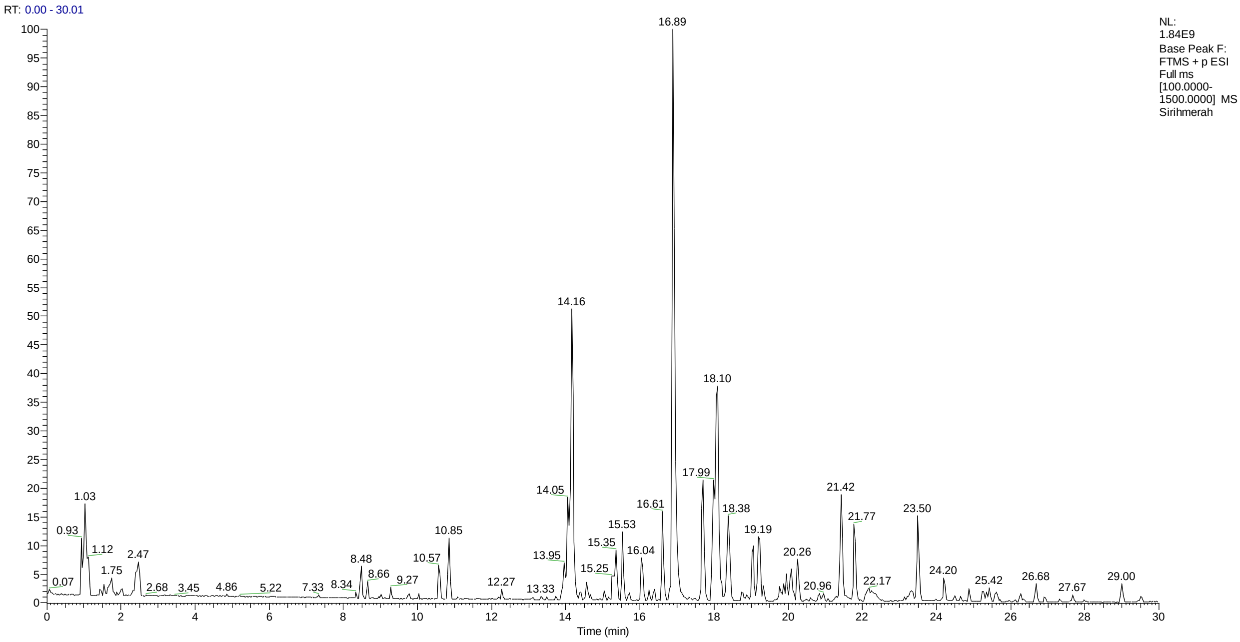
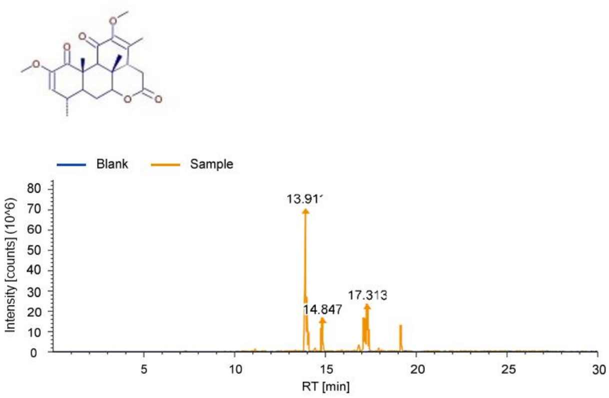
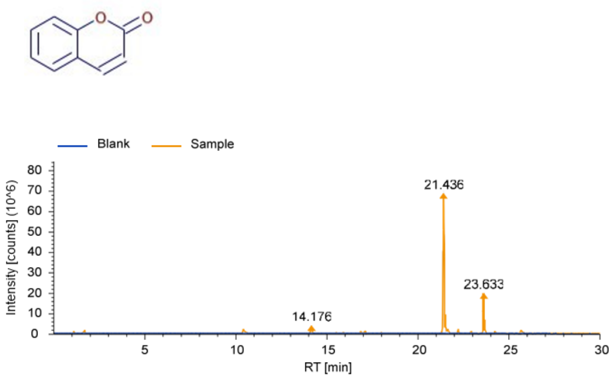


Figure 3. LC-MS chromatogram graph of red betel leaf extract



A. Quassin



B. Coumarin

Figure 4. The results of the LC-MS analysis of red betel leaf active compound (A: quassin¸ B: coumarin)

ailanthoids. C_{26}, C_{25}, C_{20}, C_{22}, C_{19}, and C_{18} are classed as six distinct classes of quassinoids (Duan et al. 2021). Quassinoids exhibit many biological effects, including anticancer, antimalarial, anti-inflammatory, antifeedant, insecticidal, amoebicidal, antiulcer, and herbicide properties, according to recent pharmacological and clinical investigations (Houël et al. 2013). Coumarin ($C_9H_6O_2$) is a phenylpropanoid containing a 2H-1-benzopyran-2-one core and a six-ring lactone ring (Isnawati et al. 2008). Several of coumarine's pharmacological activities have been thoroughly examined. Coumarin can stimulate reactive oxygen species (ROS) generation and result in a loss of cell membrane selectivity (Barbosa et al. 2018)

In vitro results

Mortality of larvae and imago, pupa weight, and hatchability were evaluated *in vitro*. Larvae weighing less than 30 mg are typically incapable of developing into pupae, but pupae weighing less than 23.5 mg do not typically hatch into adult flies (Wardhana and Muharsini 2004). Environmental variables can have an impact on pupa hatchability (Wardhana 2016). The interaction between pupa weight and hatchability can influence the mortality rate of larvae and imago.

Table 1 demonstrates that ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf are larvicidal against L1. This is shown by the fact that the larval death rate increases as the concentration of the extract increases. The administration of ethanol extract of yam bean seeds at varying dosages was able to kill all of the larvae, preventing their development into pupae. As a result, the larvae in this population never reached the imago stage.

Depending on the concentration of the preparation, red betel leaf ethanol extract was able to induce variable levels of larval mortality. The injection of increasing doses of an ethanolic extract of red betel leaf resulted in mortality rates of 80%, 82%, and 93% among L1 larvae. Larvae can develop into pupas, but as their weight and volume decrease, their hatchability diminishes. Larvae fed red betel leaf at concentrations of 1% and 2% were able to hatch into imago, however the imago's wing condition was so poor that they could not live long.

The combination of ethanol extract of yam bean seeds and red betel leaf induced 77% larval mortality at a concentration of 0.5%, whereas at concentrations of 1% and 2% it was capable of killing 100% of L1 larvae. The larvae were able to develop into pupae after receiving 0.5% ethanol extract of yam bean seeds and red betel leaf, but their weight and volume decreased, resulting in a decrease in hatchability. The larvae were unable to grow into pupae at doses of 1% and 2%. As a result, the larvae in this population never reached the imago stage.

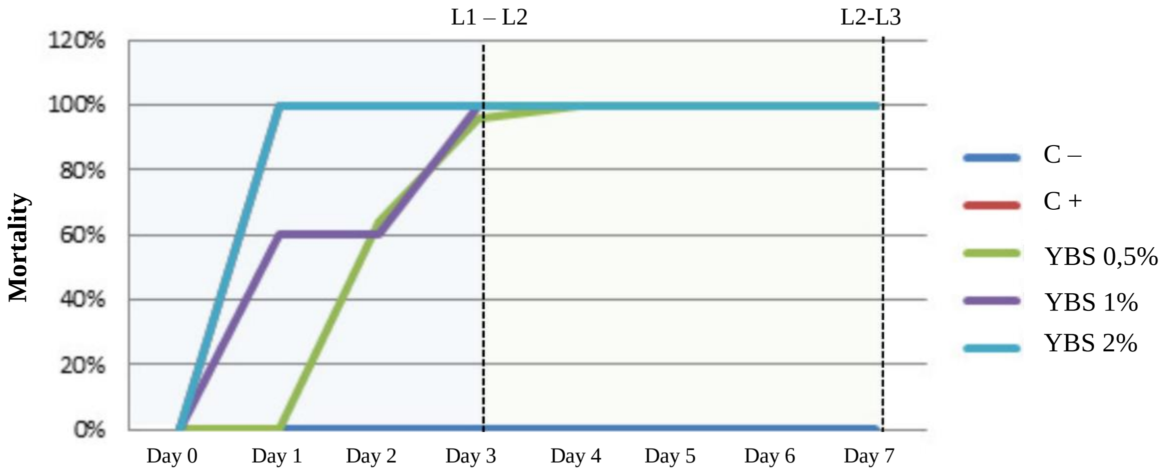
At all doses, the yam bean ethanol extract was able to eliminate all L1 larvae. Between the first and third day, larval mortality occurred (Figure 5). At concentrations of 0.5%, 1%, and 2%, the ethanol extract of red betel leaf killed L1 larvae by 80%, 82%, and 93%, respectively. From the third to the seventh day following administration of an ethanolic extract of red betel leaf, larvae died (Figure 5). At concentrations of 0,5% the ethanol extract of yam bean seeds and red betel leaf killed 77 percent of L1 larvae, while at a concentration of 1% and 2% it was able to kill 100 percent of L1 larvae. From the second to seventh day, larval mortality occurred (Figure 5).

Table 2 demonstrates that ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf has larvicidal activity against L2 and tend to have the same impact as L1. The administration of ethanol extract of yam bean seeds at varying dosages was able to kill all of the larvae, preventing their development into pupae. As a result, the larvae in this population never reached the imago stage. Depending on the concentration of the formulation, red betel leaf ethanol extract was able to induce variable levels of larval mortality. 7 %, 26 %, and 21 %, respectively, of L2 larvae were killed by increasing concentrations of ethanolic extract from red betel leaf. Larvae are able to develop into pupas, but their hatchability decreases as their weight and volume decrease. Larvae fed a 0.5% concentration of red betel leaf were able to hatch into imago, however the imago's wings were deformed, limiting its ability to survive. Different quantities of yam bean seed ethanol extract were able to kill 100 percent of L2 larvae, preventing their development into pupae. As a result, the larvae in this population never reached the imago stage.

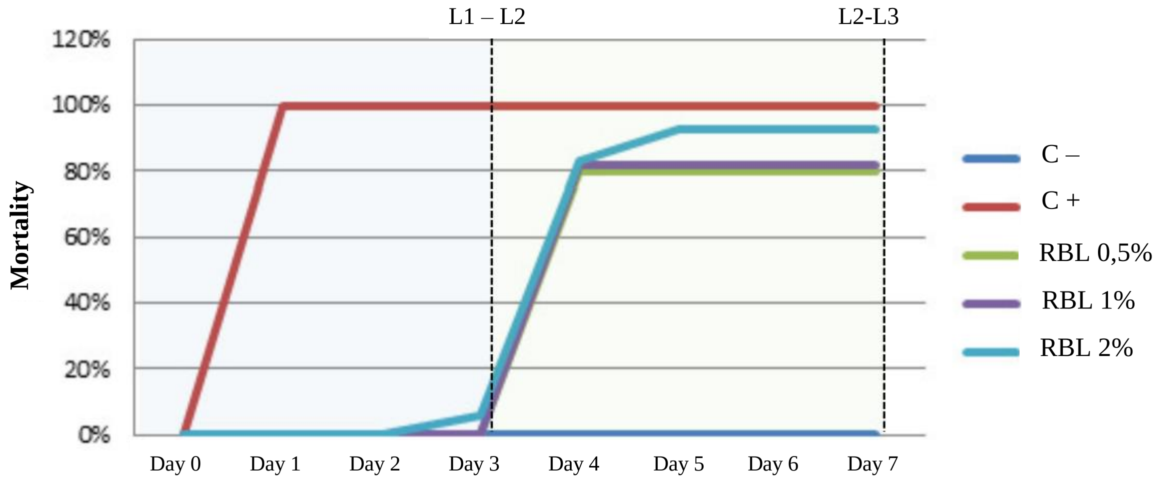
Table 1. The significance of larval mortality, average pupal weight, hatchability, and mortality of imago L1 based on *in vitro* tests using ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Treatment groups	Mortality of larvae (%)	Mean of pupal weight (g)	Hatchability rate (%)	Mortality of imago (%)
Yeam bean seed 0.5%	$100±0^a$	$0±0^c$	$0±0^c$	$100±0^a$
Yeam bean seed 1%	$100±0^a$	$0±0^c$	$0±0^c$	$100±0^a$
Yeam bean seed 2%	$100±0^a$	$0±0^c$	$0±0^c$	$100±0^a$
Red betel leaf 0.5%	$80±7.91^b$	$0.00589±0.00309^c$	$0±0^c$	$100±0^a$
Red betel leaf 1%	$82±13.51^{ab}$	$0.0073±0.01026^c$	$40±54.8^b$	$100±0^a$
Red betel leaf 2%	$93±13.04^{ab}$	$0.0064±0.00921^c$	$40±54.8^b$	$100±0^a$
Combination 0.5%	$77±24.9^{ab}$	$0.01761±0.01464^b$	$0±0^c$	$100±0^a$
Combination 1%	$100±0^a$	$0±0^c$	$0±0^c$	$100±0^a$
Combination 2%	$100±0^a$	$0±0^c$	$0±0^c$	$100±0^a$
Control -	$0±0^c$	$0.03431±0.000741^a$	$86±7.42^a$	$14±7.42^b$
Control + (Asuntol)	$100±0^a$	$0±0^c$	$0±0^c$	$100±0^a$

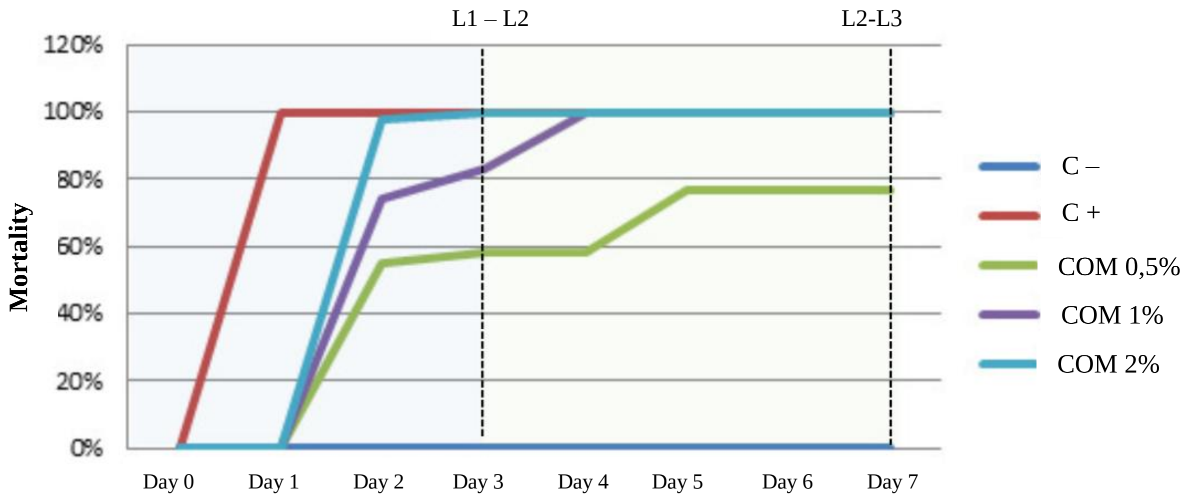
Different superscript letters on the same line showed significant differences (P<0.05)



A. Mortality time graph of L1 ethanol extract of yam bean seed



B. Mortality time graph of L1 ethanol extract of red betel leaf



C. Mortality time graph of L1 the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Figure 5. Mortality time graph of L1 ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Table 2. The significance of larval mortality, average pupal weight, hatchability, and mortality of imago L2 based on *in vitro* tests using ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Treatment groups	Mortality of larvae (%)	Mean of pupal weight (g)	Hatchability rate (%)	Mortality of imago (%)
Yam bean seed 0.5%	$100±0^{a}$	$0±0^{c}$	$0±0^{a}$	$100±0^{a}$
Yam bean seed 1%	$100±0^{a}$	$0±0^{c}$	$0±0^{a}$	$100±0^{a}$
Yam bean seed 2%	$100±0^{a}$	$0±0^{c}$	$0±0^{a}$	$100±0^{a}$
Red betel leaf 0.5%	$7±6,.71^{cd}$	$0.03665±0.00322^{a}$	$67,1±23,2^{b}$	$90±14.58^{a}$
Read betel leaf 1%	$26±19.49^{b}$	$0.01831±0.00835^{b}$	$0±0^{a}$	$100±0^{a}$
Read betel leaf 2%	$21±1.884^{bc}$	$0.012678±0.00132^{b}$	$0±0^{a}$	$100±0^{a}$
Combination 0.5%	$100±0^{a}$	$0±0^{c}$	$0±0^{a}$	$100±0^{a}$
Combination 1%	$100±0^{a}$	$0±0^{c}$	$0±0^{a}$	$100±0^{a}$
Combinantion 2%	$100±0^{a}$	$0±0^{c}$	$0±0^{a}$	$100±0^{a}$
Control -	$0±0^{d}$	$0.0396±0.001493^{a}$	$59±13,42^{b}$	$59±13.42^{b}$
Control + (Asuntol)	$100±0^{a}$	$0±0^{c}$	$0±0^{a}$	$100±0^{a}$

Different superscript letters on the same line showed significant differences ($p<0.05$)

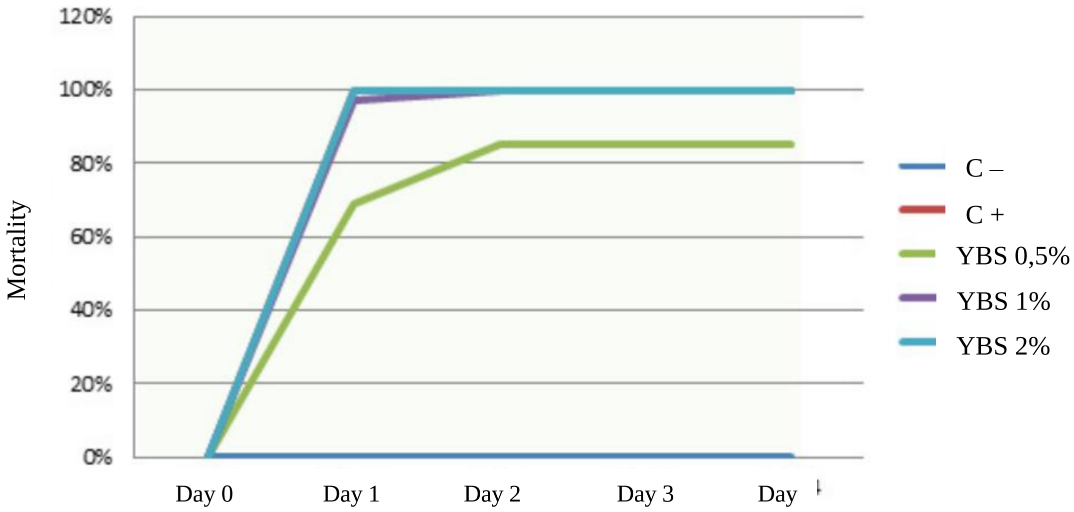
At all doses, the ethanol extract of yam bean was able to kill 100 percent of L2 larvae. Death of larvae happened between the first and fourth day (Figure 6). At concentrations of 0.5%, 1%, and 2%, red betel leaf ethanol extract was able to kill L1 larvae by 7%, 26%, and 21%, respectively. Larvae administered an ethanolic extract of red betel leaf perished between the first and fourth day (Figure 6). At all doses, the combination of ethanol extract of yam bean seeds and red betel leaf killed 100 percent of L2 larvae. Death of larvae occurred between the first and fourth days (Figure 6).

Table 3 demonstrates that the ethanol extract of yam bean seeds, the red betel leaf, and the combination of the ethanol extract of yam bean seeds and the red betel leaf have larvicidal action against L3 and tend to have the same impact as L1 and L2. The L3 larvicidal test revealed that all treatment groups (ethanol extract of yam bean seeds, red betel leaf, and a combination of ethanol extract of yam bean seeds and red betel leaf) at 10, 60, and 180 seconds of immersion resulted in smaller changes in pupa weight and were significantly different from the group negative control, but not from the group positive control.

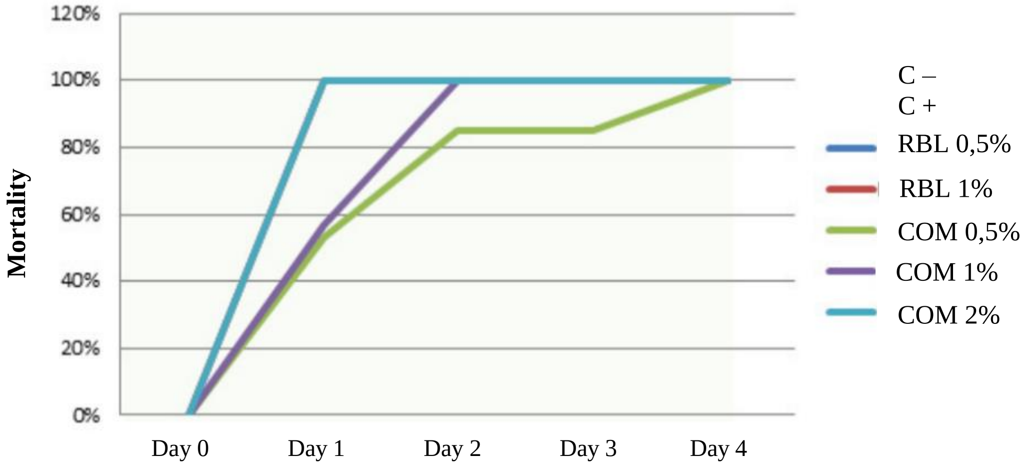
All treatment groups were able to lower hatchability at 10 seconds of immersion, based on hatchability measurements. This is evidenced by the preparation's 0% hatchability, which indicates that these three preparations did not differ significantly from the positive control. In contrast, only red betel leaf ethanol extract with a 0.5% concentration showed a 67.1% hatchability. This means that there is no substantial difference between the preparation and the negative control. The imago mortality rate at 10 seconds of immersion indicated that all preparations were able to raise imago mortality. This is evidenced by the fact that the mortality rate of imago is 100 percent in all preparations, except for the 0.5% ethanol extract of red betel leaf, where it is 90 percent (Table 3).

Table 3 demonstrates that the hatchability and mortality of imago after 60 seconds of immersion varied based on the concentration of the preparation. At 60 seconds of immersion, the concentration of 2% ethanol extract of yam bean extract and red betel leaf, which was 8%, showed the lowest hatchability. The difference between this activity and the positive control was not statistically significant. All preparations were able to raise imago mortality after 60 seconds of immersion. This is evidenced by the fact that the mortality rate of imago in each preparation was not substantially different from that of the positive control. Immersion for 60 seconds also revealed that 2% ethanol extract of yam bean extract and 2% ethanol extract of yam bean seeds and red betel leaf produced the highest imago mortality rate.

The hatchability and mortality of imago during 180 seconds of immersion varied with the concentration of the solution. The combination of ethanol extract of yam bean seeds with a concentration of 2% had the lowest hatchability after 180 seconds of immersion, at 9% (Table 3). This shows that the preparation does not differ significantly from the control sample. In general, the imago mortality rate at 180 seconds of immersion demonstrated that all preparations were able to raise imago mortality. This is evidenced by the fact that the



A. Mortality time graph of L2 ethanol extract of yam bean seed



B. Mortality time graph of L2 the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Figure 6. Mortality time graph of L2 ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

imago mortality rate of each preparation did not differ significantly from the positive control. Immersion for 180 seconds also revealed that a 2% mixture of ethanol extract of yam and red betel leaf extract had the highest mortality rate among imago.

Chemicals are frequently used to prevent or control pest infestations since they are the simplest and most cost-effective method (Hidalgo et al. 1998). However, prolonged use of chemical-based insecticides can lead to vector resistance if the use is not targeted, the dose is not appropriate, the application is not timely, and it has a negative effect on the environment and non-target organisms (Kementerian Kesehatan RI 2018). Therefore, a safe alternative to traditional insecticides is required. Plant products have been traditionally employed as pesticides throughout the world for decades. Compared to synthetic pesticides, vegetable insecticides are safer for the environment, cheaper, and easier to use by farms and small businesses (Belmain et al. 2001). This is due to the fact that bioinsecticides are effective against a restricted number of species, frequently biodegrade into non-toxic chemicals, and may be suited for use in integrated pest control, making them safer (Kim et al. 2003). Vegetable insecticides are natural substances derived from plants that contain bioactive secondary metabolites. These bioactive chemicals can be employed as synthetic pesticides.

The belief that red betel leaves and yam bean seeds are effective insecticides is backed by the fact that both plants contain naturally occurring compounds believed to be effective as insecticides. The chromatographic study of yam bean seeds revealed the presence of chemicals with rotenone and curcumin. Rotenone is an isoflavonoid with contact and gastrointestinal toxicities.

Table 3. The significance of average pupal weight, hatchability, and mortality of imago L3 based on *in vitro* tests using ethanol extract of yam bean seeds, red betel leaf, and the combination of ethanol extract of yam bean seeds and red betel leaf (1:1)

Treatment groups	Mean of pupal weight (g)	Hatchability rate (%)	Mortality of imago (%)
Immersed for 10 seconds			
Yam bean seed 0.5%	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Yam bean seed 1%	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Yam bean seed 2%	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Red betel leaf 0.5%	0.03665 ± 0.00322^{a}	67.1 ± 23.2^{b}	90 ± 14.58^{a}
Red betel leaf 1%	0.01831 ± 0.00835^{b}	0 ± 0^{a}	100 ± 0^{a}
Red betel leaf 2%	0.012678 ± 0.00132^{b}	0 ± 0^{a}	100 ± 0^{a}
Combination 0.5%	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Combination 1%	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Combination 2%	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Control -	0.0396 ± 0.001493^{a}	59 ± 13.42^{b}	59 ± 13.42^{b}
Control + (Asuntol)	0 ± 0^{c}	0 ± 0^{a}	100 ± 0^{a}
Immersed for 60 seconds			
Yam bean seed 0.5%	0.02449 ± 0.00276^{bc}	35 ± 17.68^{cde}	100 ± 0^{a}
Yam bean seed 1%	0.02741 ± 0.001989^{b}	69 ± 6.52^{abc}	98 ± 4.47^{a}
Yam bean seed 2%	0.02281 ± 0.00396^{bc}	30 ± 14.58^{de}	100 ± 0^{a}
Red betel leaf 0.5%	0.02546 ± 0.0032^{bc}	75 ± 11.73^{ab}	90 ± 7.91^{a}
Red betel leaf 1%	0.02559 ± 0.000521^{bc}	64 ± 14.75^{bcd}	98 ± 4.47^{a}
Red betel leaf 2%	0.02316 ± 0.00252^{bc}	65 ± 10.61^{abcd}	95 ± 5^{a}
Combination 0.5%	0.02138 ± 0.00498^{bc}	39 ± 25.3^{cde}	93 ± 8.37^{a}
Combination 1%	0.02343 ± 0.002222^{c}	52 ± 28.9^{bcd}	94 ± 8.22^{a}
Combination 2%	0.02124 ± 0.001984^{c}	8 ± 8.37^{e}	100 ± 0^{a}
Control -	0.03694 ± 0.000659^{a}	100 ± 0^{a}	0 ± 0^{b}
Control + (Asuntol)	0.02414 ± 0.002198^{bc}	33 ± 19.24^{de}	100 ± 0^{a}
Immersed for 180 seconds			
Yam bean seed 0.5%	0.020738 ± 0.001731^{b}	55 ± 7.91^{bcde}	96 ± 6.52^{a}
Yam bean seed 1%	0.02089 ± 0.00322^{b}	46 ± 14.32^{de}	97 ± 6.71^{a}
Yam bean seed 2%	0.02052 ± 0.001098^{b}	9 ± 12.45^{f}	99 ± 2.24^{a}
Red betel leaf 0.5%	0.02264 ± 0.00214^{b}	72 ± 13.04^{bcd}	88 ± 10.37^{ab}
Red betel leaf 1%	0.02289 ± 0.001962^{b}	73 ± 7.58^{bc}	99 ± 2.24^{a}
Red betel leaf 2%	0.02296 ± 0.00337^{b}	80 ± 11.73^{ab}	77 ± 15.65^{b}
Combination 0.5%	0.02143 ± 0.00394^{b}	68 ± 10.95^{bcd}	92 ± 10.37^{ab}
Combination 1%	0.02167 ± 0.002161^{b}	60 ± 15^{bcd}	89 ± 9.62^{ab}
Combination 2%	0.02199 ± 0.00242^{b}	50 ± 12.75^{cde}	100 ± 0^{a}
Control -	0.03818 ± 0.001015^{a}	100 ± 0^{a}	0 ± 0^{c}
Control + (Asuntol)	0.0195 ± 0.00274^{b}	33 ± 19.24^{ef}	100 ± 0^{a}

Different superscript letters on the same line showed significant differences (P<0.05)

Rotenone is a broad-spectrum cytotoxin because it can impede the mitochondrial electron transport chain. Rotenone is a respiratory enzyme inhibitor, hence it can cause respiratory failure (Ware and Whitacre 2004). Curcumin in yam bean seeds possesses insecticidal properties by suppressing nervous system enzymes (Prasad and Muralidhara 2014) and cell proliferation (Cui et al. 2016). According to the findings of the study (Sethuraman et al. 2017), curcumin compounds exhibited a cytotoxic effect on insect cell lines.

Quassin is the possible pesticide present in the ethanolic extract of red betel leaf, according to a review of the scientific literature. Quassin is a moderate pesticide with multiple modes of action, primarily preventing feeding, stomach poisoning, nervous system diseases, and contact poisoning (Jababu et al. 2019). Quassin is larvicidal against *Culex quinquefasciatus* mosquitoes by inhibiting tyrosinase activity, which impacts cuticle growth (Evans and Kaleysa 1992). Enzyme inhibition mechanism and cytotoxic activities are also present in Quassin. (Hertel et al. 2006) found that at relatively high doses, quassin had an effect on the insect circulatory system.

Chromatographic study of red betel leaf and yam bean seeds revealed the presence of additional insecticide-potential active components, particularly coumarin. As pesticides, coumarin chemicals immobilize insects through their method of action (Nicholson and Zhang 1995). The insect will undergo a slow-developing paralysis that will ultimately result in death. Disruption of muscle bioenergy has been identified as the primary mechanism underpinning surangin B's insecticidal activity (Zheng et al. 1998). In addition, homology models and docking studies reveal that coumarins and other terpene chemicals can inhibit acetylcholinesterase and block the octopamine receptor pathway, rendering them hazardous to insects (Khanikor et al. 2013). In addition to causing mortality, coumarins can have other deleterious impacts on insect populations, such as a reduction in reproductive capacity.

The synergistic impact of the active chemicals in yam bean seeds and red betel leaf as myiasis insecticides is connected. The identification of active substances revealed that the chemicals found in yam bean seeds and red betel leaf can increase imago mortality, decrease pupa weight, diminish hatchability, and reduce pupa weight. Based on the findings of this study, the most effective myiasis bioinsecticide is a 2% concentration mixture of yam bean seed ethanol extract and red betel leaf. Based on the observed parameters of larval mortality, average pupal weight, hatchability, and imago mortality, it was determined that the concentration was more effective than the control group.

CONCLUSION

According to the findings of this study, yam bean seeds contain 168 chemicals, but red betel leaf has 208. In yam bean seeds, rotenone, curcumin, and coumarin have the ability to operate as myiasis bioinsecticides, while in red betel leaf, quassin and coumarin have this capacity. The treatment's efficacy as a larvicide against myiasis *in vitro* has been demonstrated. The most efficient larvicidal action against *Chrysomya bezziana* larvae was demonstrated by the combination of ethanol extract of yam bean seeds and red betel leaf at a concentration of 2%.

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