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Nanoemulsion of the Mixture of Citronella Grass Distillation Waste and *Piper aduncum* Essential Oil to Control *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Corn production in Indonesia is challenged by the attack of the new invasive pest fall armyworm (*Spodoptera frugiperda*, Lepidoptera: Noctuidae). This pest is known to be resistant to many synthetic insecticides. Botanical insecticide with nanoemulsion formulation is an option to solve this problem because it was relatively eco-friendly, and the various active components delay insect resistance and insect resurgence. The objectives of this research are to determine the characteristics of the nanoemulsion of the mixture of spiked pepper (*Piper aduncum*) and citronella grass distillate waste (*Cymbopogon nardus*) and to test the insecticidal activity of nanoemulsion against *Spodoptora frugiperda*. The nanoemulsion formulation is made with the spontaneous emulsification method. The leaf dipping technique is applied at the nanoemulsion toxicity test on *S. frugiperda* larvae. Then, the nanoemulsion formulation is analyzed with PSA and Zetasizer Nano Malyern to measure the particle size and zeta potential. The result of the research shows that the nanoemulsion of the mixture of citronella grass waste and *P. aduncum* fruit oil has insecticide activity with LC_{50} = 0.53%. Additionally, it causes mortality and developmental delay in *S. frugiperda* larvae. The nanoemulsion particle is 273.1 nm. It has homogeneity and an even distribution.

Keywords: botanical insecticide, fall armyworm, integrated pest management, nanoemulsification, particle size

INTRODUCTION

Corn is believed as one of the strategic food and feed commodities. Both rice and corn are used as the main food commodities to achieve food self-sufficiency (Indonesian Food Security Agency 2018). However, plant-disrupting organisms such as pests, pathogens, and weeds have been hindering corn production. The pest becoming a current issue in Indonesia is the recent invasive fall armyworm (*Spodoptera frugiperda* J.E. Smith). This fall armyworm for the first time has been found attacking corn crops in West Pasaman, West Sumatra (Nelly *et al.* 2021).

Today, this pest already spread throughout Indonesia (BBPOPT 2021). *S. frugiperda* can be found in more than 100 species of host plants; consequently, it causes more intense attacks than the oligophagous and monophagous pests on plants (Sharanabasappa *et al.* 2018).

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The average population of *S. frugiperda* in Solok (West Sumatera, Indonesia) is 1.05 larvae/ 2 corn plants, whereas the average population of *S. litura* is 0.24 larvae/ 2 corn plants (Nelly *et al.* 2021). Thus, appropriate pest management is indispensable to handle this pest, one of which is to prevent resistance. The law in the Republic of Indonesia (Number 12, 1992) on crop cultivation system states that Indonesia applies integrated pest management (IPM) in order to minimize the negative impact on the non-target organisms and environment (Sodiq 2000; Yuantari *et al.* 2015). One of the available alternative options in IPM is the use of botanical insecticide utilizing chemical substances from plants, which can repel or cause toxicity to the insects. The advantage of using this botanical insecticide is that it decomposes easily. Therefore, the residual effect on the harvest is significantly small and safe for non-target organisms, and it does not cause pest resistance (Prijono 2006).

There are 235 families with 2,400 species whose active components can be used as botanical insecticides (Grange and Ahmed 1988). One of them is spiked pepper (*Piper aduncum*). The main component of *P. aduncum* is dillapiole, which belongs to the phenylpropanoids group (Lina 2014). According to Bernard *et al.* (1990) and Perry *et al.* (1998), dillapiole derived from *P. aduncum* is able to inhibit cytochrome P450 enzyme activity in microsomal preparations of the digestive tract cells of *Ostrinia nubilalis* corn stalk borer larvae. Another example of plants with active components is citronella grass also contains saponins, flavonoids, polyphenols, and terpenoids that have insecticidal activity (Syamsuhidayat and Hutapea 1991).

Lina *et al.* (2021) reported the selling price of citronella oil in Indonesia is very low – namely, IDR 140,000/L and a low yield of 0.5–1.2% of the total refined raw materials are serious problems for citronella farmers in general. In the distillation process, a large amount of liquid waste (hydrosol) is produced as much as 50–60%. Hydrosol is an essential oil emulsion that is bound to water and still contains 0.02% essential oil. When botanical insecticide is formulated in its extract form, botanical insecticide has weaknesses – including short shelf life, sensitivity to the sun, and the need to repeat spraying (Wiratno *et al.* 2013). One of the solutions to improve the performance of botanical insecticide formulation is nanotechnology, which is material manipulation on the atomic scale. The nanotechnology-based formulation is beneficial in improving the application surface area, facilitating systematic activity, reducing the waste of organic solvents, protecting the active components from decompositions by microorganisms and sunlight, increasing the solubility, prolonging the persistence of active components, and improving the stability of physicochemical formulation (Sasson *et al.* 2007).

The use of citronella distillation waste as a water phase in the nanoemulsion formulation of botanical insecticide can increase the activity of nanoemulsion against insect tests. Its hydrosol potency as a botanical insecticide can be developed further to improve its performance and sale value (Lina *et al.* 2021).

This study aims to develop botanical insecticide formula, which is more effective and efficient in the form of nanoemulsion. Specifically, the objectives of this research are [1] to identify the characteristics of nanoemulsion and [2] to determine the activity of nanoemulsion against *S. frugiperda* such as larvae mortality, development delay, and antifeedant activity of *P. aduncum* nanoemulsion against *S. frugiperda*.

MATERIALS AND METHODS

This research was conducted at the Insect Bio-ecology Laboratory of Agriculture Faculty of Andalas University in Padang, Indonesia from June–August 2021. The research was conducted in a laboratory room with a temperature of 23–31 °C. The research was supported by the research facility of Andalas University and was scientifically and technically assisted by the Bandung Advanced Characterization Laboratory of the Indonesian Institute of Sciences (Indonesian: Lembaga Ilmu Pengetahuan Indonesia; further will be referred to as LIPI).

Procurement of *Spodoptera frugiperda* Larvae

Larvae of *S. frugiperda* were collected from corn plantations in the Belimbing Area, Kuranji District, Padang City, Indonesia. The larvae were taken to the laboratory and reared in plastic containers and covered with gauze. Plastic containers are plinthed using tissue paper. Feeding larvae in the form of leaves of corn plants and replacement of tissue paper were carried out every 2 x 24 h. Larvae that have become instar 3 were transferred to a separate container with a population of 5 heads/container to avoid the cannibalistic nature of the larvae. The larvae were reared to the point of becoming pupae. Every day, the pupae that appear were transferred into gauze cages that have been coated with tissues as places to lay eggs on the inside of the cage and covered with a cloth. The imago that appeared was fed with a feed in the form of 10% honey liquid, which was dripped on a cotton swab and hung in cages. Eggs laid imago in cages were transferred into plastic containers for breeding into larvae. The larva used as the object of study is the larva of the 2nd instar because it is the weakest phase and the easiest phase to handle as a research object to control with botanical insecticides.

Production of *Piper aduncum* Essential Oil

P. aduncum fruits obtained from Bukit Lampu Padang-Painan (Indonesia) were picked directly by hand and stored in plastic bags. The scissors were used to cut 200-g plants into small sizes (± 2 cm) and were placed into the 1 L boiling flask. Next, 1 L aquadest was added to the flask containing *P. aduncum* (1:5 ratio). The fruit obtained was immediately distilled directly for 4 h after the water in the flask had boiled and the rest of the fruit was stored in the refrigerator and used for subsequent distillation. The dripping oil in the receiving flask was then carefully moved into a glass bottle. Magnesium sulfate was added to remove the water that remained in the produced essential oil.

Nanoemulsion Synthesis

There were two phases in the making of 100-mL nanoemulsion by using the spontaneous emulsification method for both the organic phase and the water phase. First, the water phase (hydrosol 78.3 mL: 2.7 mL Tween 80) was homogenized with the magnetic stirrer for 30 min at 2,500 rpm, whereas constant stirring was done using the magnetic stirrer model 79-1. Next, the organic phase (5 mL bioethanol: 5 mL *P. aduncum* essential oil) was placed and homogenized in the Erlenmeyer flask (50 mL). The organic phase consisted of *Piper aduncum* and ethanol 96% (1:1 ratio), whereas the water phase consisted of citronella grass distillate waste/hydrosol and tween 80 (87:3 ratio). After 30 min, the organic phase was mixed with the water phase by dripping it slowly. It was continually homogenized using a magnetic stirrer for 45 min (Erlina *et al.* 2020). The nanoemulsion was kept in a refrigerator until used for the test.

Characterization of Insecticide Nanoemulsion Using Particle Size Analyzer (PSA)

The nanoemulsion was observed to determine the size of its particles. The size of these particles was determined based on the droplet size measured by the particle size analyzer (PSA) of Delsa™Nano in PT. NanoTech Herbal Indonesia. PSA is a measuring method in nanotechnology research, which can analyze the particles of a sample to determine particle size and distribution in the representative samples. The zeta potential was analyzed by using zetasizer Nano ZS Malvern in Bandung Advanced Characterization Laboratory of the Indonesian Institute of Sciences (Indonesian: Lembaga Ilmu Pengetahuan Indonesia, or LIPI).

Nanoemulsion Testing

Testing of nanoemulsion of *P. aduncum* fruit was carried out by preliminary tests and follow-up tests. Preliminary tests were carried out with treatment in the form of concentrations of *P. aduncum* fruit essential oil of 0 (control), 0.5, and 1% with three replications for each treatment. Each test contained 10 larvae of 2nd instar *S. frugiperda*. Further tests were carried out with a level of five concentrations and five replications. Each test used 15 *S. frugiperda* larvae. The concentrations of advanced tests used were 0, 0.33, 0.54, 0.89 1.46, and 2.40%. The larvae of the 2nd instar were put into Petri dishes welded with tissues in an inverted position, each of which contained 10 larvae. The test was carried out using the method of leaf dipping. Corn leaves were cut to a size of 4 cm x 4 cm and dipped one by one in a solution of nanoemulsion until evenly distributed and dredged. Each Petri dish was given two leaves that have been treated. Treatment feeding is carried out every 2 x 24 h. On the third day, larvae on Petri dishes were given two untreated leaves. Furthermore, the larvae that turned into the 3rd instar were separated into other plastic containers, with a larval population of five heads per container and labeled according to treatment and given untreated leaf feed up to the 6th instar.

Feeding Inhibitor Activity Test (Antifeedant)

This observation was made to determine the feeding inhibition of *S. frugiperda* larvae by measuring the area of the treatment leaves for 2 x 24 h after treatment. The area of the treatment leaves was measured by plagiarizing the treatment leaves on millimeter paper. The part of the leaves that the larvae did not eat was shaded using a pencil. The area of the eaten leaves was calculated from the unshadowed parts. Anti-eating activity is measured by calculating the index of food inhibition with the formula (Prijono 2006):

$$AF = (Cl - Tl)/ Cl \times 100\%$$

Information:

AF = antifeedant effect

Cl = area of the control leaf eaten by the larva (mm^2)

Tl = area of the treatment leaf eaten by the larvae (mm^2)

RESULTS AND DISCUSSION

Results of Nanoparticle Characterization Test

Characteristics of nanoemulsion include the particles' z-average, distribution, polydispersity index (PdI), and zeta potential. The size and distribution of the particles were measured using the PSA. The results of the particle size characterization are shown in Table 1.

Table 1. Results of *Piper aduncum* essential oil nanoemulsion test.

Nanoemulsion	Particle size (nm)	Polydispersity index (PdI)		Zeta /PZ (mV) potential			
		Pdi	PdI standards	Uniformity	PZ	PZ standards	Stability
				√			√
P. aduncum essential oil	273.1	–0.215	< 0.5	√	–29.53	–30 < x < 30	√

Figure 1. Nanoemulsion mixture of citronella grass waste (hydrosol) and *P. aduncum* essential oil.

Physical properties can be observed to identify a compound with nano-sized particles. Phase separation should not be found in the observation. On the other hand, homogeneity and clear look (like transparency of water) should be able to be observed. Costa *et al.* (2012) argued that good nanoemulsion has clear visual and high transmittance.

The particle size of *P. aduncum* essential oil nanoemulsion was 273.1 nm, and its polydisperity index was –0.215. It shows that the nanoparticle size enables the particle to get into a cell easily. According to Jaiswal *et al.* (2015), nanoemulsion is an emulsion that has droplet submicron size of > 2–200 nm. The PdI describes the particle size distribution. The PdI is categorized into monodisperse and polydisperse. The size range of monodispersity is 0.01–0.6, whereas that of polidispersity is > 0.6 (Nidhin *et al.* 2008). The essential oil nanoemulsion of *P. aduncum* produced in this study had homogenous or uniform particle size distribution with PDI close to zero, which was –0.215. Taurina *et al.* (2017) stated that a PdI close to zero indicates a homogenous or uniform particle size distribution, whereas a PdI of > 0.6 indicates significant heterogeneity. The data shows that the particle had a physically stable particle size distribution, which does not cause aggregations of particles. According to Blackman *et al.* (2018), the stirring duration in the nanoemulsion production affects the size of the nanoparticles. The longer the stirring process is, the smaller particle size is produced since there are more particles that break down into nano size.

The zeta potential value is used to determine the particle potential. It is affected by the composition of the particle and the medium where the nanoparticle is dispersed. As reported by Mannuela (2016), a nanoparticle that has a zeta potential value > ± 30 has higher stability. The test result of the nanoemulsion of *P. aduncum* extract showed a negative surface charge of –29.53. If all particles have a high negative or positive zeta potential charge, the repulsion between particles happens, and the dispersion is stable. On the contrary, if the zeta potential charge is low, there is not enough power to prevent particle aggregation, and the dispersion is unstable. The negative zeta potential indicates that the nanoparticle formula has a negative surface charge.

Results of Nanoemulsion Toxicity Test

The preliminary test result of the nanoemulsion of the mixture of citronella grass waste (hydrosol) and *Piper aduncum* essential oil shows that the increasing concentration and the mortality number of tested insects are directly proportional. Nanoemulsion with 0.5% concentration caused 60.00% mortality in the larvae, whereas nanoemulsion with 1% concentration caused 86.67% mortality. The data in Table 2 can be used as a reference to conduct the confirmatory test so that five levels of concentration are collected.

Table 2. Mortality of *S. frugiperda* larvae results from the treatment of nanoemulsion mixture of citronella grass waste (hydrosol) and *P. aduncum* essential oil in preliminary test

Concentration (%)	Mortality (% ± SD)
0.00 (control)	0.00 ± 0.00 ^{c}
0.5	60.00 ± 1.00 ^{b}
1	86.67 ± 0.57 ^{a}

The result of confirmatory test on the mixture of citronella grass waste (hydrosol) and *P. aduncum* essential oil shows that there was a positive relation between the increasing concentration and the mortality of tested larvae. In the lowest concentration (0.33%), the mortality of *S. frugiperda* was 32.00%. The mortality of tested larvae increased as the concentration went higher. The highest number of mortality (88%) was observed at the highest concentration of 2.40%, which did not significantly differ with the concentration at 1.46%. The mortality of *S. frugiperda* larvae was also affected by the active components in citronella grass waste. The main active components of citronella grass essential oil were

30–45% aldehyde compound (citronellal or $C_{10}H_{18}O$), 55–65% alcohol compounds (citronellol or $C_{10}H_{20}O$ and geraniol or $C_{10}H_{18}O$), and other compounds such as geraniol, citral, nerol, metal, heptanone, and dipentene (Khoirotunnisa 2008). In addition, citronella grass contains saponin, flavonoid, polyphenol, and terpenoid, which also contributed to the mortality of the tested larvae (Syamsuhidayat dan Hutapea 1991).

Furthermore, the nanoemulsion affects survived larvae development time. The higher the concentration is, the longer the time needed for the larvae to develop. This was in agreement with that of Lina *et al.* (2018), who reported that the different levels of concentration can extend the survived tested larvae when compared to the control. The additional time to develop from instars 2–3 was about 0.02–1.76 d longer than the control. Time development from instars 2–4 was 0.04–2.34 d. The delay from instars 2–5 was about 0.04–3.84 d. Likewise, the delay of development from instars 3–6 was about 1.56–3.78 d compared to the control (Table 3). Table 3 shows that the development time of the larvae from instars 2–3 was not significantly affected compared to control. Lina (2014) claims that even though the insignificant increase in concentration results in significant mortality, it does not create a similar effect on the development delay of the survived tested insects. Another possible factor affecting the development time of the tested larvae is the active components in *P. aduncum* and citronella grass waste, which reduce feeding activity. The lower the feeding activity is, the longer the time they need to develop.

The mortality pattern of *S. frugiperda* larvae in Figure 2 indicates that the mortality of larvae started on the first day of treatment and increased abruptly on the second day of treatment. Moreover, there was further increase in mortality on the third and fourth day. Since the leaves with treatment were replaced to the leaves without treatment, the survived tested larvae recovered as a result of feeding on the leaves without treatment. This finding demonstrates that the nanoemulsion works better on toxicity than on nhibition of growth and development. Furthermore, the mortality pattern of *S. frugiperda* on the first day shows that when the higher concentration level is applied,

Table 3. Mortality and development time of *S. frugiperda* larvae resulting from the treatment of nanoemulsion mixture of citronella grass waste (hydrosol) and *P. aduncum* essential oil in confirmatory test.

Concentration (%)	Mortality (% ± SD)[1]	Development time (day) (X ± SD)[1]			
		Instars 2–3	Instars 2–4	Instars 2–5	Instars 2–6
0.00	0.00 ± 0.00 e	2.24 ± 0.08	4.76 ± 0.33	6.16 ± 0.08	7.22 ± 0.08
0.33	32.00 ± 0.83 d	2.26 ± 0.16	5.28 ± 0.59	6.20 ± 0.23	8.78 ± 0.26
0.54	48.00 ± 0.44 c	3.16 ± 0.37	5.04 ± 0.08	6.85 ± 0.31	9.78 ± 0.17
0.89	72.00 ± 0.83 b	3.50 ± 0.70	5.20 ± 0.27	7.18 ± 0.29	10.58 ± 3.03
1.46	84.00 ± 0.54 a	3.80 ± 1.03	7.10 ± 0.41	9.10 ± 0.22	11.00 ± 0.77
2.40	88.00 ± 0.44 a	4.00 ± 0.00	7.10 ± 0.22	10.00 ± 0.00	11.00 ± 0.00

[1]Numbers followed by the same letters are not different from LSD results (α = 0.05)
[X] average; [SD] standard deviation

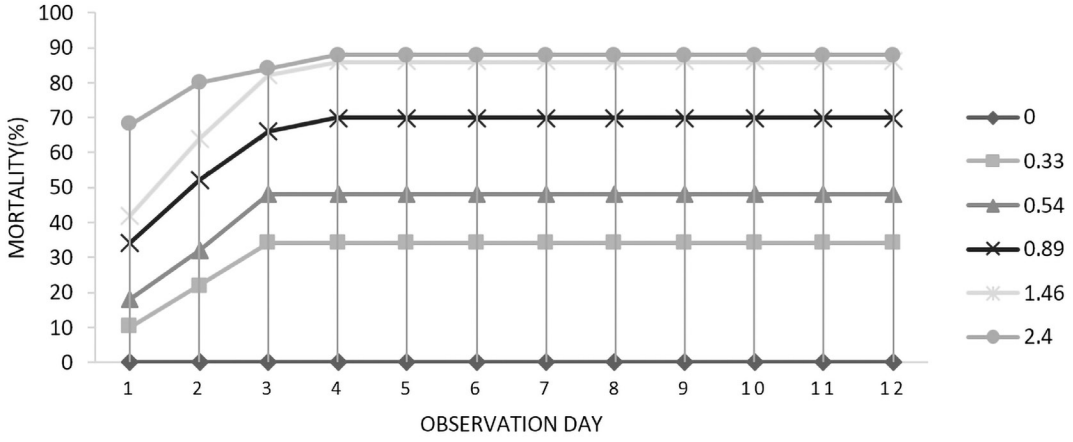

Figure 2. Mortality of *S. frugiperda* larvae results from the treatment of nanoemulsion mixture of citronella grass waste (hydrosol) and *P. aduncum* essential oil.

the greater mortality percentage of the tested larvae is attained. The results of probit analysis for nanoemulsion formulations at LC_{50} and LC_{95} were 0.53 and 2.69%, respectively, with regression slope values equal to 2.34 (Table 4).

Table 4. Nanoemulsion probit analysis of a mixture of citronella grass waste (hydrosol) and *P. aduncum* essential oil against *S. frugiperda* larvae.

Treatment	b ± SE	LC_{50}	LC_{95}
P. aduncum essential oil	2.34 ± 0.41	0.53%	2.69%

[b] regression slope; [SE] standard error

Active components contained in *P. aduncum* and citronella grass also affected the antifeedant activity. The test result shows that there was a significant difference in antifeedant activity between every treatment and the control (Table 5). The average leaf area consumed at the lowest level concentration (0.33%) was 260.70 mm^2 with antifeedant effect at 21.23%, which was directly proportional with the highest-level concentration (2.14%). The average of leaf area consumed at the highest-level concentration was 17.40 mm^2, and the antifeedant percentage was 96.74%. This result implies that the higher-level concentration is given, the smaller the average leaf area is consumed, and the higher antifeedant occurred on the tested larvae. Schoonhoven *et al.* (2005) maintains that the better the quality and quantity of insect feed is, the less delay is found in insect development. In contrast, when the feed is antifeedant, the insects do not have any options but keep feeding on the improper feed in order to survive. The consequences happened on insects – specifically the growth and developmental delay, the insect mortality, and the antifeedant effect on *S. frugiperda* larvae – were presumably caused by the active components in *P. aduncum* on the treatment leaves.

Table 5. Antifeedant effect of the nanoemulsion of citronella grass waste (hydrosol) and *P. aduncum* essential oil mixture on *S. frugiperda*.

Concentration (%)	Average of leaf area consumed (mm^2) ± SD[1]	Antifeedant effect (%)
0.00 (control)	331.00 ± 75.89 a	–
0.33	260.70 ± 22.90 b	21.23
0.54	150.80 ± 44.28 c	54.44
0.89	113.30 ± 20.53 c	65.77
1.46	32.90± 22.86 d	90.06
2.14	17.40 ± 18.10 d	96.74

[1]Numbers followed by the same letter is no different from LSD results (α = 0.05)

CONCLUSION

Nanoemulsion formulation of the mixture of citronella grass waste and *P. aduncum* essential oil is categorized as nanoemulsion because its particle size is 273.1 nm. The nanoemulsion toxicity test on *S. frugiperda* showed 88.00% mortality at the highest-level concentration (2.40%). The mortality rate of the tested larvae at concentration of 0.53% was 50%, and a 95% mortality was found at concentration of 2.69%. In addition to toxicity, the nanoemulsion also caused developmental delay on the survived larvae and affected the antifeedant activity on *S. frugiperda* larvae with antifeedant effect percentage of 94.74.

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STATEMENT ON CONFLICT OF INTEREST

There is no conflict of interest to declare.

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