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Synergism of *Tephrosia vogelii* and *Piper aduncum* Based Nanoemulation Against *Spodoptera frugiperda*

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A B S T R A C T

The botanical insecticide nanoemulsion is a pesticide having an oil phase and aqueous phase that spontaneously emulsify and range in size from 20 to 200 nm. Bio-combi extracts frequently contain intricate combinations of active ingredients that may work in concert to increase bioactivity. The goal of the study was to develop the optimal nanoformulation and investigate the physiological effects of a synergistic nanoemulsion made from *Tephrosia vogelii* leaves and *Piper aduncum* fruit extracts as a substitute pesticide for *Spodoptera frugiperda* larvae. Low-energy spontaneous emulsification with a magnetic stirrer was used to create nanoemulsions, which were then subjected to a toxicity and food absorption test. Malvern's Zetasizer Nano (ZN), which was used to analyze the insecticidal nano formulae for PSA, revealed that the particle sizes for the 1:1, 3:1, and 1:5 ratios were 204 nm, 4724 nm, and 97 nm, respectively. Only the 1:5 formula, which is classified as a nanoparticle, met the standards for a nanoemulsion and produced *S. frugiperda* mortality of 82.34% at a concentration of 0.56%, and the LC_{25}, LC_{50}, and LC_{95} values were 0.1, 0.22, and 1.59%, respectively. The analyzable results demonstrated that the mixed nanoemulsion was additive at the LC50 value was 0.95 while the LC95 value was 0.70%, meaning only marginally synergistic.

INTRODUCTION

The autumn armyworm (FAW) is a polyphagous, voracious, invasive pest and a harmful transboundary bug with a high potential for fast expansion (Sisay et al., 2019). FAW feeds in great quantities on the leaves, stems, and reproductive organs of about 350 plant species from 27 families (Montezano et al., 2018). Corn was the preferred host of FAW, which can cause significant yield losses if not well managed by more than 70% (Nboyine et al., 2020). This species can reduce corn yields by up to 57% in South American countries, depending on the crop season and hybrid used (Tambo et al., 2020). The *S. frugiperda* pest was initially discovered in early March 2019, and within four months, it spread to 12 provinces in Sumatra, Java, and certain areas of Kalimantan (Kumela et al., 2019).

The green revolution has resulted in the use of synthetic insecticides and chemical fertilizers blindly, leading to the loss of soil biodiversity and developing resistance to pathogens and insect pests (Duhan et al., 2017). According

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to estimates, synthetic pesticides used in agriculture are responsible for between 30 and 40 percent of crop losses throughout pre- and post-harvest periods. However, only 0.1% of active ingredients reached the target pests, while 99.9% leaked into the surrounding environment (Kumar et al., 2019). Lina et al. (2015) mentioned the utilization of botanical insecticides still contains many weaknesses that are easily decomposed by heat and easily broken down by the sun, often a lower efficacy and low physicochemical stability.

According to Liang et al. (2020), the synergisms can develop a new, exceptional commercial bioinsecticide formulation, supporting organic farmers to overcome the aforementioned limits, and the efficacy of the bio-combi products can outperform the flaws of each product alone. Lina et al. (2021) proved that the application of botanical insecticides made from two or more kinds of plant extracts could reduce dependence on one type of plant. The combination of two extracts with nanoemulsion technology could form nano-size particles that more easily fit into the target, which is possible to give a promising prospect to the alternative insecticide to control FAW.

METHOD

The study was carried out In Vitro in the Insect Laboratory, Department of Plant Protection, Faculty of Agriculture, Andalas University, from June until November 2022. The research method used is an experimental method with a Randomized Complete Block Design with two factors, including using five treatments of concentrations (A = 0.56%; B = 0.375%; C = 0.15%; D = 0.1%; E = 0.00%), three treatments of nanoformulation mixing ratios *Tephrosia vogelii*: *Piper aduncum* (1 = 1:1; 2 = 3:1; 3 = 1:5), and five replications. Using the SPSS Statistics 20 software, the Analysis of Variance (ANOVA) two-factor was used to assess the statistical significance of the difference or interaction between the control and treatments based on mortality and duration growth using the Least Significant Difference (LSD) at a 5% confidence interval.

Extraction

The samples of *T. vogelii* and *P. aduncum* were taken in Alahan Panjang and Padang. The extraction of two kinds of plants using the maceration method with ethyl acetate serving as the solvent. The leaves of *T. vogelii* were taken at the center of the plants to get the best leaves, and the criteria for picking fruits of *P. aduncum* were uniform fruits with green characteristics, sizes ranging from 10-13 cm, and a slightly hard texture. The raw materials, leaves, and fruits were cut into 1 cm size and placed on a bamboo tray lined with paper, and then the drying process was done without direct sunlight for approximately three weeks. The dried samples will be cut into small pieces and crushed using a blender. The crushed results will be sieved using a sieve of 0.5 mm to obtain a form of powder.

Fifty grams of each powder (*T. vogelii* and *P. aduncum*) were then added to a separate Erlenmeyer flask, submerged in 500 ml of ethyl acetate, covered, and allowed to soak in the dark for two to twenty-four hours. The liquid extract was subsequently filtered twice using a glass funnel (9 cm in diameter), the first time using regular filter paper and the second time using Whatman filter paper number 41. At a temperature of 50°C and a pressure of 240 bar, the distillate is collected in an evaporator flask and evaporated into a Rotary evaporator. The ethyl acetate solution was obtained from the evaporation of the pulp and was used to soak the plant extract three times immersions (2 further immersions for 1 x 24 hours).

Preparation of nanoemulsion insecticide

This study followed the method used to create nanoemulsion; a magnetic stirrer was used to homogenize an aqueous and organic phase (Lina et al., 2021). The two extracts, *T. vogelii* and *P. aduncum,* followed by the mixing ratios (x:x), and the solvent (bioethanol), up to 10% of the total emulsion with a composition of 1:1, were mixed to create the emulsion system. 3% Tween 80 and 87% sterile distilled water will be combined to make the aqueous phase, which was then agitated using a magnetic stirrer for 30 minutes. While the water phase is being homogenized with a magnetic stirrer, the aqueous phase should be continuously stirred. The organic phase will be dripped into the aqueous phase using a pipette after the water phase has been thoroughly homogenized using a magnetic stirrer. For 45 minutes, the homogenization procedure will be applied progressively.

Propagation *Spodoptera frugiperda*

According to Maredia, Segura, & Mihm (1992), the following components were required for efficient mass rearing and a thorough comprehension of the biology of the insect: Corn was sown for animal feed in polybags. The planting media was made up of a soil and manure mixture in a 1:1 ratio. After three seeds have been planted in the planting hole, add about two cm of dirt. Watering, weeding, and mechanical pest control are used for maintenance. The leaves of corn plants that are less than one month old are used as food for larvae.

The collection of *S. frugiperda* larvae from an untreated corn field in Padang City. *S. frugiperda* larvae were transported to the lab and kept in a plastic container that measures 30 cm in diameter and 35 cm in height, with a gauze-covered lid. Place in a plastic container with new food and corn leaves daily after that until pupae. Every day, pupae will be gathered and placed in the sawdust box.

Following the formation of the image, the object is fed a liquid honey drip of 10% to cotton, strung in a gauze cage, and placed in a sweet corn plant for egg laying. Before the II instar larvae were employed as test insects in this study, the hatched eggs were moved into a plastic lunchbox (23cm x 15cm x 7cm) filled with corn leaves.

Particle Size Analysis

A particle size analyzer was used to analyze the insecticide nanoemulsion once it was manufactured and transferred to the Bogor Post Harvest Center (PSA). The Zetasizer Nano ZS Malvern is the device used in the PSA. Particle size, polydispersity index, zeta potential, and particle distribution width of nanoemulsions can all be used to gather analytical data.

Nanoemulsion toxicity test

Four-by-four-centimeter pieces of corn leaf were cut, dipped one at a time into the appropriate nanoemulsion until evenly moist, and then dried in the air. The equivalent control solution was applied to control leaves. In a petri dish with a 9 cm diameter used for rest tissue, treated leaf parts and control leaves were arranged individually. Thirty minutes after being sprayed, the corn leaves will be picked individually and fed to the larvae. There was a piece of leaves, each measuring $4cm^2$. Furthermore, ten second-instar larvae will be released per petri dish. The insect diets (corn leaves) will be changed every day with leaf treatment without control. The bioassay was repeated. Insect mortality was noticed 24 and 48 hours after treatment application. In order to prevent cannibalism, the larva was then removed one at a time and placed in plastic containers. They were then fed leaves without further treatment until they reached the fourth instar.

Mixed Activity Nature Analysis

The concentration achieved becomes a reference for obtaining a comparison of the two constituents in the blended extract after learning the LC50 from the follow-up test. Based on the combined index of LC50 and LC95 levels, the activity characteristics of the mixture of *Piper aduncum* fruit extract and *Tephrosia vogelii* leaf extract were examined. According to the following formula by Chou & Talalay (1984), an analysis of mixed activity properties by the combination index at the LCx level was calculated:

$$CI = \frac{LCx^{1(cm)}}{LCx^{1}} + \frac{LCx^{2\,(cm)}}{LCx^{2}} + \frac{LCx^{1(cm)}}{LCx^{1}} \times \frac{LCx^{2(cm)}}{LCx^{2}}$$

In independent studies, LCX1 and LCX2 were used to extract the LCx components 1 and 2, respectively. When these components were combined, mortality x was 50% and 95%, respectively. The LC value is calculated by multiplying the mixture's LCx by the ratio of component 1 and component 2 concentrations in the mixture under laboratory conditions. Based on the inverse co-toxicity ratio, the category of mixed interaction features will be modified from (Kosman & Cohen, 1996):

(1) The mixture's components exhibit strong synergy if the CI is less than 0.5;
(2) The components of the mixture are only marginally synergistic if the CI is 0.5-0.77;
(3) If the CI is between 0.77 and 1.43, the mixture's components are additive;
(4) if it is between 1.43 and 2.43, the opposite is true.

RESULTS AND DISCUSSION

The impact of nanoformulations on the mortality of *Spodoptera frugiperda*

Four concentration types, a control, and three mixing ratios were used to assess the synergistic nanoemulsion toxicity on S. frugiperda second instar larvae. The results are shown in Table 1:

Table 1. Mortality of *Spodoptera frugiperda* larvae at various concentrations and mixing ratios *T. vogelii* and *P. aduncum*

Concentrations (%)	Mortality (%) ± SD		
	1:1	3:1	1:5
0.56	72.58 ± 0.44 a	62.86 ± 0.45 a	82.34 ± 0.45 a
0.375	50.41 ± 1.00 b	40.37 ± 1.00 b	60.23 ± 1.58 b
0.15	30.12 ± 1.00 c	20.47 ± 1.00 c	40.24 ± 1.41 bc
0.1	20.23 ± 0.71 c	20.25 ± 0.71 c	24.14 ± 0.55 c
0.00 (control)	0.00 ± 0.00 d	0.00 ± 0.00 d	10.00 ± 1.00 d
F (Nanoformulations-N)	**		
F (Concentrations-C)	**		
F (N*C)	ns		

* The 5% least significant different, SD = standard deviation, ns = no significant difference, ** = significant difference < 0.01, the numbers that were followed by the same letter in the same column did not differ substantially from one another.

The analyzable data in Table 1 showed that there was a significant difference in mortality between the three formulations and five concentrations, and treated larvae followed the treated concentrations in terms of mortality. Nothing can be more obvious than the case of the 1:1 formula, at concentrations 0.00; 0.1; 0.15; 0.375; 0.56% were 0.00; 20.23; 30.12; 50.41; 72.58%, respectively. The highest concentration of 0.375% of the three nanoformulations was applied, showing that the highest mortality was achieved at 72.58%, 62.86%, and 82.34%,

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respectively. At the least concentration of 0.00% were 0.00, 0.00, and 10.00%, respectively. These results were similar to the previous report by (Lina et al., 2021). The two-factor analysis of variance revealed a substantial difference between the highest concentration and the other concentrations, particularly a huge difference from the control. Of the three analytical formulas, formula 1:5 gave the most effective mortality, which was the significant difference between formula 1:1 and 3:1, but no significant difference between two nano-formula 1:1 and 3:1. Moreover, we found that there was a strong statistical interaction between the concentrations and the nanoformulas.

However, we found that the most efficient mortality in the test was the 1:5 nano formula at a concentration of 0.56%. The higher *P. aduncum* presence was proportional to the *S. frugiperda* mortality, which was presented in Figure 1. Phenylpropanoid was the primary chemical discovered in ethyl acetate, with the predominant compound accounting for 54.9% of *P. aduncum* essential oil and being linked to larvicidal activity on species of Diptera and Lepidoptera (Vila et al., 2005; de Almeida et al., 2009; Monzote et al., 2017). Depending on the environmental circumstances present during plant development or the age of the plant, *P. aduncum*'s variety and absence of certain chemicals have been recorded (Lucena et al., 2017). Guininsin and piperidines, two piperamide compounds from the Piperaceae family, kill the test insects by acting as neurotoxins that block the flow of nerve impulses to the axons, resulting in paralysis, and by inhibiting the function of cytochrome P450 because the secondary metabolites ingested by the insect come from a complex with major detoxification enzymes, of which dependent monooxygenase is one. These enzymes were involved in many manufacturing processes: ecdysteroid and juvenile hormone biosynthesis, as well as detoxification from plant compounds and insecticides. Additionally, methylenedioxyphenyl-containing lignans have been found to have synergistic effects when combined with other extracts, reducing the toxicity of insecticides and inhibiting the activity of cytochrome P450 enzymes (Lina et al., 2021).

According to Zhao et al. (2017), *T. vogelii* contains 80% to 90% rotenone chemicals, which are more effective than tephrosine and degueline as a stomach poison and contact poison against a variety of insects. A reduction in oxygen intake, respiratory depression, and ataxia in rotenone-poisoned insects cause convulsions, paralysis, and death by respiration arrest. The muscular and tissue systems that are involved in food digestion are known to be paralyzed by rotenone (Pantar and Pu'u, 2020). Hassanali & Lwande (1989) inhibiting the transfer of electrons between NADH dehydrogenase and coenzyme Q in complex I of the electron transport chain in the mitochondria, rotenone has been characterized as a toxin that hinders cellular respiration. Rotenone stops electrons from moving from Fe-S to the ubiquinone coenzyme, which slows cellular respiration, lowers ATP generation, harms the insects' muscles and other tissues, and ultimately results in their death.

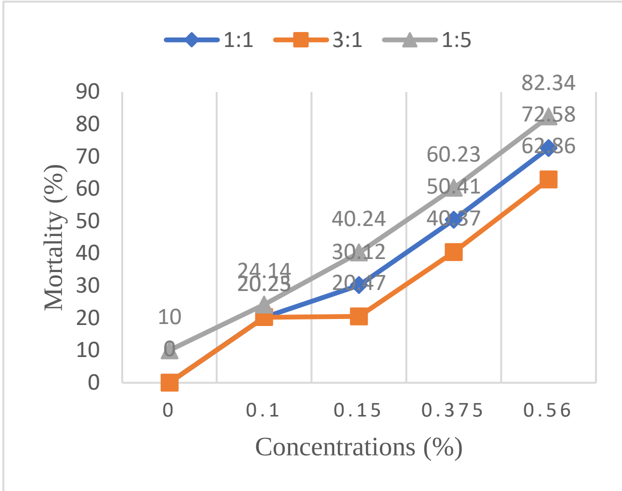

Figure 1: Mortality of *Spodoptera frugiperda* larvae at various concentrations and mixing ratios *T. vogelii* and *P. aduncum*

Tama et al. (2020) and Duhan et al. (2017) reported that other benefits of nanoparticle-sized insecticides had been observed, including greater effectiveness, durability, absence of toxicity, a decreased purpose of AIs, speeding the solubility of active ingredients, and kinetic stability to prevent creaming and aggregation during storage. Sahayaraj et al. (2018) and Fraceto et al. (2016) also believed that Nanoemulsions are easier to penetrate plant tissue due to their small size, which reduces the number of pesticides that were wasted during treatment and enabled the target pest to be killed quickly. The greatest influence on insects was provided by plant-sourced nanoformulations, but the least for fungi (Sparks, Hahn, & Garizi, 2017).

Based on the PSA results in Table 4, it was evident that 1:5 nanoformulation was more effective and efficient than non-sized nanoparticle formulations. As previously mentioned, the *P. aduncum* active component inhibits the action of enzymes that break down toxic chemicals in insect bodies. As a result, *T. vogelii*'s active component was not sufficiently degraded to allow it to reach the target and work effectively. This is how the blended extract blends together. Since plant essential oils were typically complex combinations of terpenoids, their bioactivity was probably commonly the consequence of synergy between ingredients and was not harmful to other organisms (Sparks et al., 2017). As a result, when combined with other plant extracts, *P. aduncum* extract containing dillapiole may have a beneficial synergistic effect. As demonstrated in Figure 1, the fraction of *P. aduncum*'s concentration was higher than that of *T. vogelii*, which results in a stronger reduction of the PSMO enzyme's activity, allowing the active ingredient *T. vogelii* to evade the enzyme's breakdown and continue to attack the target. Numerous researchers have demonstrated that a synergy of two or more types of plant extracts can be utilized as a botanical insecticide to lessen adverse effects on non-target organisms, people, and the environment. For instance, the *T. vogelii* and *P. cubeba* leaf extract mixture (5: 9) had synergistic effects against *C. pavonana* larvae, both at the LC50 and LC95 levels (Syahroni & Prijono, 2013). According to the previous research of Lina et al. (2021), the Limixture of *T. vogelii* and *P. aduncum* (1:5) was synergistic against *C. pavonana* larvae, both at the LC50 levels.

Duration of larval development of *Spodoptera frugiperda* second to fourth instar larvae

According to Table 2, statistical findings from the two-factor analysis of variance performed on three nanoformulations made from a combination of *T. vogelii* and *P. aduncum* extracts, there was a statistically significant difference between the level of treated concentrations and the three formulas on the length of time *S. frugiperda* took to develop from instar II to IV. Moreover, there was a strong interaction between nano formulas and concentrations. There was no significantly different in the level of treated concentrations on the duration of development.

Table 2. Duration of larval development of *S.frugiperda* second to fourth instar larvae after mixed nanoemulsion treatment at five concentrations and three mixing ratios

Concentrations (%)	Duration of larval development (days) (Mean ± SD)		
	1:1	3:1	1:5
0.56	5.48 ± 0.75 a	5.38 ± 0.69 a	6.23 ± 1.15 a
0.375	5.36 ± 0.81 a	5.22 ± 0.74 a	6.18 ± 1.27 a
0.15	5.32 ± 0.92 a	5.14 ± 0.83 a	6.11 ± 1.45 a
0.1	5.24 ± 1.10 a	5.08 ± 0.99 a	6.08 ± 1.77 a
0.00 (control)	3.68 ± 0.00 b	3.68 ± 0.00 b	3.58 ± 0.00 b
F (Nanoformulations-N)	**		
F (Concentrations-C)	**		
F (N*C)	ns		

* The 5% Least Significant Different, SD = standard deviation, ns = no significant difference, ** = significant difference < 0.01, the numbers that were followed by the same letter in the same column did not differ substantially from one another.

In contrast to controls, the results generally showed that the larval development's survival span was significantly lengthened. The duration of larval growth at a concentration of 0.00% (control) was 3.58 days for II-IV instars, which was a completely statistically different result from mixing nanoformulation at a ratio of 1:5 to the other two formulas. This is shown in Table 2 as the longest extension among the three analytical formulas. When the mixed nanoemulsion treatment was applied at the highest concentration of 0.56%, it took 6.23 days to develop for instars II to IV, which was statistically different from the control but not the other three treated concentrations. The smallest extension for II-IV instars was determined to be 3.68 days for nano-formula at a ratio of 3:1 at a concentration of 0.00% (control), whereas the longest extension for II-IV instars was 5.38 days at the greatest concentration of 0.56%, which was completely statistically different from control.

The analyzable results in Figure 2 illustrated a considerably extended survival period of larval development along with the intention of *P. aduncum* active compound and the synergy at 1:5 nano-formula among active compound of *P. aduncum* and *T. vogelii* that make this formula become statistical difference to two of other formulas. This result has been proved previously by several researchers like Erlina et al. (2019) stated that nanoemulsion of *P. aduncum* extract made slow survival development of instar II-IV *Crocidolomia pavonana* at 0.00% was 4.85 days and at 0.375% achieved 6.31 days. According to the previous study by Lina et al. (2021), the interaction between *T. vogelii* and *P. aduncum* at a ratio of 1:3 extended duration of development from instar II-IV *C. pavonana* at 0.00% was 4.89 days and at 0.375% achieved 7.10 days.

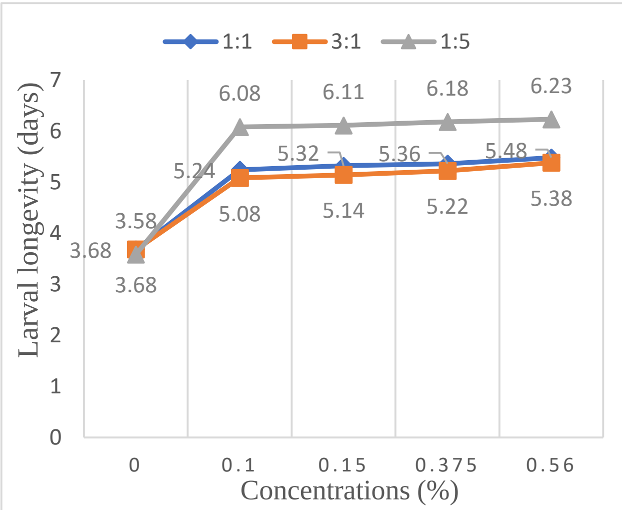

Figure 2: The extended duration of development from instar II-IV *S. frugiperda*

The active ingredients in the leaf of *T. vogelii*, which were toxic to cellular respiration when combined with the fruit of *P. aduncum*, a piperamide, work as neurotoxins to

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paralyze the muscle necessary for food digestion and have a direct impact on growth, which influences and increases the survival time of *S. frugiperda* larvae from stage II to stage IV. This study has demonstrated why there has been an increase in publications and the primary uses for *P. aduncum*, *T. vogelii* essential oils, and the synergy of botanical insecticides in recent years. These substances have a high representation in scientific journals related to pest control because of their insecticidal activities (de Almeida et al., 2009; Monzote et al., 2017; Liang et al., 2020).

Mixed activity nature analysis

The association between the concentration of nanoemulsions made from a combination of *T. vogelii* leaf and *P. aduncum* fruit extracts on the mortality of *S. frugiperda* larva was discovered using probit regression analysis, as shown in Table 3. The values of the regression slope, LC_{50}, and LC_{95} obtained by probit analysis are as follows:

Table 3. There is a concentration relationship between the LC_{50} and LC_{95} probit regression parameters. The effects of a 1:5 nanoformulation on the mortality of *Spodoptera frugiperda*

Value b ± SE	LC_{50} (%)	LC_{95} (%)	CI value at the level			
			LC_{50}	Criteria	LC_{95}	Criteria
1.93 ± 0.32	0.22	1.59	0.95	AS	0.70	SL

* b = slope of the regression; SE = standard error; CI: combined index; AS: additive synergy; SL: weak synergy

It was known that the mixed nanoemulsion's b value was 1.93>0 based on the findings of the probit analysis. The positive slope shows a positive correlation between the mortality rate and the experimental concentration, from which we saw that when the concentration was increased, the mortality rate also increased. The higher the slope, the higher the effectiveness against *S. frugiperda*, and it kills the treated insects faster than a nanoemulsion with a lower b value (Lina et al., 2021). Then, a 1.59% of 1:5 nanoformulation was needed to kill up to 95% of the *S. frugiperda* larvae. While the concentration of LC_{50} was 0.22% which can kill 50% of the treated larva. The LC_{95} and LC_{50} values of 1:5 nanoformulation were lower than the LC_{95} and LC_{50} values of the two nanoemulsions 1:1; 3:1. The mixture's components exhibit an additive indication of mixed nanoemulsion at the LC_{50} value of CI was 0.95. In contrast, the association coefficient of LC_{95} was 0.70, which means the components of the mixture were marginally synergistic.

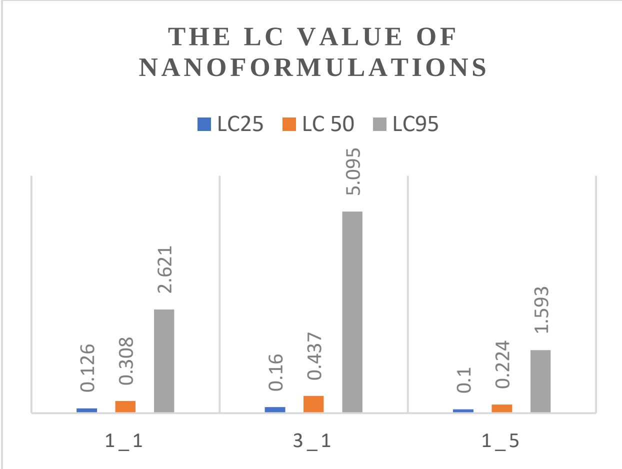

Figure 3: The LC_{95}, LC_{50}, and LC_{25} value of nanoformulations

The analyzable results showed that the percentage of mortality of treated larva in 1:5 nanoformulation was proportional to the LC value in Figure 3. Furthermore, there was an increase in concentration along with the synergy and the presence of the active compound of *P. aduncum*. The previous research of Tama et al. (2020) stated that the LC_{95} value of *C. pavonana* at single *T. vogelii* nanoformulation at a concentration of 1.29%, and Erlina et al. (2019) also reported that the LC_{95} value of *C. pavonana* at single *P. aduncum* nanoformulation at a concentration of 0.85% while Lina et al. (2021) proved that the synergy between both *T. vogelii* and *P. aduncum* nanoformulations control *C. pavonana* at LC_{95} was 0.3%. Both of them have a combination index value with differing synergistic qualities.

These results proved the idea that *P. aduncum* and *T. vogelii*'s active ingredients work synergistically to eliminate pests more effectively compared to the single/separate nanoemulsion treatment demonstrating the strong synergistic indication of mixed nanoemulsion. A similar issue was previously covered by Syahroni and Prijono (2013), and Lina et al. (2021) proved that using a combination of synergistic botanical pesticides can boost the application's effectiveness because combined insecticides are employed at a lower dose than the amount of each component alone. Lina et al. (2013) reported that a single extract has less efficiency than the mixtures of *T. vogelii*, *B. javanica*, and *P. aduncum* (1: 0.5: 2.5) and *T. vogelii* and B. *javanica* (5: 1), all 13 mixed extract combinations of *B. javanica*, *T. vogelii*, and *P. aduncum* showed substantial synergy at the LC95 level killing *S. frugiperda* larvae.

Characteristics nanoformulation

The results of the probit regression above were used as a reference for conducting particle analysis nano using the

Particle Size Analyzer (PSA) with the Zetasizer ZS Nano tool Malvern, as shown in Table 4.

Table 4. Analysis of *Piper aduncum* nanoemulsion based on Particle Size Analyzer

Formula	Size particle (nm)	Polydispersity Index/ PdI
1:5	97	0.22
1:1	204	0.352
3:1	4724	0.701

Based on PSA analyzable results in Table 4, the two nanoformulations at ratios 1:1 and 3:1 did not satisfy the nanoemulsion requirements, and the criteria were 204nm and 4724nm, respectively, which have a significant difference compared to the 1:5 nano-formula particle size 97nm. According to the earlier study by Wang et al. (2009), the requirements of the formula can be summed up as requiring a nanoemulsion with particles with a size between 0 and 200nm. Therefore, three nano formulas (1:1, 3:1, 1:5) were selected, which had the better regression parameters were 1:5 nanoformulation. Polydispersity index analysis showed uniformity between nanoemulsion particles and zeta potential, indicating the stability of nanoemulsion particles 1:5 nano formula has a polydispersity index value was 0.22. This nanoformulation was categorized as having large nanoparticles uniform and stable between particles because it meets PdI standards.

In contrast to macroemulsions, which need an oil phase concentration of around 5%, nanoemulsions only need an oil phase concentration of 3–10%, as stated in the scientific article. Additionally, Tama et al. (2020) concluded that the size distribution of nanoemulsion particles could be impacted by the oil phase's concentration. Emulsifier type and concentration also have an impact on nanoemulsion particle size, in addition to the oil phase. This suggests that the three nanoformulations were usually dangerous for farmers to use since excessively acidic or alkaline PH might hurt or dry up the skin. Most pesticides have an acidic pH. The control solution was acidic and had a low pH as well. The effectiveness of pesticides was reportedly impacted by the PH of the water used at the time of application. According to Erlina et al. (2019), water with an acidic PH is used for pesticide applications since it will increase the insecticides' usable life.

CONCLUSION

According to the findings of this study's research, the mixture of the leaf of *Tephrosia vogelii* and fruit of *Piper aduncum* extract nanoemulsion was synergistic, efficient, safe, and an excellent alternative to synthetic pesticides against *Spodoptera frugiperda* pests which should be recommended for use to spray at prevention or low pest levels. In order to optimize advantages, it is important to study more the combined nanoemulsion's efficiency against other pests, natural enemies in the field, and high concentrations or other synergistic nanoformulations to achieve more effective management.

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