JOURNAL OF AGRICULTURE AND APPLIED BIOLOGY
2023, Vol. 4, No. 2, 174 - 181
http://dx.doi.org/10.11594/jaab.04.02.07
E-ISSN: 2723-5106

Research Article

The efficacy of *Acorus calamus* L. crude extract formulated in bentonite nanoparticles against *Crocidolomia pavonana* (Lepidoptera:Pyralidae)

Purwatiningsih[1]*, Dwi Indarti[2]

[1]Biology Department, Faculty of Mathematic and Natural Sciences, Universitas Jember, Indonesia
[2]Chemistry Department, Faculty of Mathematic and Natural Sciences, Universitas Jember, Indonesia

Article history:

Submitted 17 September 2023
Accepted 29 November 2023
Published 6 December 2023

Keywords:

Antifeedant
β-asarone
Na-bentonite
Phytotoxic effect
Vulnerability to UV

***Corresponding author:**

E-mail:
purwatiningsih.fmipa@unej.ac.id

Abstract

The prospect of *A. calamus* extract to be botanical insecticides to control the insect pests is very promising. The insect control properties of the *A. calamus* are predominantly to have β-asarone, saponin and flavonoid. However, there are some factors limiting their efficacy. Short release rate at the point contact, the inherent volatility and vulnerability to oxidation and ultra-violet light are causing phytochemical changes during the application. Thus developing nanotechnology to increase their efficacy studied in this research. To develop the plant extract in nanoformulation, experiment on adsorption and desoption capacity were carried out with *Acorus* extract treated bentonite substrate analysed by GC techniques. In addition, to investigate the effectiveness of the plant extract treated bentonite were evaluated on *Crocidolomia pavonana*. Third instar larvae were used in the evaluation of antifeedant effect by using no choice methods. The results showed that Bentonite nanoparticles was effective in controlling the release of a bioactive element. Purifed clay bentonite for getting Na-bentonite increased the specific surface area of material therefore increased the adsorption site on the clay layer of the material. Crude extract of *A.calamus* can dissolve well in combination of water and organic solvents. Desorption experiments proved that Na-bentonite released more slowly than raw clay bentonite. Purification of bentonite to Na-bentonite resulted the changing of phytochemical properties bentonite which lead to an increase in the adsorption capacity of bentonite. In term of antifeedant evaluation based on damage to cabbage leaves seedling, the least damage was observed on leaves with nanoparticle plant extract formulation. The antifeedant index of *A. calamus* in nanoparticles formulation showed 25% higher that untreated cabbage plant seedling. The glasshouse trial was conducted to evaluate their efficacy on cabbage seedling. However, the brown leaves or phytotoxic effect has been found by the time the extracts nano formulation has been sprayed.

How to cite:
Purwatiningsih & Indarti, D. (2023). The efficacy of *Acorus calamus* L. crude extract formulated in bentonite nanoparticles against *Crocidolomia pavonana* (Lepidoptera:Pyralidae). *Journal of Agriculture and Applied Biology, 4*(2): 174 - 181. doi: 10.11594/jaab.04.02.07

1. Introduction

C. pavonana is a major world pest of cruciferous crops. This insects causes damage to plants 10 weeks after planting (Sembiring & Prasetia, 2019). *C. pavonana* larvae usually attack young plant parts or crops so that they can cause death to cabbage plants (Kumarawati et al., 2013; Yuliadhi et al., 2021). At present to counter this destructive insect pest, synthetic insecticides has been applied. Unfortunately, this insect has developed resistance to synthetic insecticide. Additionally, the synthetic insecticide showed unfriendly environment. Thus, the high cost-benefit ratio of synthetic insecticides pushed research towards investigating alternative insecticides. The prospect of using botanical insecticides to control the insect pests is very promising. One of the plant showed the potential insecticidal properties is *A. calamus* L. (Purwatiningsih et al., 2023; Purwatiningsih et al., 2012). However, there are some factors limiting their efficacy. Short release rate at the point contact, the inherent volatility and vulnerability to oxidation and ultra-violet light are causing phytochemical changes during the application.

The use of clay nanoparticles (bentonite) is a novel technology with their potency to maximize the efficacy of phytochemicals. Nanobiotechnology has been applied in pharmacist and biomedical application. It gives more benefit for expanding the bioavailability and for extending the therapeutic effect of drug (Murugan et al., 2018). The nanoparticles has small size and high surface area. In addition, this nanoparticle can act as delivery vehicle of phytochemicals. This characteristic can be applied as a new generation of botanical insecticides. Thus represents a revolution in integrated pest management (Murugan et al., 2018). Therefore, it would break up their limiting factors of botanical insecticides, especially when they are applied in the field. It can control the release rate at the point of contact of botanical insecticide and their inherent volatility and susceptibility to oxidation and ultra violet radiation (Pavela et al., 2019; Pavoni et al., 2019).

This current study addresses the above factor by using clay nanoparticle to control the adsorption and desorption of the *A. calamus* extract. Additionally, investigating the phytochemical efficacy in clay nanoparticles on *C. pavonana* has been done by investigating their antifeedant effect. The mechanism of action of antifeedant plant extract treated with nanoformulation such as bentonite was determined. Acceptance or rejection of potential food plants by insects is controlled by the behaviour responses of the central nervous system to various excitatory and inhibitory sensory input. Insect can identify the chemical substance in plants which can influence the decision to feed through the responses of the insect gustatory and olfactory sensila. Thus the receptors are likely to be important in this process. This would be useful in developing new insecticidal formulation based on plant origin.

2. Materials and methods

2.1. To investigate and characterize the insecticidal properties of A. calamus collecting from Java island against C. pavonana.

A.calamus has been collected from East Java in the Slawu district, Jember. Extraction process to get the extract has been done by using maceration techniques. 250 grams of rhizome powder of *A. calamus* has been extracted with 95% ethanol for 12 hours. The obtained extract, then concentrated using rotary vacuum evaporator (BUCHI, Japan) at 40-60°C. The concentrated semisolid extracts will be stored in refrigerator at 2-8°C untill further use (Purwatiningsih, 2013). Antifeedant effect from the extracts have resulted the perception of deterrent by insect gustatory receptor in the mouthpart and defensive behavior to respond the toxic substances (Schmutterer, 1990). Therefore the extracts has been characterized the insecticidal properties by its feeding behavior.

2.2. To investigate the formulation of plant extracts encapsulated in clay nanoparticles

Clay nanoparticles that we used for this project is bentonite. It composed of montmorillonite primarily which are 1nm in thickness and 0.2-2 microns in diameter. The montmorillonite has two tetrahedral layers of silicate around a central octahedral layer of aluminium oxide.This particle has ability to absorb natural organic compounds including phytochemicals as the hydration make the platelets of montmorillonite separate into a porous structure which has positive and negative charges. Adsoption of phytochemicals on clay nanoparticles can reduce photodegradation and volatilization. Therefore this character contributes to inhibit phytochemicals changes while applied in the field with UV sunlight. Thus this is very promising to be a good carrier of botanical insecticides. We has been prepared the plant extracts in clay nanoparticles and compare with the plant extracts only for their adsorption ability and desorption rate (Degefu, 2012).

2.3. Preparation of plant extracts in clay nanoparticles

The 50g of clay minerals (bentonite) has been added into $(NaPO_3)_6$ and the suspension was shaken for 2 hours and kept at room temperature overnight for further aggradation. NaCl (1M) solution was used to wash the bentonite solution 3 times and then was rinsed by deionised water for getting suspension with chloride ions free. The Na-bentonite sample was centrifuged and then the supernatant was dried at room temperature (Degefu, 2012).

The required amount of *A. calamus* extract was weighed and mixed in solvent of 30% ethanol and 50 gram Na-bentonite. The plant extract emulsion in clay nanoparticles resulted.

2.4. Adsorption isotherm

To conduct the adsorption experiments, it has been done in a water bath. Plant extract solution obtained, has been put in the centrifuge tube and shake at 200 rpm for at least 2 days until reach equilibrium and then will be centrifuged for separation. The supernatant was determined by GC. The Langmuir equation has been used to describe the equilibrium data and then produced the Langmuir isotherm. The adsorption obtained data was fitted to the Langmuir equation using non linear least square regression by means of the Microsof Excell program (Degefu, 2012). The same method was applied for plant extracts diluted in ethanol or plant extracts without bentonite.

2.5. Desorption isotherm

The experiments was conducted on a Gravimetric Rig. which based on Hook-Law. The weight of adsorbent, it was calculated from the linear calibration curve. Determination of plant extract concentration will be done by using GC as well to compare the results. Thus desorption isotherm curves will be obtained (Degefu, 2012). The same method was applied for plant extract diluted in ethanol or plant extracts without bentonite.

2.6. *To test the plant extracts of A. calamus. in clay nanoparticles for their efficacy against C. pavonana in the laboratory and under glasshouse conditions.*

Laboratory tests

Antifeedant effect was determined by using 1cm in diameter of cabbage leaf-discs. Modified leaf no choice procedures was applied as described by (Jiang et al., 2020). Third instar of *C. pavonana* was used in tests over 24 hours. The required amount of plant extracts diluted in ethanol used within the range of effective concentration of development of third instar larvae. Dipping assay methods was used in this study to get the good coverage of leaf by oil. In 9cm in diameter petridish line with moist filter paper, the assay was applied. The larvae was allowed to feed for 24 hours and the image area of damaged leaf discs was measured with digital imaging system, ImageJ software. The Antifeedant index 50 (AI_{50}) were determined and compared among untreated leaves, leaf disc with *A. calamus* extract and leaf disc with *A.calamus* formulation . Percentage of antifeedant index was calculated according to the formula form of the antifeedant index (AI_{50}). AI_{50} for the no-choice method (C-T)/C *100%, where C and T (mm^2) denoted the consumed area of control and treated discs respectively. AI_{50} has been determined in this experiment is 2.10%.

Glasshouse tests

The application of plant extracts both of *A. calamus*. in clay nanoparticles has been applied under glasshouse conditions to evaluate their efficacy. Cabbage plant treated in several concentrations of plant extract emulsions has been laid out in a randomised block design (RCBD) of 4 blocks in which each block is a replicate. The randomisation has been done in applied concentration with 10 plants in each treatment in each of block (n=40). Each plant were be infested with 10 third instar *C. pavonana* pre starved for four hours prior. 50 ml hand held sprayer with oil suspensions will be sprayed on each treated plants with the required amount of concentrations. The rate concentrations used in this project are 2.1 %. A post treatment count were be made every 24 hours for the larval mortality and abnormality.

3. Results and discussion

3.1. Characterising and investigating of A.calamus extract collected from Slawu Distric Jember, East Java

The essential oil obtained from the *A. calamus* extract was characterized by β-asarone. This has been determined from the TIC (Total ion Chromatography) analysis. As depicted in the Figure 1. β-asarone showed highest peak both in the polar (methanol) and non polar (hexane) organic solvent. Early studies by (Hossain et al., 2008) which were based on chemical studies of *Acorus* species, indicated that the rhizome contained eugenol ; methyleugenol; asarone (2,4,5-tri-methoxy-1-propenylbenzene), monoterpene hydrocarbons, sequestrine ketones and saponin. Other studies found that the major components of essential oils derived from *A. calamus* rhizomes are phenylpropenes, monoterpenes and termolabile sesquiterpenoids with asarone as the predominant compounds. It was found that asarone has two geometrical isomers, that is α-asarone (trans-1,3,4-trimethoxy-5(1-propenyl) benzene) and β-asarone (cis- 1,3,4- trimethoxi-5(1-propenyl) benzene). The most effective compound in *A. calamus* oil is β-asarone (cis-2,4,5-tri-methoxy-1- propenylbenzene) (Hossain et al., 2008).

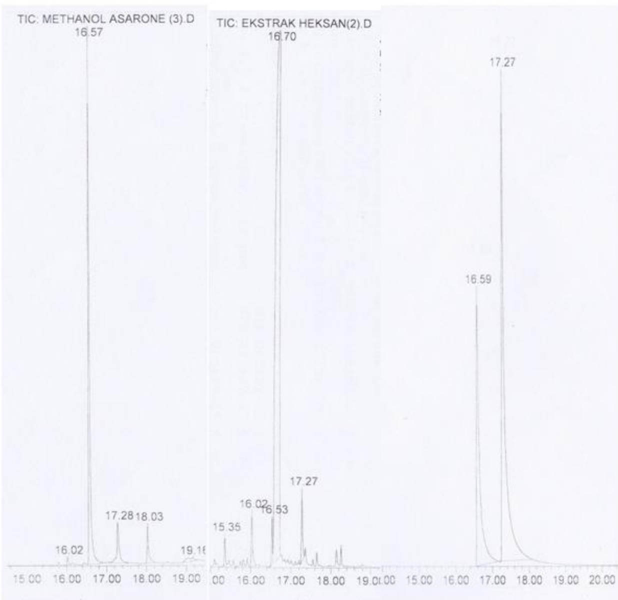

Figure 1. Total Ion Chromatography of A. calamus extract

The bentonite formulation size was found less than 1 μm (Figure 2). This size was resulted by Scanning Electron Microscope (SEM) which showing spherical shape with particle size less than 1 μm of diameter. Based on the data, β-asarone has been chosen as a standard parameter to do adsorption and desorption capacity experiments.

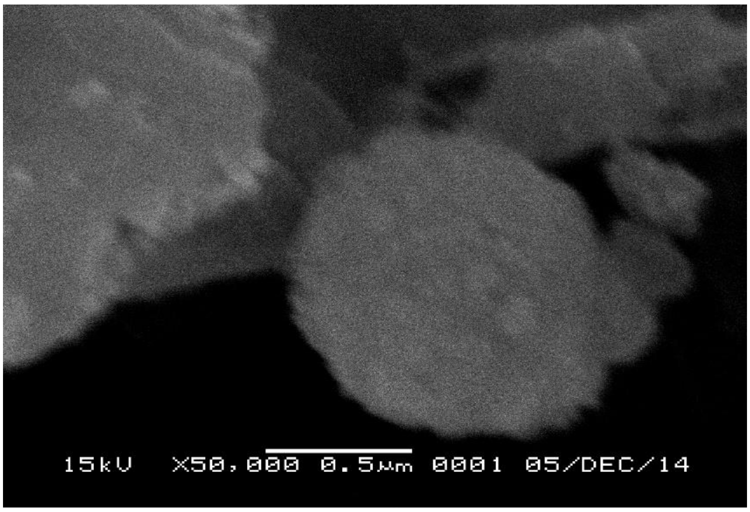

Figure 2. the SEM micrograph plant extract encapsulated with Clay Bentonite nanoparticles

3.2. Adsortion capacity of A.calamus extract in bentonite and Na Bentonite

It has been shown from the Table 1. The comparison of of mean adsorption isotherm capacities of *A. calamus* in bentonite and Na bentonite clay analysed by GC. It has been showed that Na bentonite had lower adsorption capacity than *A.calamus* in raw bentonite. At the maximum of mass of bentonite that has been reached at 3.0 grams, the adsoption capacity of raw bentonite is 17.703 % mg/g while the adsorption capacity of Na bentonite is 14.038 % (mg/g).

Table 1. The mean adsorption isotherm capacities of A. calamus in bentonite and Na bentonite clay analysed by GC

Adsorben	Adsorben mass (g)	Adsorption mass (mg)	Adsorption capacity (%) (mg/g)
Na-Bentonite	0.511	19.8	38.691
	0.998	50.1	50.164
	1.512	61.5	40.671
	2.021	62.0	30.655
	2.507	43.7	17.437
	3.009	42.2	14.038
Bentonite	3.009	53.3	17.703

Na Bentonite has a high dry bonding strength and significant swelling characteristics. The Osmotic pressure, created by sodium ion (Na+), draws water molecules into the between layers. Bentonite made of calcium (Ca) and magnesium (Mg) has a considerably lower potential to expand and show improved adsorptive qualities. When these platelets get hydrated, they segregate into a structure that is permeable and holds both positive and negative charges. It is well known that bentonite's porous nature and ionic charge allow it to absorb chemicals. Bentonite, or smectites, can absorb several naturally occurring organic substances, such as which have pesticidal properties (Masini & Abate, 2021)

3.3. Desorption capacity of A.calamus extract in bentonite and Na Bentonite

The comparison of of mean desorption isotherm capacities of *A. calamus* in bentonite and Na bentonite clay analysed by GC can be shown at Table 2. Both Na bentonite and bentonite of *A. calamus* extract showed the longest desorption capacities. The desorption capacity of *A. calamus* extract itself was only about 120 minute. It has been showed that Na bentonite had lower desorption capacity than *A.calamus* in raw bentonite. After 1560 minutes the desortion capacity of bentonite is 0.15% while the desorption capacity of Na bentonite is 2.07 %.

Table 2. The mean desorption isotherm capacities of A. calamus in bentonite and Na bentonite clay analysed by GC

t desorpsi (min)	Adsorben Mass Na-Bentonite	% desorption	Adsorben Mass Bentonite	% desorption
0	62.00	0.00	42.2	0.00
20	18.42	29.71	1.36	3.11
60	14.79	23.31	1.27	3.01
180	6.36	10.55	0.89	1.97
360	6.08	10.25	0.64	1.52
1560	2.07	3.44	0.05	0.15

A.calamus extract was released by Bentonite more slowly than by other Na bentonite adsorbents. The physiochemical characteristics of bentonite, such as its chemical composition, selectivity, porosity, cation exchange capacity, and surface area, increased the material's ability to adsorb substances (Masini & Abate, 2021)

3.4. Laboratory assay of test the plant extracts of A. calamus. in clay nanoparticles for their efficacy against C. pavonana in the laboratory and under glasshouse conditions.

The mechanism of action of *A.calamus* extracts used as antifeedants in nanoformulations, bentonite, has been identified. At first the larvae were repelled when exposed to the treated leaf substrate and moved away from the feeding arena or onto the lid of the container. After 4 hours, some of the larvae approached the feeding arena, and settled for about 30 minutes. The higher the concentration of the extract, the longer the larvae took to settle and start to feed. The assay showed antifeedant effect of *A.calamus* extract against *C.pavonana.* The antifeedant index values *A.calamus* extract +bentonite showed lower value *A.calamus* extract. This mean that *A.calamus* extract +bentonite had the more effective of antifeedant effect than *A.calamus* extract without bentonite. (Table 3). Insects' behavioural responses to stimulatory and inhibitory sensory inputs in the central nervous system determine whether they accept or reject a potential food plant. The inhibition of receptors that typically respond to phagostimulants is the cause of the antifeedant activity of bioactive compounds from plant such as citral (lemon myrtle), azadiractin (neem seed extract), tea tree oil (Schmutterer, 1990). From this point of view, it may be that β-asarone, the active constituent which is predominant in *A. calamus* responsible for the antifeedant activity.

Table 3. The comparison of antifeedant index50 of A.calamus extract and A.calamus extract+bentonite, n:4

Treatment	Antifeedant index
Untreated	00.00 ± 0.00^{a}
A. *Calamus* extract	36.15 ± 9.48^{c}
A. Calamus extract + Bentonite	25.01 ± 1.26^{b}

Values followed by the same letter(s) are not significantly different, LSD,α=0.05

During the trials, differences in the leaf disc feeding activity results were noted; these variations were likely caused by the chemical makeup of the bioactive substances that were adsorbed onto the bentonite substrates after treatment. The larvae given cabbage leaf discs treated with *A. calamus* extract survived for several days since their neural membranes and synaptic enzymes were unaffected. α-asarone, methyl eugenol is known to have potential fumigant for *Liposcelus bostrychophila* Badonnel (Liu et al., 2013), feeding deterrents for *Spodoptera litura* (Kumrungsee, 2023). The results in this study are similar to those of (Koul, 1987) that noted plant oils of *A. calamus* caused growth and feeding inhibition in *S. litura*. This was found to be due to the component β-asarone which acts as an antifeedant and growth inhibitor, The β-asarone was also found to be the main compound of *A. calamus* tested in this current study. This prospective result has been supported by the glasshouse experiments. However, the phytotoxic effect has been found by the time the extracts has been sprayed with the extracts formulation. It has been recorded that the pH level is 5. This contributed to the phytotoxicity. Thus specific nanotech materials and methodologies will be investigated to create a synergistic effect with biopesticides as screening for insecticidal activity is a very complex process. More investigation is needed on the PH level of *A.calamus* extracts without affecting the efficacy of nanoformulation

4. Conclusion

The bentonite nanoparticles with plants extract showed substantially 10 % higher of antifeedant effects against *C. pavonana*. This clay formulation increased the specific surface area of material therefore increased the adsorption site on the clay layer of the material. However the pH level indicated that the formulation had around pH 5. This need more investigation and research to solve the acidity of formulation by adding some material to reduce the acidity. Considering these findings, the work's contribution to knowledge is the enhancement of plant extract agents' longevity and efficacy in insect control. Therefore, it is anticipated that a complex composed of nanostructured phytochemical formulation will postpone pesticide resistance, shield the bioactive components from UV light, and improve the effectiveness of plant extracts. The phytochemical nanoformulations' ability to effectively increase feeding detteren in agriculturally significant pests, such as *C. pavonana*, suggests that they have a great deal of potential to aid in large-scale pest management.

Acknowledgement

The author would thanks to ITSF (Indonesian Toray Science Foundation 2015) who give support funding for this projects.

Author's declaration and contribution

The authors state that they have no conflicts of interest. All authors participated equally to this works at all procedures and approved the final version of the work

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