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Insecticidal Activity of *Tinospora rumphii* Plant Powder Against Maize Weevil, *Sitophilus zeamais*

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Abstract

The insecticidal activity of Tinospora rumphii stem powder was evaluated on corn weevil, Sitophilus zeamais M. under laboratory condition. Phytochemical analysis of the plant stem was conducted to determine the active toxic compounds. Three various concentrations (6%, 12% and 18%) of T. rumphii powder were exposed to corn grains containing the mature weevils within 120 hours. Results revealed that T. rumphii stem contain the following phytochemicals: flavonoids, alkaloids, steroids and tannins. Tinospora rumphii powder showed significant insecticidal activity against maize weevil as compared to the control group at 0.05 level of significance. In addition, the mortality of maize weevil treated with the plant extract is concentration-dependent effect since the percentage of weevil's mortality increase in relation to the increase of extract concentration. Higher concentrations of the extract had stronger insecticidal effect than the lower concentrations. The corn seeds treated with the plant extract shows the least number of holes (0.25 to 0.75 out of 10 seeds) compared to the negative control (10.25 out of 10 seeds). This indicates that the plant extract has a preventive effect against weevil from damaging the corn seeds. Makabuhay extract also showed a concentration-dependent in terms of the percentage weight loss of corn grains infected with the weevil. The significant decrease of weight loss in treatment/experimental groups as compared to the negative control could be a result of higher corn weevil's mortality. Moreover, result also reveals a significant difference, p = 0.000 on the percentage of germination among the control groups and those treated with various concentrations of the plant extract. The lowest percentage of germination was observed in the negative control (15 %) which is extremely low than those treated with the plant extract and the positive control, on the other hand, the highest concentration of the plant extract showed the highest percentage of germination (95%). Hence, T. rumphii stem powder possess insecticidal activity against maize weevil, S. zeamais.

Keywords: Mortality, *Sitophilus zeamais*, Grain protectant, Phytochemicals, *Tinospora rumphii*.

Introduction

Zea mays L. is recognized as a cheap source of food crops that are commonly cultivated in tropical countries[1]. Besides of its significance as a major source of food for both human and other animals, it is also utilized and processed into variety of useful industrial products such as starches and fuel sources[2]. In addition, maize is also processes into various products such as breakfast cereals, flour, cooking oil, maize germ and bran[3].

On the other hand, corn storage is greatly affected by insect damage that greatly influences the projected production of corn especially in the developing countries[4]. *Sitophilos zeamais* is one of the major damaging pest which causes serious damage to stored corn especially in the tropics[5]. The said pests begin to infest corn in the field and they are carried into the storehouses where population increases rapidly[6]. *S. zeamais* begin their infestation when female lay their eggs into the grain upon hatching the larvae feed the inside of the grain until pupal stage is reached[7]. Damaged corn grains are inappropriate for human and animal consumption and not suitable for commercial and agricultural uses[8].

In order to prevent the attack and further grain damage caused by the weevils, farmers rely on the use of commercial/synthetic pesticides for its control. However, using these synthetic chemicals pose some problems such as the toxic residues in food and humans, development of insect resistance strain, high cost, toxicity to non-target organisms and workers safety[9]. Hence, alternative biodegradable insecticides derived from plant products which is also effective, safe to environment as well as human health, and easy to utilize[10]. Various researches revealed that plant-derived products such essential oils and other compounds posses anti-feedant activities towards several insects[11,12]; plant powders also caused circumvent and mortality of weevils such as *S. zeamais*[13]. *Tinosphora rumphii* belongs to the family Menispermaceaeis, a climbing vine plant are commonly grown and matured in the wild places and can also be cultivated through artificial propagation like stem cuttings. It is commonly known as *Makabuhay* in the Philippines which literally means "bring backs life"[14]. It is also revealed that *T. rumphii* can be used to control worms in goats[15]. In addition, another study reported that the said plant extract is an effective pesticide used to kill larvae of *Aedes aegypti* mosquito[16].

This study aimed to determine the bioefficacy of Makabuhay, *Tinospora rumphii* stem powder against *Sitophilus zeamais.* The success of this study may lead to the revelation of new natural/organic pesticides that help control corn weevils thus aiding in preservation of cereal products.

Methodology

Adults of corn weevil were collected from farmers' stored corn grains in the province of Cebu, Philippines. These were brought to the Biology Laboratory of Cebu Normal University, Cebu City, Philippines. The weevils were reared with corn seeds in plastic containers covered with fine cloth to allow gas exchange. *Tinospora rumphii* stem was washed with tap water and then rinsed with distilled water. The plant samples were air dried for 96 hours at room temperature and pulverized using an electric blender. The phytochemical analysis was conducted following the standard procedure evaluating the qualitative determination of major phytochemical constituents[17].

The insecticidal activity of *T. rumphii* powder against corn weevils was carried out using 200ml plastic containers containing 10g of corn with three (3) different concentrations (6%, 12% and 18%) of the plant powder. Four replicates were made for each treatment concentration of the powder and the control groups. Malathion was used as the positive control. The containers were shaken for 5-10 minutes to ensure uniform mixing and coating. Twenty (20) adult insects of corn weevil were introduced into the treated and control. Weevil mortality was assessed every 24 hours for five days. The insects were confirmed dead when there was no response to probing with sharp pin at the abdomen[18]. On day 45, samples of the grains were taken for the determination of grain damage holes, weight loss and germination rate. For grain damage determination, 10 seeds were taken randomly from each replicate and examined for exit holes within the seeds, if any. Weight loss was obtained using the formula which is given by[19]:

$$\text{Percentage Weight Loss} = \frac{\text{Initial Weight - Final Weight x 100}}{\text{Initial Weight}}$$

The statistical tools that were used in this study are the following: the Arithmetic Mean to get the average number of dead of corn and rice weevils, Analysis of Variance (ANOVA), to determine the significant difference on the mortality of weevils between the control and the experimental groups, Post – Hoc Analysis using the Tukey Test to determine the degree of variability between the control and different concentrations of the plant powder.

Results and Discussion

Phytochemicals: Table-1 shows the following phytochemicals/ secondary metabolites present in *Tinospora rumphii* stem: alkaloids, flavonoids, steroids and tannins. Plant's secondary metabolites are important compounds that serve various biological action[20] and can take action as repellent, larvae insecticide, ovipositor attractant, insecticide and medicine[21,22]. In addition, that phytochemicals act as anti-herbivore and plant defense responses and other pesticidal activities that is comparable to commercial/synthetic insecticides[23]. Alkaloids and tannins possess medicinal and insecticidal activities[24]. Flavonoids in plants act as antioxidants, insecticides, antimicrobials and repellent[25]. Steroids take part in a defensive function by disrupting the insect's molting cycle when ingested by insect herbivores[26].

Mortality Rate: The bioefficacy of *Tinospora rumphii* stem powder as pesticide against corn weevil, *Sitophilus zeamais* was established through mortality test. The corn weevils' mortality was noted in three various concentrations of the plant extract after 24, 48, 72, 96, and 120 hours of exposure period. Table-2 shows the average percentage mortality of *Sitophilus zeamais* exposed to various concentrations of Makabuhay, *Tinospora rumphii* powder and the control groups. Results reveal on a variation of percentage mortality of maize weevil treated with the different concentrations of the plant powder and the control groups throughout the entire exposure periods. It is also noted that the weevils exposed to the negative/untreated control showed the lowest mortality (6.25%) compared to those treated with the various concentrations of the plant powder after 120 hours of observation. On the other hand, the highest concentration (18%) of the plant powder manifests the highest percentage of weevil's mortality which is equal to 86.25% after 120 hours of exposure. The lowest concentration (6%) of the plant powder reveals the least percentage of weevil's mortality. It is also noted that mortality of corn weevils increases in relation to the time of exposure. In addition, the powder also showed concentration-dependent effect because the percentage mortality of the weevils increases in relation to the increase of powder concentrations. Furthermore, the percentage mortality of the weevils through the application of the *T. rumphii* powder is comparable to the positive control (Malathion) and significantly different against the negative control.

Table-1
Phytochemicals present in Tinosphora rumphii stem

Plant Sample	Alkaloids	Cyanogenic glycosides	Flavonoids	Saponins	Steroids	Tannins
Tinospora rumphii	+++	-	+++	-	+++	+++

Legent: (-) absence, (+) - less abundant, (++) - average, (+++) - very abundant

Table-2
Average Percentage Mortality of Maize Weevil, *Sitophilus zeamais* M. Treated with Various Concentrations of Makabuhay, *Tinosphora rhumpii* Powder and the Control Groups

Treatments	Mean Percentage (%) Mortality									
	24 Hours	SD	48 Hours	SD	72 Hours	SD	96 Hours	SD	120 Hours	SD
Negative Control	0.00	0.00	0.00	0.00	0.00	0.00	3.75	4.79	6.25	4.79
Positive Control	27.50	15.55	52.50	26.30	68.75	29.00	73.75	25.00	77.50	23.30
6%	7.50	6.45	13.75	15.48	26.25	20.20	42.50	26.00	56.25	31.50
12%	6.25	4.79	21.25	2.50	45.00	4.08	71.25	9.46	83.75	11.09
18%	10.00	4.08	22.50	2.89	41.25	6.29	78.75	16.52	86.25	16.01

Table-3 showed a significant increased (p = 0.000) of percentage mortality of corn weevils exposed to various concentrations of *T. rumphii* powder and the control groups. In addition, results also revealed that the rising percentage of mortality is dependent on the length of time of exposure, p = 0.00. Moreover, the treatments and time of observation also revealed significant interaction with each other, p =0.001. This means that the treatments and time of observations made during the experiment affect simultaneously on the increasing percentage mortality of the weevil.

The Post–Hoc analysis results using the Tukey test (Table-4) revealed that the positive control, 6%, 12% and 18% concentrations of *T. rhumpii* power are significantly higher than the negative control group in terms of killing maize weevil as evidenced on the growing percentage mortality. However, the 12% and 18% concentrations showed a comparable effect to the positive control group on weevil's mortality except for the 6% concentration. Meanwhile, the 6%, 12% and 18% concentrations are found to have the same effect in killing the same number of maize weevil.

Table-5 shows the average number of holes of ten (10) seeds randomly selected from infected corn seeds treated with various concentrations of Makabuhay, *Tinospora rumphii* powder and the control groups. Result reveals a statistically significant difference, p = 0.000 (Table 5) on the variations of the number of holes that were observed among the control groups and those treated with the three concentrations of the plant powder. On the other hand, the highest number of exit holes was observed in the negative control (10.25 out of 10 seeds) wherein certain seeds with more than one (1) holes which is extremely high than those in the experimental groups (0.75 to 2.75 out of 10 seeds) and positive control (0.50 out of 10 seeds). The 18% concentration (highest concentration) of the plant powder reveals the lowest number of holes (0.75 out of 10 seeds) indicating that the plant extract has a preventive activity against from damaging the corn seeds.

Table-3
Two–Way ANOVA With Replications Results for the Differences on the Percentage Mortality of Maize Weevil, *Sitophilus zeamais* M. among Treatments with the Various Concentrations of Panyawan, *Tinosphora rhumpii* Powder and the Control Groups Across Different Time of Observations

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	*F*-Value	*P*-Value
Treatments	4	40,037.00	10,009.25	40.44*	0.000
Time of Observation	4	37,102.00	9,275.50	37.48*	0.000
Treatments x time of Observations	16	11,489.00	718.06	2.90*	0.001
Error	75	18,563.00	247.51		

Table-4
Summary of All Pairwise Comparisons Results Showing the Differences on the Percentage Mortality of Maize Weevil, *Sitophilus zeamais* M. Among Treatments of Various Concentrations of Panyawan, *Tinosphora rhumpii* Powder and the Control Groups Using the Tukey Test

Pairwise Comparisons (Treatments)	Difference of Means	T – Value	P – Value	Interpretation
Positive Versus Negative	58.00*	6.899	0.000	Significant Difference
6% Versus Negative	27.25*	3.241	0.014	Significant Difference
12% Versus Negative	43.50*	5.174	0.000	Significant Difference
18% Versus Negative	45.75*	5.442	0.000	Significant Difference
6% Versus Positive	-30.75*	-3.657	0.004	Significant Difference
12% Versus Positive	-14.50*ns*	-1.725	0.424	No Significant Difference
18% Versus Positive	-12.25*ns*	-1.457	0.593	No Significant Difference
12% Versus 6%	16.25*ns*	1.933	0.307	No Significant Difference
18% Versus 6%	18.50*ns*	2.200	0.189	No Significant Difference
18% Versus 12%	2.250*ns*	0.268	0.999	No Significant Difference

Table-5
Average Number of Holes of Ten (10) seeds Randomly Selected from Infected Corn Seeds Treated with Various Concentrations of *Tinosphora rhumpii* Powder and the Control Groups

Treatments	Average Number of Exit Holes per 10 Seeds	Standard Deviations
Negative Control	10.25	5.68
Positive Control	0.50	0.58
6%	2.75	1.50
12%	1.00	1.16
18%	0.75	0.96

Table-6
One–Way ANOVA Result on the Differences of the Number of Holes of Ten (10) seeds Randomly Selected from Infected Corn Seeds Among Treatments of Various Concentrations of *Tinosphora rhumpii* Powder and the Control Groups

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	*F*-Value	*P*-Value
Treatments	4	271.70	67.925	9.16*	0.001
Error	15	111.30	7.417		
Total	19	383.00			

Legend: *P* - Value < α = 0.05 - Significant at α = 0.05 (*), *P* - Value > α = 0.05 - Not Significant at α = 0.05 (*ns*)

Percentage Weight Loss and Germination: Table-7 shows the average percentage (%) of weight loss of corn seeds treated with various concentrations of Makabuhay, *Tinospora rumphii* powder and the control groups. Result demonstrates a statistically significant difference, $p = 0.000$ (Table-7) on the variations of the average percentage weight loss that were observed among the control groups and those treated with the various concentrations of the plant powder. Negative control shows the highest percentage of weight loss (15.35%) which is intensely high than those in the 18% concentration 0.78%) and positive control group (0.00%). In addition, the Makabuhay powder showed a concentration-dependent in terms of the percentage weight loss. The significant decrease of weight loss in treatment/experimental groups as compared to the negative control could be a result of higher corn weevil's mortality.

Table-9 shows the average percentage (%) germination of infested corn seeds by maize weevil after treatment with various concentrations of Makabuhay, *Tinospora rumphii* powder and the control groups. Result reveals a significant difference, $p = 0.000$ (Table-10) on the percentage of germination that were observed among the control groups and those treated with various concentrations of the plant powder. The lowest percentage of germination was observed in the negative control (15 %) which is extremely low than those treated with the plant extract and the positive control. In addition, the highest concentration showed the highest percentage of germination (95%).

Table-7
Average Percentage (%) of Weight loss of Corn Seeds Treated with Various Concentrations of *Tinosphora rhumpii* Powder and the Control Groups

Treatments	Average Percentage (%) of Weight Loss	Standard Deviations
Negative Control	15.35	3.70
Positive Control	0.00	0.00
6%	5.67	4.50
12%	3.17	4.89
18%	0.78	0.73

Table-8
One – Way ANOVA Result on the Differences of the Percentage (%) Weight loss of Corn Seeds among Treatments of Various Concentrations of Makabuhay, *Tinosphora rhumpii* Powder and the Control Groups

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	*F*-Value	*P*-Value
Treatments	4	615.00	153.75	13.19*	0.000
Error	15	174.90	11.66		
Total	19	789.90			

Table-9
Average Percentage (%) Germination of Infested Corn Seeds by Maize Weevil After Treatment with Various Concentrations of Makabuhay, *Tinosphora rhumpii* Powder and the Control Groups

Treatments	Average Percentage (%) of Germination	Standard Deviations
Negative Control	15.00	17.32
Positive Control	87.50	5.00
6%	70.00	29.40
12%	87.50	18.93
18%	95.00	5.77

Table-10
One – Way ANOVA Results for the Differences of the Percentage (%) Germination of infested Corn Seeds by Maize Weevil after Treatment among Various Concentrations of Makabuhay, *Tinosphora rhumpii* Powder and the Control Groups

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	*F*-Value	*P*-Value
Treatments	4	17,030.00	4,257.50	13.44*	0.000
Error	15	4,750.00	316.70		
Total	19	21,780.00			

The study on the insecticidal activity of *T. rumphii* plant stem powder against corn weevils, *Sitophilus zeamais* revealed that the various concentrations of the said plant manifest grain protectant effect. Results on phytochemical analysis revealed the presence of the following secondary metabolites: alkaloids, flavonoids, steroids and tannins can be the strong causative factors of the plant powder as killing chemical means against corn weevils. Natural products/phytochemicals are identified for their insecticidal/pesticidal activity. Through the years, several studies revealed strong evidences that plant's natural products perform significant environmental roles, such as protection against microbial and pests attack, together with environmental protection[26]. Alkaloids from *Piper longum* appeared very effective as larvicides against mosquito larvae[27]. A study revealed that alkaloids manifest anti-feedant, repellent and toxic to various insects[28]. Tannins are effective as larvicide against *Culex quinquefasciatus* larvae Lee (2000). Flavonoids isolated from *Tephrosia apollinea* revealed as toxic compounds to *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* insect pests[29]. Another study revealed that steroids possess insecticidal activity by interrupting the insect's life cycle specifically on their molting activity after eaten by herbivorous insects[26]. Saponins isolated from *Castanospermum australe* are also effective as plant protectant due to its pesticidal activity[30].

Acalypha fimbriata extract posse's insecticidal activity against *Sitophilus zeamays* which is manifested by a significant increased of maize weevil mortality compared to the untreated groups. The said plant contains the following phytocehmicals such as alkaloids, tannins, saponins, flavonoids, resins and glycosides[31]. *Vernonia amygdalina, Sida acuta, Ocimum gratissimum and Telfaria occidentalis* insecticidal efficacy against beans weevil (*Acanthscelides obtectus*). Phytochemicals that are present of the plant extracts investigated are alkaloids, flavonoids, saponins, steroids, tannins, phlobatannins and terpenoids[32]. In addition, it is also revealed that the various concentrations of *Brassica carinata* and *Gossypium hirsutum* oils possess pesticidal activity against *Sitophilus zeamais*[30]. Several studies proved that *T. rumphii* is very important natural insecticides used in controlling some insect pests. A study showed that *T. rumphii* leaf and stem ethanolic extract are very effective larvicides on *Aedes aegypti* mosquito larvae[2]. *T. rumphii* aqueous extract is effective as pesticide against rice stem borer, rice black bugs, brown plant hopper and green leaf hopper[33]; and the lotion originated from the said plant stem is valuable in controlling scabies[34]. In addition, a study revealed that *T. rumphii* crude extract are very effective as dewormer for goats[15].

Conclusion

The study on the grain protectant efficacy of Makabuhay, *Tinospora rumphii* powder against corn weevil, *Sitophilus zeamais* M. showed that the plant stem contains alkaloids, flavonoids, steroids and tannins. These phytochemicals exhibit insecticidal and pesticidal activities to insects and pests.

T. rumphii powder showed insecticidal activity to corn weevils which is manifested by a high percentage of mortality as compared to the non treated/negative control group. The mortality of the maize weevil treated with the three various concentrations of the plant powder is significantly different, $p = 0.000$ as compared to the control groups. The highest concentration (18%) of Makabuhay stem powder reveals the highest percentage of weevil's mortality which is equal to 86.25% after 120 hours of exposure. In addition, the corn seeds treated with the plant shows the least number of holes (0.75 to 2.50 out of 10 seeds) compared to the negative control (10.25 out of 10 seeds). This indicates that the plant powder has a preventive effect against weevils from damaging the corn seeds. *T. rumphii* powder also showed a concentration-dependent in terms of the percentage weight loss of corn grains infected with the weevil. The significant decrease of weight loss in treatment/experimental groups as compared to the negative control could be a result of higher corn weevil's mortality. Moreover, result also reveals a significant difference, $p = 0.000$ on the percentage of germination among the control groups and those treated with various concentrations of the plant powder. The lowest percentage of germination was observed in the negative control (15 %) which is extremely low than those treated with the plant powder and the positive control, on the other hand, the highest concentration of the plant powder showed the highest percentage of germination (95%).

The presence of phytochemicals of *T. rumphii* such as alkaloids, flavonoids, steroids and tannins can be attributed to the plant's grain protectant efficacy as killing agent against maize weevil.

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