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RESEARCH ARTICLE

BOTANICAL INSECTICIDE NANOEMULSION MADE BY Tephrosia vogelii J. D. HOOKER (LEGUMINOSAE) AND TRIAL TO CABAGE Crocidolomia pavonana F. (LEPIDOPTERA : CRAMBIDAE)

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Abstract

Nanoemulsion is a material which consists of oil and water phases with particle size in the range of 20-200 nm. The purpose of this research is to obtain a botanical insecticide nanoemulsion made from T. vogelii which is active against C. pavonana. Nanoemulsion made using low energy method whch is spontaneous emulsification using a magnetic stirrer. This research produced 12 formulas. Four best formulas chosen for PSA (Particle Size Analizer) analysis. Four formulas was selected based on the toxicity of each formula. Nanoemulsion was analyzed for PSA using Malvern's Zetasizer Nano (ZN). The analysis results showed nanoemulsion A.1, A.2, B.1.a and B.1.b had particle sizes in a row of 134nm, 156nm, 1292nm, and 1286nm. Formulas A.1 and A.2 have fulfilled the nanoemulsion requirements and criteria, otherwise formulas B.1.a and B.1.b have not classified as nano-sized material, so they cannot be categorized as nanoemulsions. Formulas A.1, A.2, B.1.a and B.1.b have LC_{95} values is 1.09%, 3.15%, 0.45% and 2.26%.

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Introduction:-

Nanotechnology is very beneficial for human life and contributes in advancing the state. Countries in Asia such as China, Korea and Thailand nationally have adopted a nanotechnology development strategy. Nanotechnology is still underdeveloped in Indonesia, people are still unfamiliar with this technology. Nanotechnology is technology that studies about tiny particles ($1x10^{-9}$) using a nanometer scale (nm), then manipulated to produce objects/substances with new functions with special characteristics [1].

Nanotechnology can be implemented on botanical insecticides, so as to increase the efficiency of using insecticide raw materials. Botanical insecticides contain natural active ingredients derived from plants that are easily degraded in nature and are selective so they are safe against non-target organisms. Botanical insecticides do not quickly cause resistance, can be combined with other pest control techniques and a simple application process can reduce dependence on synthetic insecticide products [2].

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Plants that are known to be used as raw material for insecticides include Tephrosia vogelii. T. vogelii leaves contain isoflavonoid group compounds such as rotenone, deguelin and tefrosin [3]. Rotenon works as a cellular respiration toxin that inhibits the transfer of electrons in NADH - the ubiquinone reductase coenzyme (complex I) of the electron transport system in the mitochondria [4]. This will eventually lead to paralysis of various muscular systems and other body tissues in insects which eventually cause death.

Materials and Methods:-

Preparation of raw materials:

The insecticide used was a botanical insecticide made from T. vogelii. The criteria for taking T. vogelii leaves are: T. vogelii has purple flowers and is already bearing fruit. The leaves are taken in the center of the plant to get the best leaves. Then the leaves are collected and put in a large plastic bag and then taken to the Insect Bioecology Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Andalas University. The leaves of T. vogelii were placed on a 60 cm diameter rattan tray that had been covered with paper and then carried out the drying and dispersing process. This drying process is carried out for approximately 3 weeks, until the leaves are dry. The dried T. vogelii is then cut into 2.5 cm and mashed using a blender. The results of the blender were sieved using a 0.5 mm sieve to obtain T. vogelii in powder form [5].

Tephrosia vogelii extraction:

The powdered T. vogelii leaves were taken as much as 100 grams then put into an erlenmeyer flask and 1000 ml of ethyl acetate solvent were added. Comparative composition of ethyl acetate with T. vogelii powder refers to previous studies by Lina et al. (2014). Soaking process is left for 2 × 24 hours. Then the extracted liquid was filtered twice, first using a glass funnel (9 cm in diameter) on a regular filter paper on the second using a glass funnel (4 cm in diameter) based on whatman filter paper number 41 on the second screening. The filter is collected in a vaporizer flask, then evaporated with a rotary evaporator at a temperature of 50ºC and a pressure of 240 mbar. The solution obtained from evaporation is used to re-soak the extracts of the plant up to three times soaking. The extract was then stored in a refrigerator at 4ºC until used for testing.

Making nanoemulsions:

The process of making nanoemulsion involves the aqueous phase and the organic phase which is homogenized using a magnetic stirer. The organic phase consists of T. vogelii extract and solvent while the water phase consists of sterile aquades and tween 80. The first thing to do is the water phase is homogenized using a hot plate magnetic strirer at a speed of 2,500 rpm while stirring constantly using strirer seeds for 35 minutes. Then made an organic phase in erlemeyer, in the form of extract of T. vogeli dissolved with 96% ethanol.

After the water phase is finished homogenized using a magnetic stirer then an organic phase /oil phase drop is carried out into the water phase using a dropper pipette, the dropping process is carried out slowly (drop by drop). When dripping the organic phase/oil phase into the water phase, the water phase must still be stirred using a magnetic stirrer. After the organic phase has been dropped, the homogenisation process using magnetic strirer is continued for another 45 minutes.

To get the best nanoemulsion composition, nanoemulsion was made using 4 different steps/methods, namely: the first step, making nanoemulsion of Formula A and B. The composition of Formula A and B are shown in Tables 1 and 2. Second step, making nanoemulsion by manipulating composition refers to the formula A, the third step, making nanoemulsion by composition manipulation refers to formula B, and the fourth step making nanoemulsion refers to Putu's method, 2018.

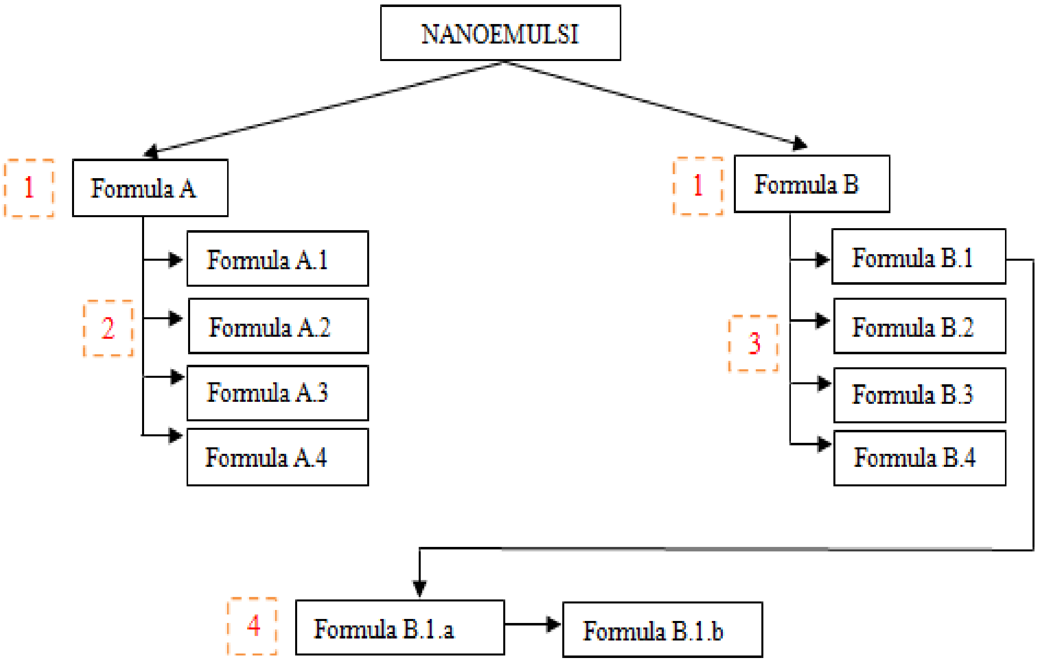

Figure 1:- Diagram of the steps for making nanoemulsions.

The first step nanoemulsion composition, namely making nanoemulsion Formula A and B are:

Table 1:- Composition of Nanoemulsoin of Formula A and B.

Composition of Nanoemulsoin				
Formula	(Ekstract :	Etanol70%)	(Tween80) (%)	(Akuadest) (%)
A. Oil phase 10%	5 :	5	3	87
B. Oil phase 20%	10 :	10	3	77

To get better nanoemulsion, then optimization of formulas A and B. Optimization is done by making a new nanoemulsion but the composition of extracts, solvents, and emulsifiers is arranged as in (Table. 2), in order to produce more optimal nanoemulsions. Optimization is made in 8 of them, 4 formulas refer to formula A consisting of 10% oil phase (second step) and 4 formulas refer to formula B consisting of 20% oil phase (third step). The nanoemulsion optimization composition of the second step, which refers to formula A, is:

Table 2:- Composition of Nanoemulsion Optimization refers to formula A.

Formula	Composition of Nanoemulsoin				
	(Ekstract + (10%)	Etanol 96%)	(Tween 80) (%)	(Agristik) (%)	(Aquadest) (%)
A.1	1 :	1	3	-	87
A.2	1 :	3	3	-	87
A.3	1 :	1	-	3	87
A.4	1 :	3	-	3	87

Third step, optimization is carried out in Formula B (Table. 1). Optimization is made in a number of 4 formulas, as follows:

Table 3:- Composition of Nanoemulsion Optimization refers to formula B.

Formula	Composition of Nanoemulsion			
	(Ekstract + (20%)	Etanol 96%)	(Tween 80) (%)	(Aquadest) (%)
B.1	1 :	3	3	77

B.2	1 :	5	3	77
B.3	1 :	3	4	76
B.4	1 :	5	4	76

To get a more optimal nanoemulsion formula, Formula B.1 nanoemulsion was re-optimized using the Putu method, 2018 by means of active ingredients, emulsifiers and solvents homogenized using magnetic strirers simultaneously for 60 minutes. Furthermore, it is dropped with distilled water while homogenizing again for 35 minutes. Nanoemulsion, the fourth step, is made in 2 formulas including:

Table 4: Composition of Nanoemulsion Optimization using methods Putu, 2018

Formula	Composition of Nanoemulsion			
	(extract + (20%)	Etanol 96%)	emulsifier (3%)	Aquadest (%)
B.1.a	1 :	3	Agristik	77
B.1.b	1 :	3	Tween 80	77

The process of making nanoemulsion Formula B.1.a and B.1.b is expected to be able to produce nanoemulsion that is more optimal than the previous formula. Overall the composition of the oil phase used is 20%. The ratio of extract versus solvent in the oil phase is 1: 3 in each formula. Agristic emulsifiers were used 3% in Formula B.1.a and Tween 80 emulsifiers were used 3% in Formula B.1.b.

Nanoemulsion toxicity test:
Broccoli (4x4) cm^2 leaf pieces are dipped one by one into nanoemulsion until they are evenly wet and then air dried, the leaves will be used as treatment feed, Control leaves are dipped in the appropriate control solution. The cuttings of the treatment feed and control leaves were placed separately in a petri dish (9 cm diameter) with a tissue. Petri dishes are placed upside down. A tissue mat is placed on the lid of the cup and the bottom of the cup is closed on the tissue. Thus, the lid and base of the dish are insulated with tissue so that the test larvae cannot escape from the cup.

The 15 instar larvae of C. pavonana newly replaced skin were put into each petri dish which contained treatment feed or control leaves, then the larvae were allowed to eat for 24 hours. Each treatment and control was repeated 5 times. After 24 hours the new treatment feed and control leaves were added again. Twenty-four hours later, the treated leaves were replaced with untreated leaves, dead larvae were counted and removed from the cup while the living ones were kept until the fourth instar larvae. Data from observations of toxicity test were analyzed by probit using POLO PC software then imago mortality data were analyzed by ANOVA using statistical software 8 and followed by Least Significant Different (LSD) test at 5% level.

Nanoemulsion evaluation:
Nanoemulsion evaluation is carried out to determine the nature of nanoemulsion that has been produced and obtain nanoemulsion that is stable, safe to use and efficient in application.

Particle Size Analizer Analysis:
nanoemulsion samples were analyzed by PSA using the Zetasizer Nano ZS Malvern located in the Bogor Post Harvest Center. This tool has a sensitivity value of 3-10,000 nm and is able to measure particles or molecules which have a range of 0.15-10µ. Nanoemulsion samples were put into vials with screw caps then labeled and then samples were packed and sent to the Bogor Post Harvest Center. Jalan Tentara Pelajar Student No.12, Cibogor, Bogor Tengah District, Bogor City, West Java 16122.

Zeta Potential Nanoemulsion:
The zeta value of nanoemulsion potential was obtained together with the results of the PSA (Particle Size Analizer) analysis sent from the Insect Bioecology Laboratory, Faculty of Agriculture, Andalas University to the Bogor Post Harvest Center.

PH Test:
PH measurements are carried out using a PH meter that has been calibrated according to the calibration instructions. Measurements were made aimed at determining the PH value and acidity level of nanoemulsion so that the safety of

the nanoemulsion is known to human skin and has no side effects or irritation. PH measurements are carried out at room temperature.

Results and Discussion:-

Manoemulsion toxicity test:

Twelve nanoemulsion formulas were tested preliminary, then four formulas with the highest mortality values were selected for further testing. Preliminary tests produce larval mortality as follows:

Table. 5:- Preliminary test results of Crocidolomia pavonana larvae mortality after nanoemulsion treatment of formulas A and B.

Formula	preliminary test		
	Concentration 0 (%)	Concentration 25(%)	concentration 50 (%)
A.	0,00	67,00	83,33
B.	0,00	100,00	100,00

Formula A at 25% (v⁄v) solubility kills larvae at 67.00% and at 50% solubility (v⁄v) is 83.33%, formula B can kill off larvae as a whole.

Table. 6:- Preliminary test results of Crocidolomia pavonana larvae mortality after nanoemulsion treatment of formulas A.1 to B.1.b.

Formula	Preliminary test		
	Concentration 0,5(%)	Concentration 0,25 (%)	Concentration 0,1 (%)
A.1	83,33*	-	56,67
A.2	66,67*	-	36,67
A.3	56,67	-	23,33
A.4	23,30	-	6,66
B.1	23,33	26,67	13,33
B.2	33,33	40,00	30,00
B.3	30,00	13,33	6,67
B.4	20,00	36,67	13,32
B.1.a	100,00*	90,00	22,67
B.1.b	89,33*	60,00	56,00

Remarks (*) = formula that meets the criteria for further testing

Four selected formulas namely formula B.1.a, formula B.1.b, formula A.1 and formula A.2 are further tested. The four formulas were selected based on the highest successive mortality values in Table (Table 6). Further tests consisted of five levels of concentration based on preliminary test observations. The results of further tests of the four formulas are as follows:

Table 7:- Mortality results and length of development of the larvae of Crocidolomia pavonana after further treatment with nanoemulsion formula B.1.a.

Consentrasion (%)	Mortality (%) ± SD		Mortality results (days) (X±SD)	
			Instar 2-3 ± SD	Instar 2-4± SD
0,13	22,66 ± 0,78	A	3,05 ± 0,06	6,10 ± 0,13
0,15	29,34 ± 1,14	B	3,05 ± 0,33	6,11 ± 1,06
0,18	44,00 ± 1,14	B	3,11 ± 0,62	6,00 ± 2,27
0,21	48,00 ± 1,48	BC	3,18 ± 0,63	6,43 ± 1,81
0,25	73,34 ± 2,50	C	3,45 ± 0,91	6,25 ± 1,70
0,00 (kontrol)	00,00 ± 0,00	D	2,96 ± 1,42	5,48 ± 1,87

Table 8:- Mortality results and length of development of Crocdolomia pavonana larvae after further treatment nanoemulsion test formula B.1.b.

Consentrasion (%)	Mortality (%) ± SD		Mortality results (day) (X±SD)	
			Instar 2-3 ± SD	Instar 2-4± SD
0,05	36,00 ± 1,55	A	3,37 ± 1,14	6,87 ± 1,87
0,09	41,34 ± 3,20	AB	3,23 ± 0,91	6,50 ± 1,06
0,17	56,00 ± 1,52	AB	3,06 ± 1,60	6,57 ± 2,27
0,31	60,00 ± 1,72	B	3,21 ± 1,16	6,60 ± 1,81
0,58	89,34 ± 0,52	B	3,10 ± 1,34	6,06 ± 1,70
0.00 (kontrol)	00,00 ± 0,00	C	2,73 ± 0,14	5,51 ± 0,13

Table 9:- Mortality results and length of development of the larvae of Crocidolomia pavonana after the further treatment of nanoemulsion formula A.1.

Consentrasion (%)	Mortality (%) ± SD		Mortality results (day) (X±SD)	
			Instar 2-3 ± SD	Instar 2-4± SD
0,05	22,67 ± 1,70	A	3,17 ± 0,48	5,94 ± 0,40
0,09	42,67 ± 2,19	A	3,09 ± 2,64	5,90 ± 3,48
0,17	62,67 ± 1,01	B	3,35 ± 2,43	6,00 ± 3,06
0,32	78,67 ± 1,73	B	3,44 ± 0,58	5,91 ± 0,73
0,59	85,33 ± 1,68	B	3,13 ± 1,06	6,07 ± 1,19
0,00 (Kontrol)	00,00 ± 0,00	C	2,96 ± 0,10	5,48 ± 0,11

Tabel 10:- Mortality results and length of development of the larvae of Crocidolomia pavonana after the further test nanoemulsion formula A.2

Consentrasion (%)	Mortality (%) ± SD		Mortality results (day) (X±SD)	
			Instar 2-3 ± SD	Instar 2-4± SD
0,05	14.66 ± 1,61	A	3,00 ± 0,80	6,42 ± 1,01
0,09	25,33 ± 2,17	A	3,09 ± 2,40	5,74 ± 2,98
0,17	36,00 ± 1,99	B	3,09 ± 3,33	5,65 ± 4,27
0,32	44,00 ± 0,89	B	3,00 ± 2,56	5,53 ± 3,24
0,59	68,00 ± 0,46	C	3,00 ± 0,79	5,88 ± 1,18
0,00 (Kontrol)	00,00 ± 0,00	D	2,96 ± 0,06	5,48 ± 0,10

The results of further tests showed the same pattern as the preliminary test, namely the death of larvae increased with increasing nanoemulsion concentration. Larval mortality in formula B.1.a and formula B.1.b is higher than larval mortality in formula A.1 and A.2. This is caused by the amount of extract or oil phase contained in formulas B.1.a and B.1.b is greater than formulas A.1 and A.2. [6] conveyed other advantages of nanoparticle-sized insecticides, namely: accelerating the solubility of active substances, kinetic stable so as to prevent creaming, and aggregation during storage. With a very small size nanoemulsion is easier to penetrate plant tissue, so as to minimize the insecticide wasted at the time of application and allows quickly kill the target pest. [7] also believes that the small grain size makes it easy to spread. It is therefore clear that nanoemulsion is more effective and efficient compared to non-sized nanoparticle formulas.

The difference in the duration of larval development that survived due to nanoemulsion treatment compared to control was that nanoemulsion treatment delayed the development of larvae to the next instar for about 1 day compared to control. The occurrence of developmental disorders of larvae treated by nanoemulsion may be caused by active compounds contained in T. vogelii. The active compound contained in T. vogelii is rotenone. Rotenon is a toxic cellular respiration that is likely to influence the survival time of larvae that survive.

Probit regression analysis was used to determine the relationship of T. vogelii nanoemulsion concentration of Formula A.1, A.2, B.1.a and B.1.b to mortality of C. pavonana larvae. Probit analysis produces the regression slope values (value b), LC50 and LC95 for each formula, as follows:

Table 11:- Probit regression parameters related to the concentration of Tephrosia vogelii nanoemulsion on the mortality of Crocidolomia pavonana larvae.

Formulasion	Value b + SE	LC_{50} (%)	LC_{95} (%)
A.1	1,71 ± 0,19	0,12	1,09
A.2	1,53 ± 0,19	0,30	3,15
B.1.a	4,54 ± 0,68	0,19	0,45
B.1.b	1,29 ± 0,87	0,12	2,26

b = slope of the regression; SE = standard error

Larvae mortality due to nanoemulsion A.1 concentration of 0.1 was 56.67%, whereas the results of larvae mortality due to the treatment of a single extract of T. vogelii instead of nanoemulsion were tested by [5]was 88.89%. If you only see the mortality value, nanoemulsion has a lower value than single extracts rather than nanoemulsion. It is not comparable, if the comparison is made according to LC_{95} of each preparation, there is a saving of extract raw material in nanoemulsion compared to single extract rather than nanoemulsion. The LC_{95} nanoemulsion A.1 value was 1.09 (Table. 11) while the LC_{95} single extract of T. vogelii was not nanoemulsion by [5] is 0.16. Nanoemulsion A.1 has a LC_{95} value of 1.09 which means that to kill larvae at 95% it takes nanoemulsion at 0.272ml, in 0.275ml (v/v) nanoemulsion there is a T. vogelii extract of 0.013 gram, while a single non-nanoemulsion extract to kill larvae is equal to 95% require an extract of 0.040 grams. There are three times the saving of extract raw material in nanoemulsion compared to non-nanoemulsion extract. This proves the theory of one of the properties of nanoemulsion which has a good penetration ability, thus saving the use of active substances.

Nanoemulsion evaluation:

This research resulted in 12 nanoemulsion formulas, 4 of the best formulas were chosen to be tested for PSA analysis, zeta potential, and pH test. The criteria for selecting the four best formulas were selected according to the toxicity of each formula in the preliminary test (Table. 6). Four formulas are:

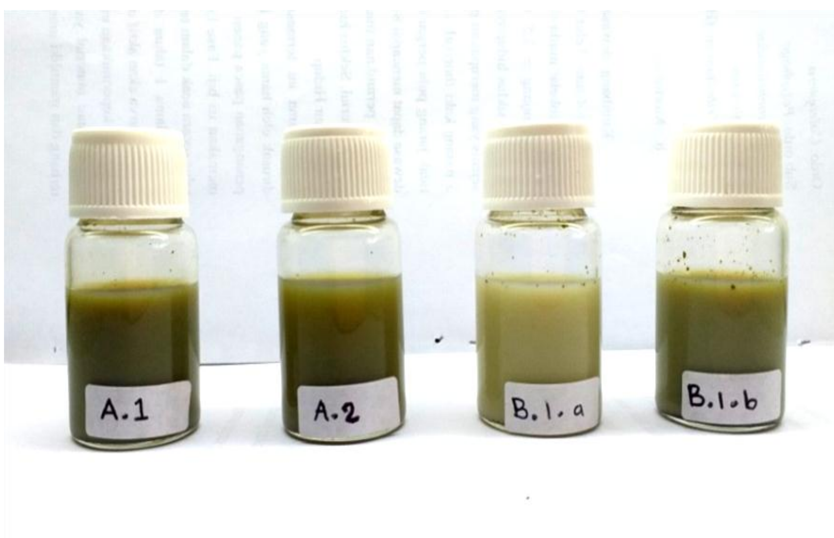

Figure 1:- Nanoemulsions selected based on the toxicity value of each formula.

Significant color differences in Formula B.1.a occur because the type of emulsifier used is different from other Formulas. Formulas A.1, A.2 and B.1.b use Tween 80 as an emulsifier, while Formula B.1.a uses agristics as an emulsifier.

Table 12:- Results of nanoemulsion analysis based on Particle Size Analyzer (PSA).

Formula	Particle Size (nm)	Zeta Potensial (mV)	PH nanoemulsion	Polydispersit index (PDI)
A.1	134	-29,2	3,0	0,238
A.2	156	-24,3	3,3	0,142
B.1.a	1292	-16,3	2,8	0,850

B.1.b	1286	-24,2	2,9	0,804

Four formulas produced were only two formulas that met the nanoemulsion requirements and criteria. Formula that meets the requirements is formula A.1 and A.2. The particle size of formula A.1 is 134 nm and formula A.2 is 156 nm. Formula B.1.a and formula B.1.b do not meet the nanoemulsion requirements because they have particle sizes of 1292 nm and 1286 nm, respectively. The terms of the formula can be said nanoemulsion that must have a particle size in the range 0-500 nm [8].

Formula B.1.a and B.1.b. the unsuccessful nanoemulsion was thought to be due to the composition of the oil phase which was too large. Formula B.1.a and B.1.b. consists of 20% oil phase (Table. 4). In contrast to formulas A.1 and A.2 which consist of 10% oil phase (Table. 2). The oil phase is a homegene solution consisting of a combination of extract and 96% ethanol solvent in different ratios in each formula. In accordance with the writings of [9] in his scientific journal that is to produce nano emulsions, it is sufficient to use 3-10% oil phase, in contrast to macroemulsions which require high concentrations in the oil phase of around 20%. Furthermore [10] concluded that the concentration of the oil phase can affect the size distribution of nano emulsion particles. In addition to the oil phase, the composition and type of emulsifier also affect nanoemulsion particle size. [11] reports that in the process of nanoemulsion formation, emulsifiers play an important role in the process of solving droplets / particle size.

Based on the Polydispersity index (PDI) values of the four selected formulas it can be categorized that formulas A.1 and A.2 are homogeneous because they have Polydispersity index (PDI) values respectively 0.238 and 0.142 while formulas B.1.a and formulas B.1 .b is heterogeneous because it has a Polydispersity index (PDI) value of 0.850 and 0.804, respectively. The provisions of the Polydispersity index (PDI) are in line with the statement of [12] that if the value of the Polydispersity index (PDI) starts from 0.01 to 0.5 for particles categorized as monodispersion. Monodispersion system shows the distribution of particle size which tends to be narrow which indicates that the monodispersion system has a good uniformity level (homogeneous). A Polydispersity index (PDI) of greater than 0.7 indicates a very broad particle size distribution indicating that it does not have uniformity in other words, that is, heterogeneous.

Based on the overall potential zeta value of the resulting nanoemulsion charge the negative value (-) shows that the formula has a globul surface which is negatively charged (-) as well and is relatively stable because it is less than (-) 30mV. [13] confirms the negative potential zeta value (-) shows the surface charge of globally negatively charged (-) as well, and vice versa the positive zeta value (+) indicates the globul surface charge which is positively charged (+) as well. Added by [14] zeta potential values above (+) 30 mV or below (-) 30 mV indicate a stable colloidal system.

PH test results showed overall nanoemulsion was acidic. This means that the four selected formulas are relatively unsafe to use by farmers, because PH that is too acidic can irritate the skin while PH that is too alkaline can make skin flaky. [15] argue that the PH that is suitable for human skin is 4.6 - 6.5. Besides that [16] believes that good pH tolerance used for skin is 5.5 - 6.5. Pesticides are generally acidic. Nanoemulsion produced in this research was acidic, presumably due to autocidation that occurred in tween 80 and agristic which caused a decrease in PH value, according to the control solution which also had a low pH and was acidic. [17] expressed a decrease in PH value of nanoemulsion preparations usually caused by oxidation in the presence of oxygen from the atmosphere and light, and the presence of microorganisms. The PH value can increase due to the release of hydroxyl ions slowly. Temperature is one of the factors that can affect the PH value of nanoemulsion. [18] explained the water PH used at the time of the application affected the effectiveness of pesticide spraying. Water PH that has acidic properties is good for pesticide applications because it will extend the duration of effective use of pesticides. Pesticides will be more effectively applied if they are acidic.

References:-

1. Wulan, T. 2016. Nano Technology, Important But Not Yet Developing in Indonesia.https://www.voaindonesia.com/a/teknologi-nano-pentingnamun-belum-berkembang-di-indonesia/3657248.html. [accessed January 10, 2020].
2. Widawati, M. 2014. Utilization of Nanotechnology in the Development of Organic Fertilizers and Pesticides. Researchgate. 11 things.
3. Lambert, N., M. F. Trouslot, C. N. Campa, H. Chrestin. 1993. Production of rotenoids by heterotrophic and photomixotrophic cell cultures of Tephrosia vogelii. Phytochemistry 34: 1515-1520.

4. Hollingworth, R. M. 2001. Inhibitors and uncouplers of mitochondrial oxidative phosphorylation. Inside: Krieger R, Doull J, Ecobichon D, Gammon D, Hodgson et al., Editors. Handbook of Pesticide Toxicology. Vol 2: 1169-1227. San Diego (US): Academic Press.
5. Lina, E. C., Dadang, S. Manuwoto, G. Syahbirin. 2016. Physiological and Physical Disorders of Crocidolomia pavonana (F.) (Lepidoptera: Crambidae) Due to the Treatment of Mixed Extracts of Tephrosia vogelii and Piper Aduncum. Indonesian Entomology Journal 12 (2) 94-101.
6. Rahmaniyah, D. 2018. Comparison of the Formulation of Hydroctison Gel Nanoemulsion and Nanoemulsion Systems with Variations in the Palm Oil Oil Concentration. [thesis]. Poor. Faculty of Pharmacy. Maulana Malik Islamic University of Malang. 193 things.
7. Acosta E. 2009. Bioavailability of nanoparticles in nutrient and nutraceutical delivery. Current Opinion in Colloid & Interface Science. 14 (1): 3-15.
8. Shakeel, F., Baboota, S. Ahuja, A. Ali, J. Faisal, M. S. Shafiq. 2008. Stability evaluation of celecoxib nanoemulsion containing tween 80. Thai Journal Pharm. Sci 32 (1): 4-9.
9. Bouchemal, K. 2004. Nano-emulsion formulation using spontaneous emulsification: optimization of solvents, oils and surfactants. International Journal of
10. Seijo., B., Fattal, E. Treupel, L. Couvreur. 1990. Design of nanoparticles of less than 50 nm diameter: preparation characterization. Int. J. Pharm. 62-88.
11. McClements, D. J., 2004, Protein-Stabilized Emulsions, Current Opinion in Colloids and Interface Science. 9 (5), 305-313.
12. Wu, Q., H. Yangdong, Y. Hanbo, L. Yongzi. 2012. Changes in active-layer thickness and near-surface permafrost between 2002 and 2012 in alpine ecosystems, Qinghai-Xizang (Tibet) Plateau, China. Global and Planetary Change. 124 (3): 149-155.
13. Mardikasari, S., Aulia, Jufti, M. djajadisastra, Jositha. 2016. In-Vitro Formulation and Penetration Test of Topical Genistein Nanoemulsion from Sophora japonica Linn. Indonesian Journal of Pharmaceutical Sciences. 2614-6495.
14. Preeven, S., D. V. Gowda, A. Srivasta and R. A. M. Osmani. 2016. Formulation and Evaluation of Nanostuctured Lipid Carrier (NLC) for Glimepiride. Der Pharmacia Lettre. 8 (7): 251-256.
15. Naibaho, OH, Yamlean, PVY Wiyono, W., 2013, Effect of Ointment Base on Ointment Formulation of Basil Extract (Ocimum sanctum L.) on Rabbit Back Skin Made by Staphylococcus aureus, Pharmaceutical Scientific Journal, 2 (02) : 32-34.
16. Gotaro, D. 2016. Optimization of Span 60 and Tween 80 Emulgator Composition on Physical Stability of Cold Cream Preparation of Ethanol Extract Ambon Yellow Banana (Musa paradisiacal L.). [Thesis]. Faculty of Pharmacy. Sanata Dharma University. 92 things.
17. Martin, A., James, Arthur. 1993. Physical Pharmacy: Fundamentals of Physical Chemistry in Pharmacetic Sciences Edition 2. Ui Press. Depok. 87-88.
18. Prabaningrum, L., T. K. Moekasan. 2016. Effect of Solvent Water pH and the Age of Spray Solution on the Efficacy of Pesticide in Potatoes. Horticultural Journal. 26 (1): 113-120.