The Using Of Fenolic Compounds Of *Pluchea indica* (L.) Less. Leaves Extracts As A Bioinsecticide And Bioherbicide

**Yuliani**, Y S **Rahayu**

* * Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Indonesia

Corresponding author’s: yuliani@unesa.ac.id

**Abstract.** *Pluchea indica* (L.) Less. produces secondary metabolites such as lignans, terpenes, phenylpropanoids, benzoids, tannins, flavonoids, and hydroquinone phenols, used to protect plants against various herbivores, and pathogenic microorganisms, and are used as bioherbicides for weed control. The purpose of this study is to describe the effect of phenolic compounds of leaf extract of *Pluchea indica* (L.) Less. to the mortality of *Spodoptera litura* (LC 50 and LC 80) and its effect for seed germination inhibitor of *Amaranthus spinosus*. The research design was Randomized Completely Design (RCD) with one factor i.e. concentration. Test of methanol extract bioactivity on instar two *S. litura* using five levels of concentrations 0%, 6%, 8%, 10% and 12%, whereas in plants *Amaranthus spinous* use lower concentrations 0%, 0.125%, 0.50% and 1%. The parameters measured the mortality of *S. litura* and the seed germination of *A. spinosus* which included percentage of germination and germination rate. 

The results show that the biopesticide developed from plants *Pluchea indica* can result in mortality 81.90% of *S. litura* at a concentration of 12%, with LC 50 of 4.00 ± 0.60% and LC 80 of 9.88 ± 0.61%. As for the seed germination of plants, the higher concentration of *P. indica* leaf extract resulted higher inhibition on seed germination of *Amaranthus spinosus*

**Keywords** : Fenolic compound, leaves extracts, *Pluchea indica*, Bioherbicide, Bioinsecticide.

1. **INTRODUCTION**

Pests and weeds that are around cultivated plants, look very harmful and become a problem because it can inhibit the growth of cultivated plants and decrease end products. All this time, pests and weeds control used synthetic pesticides and its use increases because in some way is more effective and efficient (1). Nevertheless, using of insecticides or herbicides cause side effects, because the residue or residual active matter are toxic in the soil. Pesticides effect to environmental pollution, and also affect non-target organisms ie plant or animal that are sensitive to inhibitors. Other negative effects are pest resistance, pest resurgence, and secondary pest explosions (2).

Based on this problem, pest and weed control strategies are diverted to search alternative herbicides and natural insecticides that can control weeds and pests but do not antagonistic to environment or non-target organisms. This biopesticide based on the discovery of various chemical compounds produced by a plant that can inhibit the germination of plants and control pests (3).

Biopesticides are secondary metabolite compounds produced by plants such as phenolic compounds, alkaloids, terpenoids, and sulfur compounds. This secondary metabolite is a plant defense against pest attack (4), because it has a mechanism that can inhibit insect metabolism (5). The effects of secondary metabolite compounds which is insecticide is the occurrence of death at an early age, and the rate of growth decreases. The use of bioactive compounds derived from plants is more developed because it is safe and environmentally friendly, besides it is one of the traditional pest control methods that have long been known by Indonesia people as life experience, indigenous knowledge and local wisdom.

Control of weeds known as the phenomenon of Alelopati (6). The phenomenon of allelopathy includes all types of chemical interactions between plants, between microorganisms, or between plants and microorganisms. According to Rice (7) and Mandava (8) the interaction involves inhibition and stimulate directly or indirectly a chemical compound formed by an organism to the growth and development of other...
organisms. In agriculture, the allelopathic mechanism for weed control is applied through the use of allelopathic non-production to production weeds plant as cover crop, or mulch, or as a second plant in the plant rotation system. Beside of that, by isolating alelochemistry to be used as an active ingredient of natural pesticides (9,10).

Pluchea indica (L.) Less. is a compositae plant, grown in sub tropical climate and spread in Indonesia, is known to contain allelopathic compounds/secondary metabolite released into the environment, both in the form of vaporized compounds from leaves and in the form of decomposition compounds in the soil (11). P. indica is known to contain secondary metabolite compounds such as alkaloids, flavonoids, hydroquinone phenols, tannins, and essential oils that can be used as herbicides or insecticides. Other Pluchea genus content is terpen, benzenoid, phenylpropanoid, lignin and steroid (12,13). P.indica is also known to have antioxidant activity, besides it contains tannins, alkaloids, flavonoids, hydroquinones (14). Inderjit and Dakshini (15) suggest that phenolic compounds from Pluchea causes affect seed germination and growth of various plant species

Research about the use of leaves of Pluchea as bioherbisia shows the presence of phenol compounds namely coumarin, benzoic acid, salicylic acid and vanilic acid. Bioherbicides Pluchea are treated on weed seeds Mimosa pudica and Ruellia tuberosa. shows obstacle of germination and growth (16). Biswas et al. (17) isolated pure compounds from Pluchea root extract and identified as having an antimicrobial compound. The extract of methanol leaves of Pluchea indica had total phenol content (equivalent of gallic acid) of 304.42 mg / 100 dry weight and total flavonoid 116.38 mg / 100 g dry weight (18). Yuliani et.al (19) found the content of phenolic compounds and flavonoids from Pluchea indica plants that obtained from three high altitude regions (low, medium and high plains). The total phenolic content of Pluchea indica lowland higher (1.763 ± 0.047 mg / m L) is compared with Pluchea indica which grows in the middle-highland (1.455 ± 0.295 mg / mL) and highland (1.212 ± 0.608 mg / mL). The total flavonoid contents in Pluchea indica showed no mean difference content of flavonoids in the highlands (3.2 mg / mL), middle-highland (3.0 mg / mL) and lowland (3.1 mg / mL). It shows that secondary metabolite content is influenced by environmental factors. It is further said that the influence of environmental factors interacts with genetic factors in secondary metabolite expression, so that the production and excretion of secondary metabolite compounds is affected by temperature, light, soil conditions, microorganisms and nutrient status (20, 21)

From the above background, a study aimed to describe the effect of phenol compounds from Pluchea indica leaf extract (obtained from lowland) to Spodoptera litura mortality and its impact on seed germination of Amaranthus spinosus which includes percentage of germination and seed germination rate. This biopesticide research is different from another research because the biopesticide developed in animal and plants testing. The use of biopesticides in agriculture is expected to maintain natural resources and agricultural productivity in long-term, maintain minimal environmental impact, optimum plant production with minimal chemical input, provide economical benefits which is commensurate to the farmers.

2. MATERIAL AND METHOD

The subjects of this study were Pluchea indica plant obtained from lowland, Amaranthus spinosus as tested weed plant and instar larvae of two Spodoptera litura as tested animals. For bioactivity test with S. litura or A.spinensis plants used Randomized Completely Design (RCD) with one factor that is concentration. The concentration used for A.spinosis plant test consists of 5 levels ie 1%, 0.50%, 0.25%, 0.125% and 0% (with aquadest solvent), while for the test of animal of S. litura using the concentration of 12% 10%, 8%, 6% and 0% (with DMSO solvent). Determination of the concentration based on preliminary test that shows no seed germination of plants when the concentration is equalized, and otherwise. So the concentration for the plant is made different from the animal. The parameters measured were mortality of S. lituras and seed germination of A. spinosus which included germination percentage and germination rate.

2.1. Making Extraction

Extraction procedure (22,16,19) is a leaf flour Pluchea indica (dried leaf flour powder with size 40 mesh) in maceration with petroleum ether at room temperature for 24 hours, then the dried residue is extracted with methanol using soxhlet extraction at 65°C for 3 hours. The methanol solvent is evaporated with a rotary evaporator to obtain a dry methanol extract. The next step is to partition the extracts successively with n-butanol, and ethyl acetate to separate the compounds with each other. The obtained white crystals of P.indica are then tested to A. spinosus and S. litura.

2.2 Bioactivity test on larvae and seeds
The seeds of the test plant were sterilized with 1% sodium hypochlorite for 30 minutes then washed with aquades, ten A. spinosus seeds placed in petridish that have been coated filter paper, then given *P.indica* leaf extract (4 ml) to be tested with variation of concentration different. Incubation is done at room temperature. The observation of seed germination was calculated until day10. For the animal test, the treatments were as follows: leaves (as feed) were cut with circle shape, and weighed 0.1 gram and then placed on prepared container, on it was placed *S. litura* as many as 10 larvae. After that given the extract to be tested (0.2 ml), and observed every day up to 10 day. Analyze data to known the effectiveness of each biopesticide by ANOVA test followed by Tukey test multiple range test conducted with the SPSS program package for Windows release 16. For determination of LC 50 will be analyzed by using probit analysis.

3. RESULT AND DISCUSSION

3.1 The percentage of mortality larvae *S. litura*

The percentage of mortality was calculated after 24 h of treatment until 10 days after treatment, which results can be seen from table 1. In the concentration of 12% of *P.indica* extract was shown the best concentration in causing *Spodoptera litura* mortality.

Table 1. Average of percentage *S.litura* mortality with different concentration of *P.indica* extract treatment

| Concentration treatment (K) | % average of *Spodoptera litura* Mortality that given *P.indica* extract |
|-----------------------------|--------------------------------------------------------------------------|
| 7 day                       | 10 day                                                                   |
| K 0%                        | 16.67 ±8,16a                                                              |
| K 6%                        | 69.99 ±8,36 b                                                             |
| K 8%                        | 72.00 ±16,33 bc                                                           |
| K 10%                       | 72.00±15,05 c                                                             |
| K 12%                       | 81.90±8,36 c                                                             |

Description: The number followed by the same alphabet in the column is not significantly different at the 5% level.

The variance analysis test for the 7th day showed that were significant mean differences between concentration of *P.indica* extract (0%, 6%, 8%, 10% and 12%) on mortality of *S. litural* larvae with F arithmetic (50,259) > F table (2,493). Tukey test also showed that the concentration of *P.indica* extract 0% had significantly different mortality rate with mortality at extract concentration of 6%, 8%, 10% and 12%. Mortality with *P.indica* extract at 6% concentration was similar to mortality at 8% concentration. The concentration of *P.indica* extract 8% had the same mortality rate as mortality at 10% and 12% *P.indica* concentration, but mortality in *P.indica* extract concentration 10% and 12% was different with mortality at 6% concentration. The variance analysis test on day 10 also showed that there were significant differences between the concentration of *P.indica* extract (0%, 6%, 8%, 10% and 12%) on the mortality of *S. litural* larvae with F count (33,386) F table (2,493). The lowest *S. litural* mortality occurred at 0% extract concentration, when concentration of *P.indica* extract increased, larval mortality also increased.

Probit analysis on mortality of *Spodoptera litura* given *P.indica* plant extract is feasible to be used in determining the concentration of LC 50 and LC80 because the significance level of both methods is more than 0.05. Based on goodness of fit, chi square values obtained from pearson and deviance methods, respectively were 12.7159 and 13.45 with significance level of 0.312 and 0.265. The result of probit analysis is seen in table 2.

Table 2. Result of extract concentration LC 50 and LC 80

| LC50 extract concentration | Interval Confidency (LC 50) | LC80 extract concentration | Interval Confidency (LC 80) |
|----------------------------|-----------------------------|----------------------------|-----------------------------|
| 4.001 ± 0,607             | 2.718 - 5.135              | 9.886 ±0.611              | 8.747 - 11.179             |
Based on table 2, it can be explained that *P.indica* extract with a concentration of 4.001% is capable of killing *S.litura* larvae by 50% with a concentration interval between 2.718% up to 5.135%. Similarly, the concentration of 9.886% is able to kill the larvae of 80% with the concentration interval between 8.747% to 11.179%

3.2 Inhibition on seed *A.Spinosus* germination

Inhibition on seed *A.Spinosus* germination is shown in table 3. The results of variance analysis test showed significant mean differences between concentration of *P.indica* extract on seed germination percentage and seed germination rate. The results show that the greater the concentration given, the greater the inhibition of seed germination. Percentage of seed germination range from 73.33% to 6.67%. The lowest percentage of germination at K 1% was 6.67%. Seed germination index for *A.Spinosus* ranged from 2.41 to 0.167. The lowest IKP is at K 1% of 0.167

Table 3. Avarage of germination seed after given extract of *Pluchea indica* leaf

| Concentration(K) extract | Germination percentage | Germination rate (IKP) |
|--------------------------|------------------------|------------------------|
| K 0 %                    | 73.33 ± 15.28          | c                      |
| K 0.125 %                | 56.67 ± 15.28          | bc                     |
| K 0.25 %                 | 53.33 ± 25.17          | bc                     |
| K 0.5 %                  | 23.33 ± 11.55          | ab                     |
| K 1 %                    | 6.67 ± 11.55           | a                      |

Adduction of allelochemical compounds from *P.indica* with various concentrations on *A.spinosus* seed can inhibit seed germination. When the concentration given is high, then inhibit of seed germination is high. Phase of seed germination is a very critical phase in some plants, so it is very susceptible to the influence of environmental factors. One is secondary metabolite which is inhibiting (allelochemi). Altieri and Doll (23) argue that the common symptoms caused by the inhibitory effect on a plant species are the inhibition of germination and plant growth. In addition there are also symptoms of growth abnormalities and germination death, this is due to inhibiting substances can affect the growth of plants through several ways that inhibit cell division and opposition, inhibit the growth hormone induction, inhibit respiration and photosynthesis, reduce membrane permeability, affect the enzyme work, and inhibit synthesis of various essential compounds needed by plants (24; 25)

Similarly, in the larvae of *S. litura*, secondary metabolite compounds of Pluchea indicants such as flavonoids, hydroquinone phenols, tannins and sterols (18) can affect the physiology and growth of insects (26,27,28). As contact poison, the active compound enters the body of the insect through the body wall, skin surface, and nervous system located on the surface of the skin. Insecticides enter the body of the insect when the insect is in contact or walks on the surface of the plant containing insecticide (29). Contact poison has a fast killing power after the poison attached on body part of insect. Penetration of chemicals into the body of the insect through the epiciles causes the destruction of the wax substance in the cuticle layer, thus experiencing of water loss and causing death (30).

The active compound enters the tissue under the integument to the target area ie the neurosecretory cells, then enters the cardiac corpora through axonal transport. In corpora cardiaka, active compounds will inhibit the protoraks gland to excrete alpha ekdison (molting hormone) into hemolimfe, if the alpha ecdison secretion is disrupted then beta ecdison is disrupted resulting in inhibition of new cuticle formation, and the old cuticle will remain attached to the body of the insect causing insect body can not develop and grow into adult insects (31,32).

4. CONCLUSION

4.1. *Pluchea indica* leaf extract effect on larva mortality of *Spodoptera litura*. Concentration of 12% gives an optimal mortality effect of 81.90%

4.2. *Pluchea indica* leaf extract effect on seed germination (percentage and rate of germination) *Amaranthus spinosus*. The higher the concentration (1%), the germination inhibitionis greater.

4.3. *Pluchea indica* leaf extract can be developed as bioherbicide and insecticide.
5. ACKNOWLEDGMENT

The author would like to thank all the laboratory staff of Biology Laboratory, Biology Department, Mathematic and Natural Sciences Faculty, Universitas Negeri Surabaya, and Ministry of Research Technology and Higher Education.

References

[1] Zimdahi, R.L. 1993, Fundamentals of Weed Science. Academic Press. San Diego.
[2] Metcalf, R.L. 1986. The Ecology of Insecticides and The Chemical Control of Insects (Kogan M, editor) Ecological Theory an Integrated Pest management Practice.New York: J Wiley.
[3] Heisey, Rod M. 1996. Indentification of an Allelophobic Compound from Ailanthus altissima (Simaroubaceae) and characterization of its herbicidal activity. America Journal Botany, 83(2): 192-200.
[4] Lambers, H, F. Stuart Chapin, Thijs L. Pons. 1998. Plant Physiological Ecology. New York: Spinger-Verlag.
[5] Pedigo, L.P. 1989. Entomology and Pest Management New York : Macmillan Publishing Company
[6] Inderjit and Keating KI. 2003. Allelopathy: Principles, Procedures, and Promises for Biological Control. dalam: Sparks DL (ed) Adv. Agron Vol. 67 San Diego: Acad Pr : 141-231.
[7] Rice, E.L. 1994. Allelopathy. Academic Press Inc: London.
[8] Mandava, N.B. 1985. Chemistry and Biology of Allelopathic agent dalam A.C. Miller, R.W. and R.L. Donahue. 1990. Soil. An Introduction to Soil and Plant Growth. Prentice Hall International Edition .New Jersey.
[9] Rizvi, S.J.H. and V.Rizvi. 1984. Allelopathy : A New Strategy in Weed Control. The Frist Tropical weed Science Conference. 2: 393-400.
[10] Mazid, M, Khan TA, and Mohammad F. 2011. Role of Secondary Metabolites in Defense Mechanisms of Plants. Biology and Medicine 3 (2): 232-249
[11] Uchiyama, Taketo, T. Miyase, A. Uena and K. Usmanghani, 1989. Terpenic Glycosides from Pluchea Indica. Phytochemistry, 28 (12): 3369 – 3372.
[12] Luger P. 2000. The Crystal Structure of Hop-17(21) en Assat of P. indica Pteropoda Hems1 from Vietnam. Crystal Res Technology 35(3) :355-362.
[13] Traithip, A. 2005. Phytochemistry and antioxidant activity of P. indica Mahidol University
[14] Andarwulan, N. 2010. Flavonoid Content and Antioxidant Activity of Vegetables from Indonesia. Food Chemistry, 12:1231-1235.
[15] Inderjit and K.M.M. Dakshini,1994. Allelopathic Potential of the Phenolics from the roots of Pluchea lanceolata. Physiologi Plantarum, 92 : 571 – 576
[16] Yuliani, Yuni Sri rahayu, Evie R., Mitarlis.2009. Potensi senyawa Alelokemi Daun Pluchea indica (L.) Less sebagai penghambat perkecambahan biji gulma secara hayati. Berkala Penelitian Hayati (journal of biological Researches) No 3A (12).
[17] Biswas R, Dutta PK., Achari B, Bandypadhyay D, Mishra M, Pramanik KC, and Chatterjee TK. 2007. Isolation of pure compound R/J/3 from P. indica (L.) Less. and its antiamebic activities against Entamoeba histolytica. Phytotherapy Res (Jena) 14, 534-7.
[18] Widyawati, Paini Sri, C. Hanny Wijaya, Peni Suprapti Harjosworo dan Dondin Sajuthi. 2010. Pengaruh Ekstraksi dan Fraksinasi Daun Pluchea indica (L.) Less sebagai penghambat perkecambahan biji gulma secara hayati. Berkala Penelitian Hayati (journal of biological Researches) No 3A (12).
[19] Olofsdotter, M. 2001. Rice-a-Step Toward Use Allelopathy. Agron J. 93:3-8.
[20] Khan, Taqi Ahmed, Mohd Mazid and Firoz Mohammad. 2011. Status of Secondary Plant Product Under Abiotic Stress: an Overview. Journal of Stress Physiology & Biochemistry, 7 (2) : 75-98.
[21] Dorman, H.J.D., and Hiltunen R.2004. Fe(III) reductive and free radical-scavenging properties of summer savory (Satureja hortensis L.) extract and subfractions. Food Chemistry 88: 1887-1892
[22] Altieri, M.A and J.D. Doll. 1978. The Potential of Allelopathy as a Tool for Weed Management in Crop Fields. PANS 24 (4): 495-502.
[23] Einhellig, F.A, 1986. Mechanics and Modes of Action of Allelochemicals in Putnam and Tang (ed.) The Science of Allelopathy. John Wiley and Sons. New York.
[24] Seigler, D.S, 1996. Chemistry and Mechanisms of Allelopathic Interaction. Agronomy Journal. 88 : 876 - 885.
[26] Kardinan, A. 2000. *Pestisida Nabati Ramuan dan Aplikasi*. Jakarta: Penebar Swadaya.
[27] Kusnaedi. 2003. *Pengendalian Hama Tanpa Pestisida*. Jakarta: Penebar Swadaya.
[28] Bidlack, Wayne R, Stanley T. Omaye, Mark S. Meskin, and Debra K.W. Topham. 2000. Phytochemicals as Bioactive Agents. Lancaster, Pennsylvania USA: Tehnomic Publishing Company, Inc.
[29] Untung, K. 2006. *Pengantar Pengololaan Hama Terpadu (edisi kedua)*. Yogyakarta: Gadjah Mada University Press.
[30] Cottrell, H.J. 1987. *Pesticides on Plant Surfaces*. New York: Society of Chemical Industry.
[31] Baskar, K.R. Maheswaran, S. Kingsley, and S. Ignacimuthu. 2010. Bioefficacy of Couroupita guianensis (Aubl) against Helicoverpa armigera (Hub. (Lepidoptera:Noctuidae) Larvae. *Spanish Journal of Agricultural Research* 8(1).135-141.
[32] Isnaeni, W. 2006. *Fisiologi Hewan*. Yogyakarta: Kanisius.
