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Biopesticidal Effects of Madre De Cacao (*Gliricidia sepium*) and Makabuhay (*Tinospora rumpii*) Extracts on Arugula (*Eruca sativa*)

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Abstract

Considering the cost, health and environment hazards of synthetic pesticides, there is a need to find and develop practical, safe and effective alternatives. The study aimed to determine the biopesticidal effects of *Gliricidia sepium* and *Tinospora rumpii* extracts on *Eruca sativa* under different frequency of application. A 4 × 3 factorial experiment in Split Plot Design in Randomized Complete Block Design (RCBD) was used in the study. Data obtained was analyzed using STAR. Significant results were subjected to further analysis using Least Significant Differences (LSD). Sensory evaluation was analyzed using Duncan's Multiple Range Test (DMRT). Results revealed that different pesticides used in the study have significantly affected the width of leaves, shoot root ratio, biological, economic yields and sensory evaluation. No significant differences were noted on the biopesticide and frequencies observed in the parameters used in the study. The population of green peach aphids and striped flea beetles declined on the plants sprayed with the botanical extracts and commercial pesticide noticed on *Eruca sativa* sprayed with *Tinospora rumpii* extract thrice a week. The application of fermented makabuhay extract twice a week is the most economical for arugula production. The untreated plants stood out in terms of odor, crispiness, taste and passed the preferences of the respondents, while the commercial pesticide obtained the highest score average in terms of appearance and aftertaste. Twice a week application of fermented makabuhay extract is recommended for high yield and net income.

Keywords: Biopesticide effects; *Gliricidia sepium*; *Tinospora rumpii*; *Eruca sativa*

Introduction

Arugula (*Eruca sativa*) is a tangy green in the mustard family (*Brassicaceae*) also known as rocquette, rocket, garden rocket, rocketsalad or Italian cress. It is native to the Mediterranean east to Turkey and Northern India, where it grows on dry, disturbed ground. It has been cultivated at least since Roman times, and is still common in Italian cuisine, and the seeds were once used in aphrodisiac concoctions. It is a cool-season crop, grown like lettuce or spinach, use raw in salads or cooked as an accent in a variety of dishes. Although arugula has been around for a long time, it only became popular in the United States in the 1990's as a trendy leafy green [1]. Arugula is usually harvested when it is in a 'baby' stage, thus, it does not experience many insect pressures. Flea beetles, crickets, grasshoppers, ants, aphids, thrips, leafminers, whiteflies and lepidopterous larvae have been noted as pests to arugula [2].

Worms and other pests love leafy greens and to keep these away for human consumption, pesticide is often sprayed. It's often difficult to wash these chemicals off the nooks and crannies of each leaf, so they end up getting inside the body. As the arugula grows, pest can also afflict it. Flea beetles are a common problem encountered when growing arugula, as are slugs, some caterpillars, aphids and even birds [3]. Pesticides are widely used by developing countries such as the Philippines for agricultural purposes. Unfortunately, chemicals used to treat greens are suspected of causing cancer and interfering with hormone production. Arugula and other leafy greens have all been found to have high levels of pesticide residues [4]. Pesticide poisoning is still one of the global health problems. It is estimated that every year, five million people die as a result of intentional, accidental and occupational pesticide exposures worldwide [5].

Due to lack of effective means of controlling pests, most farmers resort to synthetic pesticides that are both hazardous to human health and the environment. Compared to other forms of control, synthetic pesticides are highly effective, and these pesticides are not only toxic to the target organisms but are also toxic or hazardous to a certain degree to man, plants and the environment. Moreover, continued use of pesticides can contribute to the development of resistant pest populations.

The use of plant derivatives has been studied throughout the world. Over 2,000 plant species have been reported to possess pest control characteristics. According to Wilbraham et al. (2002), certain plants contain chemical compounds with insecticidal properties. These compounds are used by the plants that contain them as protection against herbivorous insects. Such compounds therefore have chemical properties useful to humans. Some plants produce a distinctive odor or chemicals that are repulsive to insects. Realizing the potential of plants in controlling insects, botanical insecticides are now being considered instead of the wide spread use of synthetic insecticides. Botanicals are safer to the user and to the environment because they break into harmless compounds within hours and days in the presence of sunlight. Among these plants are madre de cacao and makabuhay.

Madre de cacao (*Gliricidia sepium*) is a nitrogen-fixing tree. The toxic properties of its seeds and bark give rise to the generic epithet

"*Gliricidia*=mouse killer". According to Duke and Wain (1981) as cited by Abulude et, al (2009), *Gliricidia sepium* has said to be expectorant, insecticidal, rodenticidal, sedative and suppurative [6,7]. Rabena (2007) found out that the madre de cacao leaves are good source of coumarins, a toxic substance that can kill almost all types of pests and insects [8]. On the study conducted by Tacio (2009), madre de cacao has distinctive aroma that could attract the rodents that eventually, with the right amount could terminate them [9]. Indigenous knowledge also indicates that madre de cacao has broad spectrum of uses against pests and as an herbal medicine both for humans and animals. On the other hand, research has been conducted on both the antifungal and antimicrobial properties of *gliricidia* extracts. In a brine shrimp toxicity test, a general screening method indicative of cytotoxicity and pesticidal activity, the LC50 was 454 ug.ml (CI 328-608)-medicarpin, one of the compounds in the leaves and heartwood of *gliricidia*, is supposed to be antifungal *Gliricidia sepium* Essays (2007).

Moreover, makabuhay (*Tinospora rumphii*) is a climbing, dioecious vine reaching a height of 4 to 10 meters. It grows in and nearby towns in thickets in most or all islands of the Philippines. It is considers ferfiburage, vulnerary, tonic, antimalarial, parasiticide and insecticidal. The ethanolic stem extract of *T. rumphii* contains phytochemicals such as alkaloids, flavonoids, steroids and tannins. These phytochemicals possess pesticidal activities against insects and other pests [10]. Various studies showed that this kind of plant possesses antimicrobial and parasitical activity. A good example is the study conducted by Salazar et al. (1988) as cited by Guttierez (2015), on the affectivity of the lotion from the ground stem of makabuhay in the treatment of scabies which established the acaricidal property of the said plant [11].

In addition, phytochemicals found in the ethanolic stem of makabuhay such as alkaloids, flavonoids, steroids and tannins manifest strong insecticidal effect and therapeutic roles [12]. Lee (2000) also stated that tannins can be used as insecticide against *Culex quinquefasciatus* larvae [13]. In addition, Azmathullah et al. (2011) said that alkaloids and tannins are effective as pesticides [14]. On the other hand, flavonoids possess bioactive properties such as antibacterial and insecticidal effects. Hopkins and Huner (2009) stated that steroids play a protective function by disrupting the insect's molting cycle when ingested by insect herbivores [15].

Fernandez (1996) as cited by Guttierez (2015) stated that *T. rumphii* is essential in controlling intestinal worms of goats [16]. It provides an alternative to costly drugs and potentially toxic chemical pesticides. In the study conducted by Baloc and Bulong (2013), the fermented botanical plant extracts of madre de cacao and makabuhay are as effective and comparable to the synthetic pesticide in terms of growth characteristics, average number of whiteflies and 28-spotted beetle reduced after spraying [17]. Furthermore, there is no significant difference as to the effects of the botanical plant extracts on the number of days to flowering and fruit setting of tomato as compared to the synthetic pesticide.

Re Jesus et al. (1987) as cited by Guttierez (2015), the use of makabuhay aqueous extract for seedling root soaking and spreading of chopped vines are efficient in controlling pest such as brown plant hopper, green leaf hopper and rice stem borers. As natural pesticides are prepared from natural products, application of these materials has a less unfavorable impact to the environment than chemical pesticides. Once proven that certain botanical plants are effective and comparable to their synthetic counterparts, more farmers will adopt these alternatives without reservations. This information encouraged the researcher to determine the effects of *G. sepium* and *T. rumpii* as biopesticide on arugula.

Materials and Methods

Materials

Eruca sativa seeds were used in the study particularly Astro variety. Leaf extracts of *Gliricidia sepium* and stem extracts of *Tinospora rumpii* were used as biopesticides. Plant extract was done through fermentation adopting the methodology established by Baloc and Bulong (2013). The botanical pesticides were prepared following a modified procedure of Oriental Herbal Nutrient.

The botanicals were freshly chopped separately. Proportions were one kilogram of chopped madre de cacao leaf and makabuhay stem were put in labeled container. Primary fermentation was done through adding 700 mL coconut vodka to each container, covered with pasteurized manila paper and placed in a cool and dark place for 14 days. One (1) kilogram of molasses was poured into each container and mixed thoroughly after 12 hours of fermentation. Second fermentation was undertaken for 10 days. After 10 days, 2.5 liters of coconut vinegar was added to each container and allowed to ferment for another 10 days. The chopped botanicals were strained to separate the juice. Extracts from each container was put in separate marked bottles and used as stock solution. A 10 ml stock solution was added to 5 liters of water and sprayed to the samples.

Methods

Soil samplings were randomly collected in ten spots following zigzag method. Composite soil samples were mixed, air dried and pulverized. The composite soil samples were brought to soil laboratory to determine the pH, NPK contents using corresponding laboratory analytical procedure.

A four by three (4 × 3) factorial experiment following the Split Plot Design in Randomized Complete Block Design (RCBD) wherein treatments A1-control, A2-*Gliricidia sepium*, A3-*Tinospora rumpii* and A4-commercial pesticide. Frequency of application were undertaken B1-once a week, B2-twice a week and B3-thrice a week. The totals of four hundred eighty (480) arugula plants were subjected for experimental units. Collection of data based on the parameters used was done weekly. Sample arugula plants were randomly selected from the harvest area per plot for sensory evaluation.

Results and Discussions

Height at maturity

The result of the analysis on the height at maturity of arugula at different pesticides and frequency of application was presented in Table 1. No significant interaction effect between the different pesticides and frequency of application has been observed. Irrespective of the pesticides used, arugula at A2 have been found to have higher measurement in its height at maturity, on the other hand, irrespective of the frequency of application, the arugula sprayed thrice a week (B3) have obtained higher.

Data contradicts with the findings of Tapo et al. (2013) that the height of eggplants treated with botanical pesticides, including madre de cacao, shows an evident height out performance over other

treatments [18]. This may be explained by the fact that botanicals also contain natural fertilizers which are applied in foliar form.

Frequency of Application				
Different Pesticides	**B1**	**B2**	**B3**	**Pesticide Mean**
A1	25.35	25.31	26.7	25.78^{ns}
A2	27.01	28.12	27.88	27.67^{ns}
A3	27.81	27.76	27.26	27.61^{ns}
A4	26.94	27.24	27.23	27.14^{ns}
Frequency Mean	26.78	27.11	27.26	-

Table 1: Mean height at maturity of arugula at different pesticides frequency of application.

Yield components

Length of leaves: Table 2 shows the analysis of mean length of leaves of arugula at different pesticides and frequency of application. Data show that different types of pesticides, their frequency of application and interaction failed to show significant effect on the length of arugula leaves.

Frequency of Application				
Different Pesticides	**B1**	**B2**	**B3**	**Pesticide Mean**
A1	25.25	24.98	26.6	25.61^{ns}
A2	27.82	27.71	27.98	27.84^{ns}
A3	27.91	27.93	27.82	27.89^{ns}
A4	27.1	26.81	27.21	27.04^{ns}
Frequency Mean	27.02	26.86	27.4	-

Table 2: Mean length of leaves of arugula at different pesticides and frequency of application.

Number of leaves: Table 3 shows the number of leaves of arugula per plant. Data show that the highest number of leaves was observed among arugula with commercial pesticide (A4) applied twice a week while the least was noted on arugula plant sprayed with fermented makabuhay extract applied once a week. A highly significant interaction effect between different pesticides and frequency of application has been observed.

Statistical analysis using the Least Significant Difference show that the effect of fermented madre de cacao extract applied once a week and in the control was not significantly different from the effects of commercial pesticide applied at once, twice and thrice a week. This result confirms the report given by Kamp (2006) that arugula's spicy aroma and flavor make it naturally resistant to pests [19].

Frequency of Application				
Different Pesticides	**B1**	**B2**	**B3**	**Pesticide Mean**
A1	21.03^{b}	20.80^{c}	24.69^{a}	22.17
A2	25.50^{a}	22.63^{bc}	20.52^{b}	22.88
A3	17.98^{c}	25.28^{b}	20.48^{b}	21.25
A4	23.25^{ab}	31.36^{a}	26.97^{a}	27.19
Frequency Mean	21.94	25.02	23.16	-

Table 3: Mean number of leaves of arugula at different pesticides and frequency of application.

Width of leaves: The width of arugula leaves is presented in Table 4. Data showed that the widest leaves were noted on arugula plants sprayed with fermented makabuhay extract. The least was observed on the control plants. Analysis showed significant differences among the different pesticides used in this study. However, the frequency of application and its interaction shows no significant effects.

Statistical analysis showed that the effect of fermented madre de cacao and makabuhay extracts were not significantly different from each other but significantly different from the rests of the treatments.

This result indicates that the width of arugula leaves depend on the different pesticides used, that the effect of fermented makabuhay extract was comparable with the effect of fermented madre de cacao extract.

Frequency of Application				
Different Pesticides	**B1**	**B2**	**B3**	**Pesticide Mean**
A1	8.11	8.68	8.7	8.50^{c}
A2	8.73	9.15	8.73	8.87^{ab}
A3	8.9	8.96	8.9	8.92^{a}
A4	8.57	8.65	8.49	8.57^{bc}
Frequency Mean	8.58	8.86	8.71	-

Table 4: Mean width of arugula leaves at different pesticides and frequency of application.

Biological yield

Table 5 revealed that the highest biological yield was found on arugula sprayed with makabuhay extract (A3) and the least was observed on the untreated plants (A1). Analysis showed highly significant differences among different pesticides used in this study but no significant differences among frequency of application and their interactions.

Result indicates that the effect of fermented makabuhay extract on the biological yield of arugula was comparable with the effect of commercial pesticide. This result is similar to the study of Tapo et al. (2013) that makabuhay leaf and stem extracts significantly increased the number of eggplant fruits per plant.

Frequency of Application				
Different Pesticides	**B1**	**B2**	**B3**	**Pesticide Mean**

A1	57.73	62.89	59.22	59.95[c]
A2	66.88	82.27	76.95	75.36[b]
A3	78.05	84.84	84.22	82.37[a]
A4	75	72.03	81.41	76.15[ab]
Frequency Mean	69.41	75.51	75.45	-

Table 5: Mean Biological Yield of arugula at different pesticides and frequency of application.

Economic yield

Highest economic yield was noted on arugula plants sprayed with makabuhay extracts (A3) while the least was observed on the control plants (A1) was shown in Table 6. However, no significant differences was noted among frequency of application and their statistical analysis showed that economic yield of arugula sprayed with fermented makabuhay extracts and commercial pesticide was not significantly different from each other but significantly different from the rests of the treatments. This result indicates that the effect of fermented makabuhay on the economic yield of arugula was comparable with the effect of the commercial pesticide. Result conforms to the study of Tapo et al. (2013) that the harvested eggplant treated with madre de cacao and makabuhay leaf and stem extracts was the highest among other treatments.

Frequency of Application				
Different Pesticides	**B1**	**B2**	**B3**	**Pesticide Mean**
A1	53.83	57.66	53.83	55.11[c]
A2	61.64	75.86	71.02	69.50[b]
A3	72.19	78.36	78.2	76.25[a]
A4	69.53	66.64	75.39	70.52[ab]
Frequency Mean	64.3	69.63	69.61	-

Table 6: Mean Economic Yield of arugula at different pesticides and application frequency.

Shoot root ratio

The result of the analysis on the shoot root ratio of arugula at different pesticides and frequency of application is presented in Table 7. No significant interaction effect but significant effects at different pesticides have been observed. Irrespective of the frequency of application, arugula sprayed with fermented madre de cacao, fermented makabuhay extract and commercial pesticide has been found to have significant effect over the control. Analysis shows significant differences among the different pesticides used. It implies that the effect of fermented madre de cacao and makabuhay extracts is comparable with that of commercial pesticide in terms of shoot root ratio.

Frequency of Application				
Different Pesticides	**B1**	**B2**	**B3**	**Pesticide Mean**
A1	11.15	11.06	9.57	10.59[b]
A2	11.76	12.14	12.93	12.28[a]
A3	12.04	12.72	13.52	12.76[a]
A4	12.9	11.72	12.65	12.42[a]
Factor B Mean	11.96	11.91	12.17	-

Table 7: Mean Shoot Root Ratio of arugula at different pesticides and frequency of application.

Average weekly population of green peach aphids after application

The effects of the treatments in terms of average weekly population of green peach aphids after application of pesticides were shown in Figure 1.

The average weekly population of the said insect pest found on Figure 1. The samples sprayed with commercial pesticides once a week had the lowest average population during the first week and fluctuated on the A3B3 and A3B3. However, on the second and third week lowest average population was noted on arugula plants sprayed with fermented makabuhay extract thrice a week. Average weekly population of green peach aphids has declined on the plants sprayed with the botanical extracts and commercial pesticide. This conforms to the study of Tapo et al. (2013) that madre de cacao and makabuhay extracts had the same effect as that of the synthetic formulation in reducing insect pests on eggplant.

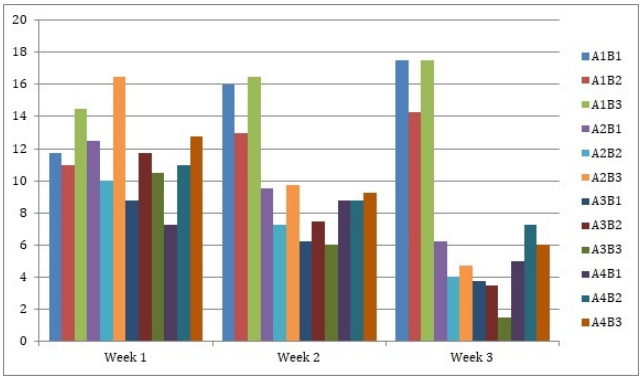

Figure 1: Average weekly population of green peach aphid after spraying.

Average weekly population of striped flea beetle after application

The effects of the treatments in terms of average weekly population of striped flee beetle after spraying were shown in Figure 2. It can be gleaned in Figure 2 that the average weekly population of the said insect pest found on control plants decreases from week 1 to week 2 then increased on the last week. However, the average weekly population of striped flea beetle declined on the plants sprayed with the botanical extracts and commercial pesticide. Lowest population of striped flea beetle on the third week was noted on plants sprayed with

fermented makabuhay thrice a week. This result agrees with the study of Allig (2009) that the effect of botanical plants as control agent against insect pests of snap beans is comparable to the synthetic pesticide used [20].

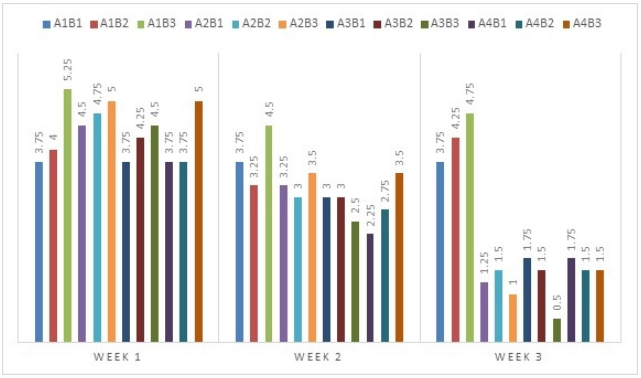

Figure 2: Average weekly population of striped flee beetle after spraying.

Sensory evaluation

Using 9-point hedonic scale of sensory evaluation, Table 8 presents the mean sensory characteristics of arugula in terms of appearance, odor, crispiness, taste (degree of bitterness), aftertaste and general acceptability of fresh arugula leaves obtained from the different treatments, respectively.

In general, the average scores fell from 4 to 7 ("disliked slightly" to 'like moderately"). With regard to the attribute appearance, A4B1 obtained the highest sensory score average of 7.60 differed from the rest of the samples. This implies that the samples sprayed once a week of commercial pesticide stood out in terms of appearance [21,22].

As for odor, A1B2 (control) which attained the highest score average of 6.87 presents significant difference among other samples. This result implies that the sample pass the preference of the respondents. With regards to the crispiness attribute, A1B1 and A1B2 (control) a score of 6.93 and 6.97, respectively, were considered statistically different from the rest of the other treatments. This infers that the untreated plants were the crispiest among the samples.

For the taste attribute (degree of bitterness), A1B3 (control) which obtained the highest score average of 4.60 differed among the remaining samples. This implies that the untreated plants have the highest degree of bitterness among the samples.

In terms of aftertaste attribute, A4B3 and A1B1 (control) that achieved the highest score average of 5.03 and 4.96 were considered as statistically different from the rest of the remaining samples. This implies that thrice a week application of commercial pesticide has different aftertaste among the remaining samples. The general acceptability values indicate that the control plants passed the preference of the respondents.

Treatment	Average Sensory Scores					
	Appearance	Odor	Crispiness	Taste	Aftertaste	General Acceptability
A1B1	7.22^{ef}	6.57^{d}	6.93^{a}	4.85^{b}	4.96^{a}	6.11
A1B2	7.50^{b}	6.87^{a}	6.97^{a}	4.85^{b}	4.85^{b}	6.21
A1B3	7.23^{ef}	6.34^{f}	6.56^{f}	4.60^{a}	4.73^{cd}	5.89
A2B1	7.27^{e}	6.73^{b}	6.63^{e}	4.33^{e}	4.33^{f}	5.86
A2B2	7.16^{g}	6.43^{e}	6.33^{g}	4.57^{d}	4.56^{e}	5.81
A2B3	7.40^{c}	6.23^{g}	6.73^{c}	4.20^{f}	4.20^{g}	5.75
A3B1	7.34^{d}	6.34^{f}	6.57^{f}	4.84^{b}	4.53^{e}	5.92
A3B2	7.00^{h}	6.30^{f}	6.34^{g}	4.75^{c}	4.64^{d}	5.81
A3B3	7.47^{b}	6.65^{c}	6.68^{d}	4.92^{c}	4.23^{g}	5.99
A4B1	7.60^{a}	6.53^{d}	6.20^{h}	4.73^{d}	4.60^{e}	5.93
A4B2	7.20^{fg}	6.67^{c}	6.87^{b}	4.83^{b}	4.83^{bc}	6.08
A4B3	7.23^{ef}	6.33^{f}	6.70^{cd}	4.84^{b}	5.03^{a}	6.03
Grand Mean	7.3	6.5	6.62	4.69	4.62	-
F=	112.05	137.71	369.06	86.67	64.46	-
P=	0.0000**	0.0000**	0.0000**	0.0000**	0.0000**	-
CV=	0.43%	0.53%	0.38%	1.04%	1.56%	-

Table 8: Scores of sensory characteristics of fresh arugula leaves.

Production income

The production income is presented in Table 9. The weight of harvested arugula per plant was converted into production basis and into peso.

Plants applied with fermented makabuhay extract twice a week gained a net income of Php 400,040.00. This implies that arugula production utilizing fermented makabuhay as biopesticide lessen the expenses in purchasing inorganic pesticides.

Conclusion

Biopesticides used in the study significantly affect the width, shoot root ratio, biological and economic yield, and average population of green peach aphids and striped flea beetles. Interaction between biopesticides and frequency of application does not show significant differences. Application of fermented *T. rumpii* extract twice a week is the most economical biopesticide for arugula production. It is also concluded that untreated plants stood out in odor, crispiness, taste which passed to the preferences of the respondents.

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