SYNERGISM ACTIVITY BETWEEN *Moringa oleifera* AND *Azadirachta indica* CRUDE EXTRACT AGAINST *Nilaparvata lugens* (HEMIPTERA: DELPHACIDAE)

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ABSTRACT

Extract seeds of *Moringa oleifera* and *Azadirachta indica* were tested separately and in mixture (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) in the laboratory for their insecticidal activity against *Nilaparvata lugens* (Hemiptera: Delphacidae). *Moringa oleifera* and *A. indica* plant materials were extracted with hexane using maceration technique. Insecticidal bioassays were done by leaf-dip bioassays method. Second to fourth instar nymph of *N. lugens* were fed extract treated paddy seedling and were observed for 24, 48 and 72 hours. The data were analyzed using PoloPlus V.2 program, analysis of variance (ANOVA) and CompuSyn software. The results showed that the yield of extraction for *M. oleifera* was 26.7%, while for *A. indica* was 3.0%. Based on LC_{50} value, mixture extract (3:7) of *M. oleifera* and *A. indica* (LC_{50}=52.24 mg/L) were more toxic compared to other mixture ratios and single crude of *M. oleifera* (98.03mg/L) and *A. indica* (87.45mg/L). Based on mortality percentages at 72 hours, 0.05% mixture of *M. oleifera* and *A. indica* showed good efficacy against nymph of *N. lugens* with 74.81% compared to 0.025% and 0.01% mixture of *M. oleifera* and *A. indica* at 68.15% and 49.63%, respectively. Based on the combination index (CI), all mixtures have synergistic joint action against *N. lugens* nymph except ratio 8:2 (C1=0). For ratio 1:9, 2:8, 3:7, 4:6, 5:5 6:4, 7:3 and 9:1 the CI were 0.0048, 0.0086, 0.0091, 0.019, 0.0133, 0.047, 0.0163 and 0.0379 respectively.

Keywords: *Moringa oleifera*, *Azadirachta indica*, mortality, combination index, brown planthopper

ABSTRAK

Ekstrak biji *Moringa oleifera* dan *Azadirachta indica* telah diuji secara berasingan di makmal menggunakan campuran nisbah (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) bagi mengesan kesan racun terhadap nimfa *Nilaparvata lugens* (Hemiptera: Delphacidae). *Moringa oleifera* dan *A. indica* telah diekstrak menggunakan heksana melalui kaedah rendaman. Bioasai dijalankan menggunakan kaedah rendaman daun. Nimfa *N. lugens* pada instar kedua hingga keempat dibekalkan dengan pokok padi yang telah dirawat dan diperhatikan selama 24, 48 dan 72 jam. Kajian ini dianalisis menggunakan Program PoloPlus V.2 dan analisis statistik ANOVA serta perisian CompuSyn. Hasil kajian menunjukkan bahawa hasil pengekstrakan untuk *M. oleifera* adalah 26.7%, sementara untuk *A. indica* adalah 3%. Berdasarkan nilai LC_{50}, campuran ekstrak (3:7) *M. oleifera: A. indica* (LC_{50}=52.24mg/L) adalah lebih toksik berbanding campuran nisbah yang lain dan rawatan kawalan positif *M. oleifera* (98.03mg/L) dan *A. indica* (87.45mg/L). Berdasarkan peratusan kematian selepas 72 jam, campuran *M. oleifera* dan *A. indica* 0.05% lebih toksik pada (74.81%) daripada campuran *M. oleifera* dan *A. indica* 0.025% (68.15%) dan campuran *M. oleifera* dan *A. indica* 0.01% (49.63%). Berdasarkan hasil ujian indeks kombinasi (CI), campuran ekstrak mempunyai tindakan bersama sinergistik terhadap nimfa *N. lugens* di mana pada nisbah 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3 dan 9:1, CI menunjukkan kesan sinergistik tetapi pada nisbah 8:2 (CI=0). Manakala untuk nisbah 1:9, 2:8, 3:7, 4:6, 5:5 6:4, 7:3 dan 9:1 keputusan indeks kombinasi adalah seperti berikut 0.0048, 0.0086, 0.0091, 0.019, 0.0133, 0.047, 0.0163 dan 0.0379 mengikut turutan.

Kata kunci: *Moringa oleifera, Azadirachta indica*, kematian, indeks percampuran, bena perang

INTRODUCTION

Brown Planthopper (BPH), *Nilaparvata lugens* is an important and notorious pest in a rice field in Malaysia. Most of the world considered that *N. lugens* as one of the most insect pests in Asia (Chaudhary et al. 2017). This insect pest is dangerous not only because it is monophagous, but it can also spread viruses such as rice grassy stunt (RGSV) and rice ragged stunt (RRSV) viruses (Choi et al. 2010). Due to the masses of introduction to synthetic chemicals to control insect pests, the environment takes some effects such as chemical pollution. To overcome these problems, scientists found that pesticide activity has been found in plant extract and essential oil (Fauziah Abdullah et al. 2020; Rahman et al. 2016).

Many species of plants in the world were prove to have chemically compound that can affect insect growth and behaviour. One of the plants is *Azadirachta indica* from family Meliaceae, which has the potential to become biopesticide and medicinal agents (Chaudhary et al. 2017). The components in *A. indica* act as a disruptor in the molting process of insects and severe growth (Mordue & Nisbet 2000). Previous research by Shannag et al. (2014) found that eventhough the neem-based formulations unable to completely prevent food intake, but they were highly effective in suppressing green peach aphid, *Myzuz persicae* population.

On the other hand, *Moringa oleifera* can be utilized as a biopesticide because of its insecticidal effect. Although it is one of the most multipurpose plant, many people are unaware of its potential as a medicinal treatment, supplement, and fungicide and insecticide (Frank et al. 2013). *Moringa oleifera* tissues contain proteins with various biological activities, including lectins, chitin-binding protein, trypsin inhibitors, and protease, and lectins have been reported to contain insecticide (Santos et al. 2015). in. A study by Frank et al. (2013) concluded that *M. oleifera* is proven to be effective in controlling store pest insects of cowpea.

Azadirachta indica and *M. oleifera* have been selected as these plants are easy to find, cause no harm to human being, safe to environment and most importantly have insecticidal compound. Thus, with synergistic interaction between these plants' extract can enhance effectiveness in controlling *N. lugens* populations. From the study of Coelho et al. (2009) water-soluble M. oleifera lectins (WSMol) show that it had insecticidal activities against *Aedes aegypti* fourth instar stage. Koul et al. (2004) mentioned that three compounds of *A. indica*, which is 3-O-acetylsalannol, salannol and salannin ensured reduction of consumption of growth when fed to the insects.

Synergism is an integration of toxicity between two chemicals combined and enhanced toxicities than the amount of the toxicities of these chemicals (Zhu 2008). Synergistic insecticides can inhibit insecticide detoxification, increase insecticide spread within the insects cuticle, or function as a binding site for the receptor protein in the target insect. The purpose of this study was to determine the insecticidal activity of extracts from *M. oleifera* and *A. indica* against *N. lugens* and to evaluate the activity of the mixed extract mixture from two types of plants.

MATERIALS AND METHODS

Plant Materials

The research was conducted at the Laboratory of Toxicology, Faculty of Agriculture, Universiti Putra Malaysia (UPM). *Oryza sativa* (paddy) seedlings from varieties of MR22-CL2 were transferred into a round plastic container and grown in a rain glasshouse at Ladang 15, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The seedlings propagation for insect rearing and bioassay were rearranged in a cage 50 x 30 x 20 cm of size, then covered with muslin cloth to prevent others insect pests make through inside the cage. Maintenance was done daily, including watering the paddy seedlings and mechanical pest control. The seedlings aged approximately 18 to 30 days old were used to feed *N. lugens* nymph maintenance and treatment. Wijayanti et al. (2018) used the same technique, transplanting rice seedlings at 20 days old and adding BPH to the seedling 25 days later.

Insect Rearing

Second to fourth instar of *N. lugens* nymphs were collected at Sungai Besar, Sekinchan and Sawah Sempadan Selangor (3.507239, 101.177459), Malaysia. They were placed in a container of 60cm x 60cm and covered with muslin cloth to prevent the *N. lugens* nymph escaped from the container. The *N. lugens* nymphs were then introduced with paddy seedlings aged approximately 18 to 30 days old. The condition and health of plants were observed every day and replaced if the plant was fully consumed by *N. lugens* nymph. The conditions of the tanks were maintained at temperature of 28°C with 70-80% humidity and 12 hours daylight and 12 hours dark.

Plant Extraction

Plant materials used in this research are *M. oleifera* and *A. indica* seeds. Each plant material were dried up in an oven for three days in temperature of 45±2°C to reduce the remaining moisture before ground into fine powder using a Panasonic MK-5087M electric blender. The extract were prepared using hexane solvent. One hundred grams of the fine powder was soaked in 1000 ml of hexane for three days and shaked for every 24 hours. The extracted solvent was filtered using BUTCHI V-700 vacuum pump and concentrated using BUTCHI R-215 rotary evaporator at 60 rpm with 40ºC water bath temperature. The extract has been calculated by using yield of extraction formula to get the extraction yield;

$$\frac{\text{Weight of crude extract}}{\text{weight of grind}} \times 100$$

The extract was stored in the refrigerator at -20±2°C until used for further testing.

Extracts Toxicity Test

The extracted *A. indica* and *M. oleifera* were used to evaluate each crude extract's efficacy levels based on the concentration of dose of the extract. Each extract was diluted with distilled water. The concentration of test mixture *M. oleifera* and *A. indica* were 0.01%, 0.025% and 0.05%. There were prepared for nine ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) of *M. oleifera* and *A. indica*, respectively. Treatments using *M. oleifera* (1%) and *A. indica* (1%) crude extract used as a positive control.

The treatment using leaf-dip bioassay method following Insecticide Resistance Action Committee (IRAC) Susceptibility Test Method Series No. 005. The treated seedlings were placed in a small round container. Each tumbler contained 10 to 15 seedlings of paddy plant. Paddy plants were fully dipped in tests mixture for 10 seconds to ensure the mix reached all parts of the plants. The paddy plants were allowed to dry for about 10 to 15 minutes before they were covered in a clear plastic tumbler and five pairs of *N. lugens* nymphs were inserted into the tumbler.

Each treatment had five replications. The observations were recorded every 24 hours up to 3 days after treatment at temperature of 28°C with 70 to 80% humidity to determine the mortality of the *N. lugens* nymph after treatments (Heong et al. 2013).

Data Analysis

Nymph mortality data were analyzed using SAS as a Complete Randomized Design (CRD) with an analysis of variance (ANOVA). While means of the data were compared to determine significant differences using Tukey analysis with probability ($P \leq 0.05$). PoloPlus V.2 program estimated the dose mortality regression base on the normal distribution (Robertson et al. 2007). PoloPlus calculates lethal concentration (LC_{50}) with the most toxic and compared to other concentrations and ratios.

Analysis of Combination Action of Extract Mixtures

Properties of these extract mixtures were analyzed by the following formula and were calculated using a combination index at rate of LC_{50} and LC_{95}. The combination index was calculated using Compusyn Software, software used to analyze drug combination and dose effect developed by Ting-Chao Chou and Nick Martin in 2005.

The mixture ratio was interpreted based on combination index; (1) CI<0.5, the mixture components were strongly synergistic, (2) CI 0.5 to 0.77, the mixture components were less synergistic, (3) CI>0.77 to 1.43, the mixture components were additive and, 4) CI>1.43, the mixture components were antagonistic (Gisi 1996; Kosman & Cohen 1996).

RESULTS AND DISCUSSION

Plant Extraction

The yield of extracted *M. oleifera* using BUTCHI R-215 rotary evaporator was 26.7%. Meanwhile, yield for *A. indica* was 3%. Both extracts were used for further experiments.

Extract Toxicity Test

The results showed that *M. oleifera* and *A. indica* have a positive relationship with increasing concentration and the number mortality of *N. lugens*. The mixture of *M. oleifera* and *A. indica* (3:7) had the lowest LC_{50} value, 52.24 mg/L compared to single *M. oleifera* (98.03 mg/L) and *A. indica* (87.45 mg/L) extract (Table 3). At 72 hours after treatments, the highest LC_{50} value was 254.53 mg/L when *M. oleifera* and *A. indica* were combined in an 8:2 ratio. Other ratios showed LC_{50} values ranging from 77.51 mg/L to 218.15 mg/L. At 24 hours after treatments, the lowest LC50 recorded was 2.69 mg/L at ratio 7:3 (Table 1) and at 48 hours after treatments, lowest LC_{50} was 116.49 mg/L at ratio 3:7 (Table 2). According to Chaudhary et al. (2017), because the chemical properties of *A. indica* contained more chemical properties for regulating *N. lugens* nymph, the ratios of mixture that had more *A. indica* crude extract performed better than *M. oleifera*. Multiple limonoids found in *A. indica* plant extracts provide a long-term pest control mechanism while also preventing plant disease resistance to a variety of synthetic insecticides (Chaudhary et al. 2017).

Table 1. LC_{50} values of the plant extract against the 2-4th instars of *N. lugens* nymphs at 24 hours after treatment

Treatment Moringa:Neem	24 hours LC_{50} (mg/l)	Slope	Chi Square	Lower limit	Upper limit
(M:N=1:9)	13723.99	0.78	3.65	≤13723.99	≥13723.99
(M:N=2:8)	940.11	0.84	0.01	≤940.11	≥940.11
(M:N=3:7)	294.13	0.69	0.32	130.69	4849.79
(M:N=4:6)	462.76	0.84	0.00	302.17	2038.47
(M:N=5:5)	940.01	0.72	1.30	≤940.01	≥940.01
(M:N=6:4)	1185.75	0.78	1.71	≤1185.75	≥1185.75
(M:N=7:3)	2.69	0.69	0.04	≤2.69	≥2.69
(M:N=8:2)	12.28	0.84	0.04	≤12.28	≥12.28
(M:N=9:1)	56330.72	0.77	0.06	≤56330.72	≥56330.72

*M :*M.oleifera* while N : *A.indica*

Table 2. LC_{50} values of the plant extract against the 2-4th instars of *N. lugens* nymphs at 48 hours after treatment

Treatment Moringa:Neem	48 hours LC_{50} (mg/l)	Slope	Chi Square	Lower limit	Upper limit
(M:N=1:9)	220.185	0.68	0.00	≤220.19	≥220.19
(M:N=2:8)	223.891	0.70	0.00	96.70	461.63
(M:N=3:7)	116.498	0.71	3.30	1.57	205.28
(M:N=4:6)	285.347	0.67	0.01	≤285.35	≥285.35
(M:N=5:5)	261.139	0.66	2.66	≤261.14	≥261.14
(M:N=6:4)	857.936	0.67	0.67	≤857.94	≥857.94
(M:N=7:3)	835.954	0.66	0.03	≤835.95	≥835.95
(M:N=8:2)	1555.36	0.68	0.00	≤1555.36	≥1555.36
(M:N=9:1)	764.253	0.67	0.00	≤764.25	≥764.25

Table 3. LC_{50} values of the plant extract against the 2-4th instars of *N. lugens* nymphs at 72 hours after treatment

Treatment Moringa:Neem	72 hours LC_{50} (mg/l)	Slope	Chi Square	Lower limit	Upper limit
(M:N=1:9)	84.44	1.02	0.51	14.22	129.74
(M:N=2:8)	96.97	1.04	0.39	32.94	140.18
(M:N=3:7)	52.24	0.89	1.85	0	112.07
(M:N=4:6)	128.49	0.74	1.57	25.94	206.41

(M:N=5:5)	80.17	0.66	1.50	≤80.17	≥80.17
(M:N=6:4)	218.15	0.67	0.53	≤218.15	≥218.15
(M:N=7:3)	77.51	0.66	1.06	≤77.51	≥77.51
(M:N=8:2)	254.53	0.66	0.09	≤254.53	≥254.53
(M:N=9:1)	124.76	0.66	0.01	≤124.76	≥124.76
PC Moringa	98.03	0.91	1.04	≤1	≥1
PC Neem	87.45	1.44	0.46	≤1	≥1

*M: Moringa, N: Neem, PC: Positive Control

Azadirachtin, a tetranortriterpenoid limonoid, is the primary insecticidal active ingredient found in many parts of the *A. indica* tree. It has antifeedant and growth-disrupting effects, among other things (Tewari &Tiwari 2018). *A. indica* has also proven to have a better capacity to cause cellular lysis of the bacteria compared to *M. oleifera* at lower concentrations (Lucía et al. 2018). According to Nwokafor et al. (2020), the aqueous extract of *A. indica* demonstrated the strongest antibacterial activity against *Escherichia coli* compared to aqueous extracts of *M. oleifera* and ginger. However, as opposed to positive control (*M. oleifera* 1%), positive control (*M. oleifera* 1%) showed faster mortality (*A. indica* 1%). According to this, *A. indica* crude extract inhibits *N. lugens* slower than *M. oleifera*. Lucía et al. (2018) found that the methanolic extract of *M. oleifera* had a more significant antibacterial effect than *A. indica* in a study.

Toxicity of Extracts Mixture Concentration

The result showed that the extract mixture of *M. oleifera* and *A. indica* has positive relationship in terms of mortality of *N. lugens* nymphs and time exposure. Paiva et al. (2011) reported that there are had two chitin-binding lectins isolated from *M. oleifera* seeds and had shown insecticidal activities. According to Agbo et al. (2019), almost 200 insect pest species can be controlled using *A. indica* extract. The concentrations used in the mixture were 0.01%, 0.025% and 0.05% less than Positive control (*M. oleifera* 1% and *A. indica* 1% alone) concentration. The mortality of *N. lugens* treated with both extract mixtures with combination ratios (*M. oleifera*: *A. indica*) of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 at 24, 48 and 72 hours.

Based on Table 4, at 0.01% concentration of all mixture ratios gave 14.81% *N. lugens* nymph mortality after 24 hours. The mortality of *N. lugens* nymphs was increased with mortality percentage 34.81% and 49.63% after 48 and 72 hours of treatments respectively. The percentages of mortality are increasing from 26.67% (24 hours) to 48.89% (48 hours) and 68.15% (72 hours) at 0.025% concentration (Table 5). Based on Table 6, at concentration 0.05% treatment, the mortality percentage for 24, 48 and 72 hours are 31.11%, 57.04% and 74.81% respectively. The test using the 0.05% concentration indicated the more significance of mortality compared to other percentages concentration and was significantly higher at 72 hours after treatment. Test of mass ratio of these concentrations showed that the mortality percentages were increased by hours or days. Result of disrupted feeding behavior can influence insects to transmit diseases (Mordue & Nisbet 2000). On the other hand, Manikandan et al. (2015) reported that it took 20.1 and 6.3 days after incubation at 28.0°C and 36.0°C, respectively to reach 50% mortality. At 28.0°C, 50% fecundity was noted at 36^{th} days after incubation while 24.3 days at 36.0°C. It is also supported by Horgan et al. (2020) in their study that revealed *N. lugens* produced more egg clusters at between 20 and 30°C, compared to other temperatures.

Table 4. Mean % Mortality (±SE) and period of *N. lugens* nymph in the treatment with 0.01% mixture concentration

Treatments (Hour)	Mortality (%)	Mean Comparison of Mortality±SE
		Instar 2-3
0 (control)	0.00	0
T1	14.81	6.67±0.94
T2	34.81	15.67±0.94
T3	49.63	22.33±0.94

*T1: 24 hours, T2: 48 hours, T3: 72 hours, SE: Standard Error

Table 5. Mean % Mortality (±SE) and period of *N. lugens* nymph in the treatment with 0.025% mixture concentration

Treatments (Hours)	Mortality (%)	Mean Comparison of Mortality±SE
		Instar 2-3
0 (control)	0.00	0
T1	26.67	12.00±0.96
T2	48.89	22.00±0.96
T3	68.15	30.67±0.96

Table 6. Mean % Mortality (±SE) and period of *N. lugens* nymph in the treatment with 0.05% mixture concentration

Treatments (Hours)	Mortality (%)	Mean Comparison of Mortality ± SE
		Instar 2-3
0 (control)	0.00	0
T1	31.11	14.00±1.51
T2	57.04	25.67±1.51
T3	74.81	33.67±1.51

Moringa oleifera and *A. indica* at certain ratios of mixture concentration developed characteristics in controlling and as a cause for mortality of *N. lugens* after being treated by these mixture concentrations after 72 hours. A higher rate of mortality was obtained when a higher mixture concentration was used. Bello et al. (2019) reported that egg hatch of *Meloidogyne incognita* decreased as the concentrations of the *M. oleifera* and *A. indica* leaf extract increased. This can be caused by some nematicidal properties such as alkaloids, amides, flavonoids and ketones associated with *M. oleifera* and *A. indica*. An experiment also proved that insects treated in the third larval instar had mortality rates statistically different from the control group and significantly higher at the higher concentration (Martinez & Emden 2001).

Properties of Mixture Activity *M. oleifera* and *A. indica* to *N. lugens*.

Based on present results, the mixture of *M. oleifera* and *A. indica* at 3:7 ratio gave the most toxic compared to other mixtures (LC_{50}=52.24 mg/L), and the synergistic effect was great combination index (CI=0.0091), although the most significant synergistic effect is on mixture 1:9 (CI=0.0048) (Table 7). The other mixtures of *M. oleifera* and *A. indica* gave a combination index ranging from 0.0086 to 0.0379. The level of synergism is usually evaluated and calculated by a quantitative measure of plant extract combination effects, the combination index (CI) to discover and explore synergistic combinations (Huang et al. 2017). The findings of the current study indicate that the patterns of interaction (synergistic or antagonistic) in insecticide combination will depend on both the combination and the ratio of insecticides (Levchenko & Silivanova 2019).

Table 7. Combination index values of the plant extract mixtures

Moringa:Neem	**LC_{50} at 72h (mg/l)**	**CI @ LC_{50}**	**Mortality at 72h (%)**			**Effect**
			0.01	**0.025**	**0.05**	
1:9	84.442	0.0048	60	87	100	Synergistic
2:8	96.967	0.0086	53	87	100	Synergistic
3:7	52.245	0.0091	73	80	100	Synergistic
4:6	128.488	0.019	47	60	93	Synergistic
5:5	80.167	0.0133	47	73	60	Synergistic
6:4	218.154	0.047	33	60	60	Synergistic
7:3	77.509	0.0163	47	67	53	Synergistic
8:2	254.534	0	40	40	40	No effect
9:1	124.758	0.0379	47	60	67	Synergistic

The synergism of insecticides in the mixtures depended on the ratio of active ingredients (Lucía et al. 2018). From this result, a mixture with a larger number of *A. indica* had a more excellent synergistic value, compared to the mixture with larger number of *M. oleifera*, even though all still produce synergistic effect. When a combination has a synergistic effect, the total concentration of extract applied to achieve a certain effect is decreased, thus lowering the side effects and unwanted off-target interactions (Doldan-Martelli & Miguez 2015).

Based on the combination index (CI) observed at 72 hours, the synergistic properties between *M. oleifera* and *A. indica* mixtures that been shown based on the mortality rate of *N. lugens* were was compatible with each other. Analysis of the mixture extracts of *M. oleifera* and *A. indica* showed that the index combination value was smaller than 0.5 on level LC_{50}. Synergistic effect is when the combination of compounds results in Fractional Inhibitory Concentration Index (FIC) value of <0.5, then the combination of the compounds increases the inhibitory activity of one or both compounds than the compounds alone (Meletiadis et al. 2010).

These results indicate strong synergistic interaction properties. It showed that the combination of *M. oleifera* and *A. indica* is more effective and selective than each extract separately. Even though the mixtures with ratios 4:6, 5:5, 6:4, 7:3 and 9:1 were not reaching 100% mortality at 0.05% concentration, the synergistic value showed a strong synergistic effect. A study by Aungtikun and Soonwera (2021) also revealed that essential oils from *C. verum*, *C. cassia*, and *C. loureiroi* and their combinations against females of *Ae. Aegypti* and *Ae. Albopictus* showed synergistic effect even in some combinations not reaching 100% mortality.

CONCLUSION

At LC_{50} level, all ratio mixtures except for (8:2) of *M. oleifera* and *A. indica* extracts were classified as synergistic against *N. lugens* nymph. Thus, these extracts can be used for formulation and may play a significant role in controlling agricultural insect pests as sources of biopesticide because they do not accumulate and affect the ecosystem.

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