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Effects of Curcumin on Stability and Efficacy of Neem Leaves Extract as Botanical Insecticides

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ABSTRACT

Botanical insecticides are considered biodegradable, environmentally compatible, and less toxic to non-target organisms than synthetic insecticides. Unfortunately, they are not attractive to Indonesian farmers due to its instability against light, temperature, and microbes, as well as they, have short storage time. This research was intended to produce a distinctive botanical insecticide that is resilient against the light, microbes, and durable. The role of curcumin in the stability and efficacy of neem (*Azadirachta indica*) leaves extract as a botanical insecticide against soybean pod sucking bug, *Riptortus linearis* was evaluated in the laboratory. This research was conducted at the Organic Chemistry Laboratory of Chemistry Department, Faculty of Science and Technology Airlangga University and the Plant Protection Laboratory, Faculty of Agriculture University of Wijaya Kusuma Surabaya, Indonesia, from March to October 2016. The results explained that curcumin is no effect on increase the stability of neem leaves extract solution towards UV light irradiation. However, it can increase the insecticide activity of neem leaves extract solution up to 96% mortality against soybean pod sucking bug, *R. linearis*. The increase of the bioactivity refers to the anti insecticidal activity of ferulic acid formed from the degradation of curcumin.

INTRODUCTION

Botanical insecticides have long been touted as more attractive alternatives than chemical insecticides in pest management since botanicals reputably possess little impact on the environment and human health. The application of botanical insecticides to reduce chemical insecticides to date is still low in Indonesia. This is due to the instability of botanical insecticides against sunlight, temperature, and microbe. Consequently, it shows low efficacy, can not be kept for a long time and has to be applied frequently. The quality of botanicals formulation is become a critical point should really be considered (Lina, Yulianti, Ernis, Arneti, & Nelly, 2018). Therefore, the use of botanical insecticide is impracticable although Indonesia is known for its plant biodiversity, which is a potential natural source to find an active and environmentally friendly botanical insecticide. It is so unfortunate, research on botanical insecticide has not yet reached the level of the formula that is practical, durable, and not easily damaged. Botanical insecticides are highly biodegradable and relatively harmless to non-target organisms and the environment. The utilization of ecofriendly-botanical insecticides plays a big role to implement the Sustainable Agriculture Programme in Indonesia.

Neem (*Azadirachta indica*) is a plant that is used as a botanical insecticide, due to its active ingredient azadirachtin found in leaves (Abdul Razak, Santhakumar, Mageswari, & Santhi, 2014; Asogwa, Ndubuaku, Ugwu, & Awe, 2010; Rashid & Ahmad, 2013) and seed (Asogwa, Ndubuaku, Ugwu, & Awe, 2010; Esparza-Díaz, Villanueva-Jiménez, López-Collado, & Osorio-Acosta, 2011). Furthermore, it is stated, the other insecticidal compounds

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contained in neem leaves and seed are salannin, meliantriol, and neem. The four compounds have many functions such as inhibit the laying of eggs by female insects, inhibit the development of pest, inhibit molting process due to the blocking of the hormone ecdysone, inhibit chitin formation, disrupt sexual communication, and make infertile on male insects. These chemical compounds also have other functions as antifeedant and repellent against pests (Castillo-Sánchez, Jiménez-Osornio, & Delgado-Herrera, 2010; Ghoneim & Hamadah, 2017). Recent research reports by Diaz-Najera et al. (2019) indicated that a mixture of *A. indica and Cinnamomum spp* effectively able to inhibit the growth of *Rhizoctonia solani in vitro*, the root pathogenic fungi, up 100%. According to Campos, de Oliveira, Pascoli, de Lima, & Fraceto (2016) and Roychoudhury (2016), the neem botanical insecticide is best known for its wide spectrum activities and very promising for the control of many pests. However, the effectiveness of this botanical insecticide is constrained by its instability against sunlight. Therefore, an effort to protect the bioactive azadirachtin keeps its efficacy high and durable is in demand.

The addition of chemicals that function as stabilizers of botanical insecticides like *ter. butyl-p-cresol*, *8-hydroxy quinoline*, and *ter.butyl hydroquinone*, has been carried out even patented in India (Johnson, Dureja, & Dhingra, 2003). It has been proven that these chemical stabilizers can extend the half-life and increase azadirachtin stability against sunlight. Unfortunately, the addition of chemical stabilizers turned out to harm human health. In 2002, the Assessment Report stated that chemical residues carried on various agricultural products and then consumed by humans can irritate skin and eyes, respiratory allergies, liver and fetal disorders, and are very toxic to aquatic organisms. Based on these conditions, the availability of natural stabilizers which are safe for agricultural products and human health and able to protect botanical insecticides from the photodegradation process is strongly necessary. One of the natural stabilizers possessing these criteria is curcumin.

Curcumin is a phenolic secondary metabolite of *Curcuma domestica*. Its bioactivity, such as antioxidant, anti-inflammation, antibacterial, and anticancer has been reported intensively (Borra et al., 2013; Danciu et al., 2015; Gul & Basheer, 2016). Based on its molecular structure, curcumin is composed of two phenolic groups and conjugated double bond, which contribute its potential antioxidant, antibacterial activity, and stability against the light. This ability of curcumin makes it widely used as a natural preservative for food and medicine. The study on curcumin as antioxidants also have been often conducted. Aznam (2004) reported that the antioxidant activity is concentration-dependent which means that the higher the curcumin concentration, the higher the antioxidant activity will be. 54.31% is the highest antioxidant activity which was gained from 25% curcumin extract. In this study, the addition of curcumin to neem botanical insecticide is expected to increase its efficacy, protect it from photodegradation, and prolonged its durable. Herein we reported the effect of curcumin extract toward photo-stability of neem leaves extract and its efficacy against soybean pod sucking bug.

Among pests that can be controlled by using neem botanical insecticides namely *Riptortus linearis*, the main pests which attack soybean crop and cause a by up 80% production decrease. *Riptortus linearis* controlling is mostly conducted by using chemical insecticides which are known to have many negative effects on humans and the environment. Therefore, safe and eco-friendly methods by using neem botanical insecticides are needed to pests control.

MATERIALS AND METHODS

The neem leaves were collected from two districts in Gresik i.e. Menganti and Sidayu, through May to June 2016. The solvent used for extraction was technical grade and redistilled, while for analysis pro analysis grade. The UV-vis spectrum was recorded on UV-spectrophotometer Shimadzu type UV-1800. Thin-layer chromatography was conducted on silica gel $60F_{254}$ on aluminum sheets (E. Merck). The spot of TLC was detected by a UV lamp (λ 254 nm).

Isolation of Curcumin from the Rhizome of *Curcuma domestica*

The rhizome of *Curcuma domestica* (500 g) was washed with flowing water, sliced and wind dried for three days. The sliced *C. domestica* was then ground and soaked with re-distilled ethanol in a maceration flask for two days. The mixture was filtered off with Buchner funnel, the filtrate separated from the residue. This process was repeated 3 times. The filtrate was collected, and concentrated by vacuum evaporator and gave a viscous brown-

yellowish extract. To the viscous extract, *n*-hexane was added, then the curcumin extract was filtered off, washed with cold *n*-hexane, and dried (Nabati, Mahkam, & Heidari, 2014; Paulucci, Couto, Teixeira, & Freitas, 2013).

Curcumin Purity Test

The purity of the curcumin was determined by TLC employing the following mobile phase: 1) *n*-hexane:ethylacetate = 2:3; 2) chloroform:ethanol = 9:1. The spot was detected by a UV lamp (λ 254 nm). Besides, the UV spectrum of curcumin in ethanol was recorded on a UV-vis spectrophotometer in range 200–700 nm (Nabati, Mahkam, & Heidari, 2014).

Preparation of Neem Leaves Extract

The neem leaves were washed with tap water, wind-dried, and then powdered. The neem leaves powder was then soaked in ethanol, and kept for a night. The mixture was then filtered off and separated between the residue and filtrate. The process was repeated 3 times, and the filtrate was collected and concentrated under reduced pressure (Rashid & Ahmad, 2013).

Photostability Test of Curcumin and Neem Leaves Extract

The stock solution of curcumin (100 ppm) was prepared by dissolved 10 mg curcumin in 100 ml ethanol. For the photostability test, a curcumin solution (4 ppm) was used, which was obtained by dilution of the stock solution. The test solution (curcumin 4 ppm) was recorded its UV spectrum and the maximum wavelength was noticed. The photostability was determined by the alteration of absorbance at the maximum wavelength and recorded every 10 minutes irradiation with a UV lamp (λ 254 nm) for 60 minutes (Kumavat et al., 2013).

The photostability of neem leaves extract was determined using neem leaves extract solution (100 ppm). First, the maximum wavelength was recorded using a UV spectrophotometer. The change of absorbance was then recorded after irradiation using a UV lamp (λ 254 nm) every 10 minutes for 60 minutes. All the procedure was conducted triple (Johnson, Dureja, & Dhingra, 2003).

Photostability Test of the Mixture Solution of Curcumin and Neem Leaves Extract

To study the effect of curcumin toward neem leaves extract photostability, we used curcumin solution in water (8 ppm) and neem leaves extract solution (20%). We varied the volume of the curcumin and neem leaves extract solution. The changes of the absorbance of the mixture solution were observed at the maximum wavelength of the neem leaves extract solution every 30 minutes for 3 hours, for the treatment with or without irradiation. It was done in triplicate (Johnson, Dureja, & Dhingra, 2003).

Efficacy Test of the Formulated Botanical Insecticide in Laboratory Scale

The formulated botanical insecticide is the mixture of curcumin solution and neem leaves extract solution as mentioned above. The test was performed by sun irradiation of the solution at 11.00 hours for 0, 1, 2, and 3 hours. The irradiated solution was then tested its efficacy against adults of pod sucking bug. Completely Randomized Design with five replications was used in this experiment. Twenty adults of pod sucking bugs were infested to each cage, so a total of 400 adults of test insects were needed (Massaguni & Md Latip, 2012). The mortality of adults of pod sucking bugs was observed, and the percentage of mortality was calculated using Abbott's formula (Abbott, 1925):

$$M = \frac{X}{Y} \times 100\% \quad \text{..... 1)}$$

where:

M = percentage of mortality (%);
X = amount of all the dead pod sucking bugs;
Y = amount of pod sucking bugs totally

The data were statistically analyzed using ANOVA (analysis of variance) and HSD (Honestly Significant Difference)-test to determine the statistically significant differences (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

Curcuma domestica contains three curcumin derivatives, those are curcumin, dimethoxy curcumin, and bis dimethoxy curcumin (Kumavat et al., 2013). The TLC analysis of the curcumin extract used in this research resulted in spot with Rf value in accordance with the result of Kumavat et al. (2013), which were 0.83 and 0.57 (*n*-hexane:ethylacetate = 2:3), and 0.74 and 0.36 ($CHCl_3$:methanol = 9:1). Spot with Rf value 0.74 indicated the existence of curcumin, while Rf 0.36 the existence of dimethoxy curcumin.

Curcumin is a phytochemical polyphenol and stable in solid (powder) state. According to Kumavat et al. (2013) and Zebib, Mouloungui, & Noirot (2010), in solution, curcumin is degraded easily in neutral or in basic medium. It is insoluble in water

under acidic or neutral conditions but dissolves in alkaline conditions. Furthermore, it is explained that at pH above neutral i.e. when dissociation takes place, curcumin will undergo a rapid hydrolytic degradation. Lee, Choi, Kim, & Hong (2013) added that curcumin, diferuloylmethane, has an unstable structure and a variety of factors has been reported to affect the chemical stability of curcumin. Curcumin is unstable undergoing rapid hydrolytic degradation in neutral or alkaline conditions. Light and presence of metal ions are also known to promote curcumin degradation, especially by ultraviolet light (Waseem & Amit K., 2020). The main decomposition products have previously been identified as feruloyl methane and ferulic acid.

The observation showed that UV irradiation affected the stability of curcumin solution linearly in which the longer the irradiation time, the more the degraded curcumin will be in solution. The data of irradiation demonstrated that irradiation for 60 minutes, The observation of UV irradiation to curcumin solution is tabulated in Table 1.

Table 1. Effect of UV irradiation toward stability of curcumin solution in ethanol (Mean ± s)

Duration of irradiation (minutes)	Percent of the remaining curcumin solution
0	100.00 ± 0.00
10	92.48 ± 1.71
20	82.64 ± 1.60
30	77.52 ± 1.79
40	69.45 ± 1.97
50	62.79 ± 1.54
60	54.91 ± 1.79

The difference of remaining curcumin between irradiation and without irradiation curcumin solution was compared by using the independent sample t-test at the 5% level of significance. There were statistically significant differences *(t-test, t = 3.46, df = 6, p = 2.45*) instability toward UV light between both curcumin groups. The result revealed that UV irradiation obviously decreased the stability of curcumin. Several studies documented similar results and strongly recommend techniques to shield curcumin against UV light degradation.

Previously, Lee, Choi, Kim, & Hong (2013) investigated changes in chemical stability and bioactivities of curcumin by ultraviolet radiation. It is reported that curcumin degradation in water was accelerated under UV irradiation (λ 254 nm) with the residual levels of curcumin were 36.9% after 24 hours radiation. In all pH conditions during research, UV irradiation caused significantly less residual levels of curcumin and then it is known that UV radiation dramatically accelerated curcumin decomposition. After 2 hours UV radiation, about 70% of curcumin was destroyed. Based on the result, it is claimed that the powder status of curcumin is much more resistant to UV radiation. UV light declines the chemical stability of curcumin in aqueous solvents, more effectively at higher pH. Certain bioactivities of curcumin including antioxidant activities can also be altered by UV radiation. Zebib, Mouloungui, & Noirot (2010) added that curcumin cannot be widely used in the food and pharmaceutical processing industry since it is insoluble in aqueous medium and has poor stability towards oxidation, light, alkalinity, enzymes, and heat. Therefore, curcumin should be protected in certain forms from physical and chemical damage. These effects should be considered carefully during the processing and storage of curcumin-contained products.

Both result are in accordance with the results reported by Mirzaee, Kooshk, Rezaei-Tavirani, & Khodarahmi (2014) that UV irradiation (λ 254 nm) caused curcumin degradation. The identified product of curcumin degradation are *trans*-6-(40-hydroxy-30-methoxyphenyl)-2,4-dioxo-5-hexenal), ferulic aldehyde, ferulic acid, feruloyl methane, and vanillin.

The stability test of neem leaves extract against UV irradiation at λ 257.5 nm showed that this extract possessed good stability towards UV irradiation. After irradiation for 1 hour, 93.32% of the extract remained in solution. It means that UV radiation for 1 hour causes degradation only of 6.77% and indicated that neem leaves extract is stable enough towards UV irradiation. In other words, the neem leaves can protect themselves from UV irradiation exposure. The entire data of neem leaves extract stability towards UV irradiation is showed in Table 2.

There was no statistically significant difference between 60 minutes and 30 minutes duration of UV irradiation in remaining neem leaves extract, but it was statistically different from the control treatment (without irradiation) until 20 minutes duration. It can be approved that the exposed neem leaves extract to UV for up to 60 minutes will remain stable and they do not lose insecticidal activity even effective sufficiently cause 78.7% mortality of *R. linearis.*

Table 2. Effect of UV irradiation towards the stability of neem leaves extract (Mean ± s)

Duration of irradiation (minutes)	Absorbance	Percent of the remaining neem leaves extract
0	0.794	100.00 ± 0.00^{a}
10	0.779	98.11 ± 1.58^{ab}
20	0.769	96.85 ± 1.39^{bc}
30	0.757	95.34 ± 1.05^{bcd}
40	0.753	94.84 ± 0.77^{bcd}
50	0.745	93.83 ± 0.77^{d}
60	0.741	93.32 ± 0.72^{d}

Remarks: The numbers followed by the same letters on the same columns were not significantly different at HSD-test 5%

This research assumed that the stability of neem leaves extract related to containing active ingredients namely azadirachtin, nimbin, and salannin. This is in accordance with Jarvis, Johnson, & Morgan (1998) who evaluated the stability of azadirachtin in aqueous and organic solvents several years ago. It is stated that the two compounds of neem i.e. nimbin and salannin are frequently present in the greatest quantity. Nimbin is essentially a very stable substance, salannin less so, but both are more stable than azadirachtin to heating in solution while azadirachtin is most stable in mildly acidic medium. So it is suggested, solutions should never be permitted to become alkaline. Aqueous solutions should be avoided for the storage of azadirachtin unless they are deep-frozen. Azadirachtin solutions in organic solvents are stable at room temperature. Madaki (2015) added, storage in brown bottles is preferred to plain bottles and it is considered as the best method of preserving azadirachtin.

A similar result was obtained by Olfat & El-Shiekh (2012) who analyzed the active ingredient degradation of neem oil 90% EC at different storage conditions. It is reported that the active ingredient of neem oil 90% EC was degraded due to sunlight storage (outdoor) for 14 days by 16.6%. Neem formulations retain their active ingredients for at least a year when stored at 25^{o}C. Nevertheless, factors that cause active ingredient stability towards sunlight are not explained.

According to Wilson, Thompson, Huner, & Greenberg (2001), in the field, neem trees resistant to sunlight since they involve several repair and protection mechanisms such as increase biosynthesis of UV-B screening compounds, antioxidant activity, and the rates of DNA repair. All trees can acclimate quite effectively to the environment by increasing UV-B through the accumulation of specific flavonoids in the leaf epidermis. UV-B radiation enhanced the total flavonoid content of the leaf extracts, without affecting the antioxidant activity. The increased flavonoid is a response to reducing radiation damage. Likewise, a marked increase in proline in UV-B and UV-C represents adaptive responses against oxidative damage induced by UV radiation (dos Santos Nascimento, dos Santos Moreira, Leal-Costa, Costa, Tavares, 2015; Mahdavian, Ashjari, & Mobarakeh, 2008; Zheng, Gao, Slusser, Grant, & Wang, 2003). Compared to other natural insecticides like pyrethrin and rotenone, azadirachtin is a rather stable substance. Its relative stability, together with its high potency and systemic action gives high hopes for its future wide-spread use (Johnson, Dureja, & Dhingra, 2003).

To study the stability of mixture neem leaves extract solution 20% and curcumin solution 8 ppm (called formulated-botanical insecticide) towards UV irradiation, the research used various volume ratios of neem leaves extract to curcumin 5:1, 4:1, and 3:1, and the results are displayed in Table 3.

Based on the data, it is shown that UV irradiation until 60 minutes did not affect the stability of the formulated-botanical insecticide. However, after 60 minutes of UV irradiation, the stability of the formulated-botanical insecticide decreased gradually and then after 120 minutes, the remain formulated-botanical insecticide treatment tends to be stable. It can also be seen that UV irradiation for 180 minutes caused a 10.6% decrease in the stability of formulated-botanical insecticide at all ratios. The result of the stability test presented in Table 1 and Table 2 showed that the stability of neem leaves extract is twice as much as curcumin's stability towards UV irradiation. Based on that data, we assumed that the stability of formulated-botanical insecticide is more supported by neem leaves

extract which is containing stable compounds such as azadirachtin, nimbin, and salannin. The Honestly Significant Difference (HSD)-test 5% showed that the most stable volume ratio towards UV irradiation can not be found, hence ratio 3:1 of formulated-botanical insecticide is strongly recommended because of efficiency considerations.

The result of this study showed that a mixture of curcumin and neem leaves extract solution in ratio 3:1 after 120 minutes irradiation was considered as the best choice. It can be clarified that the mixed solution exposed to UV irradiation up to 120 minutes no loss of insecticidal activity. By applying this formula, we obtained 96% as mortality average of the *R. linearis* such as presented in Table 4. However, when the total mortality of *R. linearis* was compared using ANOVA there was no significant difference between them (*df = 3, F = 1.17, p = 3.24*). It can be stated that formulated-botanical insecticide that is exposed to UV light up to three hours will not lose their efficacy for controlling *R. linearis.*

Johnson, Dureja, & Dhingra (2003) also obtained a similar result when exposed neem extracts to UV light up to 2 hours by adding chemical stabilizers such as ter.butyl-p-cresol, 8-hydroxy quinoline, and ter.butyl hydroquinone. It is assumed that the persistence of bioactivity indicated that any change in the tigloyl moiety does not alter the bioactivity of neem extract. This shows that the tigloyl moiety is involved only in transport phenomena and is not involved in binding to the ultimate azadirachtin receptor. Rashid & Ahmad (2013) had evaluated the effect of neem leaves extracts in different solvents i.e. petroleum ether, ether, and ethanol against mosquito *Culex pipiens fatigans*. The result showed that the 1% petroleum ether extract possessed 100% mosquito larvicidal activity and it also had good residual activity namely 0.2% for 144 hours. Furthermore, it can be said that conclusively neem leaves extract has some biologically active components which show insecticidal activity. Chattree, Mishra, & Srivastava (2016) obtained the mortality percentage of *Lipaphis erysimi* larvae by 40.73% on neem leaves extract 20% concentration. Based on the result of this study, it can be said that the formulated-botanical insecticide possessed better insecticide activity compared to neem leaves extract without curcumin addition.

Abdul Razak, Santhakumar, Mageswari, & Santhi (2014) conducted efficacy test in the laboratory on neem products consisting of neem oil (NO), neem seed kernel extract (NSKE), neem cake extract (NCE), neem leaves extract (NLE) and one commercial product neem against third instar larva of tobacco *Spodoptera litura*.

Table 3. Effect of UV irradiation toward stability of the formulated-botanical insecticide with various volume ratio (Mean ± s)

Duration of irradiation (minutes)	Percent of the remaining formulated-botanical insecticide at various volume rasio		
	5 : 1	**4 : 1**	**3 : 1**
0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
30	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
60	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
90	93.87 ± 0.02	93.85 ± 0.02	89.42 ± 0.05
120	89.27 ± 0.36	89.48 ± 0.00	89.47 ± 0.02
150	89.47 ± 0.02	89.47 ± 0.02	89.45 ± 0.00
180	89.45 ± 0.00	89.43 ± 0.00	89.43 ± 0.00

Table 4. Mortality of *R. linearis* on the treatment of formulated-botanical insecticide volume ratio 3:1 with UV irradiation

Duration of irradiation (minutes)	The total number of *R. linearis* mortality	Percentage (%)
0	56	74.7
60	59	78.7
120	72	96.0
180	65	86.7

The result showed that NLE exhibited the moderate values Antifeedant Index of 36.17 and larvae mortality by 10% on the fifth day after treatment. Meanwhile, the prospects and utilization of neem leaves extract as botanical insecticides for controlling major cocoa insect pests in Nigeria by taking into cognizance the formulation, dosage, and mode of application has been also extensively reviewed by Asogwa, Ndubuaku, Ugwu, & Awe (2010).

The above several research results indicate that the efficacy of neem leaves extract is never higher than formulated-botanical insecticide used in this study. Perhaps, curcumin contributed to the efficacy enhancing. We assumed that ferulic acid as a degradation product of curcumin increased the insecticidal activity of the neem leaves extracts synergistically. This assumption is based on a report of Huang et al. (2013), which found that ferulic acid derivatives possessed potential insecticide activity against *Aphis fabae Scopoli, Tetranychus cinnabarinus,* dan *Culex pipiens pallens*. It is also reported that the substituted ferulic acid amide derivatives 7 and the corresponding hydrogenated ferulic acid amide derivatives 13 possess excellent levels of antiviral activity, together with good levels of insecticidal activity. Furthermore, it is explained that these compounds displayed good insecticidal activities against insects with piercing-sucking mouthparts, which spread plant viruses both are between and within crops. According to Huang et al. (2013), ferulic acid A is a natural phenolic compound that can be isolated from many staple foods, including fruits, vegetables, cereals, and coffee. This compound and its derivatives exhibit a wide range of therapeutic effects (Gohil, Kshirsagar, & Sahane, 2012) with applications including anticancer (Kim et al., 2011), antidiabetic (Balasubashini, Rukkumani, Viswanathan, & Menon, 2004), cardioprotective and neuro-protective (Cheng, Yang, Yang, Chen, & Lin, 2011), as well as anti-inflammatory activities (Ou, Kong, Zhang, & Niwa, 2003). In addition, Patzke & Schieber (2018) used and screened five phenolic compounds as active ingredients of bioactive emulsion to inhibit the growth of four phytopathogenic fungi i.e *Aspergillus niger*, *Botrytis cinerea*, *Penicillium expansum,* and *Fusarium culmorum.* The result showed that ferulic acid was identified as posses highly effective against the growth of *Botrytis cinerea*. The growth-inhibitory effect of this emulsion was enhanced by adapting the ferulic acid 0.085% (m/v) concentration with the help of response surface methodology.

Unfortunately, the study on ferulic acid as anti insecticide has not been done much. Also, the study of the mixture containing neem leaves extract and curcumin as botanical insecticide constitutes the first time has been done. Overall, this study described an excellent synergy between two active ingredients of plants in order to improve botanical insecticide performance.

CONCLUSION AND SUGGESTION

This research has demonstrated that curcumin has no effect in increasing the stability of neem leaves extract solution towards UV light irradiation. However, it can increase the insecticide activity of neem leaves extract solution up to 96% mortality against soybean pod sucking bugs R. linearis.

A scientific evaluation to the formula of botanical insecticides should be conducted intensively and sustainable in order to obtain qualified and high competitive natural products. Surely, those would be enthused by farmers.

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