

Investigating the Effects of *Solanum nigrum* Linn. against *Spodoptera frugiperda* in *Nicotiana tabacum*

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ABSTRACT

Control, management, and eradication of *Spodoptera frugiperda* or Fall armyworm (FAW) are challenging without the extensive use of inorganic pesticides. However, extensive application of inorganic pesticides threatens human health, animals, and the environment. Hence, natural, organic, and eco-friendly substances are needed to control the proliferation of FAW. The main objective of this study was to determine the effects of *Solanum nigrum* Linn., or Black nightshade or *Am-amsi* leaf and fruit aqueous extract, on FAW found in *Nicotiana tabacum*, or Burley tobacco plants. A field experimental method using a completely randomized design was employed. Data gathered were analyzed through descriptive statistics such as mean and ANOVA. Phytochemical analysis revealed that black nightshade contains flavonoids, saponins, steroids, and terpenoids. Secondly, FAWs were irritable upon application of *Am-amsi* aqueous extract and became immobile after 12 hours of application. Moreover, there was an increasing mortality of FAW in the four treatments after 24 to 72 hours of application. In addition, there was a significant difference between T₁ (25% concentration) and T₄ (100% concentration) after 36 hours; there was a significant difference between T₂ (50% concentration) and T₄ after 60 hours; and there was a significant difference between T₁ and T₄ after 72 hours. Finally, post hoc analysis showed that T₃ (75% concentration) was comparable to T₄ when compared to a synthetic insecticide. It has been demonstrated that T₄ of the aqueous extract was the most effective against FAW, while T₃ was comparable to T₄ concentration. The phytochemical components of Black nightshade contributed to the irritability, immobility, and mortality of FAW. These findings suggest that Black nightshade is a potential natural and organic larvicide against FAW. Through the use of leaf and fruit extract of *Am-amsi*, tobacco farmers can control FAW which is a safer, more cost-effective, and more eco-friendly pesticide.

KEYWORDS: Burley tobacco, organic pesticides, phytochemical analysis, *Solanum nigrum* Linn, *Spodoptera frugiperda*.

Introduction

Tobacco farming is a profitable agricultural crop because it serves as one of the sources of income for the farmers in Isabela. Tobacco farming provided employment for 43,960 tobacco farmers in the Philippines with 18.17% tobacco farmers originating from Isabela (NTA, 2023a). Tobacco farming contributes employment opportunities in the agriculture sector. The agriculture sector in the Philippines provides a total employment of 23.87% or 10.36 million in 2020-2022. These data imply that almost one-fourth of the employed

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44 population are engaged in agriculture. This population considers farming as a primary source
45 of income among farmers (Baclig, 2022).

46 There are several varieties of tobacco plants, but only three varieties are commonly
47 grown by tobacco farmers in Isabela. These include *Nicotiana rustica*, or Native tobacco;
48 *Nicotiana tabacum* or Burley; and *Nicotiana tabacum* Linn. or Broadleaf (NTA, 2023b). In
49 Quirino, Isabela, Burley is usually grown by most tobacco farmers. The following are the
50 photos of tobacco species grown in Isabela.



51
52 **Fig. 1. Photos of *Nicotiana rustica*, *Nicotiana tabacum*, and**
53 ***Nicotiana tabacum* Linn., respectively (NTA, 2023)**

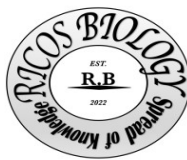
54 However, cultivating tobacco plants requires intensive care and management. Many
55 pests grow along with the growing tobacco plants. The existence and rapid population growth
56 of pests such as *Spodoptera frugiperda* or Fall armyworm (FAW) is one of the major problems
57 among tobacco farmers in the country (DA, 2020). Matova *et al.* (2020) mentioned that control,
58 management, and eradication of FAW was challenging. This kind of pests greatly affect the
59 growth and quality of tobacco plants. The widespread occurrence of FAW occurs during the
60 vegetative and flowering stage of the tobacco plants. For this reason, tobacco farmers employ
61 scouting where they manually pick the larvae out of the tobacco plants. This requires a great
62 manpower, time, and cost. Conveniently, the majority of the tobacco farmers sprayed inorganic
63 pesticides in tobacco plants to manage the proliferation of FAW. Accordingly, Lu (2022)
64 revealed that farmers utilized pesticides on an average 2.31 days per week and were exposed
65 for 3.46 months per cropping season. Tobacco farmers spray inorganic pesticides more than
66 twice a week on the onset of vegetative stage of tobacco up to flowering stage. Consequently,
67 FAW larvae may have developed pesticide resistance due to intensive use of pesticides. This
68 may often lead to the disruption of tobacco growth resulting in decreased tobacco production
69 and income. Additionally, Huyen *et al.* (2020) reported that overapplication of inorganic
70 pesticides on crops threatens human's health and the environment.

71 To lessen the risks of inorganic pesticides, we can maximize the potentials of medicinal
72 plants found in the backyards. *Solanum nigrum* or Black nightshade can be a potential natural
73 and organic pesticide in controlling the population of FAW, which are cost-effective, harm-
74 free, and eco-friendly.

75 Black Nightshade is an erect, branched and smooth herb and one meter or less in height.
76 Stems are green and leaves are oblate to oblong and have pointed ends. Its flowers are white
77 and fruits or berries are smooth, round and green when unripe and turn dark purple when ripens
78 and seeds are yellow. Figure 2 shows illustrations of the *Am-amsi* plant.

79 In the Philippines, it is called *Am-amsi* in Ilocano, *Lubi-lubi* in Tagalog, and *Amti* in
80 Ifugao. For Ilocanos, the young leaves of *Am-amsi* are edible and usually cooked as a fermented
81 fish sauce soup-based dish, steamed or as a salad. They can grow anywhere.

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The *Solanum nigrum* Linn. belongs to Kingdom Plantae, Phylum Magnoliophyta, Order Solanales, Solanaceae family, and Genus Solanum. It is widely spread in tropical regions and subtropical regions. It has beautiful and whitish flowers.



Fig. 2: Photos of whole *Am-amsi* plant and gathered whole *Am-amsi* plant taken by the researcher, respectively

It has 39 species and 14 varieties in China. In Southeast Asia, it is a natural and common edible medicinal herb. It is also widely distributed in the temperate regions of Europe, Asia, and America. Traditionally, it has been used by people to treat cancers, acute nephritis, urethritis, leucorrhea, sore throat, toothache, dermatitis, eczema, carbuncles, and furuncles (Chen *et al.*, 2022). Additionally, Khan, Qais, and Ahmad (2019) described it as a herbaceous plant with small, green, rounded berry fruits that turns purple to black when ripe and are used to treat various diseases, including cancer and tumors.

There are various benefits of black nightshade, which include sources of food and medicine due to its various nutritional and medicinal characteristics. Mandal *et al.* (2023) reported that it served as a food supply in Indian cultures. Moreover, it was used to treat infectious diseases such as cancer, acute nephritis, leucorrhea, sore throats, toothaches, dermatitis and eczema. It was also reported that it has anti-analgesic and anti-microbial activity. Additionally, Jain *et al.* (2011) found out to have antiproliferative activity in preventing the spread and growth of tumor cells in the liver, colon, and breast, as well as antiseizure, antioxidant, anti-inflammatory, and antifungal activity.

Various studies revealed that it contains natural phenolic and flavonoid compounds (Campisi *et al.*, 2019; Alam *et al.*, 2022; Callano, 2021; Thejaswini *et al.*, 2023). These two substances promoted antioxidant activity in preventing and managing neurodegenerative diseases and reduced liver enzymes and oxidative stress. In addition, Bibon (2021) reported that it contains solasodine. Solasodine has antibacterial activity against *Escherichia coli*, which causes diarrhea and vomiting once it enters the human body.

Apart from the nutritional and medicinal value of Black nightshade, it possesses pesticidal activity on various test insects. Spochacz *et al.* (2020) experimented with the extract of *S. nigrum* and found out that it could increase the toxicity of fenitrothion against *Tenebrio molitor* larvae. Moreover, Rahat *et al.* (2021) evaluated the insecticidal activity of *S. nigrum* on 2nd instar larvae and adults of *Drosophila melanogaster*. When the treatment was applied and ingested by the test subject, the larvae showed mortality. Malformation of adults' wings was observed after treating the different concentrations.

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126 In another study, Rahman (2022) evaluated the efficiency of alcoholic and alkaloid
 127 extracts of leaves and fruits of *S. nigrum* against immature larvae of blue fly with varying
 128 concentrations. Alcoholic extracts have the highest effect on killing the eggs of blue flies with
 129 89.11%, while alkaloid extracts have 88.83% mortality rates. Alcoholic extracts were more
 130 effective than alkaloids in killing the larvae of blue flies. Anti-larvicidal activity of *S. nigrum*
 131 was also explored by Mandal *et al.* (2023). 100 ml extracts of each plant part were added with
 132 ethyl acetate solvent. The larval food with concentrations was fed to the larvae. It was observed
 133 that the larvae were immobile and eventually died.

134 Based on the foregoing studies, the larvicidal activity of *S. nigrum* is a potential
 135 alternative and organic pesticide in controlling pests found in agricultural crops. Maximizing
 136 the use of this plant is beneficial for human health, environmental protection, and minimized
 137 use of synthetic insecticides.

138 FAW has the following taxonomic classification: Kingdom Animalia, Phylum
 139 Eukaryota, Class Insecta, Order Lepidoptera, Family Noctuidae, Genus *Spodoptera*, and
 140 Species *Spodoptera frugiperda*. It is a lepidopteran and polyphagous pest, which has caused
 141 major damage to maize, rice, sorghum, sugarcane, and wheat. It usually feeds on different parts
 142 of the crop, such as leaves, stems, and even fruits (Rwomushana, 2019). The first three larval
 143 stages feed on the leaves, while the older instars feed on the whorl, tassel, and ear. Pupae are
 144 oblong, whitish-green turning brown and darkened nearing adult eclosion (Navasero &
 145 Navasero, 2020). Moreover, Navik *et al.* (2021) reported that the most destructive and
 146 damaging stage of FAW is in its growing larval stage. They directly feed on stems and young
 147 shoots and leaves, which leads to slow growth and development and even death of the crops.
 148 Figure 3 presents the life cycle of FAW.

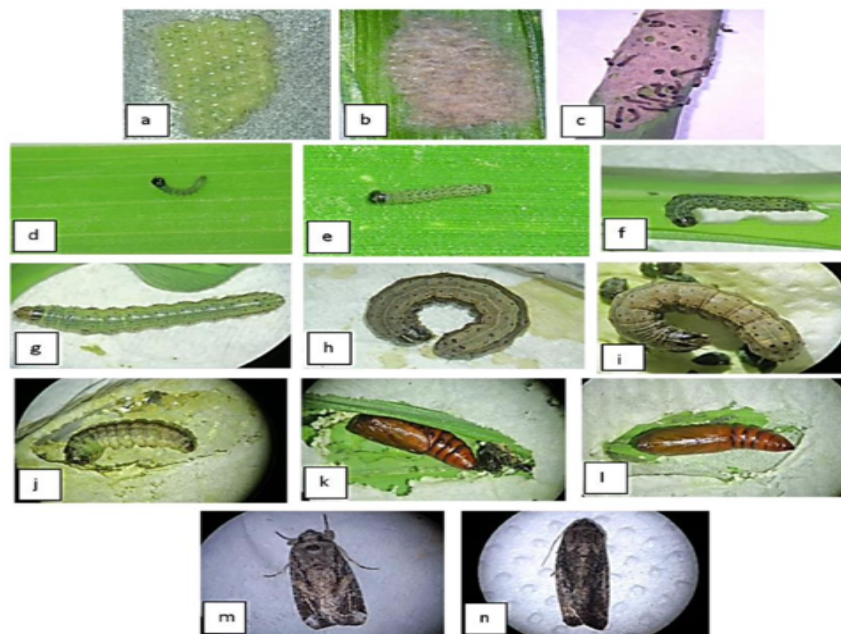
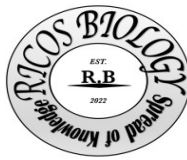


Fig. 3: The life cycle of FAW (Navasero & Navasero, 2020)

149 FAW is native in America and Europe, but its damage was first reported in Africa in
 150 2016 (Rwomushana, 2019; Hruska, 2019). Additionally, Montezano *et al.* (2018) identified it
 151 as the most important noctuid pest, which became an invasive pest in Africa, while Sisay *et al.*
 152 (2018) reported it as the major pest in maize in North and South America. In 2019, Mian (2022)
 153 reported it as the major pest in maize in North and South America. In 2019, Mian (2022)
 154 reported it as the major pest in maize in North and South America.

126-137: https://doi.org/10.24327/ricosbiology.v3i5.126-137
 138-148: https://doi.org/10.24327/ricosbiology.v3i5.138-148
 149-154: https://doi.org/10.24327/ricosbiology.v3i5.149-154
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155 stated that it is the most destructive species for several agricultural crops in Pakistan. In India,
156 it was reported that FAW damaged maize fields with fields with 44-100% field infestation
157 (Navik *et al.*, 2021).

158 In the Philippines, it was first reported in Piat, Cagayan Valley, and nine more provinces
159 in Luzon. The widespread attack of FAW drew attention for appropriate and sustainable
160 methods of control (Navasero *et al.*, 2019). Similarly, DA (2020) reported its proliferation in
161 Mindanao in 2019.

162 Because of the proliferation of FAW, farmers had decreased their yield, but it led to the
163 discovery of its sustainable management. In America, they control FAW through planting
164 genetically modified maize, while in Africa and Asia practiced intercropping, handpicking,
165 application of wood ash, soils, and tobacco extracts (Hruska, 2019). On the other hand,
166 Montecalvo *et al.* (2022) experimented with the use of wettable powders such as bentonite,
167 kaolin clay, sodium carbonate, and talcum powder. It was found out that the larvae can hardly
168 move, growth discontinued, and reduced feeding. Kaolin clay was the most effective in
169 controlling FAW. However, the use of wettable powders may harm farmers when ingested. It
170 is still safe to use organic pesticides. Furthermore, Phambala *et al.* (2020) recommended
171 farmers utilize pesticidal plants, which are effective, sustainable, and cost-beneficial in
172 managing FAW in Africa.

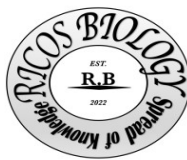
173 Despite the introduction of sustainable management of FAW, many farmers utilized
174 commercial pesticides for convenience. Mian (2022) reported that farmers sought management
175 strategies to eradicate the proliferation of FAW through the use of various insecticides. In
176 addition, Flanders, Ball and Cobb (2019) shared that the best time to apply pesticide is early
177 morning or late afternoon, when FAW is active and usually grazes in the tobacco buds.
178 However, frequent and intensive use of pesticides results in pest resistance (Phambala *et al.*
179 2020). Moreover, controlling and managing FAW through the use of pesticides is risky and
180 harmful because it is detrimental to the farmers' health and environment (Mian *et al.*, 2022).

181 The results of the study would offer benefits to the farmers, pharmaceutical industries,
182 national government agencies (NGAs) such as the Department of Agriculture (DA) and the
183 Department of Environment and Natural Resources (DENR), public, and future researchers.
184 The results of the study would be utilized by the NGAs and pharmaceutical industries for the
185 development of eco-friendly pesticides that will help the management of FAW and promote
186 the use of eco-friendly, cost-efficient, and sustainable pesticides. The community would be
187 provided with insights and perspectives with the cultivation of *Am-amsi* as a food source and
188 the use of bio-insecticides to lessen the use of synthetic pesticides. Finally, farmers would
189 effectively control and manage the rapid population growth of FAW that is both economical
190 and environmental.

191 This study generally aimed to investigate the potential use of *Am-amsi* as a larvicide
192 against FAW. Specifically, it aimed to answer the following questions:

- 193 1. What are the phytochemical compositions present in the fruit and leaf extracts of
194 *Solanum nigrum* Linn?
- 195 2. What are the effects of the varying concentrations of fruit and leaf extracts of *Solanum*
196 *nigrum* Linn. on *Spodoptera frugiperda* found in Burley tobacco plants?
- 197 3. Is there a significant difference in the varying concentrations of fruit and leaf extracts of
198 *Solanum nigrum* Linn. against *Spodoptera frugiperda*?

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Materials and methods

Gathering of *Am-amsi* and Phytochemical Screening of *Am-amsi* Aqueous Extracts

The proper identification of *Am-amsi* was done through the assistance of agriculturists from the Municipal Agriculture Office of Quirino, Isabela, Philippines, in the absence of the botanist in the area. The plant samples were collected in Manaoag, Quirino, Isabela, Philippines. They were washed with tap water to remove the dirt and were washed twice using distilled water to further remove the impurities. After washing and draining, the leaves and fruits were weighed, chopped into smaller pieces, and placed in the sterilized blender to attain a more refined particle. It was then filtered using sterilized cheesecloth and filter paper. The fresh extracts were placed in a beaker and measured with respective volume of 25% concentration or 15g /ml of the aqueous extract for Treatment 1 (T₁), 50% concentration or 30g/ml for Treatment 2 (T₂), 75% concentration or 45g/ml for Treatment 3 (T₃), and 100% concentration or 60 g/ml for Treatment 4 (T₄). Samples of *Am-amsi* aqueous extract were tested for the possible presence of phytochemical constituents such as flavonoids, saponins, phenols, steroids, terpenoids, anthocyanins, quinones, and tannins. The researchers sought the assistance of the Central Analytical Laboratory of Cagayan State University-Andrews Campus, Philippines for the confirmatory test of phytoconstituents due to the unavailability of laboratory equipment and chemicals in the school where the primary researcher is teaching. The different methods employed in determining the presence of phytochemical constituents were presented in Table 1.

Collection of FAW Larvae

FAW egg masses were observed in the tobacco field and were put under observations until they became larvae. The FAW larvae of the same stage, 3rd- 4th instar larvae, were collected and placed on the experimental plants. The proper identification of the test insects was conducted through the assistance of agriculturists from the Municipal Agriculture Office of Quirino, Isabela, Philippines, in the absence of the entomologist in the study site.

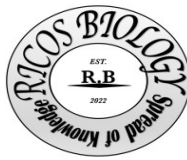
Land Preparation and Experimental Lay-out

The experimental area was harrowed two weeks before transplanting Burley tobacco, followed by the second harrowing five days before planting. The area was divided into five blocks representing the treatments with one meter distance from each block. Each block was subdivided into three plots 0.50 meter apart, representing replications.

The Burley plants were 60 days old when the second harvesting of leaves was taken. In this stage, the attack of FAW was rampant. The experimental Burley plants were not sprayed with commercial insecticides along with its neighboring Burley plants to ensure that the test insects were not affected and contaminated.

Data Gathering Procedure

To understand the relationship between two or more variables, the field experimental method was employed. A total of 450 FAW larvae were used and grouped into three consisting of three replicates with three plants per replication with 10 FAW larvae each. There were four treatments using the *Am-amsi* aqueous extracts with a volume of 60ml as the baseline: T₁ with 25% concentration or 15 ml of aqueous extract and 45 ml of distilled water, T₂ with 50% concentration or 30ml of aqueous extract and 30 ml of distilled water, T₃ with 75% concentration or 45 ml of aqueous extract and 15 ml of distilled water, and T₄ with 100% concentration or 60ml of pure aqueous extract. In addition, the fifth treatment, or T₀, for the commercial larvicide serves as the positive control.



There were three replicates per treatment. The different concentrations were sprayed with 5ml to the FAW early in the morning and were observed for 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, and 72 hours. A completely randomized design (CRD) was used. The Least Significant Difference (LSD) Test was used to test the treatment mean difference. The data were gathered in terms of the number of mortalities at a given time interval. Observed responses such as immobility, irritability, and non-feeding were also recorded. In describing the data, mean and standard deviation were used. One-way ANOVA was used to test if there was a significant difference in the larvicidal activity of *Am-amsi* leaf and fruit aqueous extract. The statistical tool used was Statistical Package for the Social Science (SPSS) software.

Results and discussion

Phytochemical Constituents present in *Am-amsi*

Based on the results obtained from the phytochemical screening through the assistance of the Central Analytical Laboratory of Cagayan State University, Philippines, it showed that *Am-amsi* fruit and leaf aqueous extracts were proven to contain secondary metabolites of flavonoids, saponins, steroids, and terpenoids. However, anthocyanin, phenols, quinones, and tannins were absent. Table 1 shows the various phytochemical constituents present in the aqueous extract of *Am-amsi*.

Table 1: Phytochemical Constituents Present in *Am-amsi* Fruit and Leaf Aqueous Extract

Parameter	Method Used	Result
Anthocyanin	Alkaline Reagent Test	-ve
Flavonoids	Shinoda Test	+ve
Phenols	Ferric Chloride Test	-ve
Quinones	Munoz <i>et al.</i> (2021)	-ve
Saponins	Froth Test	+ve
Steroids	Liebermann-Burchard Test	+ve
Tannins	Braymer's Test	-ve
Terpenoids	Salkowski Test	+ve

Results of this study confirmed the study conducted by Jain *et al.* (2011), Callano (2021), and Thejaswini *et al.* (2023) that *Am-amsi* contained flavonoids. This phytochemical constituent has antioxidant anti-inflammatory, anticancer and antiviral property, which could be a potential larvicide against FAW.

Meanwhile, saponins, steroids, and terpenoids found in plants are also potential larvicides against FAW. Marrelli *et al.* (2016) reported that saponins have pharmacological properties such as anti-inflammatory, antifungal, and cytotoxic. Steroids present in plants have anti-cancer, anti-inflammatory, and anti-viral properties (Yerlikaya *et al.*, 2023). Finally, Boncan *et al.* (2020) discovered that terpenoids have toxic and repellent effects on insects.

The wide array of potentials of *Am-amsi* plants due to their different pharmacological properties provides various benefits, such as medicine and natural pesticides, due to the presence of the aforementioned phytochemical constituents.

The Effects of *Am-amsi* Aqueous Extract on FAW

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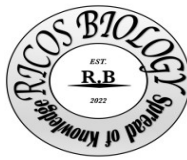
277 After 12 hours of exposure of FAW to the different treatments, only T₂ and T₄ had an
 278 effect on FAW in terms of mortality, while T₁ and T₃ had no FAW mortality. However, upon
 279 application, FAW had noticeable responses such as irritability and immobility. After 24 hours,
 280 36 hours, 48 hours, 60 hours, and 72 hours of exposure, it was noticeable that there was an
 281 increased mean mortality of FAW on all the treatments. The FAW began to be immobile and
 282 paralyzed as time went by. They did not migrate from where they were placed before the
 283 application of the treatment. There was a significant mortality within the given population after
 284 72 hours. Table 2 presents the increasing mortality rate of FAW in the different time intervals.

285 **Table 2: Mortality Rate of FAW in the Different Time Intervals**

Time	Treatment	Mean Mortality Rate of FAW
After 12 hrs	T ₁	0.00
	T ₂	1.10
	T ₃	0.00
	T ₄	2.23
After 24 hrs	T ₁	1.10
	T ₂	3.33
	T ₃	2.33
	T ₄	5.57
After 36 hrs	T ₁	1.10
	T ₂	4.43
	T ₃	5.57
	T ₄	7.77
After 48 hrs	T ₁	4.43
	T ₂	5.57
	T ₃	8.90
	T ₄	10.00
After 60 hrs	T ₁	7.77
	T ₂	8.90
	T ₃	11.10
	T ₄	14.43
After 72 hrs	T ₁	10.00
	T ₂	12.23
	T ₃	15.57
	T ₄	17.77

287 In relation to the mortality, FAW were considered dead due to the total immobility
 288 (Mandal *et al.* 2023), had dried out body, and noticeable black large spots on their head and
 289 body (Navasero and Navasero, 2020). This confirmed that the black large spots on FAW's head
 290 and body was an indication of death.

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Significant Difference of the Varying Concentration of *Am-amsi* Aqueous Extract

Analysis of variance revealed that there was no significant difference among all the treatments after 12 hours, 24 hours, and 48 hours. Additionally, there is a significant difference in at least two treatments after 36 hours, 60 hours, and 72 hours. Table 3 shows mean mortality rate of FAW, standard deviation, ANOVA Test Results. In relation to this, the Post Hoc tests after 36 hours, 60 hours, and 72 hours are shown in Table 4, 5, and 6, respectively. On the other hand, T₄ had the highest mortality rate after 72 hours of exposure.

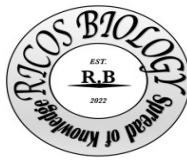
Table 3: Means, Standard Deviation and ANOVA Test Results of the Four Treatments

Time	Treatment	Mean	Standard Deviation	ANOVA Result	Interpretation
After 12 hrs	T ₁	0.00	0.00	F (3, 8) = 1.83 p = 0.22	No significant difference in at least two treatments
	T ₂	1.10	0.58		
	T ₃	0.00	0.00		
	T ₄	2.23	0.58		
After 24 hrs	T ₁	1.10	0.58	F (3, 8) = 1.94 p = 0.20	No significant difference in at least two treatments
	T ₂	3.33	1.00		
	T ₃	2.33	0.58		
	T ₄	5.57	0.58		
After 36 hrs	T ₁	1.10	0.58	F (3, 8) = 6.25 p = 0.02*	Has significant difference in at least two treatments
	T ₂	4.43	0.58		
	T ₃	5.57	0.58		
	T ₄	7.77	0.58		
After 48 hrs	T ₁	4.43	0.58	F (3, 8) = 3.78 p = 0.06	Has significant difference in at least two treatments
	T ₂	5.57	0.58		
	T ₃	8.90	0.58		
	T ₄	10.00	1.00		
After 60 hrs	T ₁	7.77	0.58	F (3, 8) = 7 p = 0.01*	Has significant difference in at least two treatments
	T ₂	8.90	0.58		
	T ₃	11.10	0.58		
	T ₄	14.43	0.58		
After 72 hrs	T ₁	10.00	1.00	F (3, 8) = 6.44 p = 0.02*	Has significant difference in at least two treatments
	T ₂	12.23	0.58		
	T ₃	15.57	0.58		
	T ₄	17.77	0.58		

* There is a significant difference.

Table 4 shows the Post Hoc Test after 36 hours of exposure of FAW to *Am-amsi* aqueous extract. It shows that only T₁ and T₄ had significant differences. The difference was

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attributed to the concentration of the applied extract because T₁ contained lesser concentration with 25% while T₄ had the highest concentration with 100%. The higher concentration of *Am-amsi* aqueous extract contributed to the higher mortality rate of FAW due to its higher concentration of phytochemical constituents.

Table 4: Post Hoc Test after 36 hours

Treatment	Other Treatments	Mean Difference	p – value	Interpretation
T ₁	T ₂	-3.33	0.23	Not significant
	T ₃	-4.47	0.09	Not significant
	T ₄	-6.67	0.01*	Significant
T ₂	T ₁	3.33	0.23	Not significant
	T ₃	-1.13	0.89	Not significant
	T ₄	-3.33	0.23	Not significant
T ₃	T ₁	4.47	0.09	Not significant
	T ₂	1.13	0.89	Not significant
	T ₄	-2.20	0.53	Not significant
T ₄	T ₁	6.67	0.01*	Significant
	T ₂	3.33	0.23	Not significant
	T ₃	2.20	0.53	Not significant

* There is a significant difference.

The Post Hoc Test after 60 hours is presented in Table 5. It shows that T₁ and T₄ had significant differences. Additionally, T₂ and T₄ also showed significant differences. These data

Table 5: Post Hoc Test after 60 hours

Treatment	Other Treatments	Mean Difference	p – value	Interpretation
T ₁	T ₂	-1.13	0.89	Not significant
	T ₃	-3.33	0.23	Not significant
	T ₄	-6.67	0.01*	Significant
T ₂	T ₁	1.13	0.89	Not significant
	T ₃	-2.20	0.53	Not significant
	T ₄	-5.53	0.03*	Significant
T ₃	T ₁	3.33	0.23	Not significant
	T ₂	2.20	0.53	Not significant
	T ₄	-3.33	0.23	Not significant
T ₄	T ₁	6.67	0.01*	Significant
	T ₂	5.53	0.03*	Significant
	T ₃	3.33	0.23	Not significant

* There is a significant difference.

imply that T₁, T₂, and T₃ had no significant difference on the potential of controlling FAW after 60 hours of exposure to *Am-amsi* aqueous extract.

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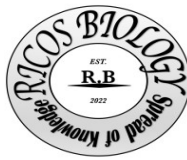


Table 6 presents the Post Hoc Test after 72 hours. It shows that T₁ and T₄ had significant differences after 72 hours of FAW exposure to the *Am-amsi* aqueous extract. These data suggest that T₄ was more effective than T₁ and T₂ at some points. On the other hand, T₃ was comparable to T₄ because they did not have a significant difference in any time tested. Hence, T₃ and T₄ were compared to T₀ to determine if the *Am-amsi* aqueous extract was comparable to the synthetic insecticide.

Table 6: Post Hoc Test after 72 hours

Treatment	Other Treatment	Mean Difference	p – value	Interpretation
T ₁	T ₂	-2.23	0.67	Not significant
	T ₃	-5.57	0.08	Not significant
	T ₄	-7.77	0.02*	Significant
T ₂	T ₁	2.623	0.67	Not significant
	T ₃	-3.33	0.37	Not significant
	T ₄	-5.53	0.08	Not significant
T ₃	T ₁	5.57	0.08	Not significant
	T ₂	3.33	0.37	Not significant
	T ₄	-2.20	0.67	Not significant
T ₄	T ₁	7.77	0.02*	Significant
	T ₂	5.53	0.08	Not significant
	T ₃	2.20	0.67	Not significant

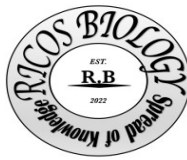
Table 7 presents the Means, SD, and ANOVA Test Results of T₀, T₃, and T₄. Based on the table, it showed that there was a significant difference between T₀, T₃, and T₄ in the different time intervals. Post Hoc Test in the different time intervals showed that T₀ had significant differences with T₃ and T₄. This means that there is a difference in the effect of T₀ with T₃, and T₄ to the FAW. Meanwhile, T₃, and T₄ did not show significant differences. This implies that these two treatments had the same effect on the FAW in the different time intervals of exposure to the *Am-amsi* aqueous extract. Additionally, T₄ was the most effective in killing FAW in comparison with T₁, and T₂.

Rahat *et al.* (2021) reported that *Am-amsi* is toxic and has great potential as an insecticidal agent. Accordingly, the potential of *Am-amsi* as a natural pesticide is attributed to the presence of phytochemical constituents such as flavonoids, saponins, steroids, and terpenoids. The phytochemical constituents present in the *Am-amsi* potentially contributed to the gradual mortality of the FAW. Terpenoids repelled FAW, as evidenced by their frequent migration from one area to another of the tobacco plant during the time interval of observation. Moreover, it could be assumed that saponin contributed to the feeding inability, paralysis, and immobility of the FAW and eventually led to death. The toxicity content of *Am-amsi* contributed to the total immobility, dried-out body, and noticeable black color on the FAW head and body.

Although synthetic insecticide was not comparable to the T₃ and T₄ of *Am-amsi* aqueous extract, intensive use of synthetic insecticides on *Nicotiana tabacum* poses hazards to humans

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as well as to animals and the environment because of the harmful chemical components (Huyen *et al.* 2020).

Table 7: Means, Standard Deviation and ANOVA Test Results of the Three Treatments

Time	Treatment	Mean	Standard Deviation	ANOVA Result	Interpretation
After 12 hours	T ₃	0.00	0.00	F (2, 6) = 19.50 p= 0.002	Has a significant difference in at least two treatments
	T ₄	2.23	0.58		
	T ₀	7.77	0.58		
After 24 hours	T ₃	2.23	0.58	F (2, 6) = 39 p = 0.00	Has a significant difference in at least two treatments
	T ₄	5.57	0.58		
	T ₀	15.57	0.58		
After 36 hours	T ₃	5.57	0.58	F (2, 6) = 66.33 p = 0.00	Has a significant difference in at least two treatments
	T ₄	7.77	0.58		
	T ₀	22.23	0.58		
After 48 hours	T ₃	8.90	0.58	F (2, 6) = 85.75 p = 0.00	Has a significant difference in at least two treatments
	T ₄	10.00	1.00		
	T ₀	30.00	0.00		
After 60 hours	T ₃	11.10	0.58	F (2, 6) = 123.50 p = 0.00	Has a significant difference in at least two treatments.
	T ₄	14.43	0.58		
	T ₀	30.00	0.00		
After 72 hours	T ₃	15.57	0.58	F (2, 6) = 73.50 p = 0.00	Has a significant difference in at least two treatments
	T ₄	17.77	0.58		
	T ₀	30.00	0.00		

Therefore, farmers can use *Am-amsi* aqueous extract against FAW as a substitute to synthetic insecticide. This natural pesticide is safer to use, more eco-friendly, and more cost-effective than synthetic insecticides. In using this, it eventually helps in the decreased utilization of synthetic insecticide in controlling FAW (Spochacz *et al.* 2020), decreasing pesticide emissions, and protecting farmers' health, animals, and the environment.

Conclusions and recommendations

Based on the foregoing findings, it has been demonstrated that *Am-amsi* aqueous fresh extract has a larvicidal activity against FAW. T₃ and T₄ were effective larvicidal treatments against FAW. Thus, *Am-amsi* aqueous extract could be an alternative, organic, and effective larvicide that can control FAW in *Nicotiana tabacum* or Burley.

The results of this study can help farmers to effectively and sustainably control and manage the rapid population growth of FAW through an environment-friendly method. It can also benefit farmers by reducing the hazards posed by synthetic pesticides. Additionally, it can be utilized by pharmaceutical industries in the production and manufacture of cheaper, safer, and more eco-friendly larvicidal products.

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363 It is recommended that tobacco farmers should cultivate *Am-amsi* to have accessibility
364 of organic pesticides to preserve and perpetuate indigenous bio-larvicidal plants. Utilization of
365 organic pesticides such as *Am-amsi* aqueous extract can lessen synthetic pesticides that are
366 detrimental to humans, animals, and the environment. Through this way, it can cut the cost of
367 pesticide inputs while promoting the health and wellness of the environment and curbing
368 greenhouse gas emissions from pesticides.

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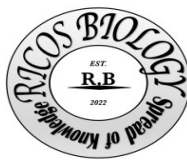
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