

Original Research Article **Formulation of *Biduri* Leaf Extract** **(*Calotropis gigantea* L.) and Its Potential as** **An Environmentally Friendly Botanical** **Insecticide for Controlling Fall Armyworm** **(*Spodoptera frugiperda* J.E.Smith.) on Corn** **Plant**

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Comment [A1]: Efficacy of *Biduri* Leaf Extract (*Calotropis gigantea* L.) for Management of Fall Armyworm (*Spodoptera frugiperda* J.E.Smith.) on Corn

ABSTRACT

Aims: This research aims to assess the effectiveness of the *C. gigantea* extract formulation as an environmentally friendly botanical pesticide for controlling the armyworm *Spodoptera frugiperda* on corn plants.

Study design: Mention the design of the study here.

Place and Duration of Study: The research was carried out in the Plant Pest and Disease Laboratory at the Faculty of Agriculture, Tadulako University, and on corn plantations in Sigi Regency, from May 2023 to November 2023.

Methodology: Making formulated *C. gigantea* extract ~~is-was~~ done by adding emulsifier and adhesive to the extract. Material in the form of kaolin flour is used as a carrier. The formulated extract was evaluated. Application ~~is-was~~ carried out on 21 days old corn plants, with an application interval of 5 days for 5 applications. Observation variables included larval population density, percentage of *S. frugiperda* attacks, presence of natural enemies of the pest, and corn production. The toxicity test uses probit analysis and the significant difference test uses the T test.

Results: Toxicity of the formulated *C. gigantea* extract was LC_{50} of $0.522 \mu\text{g l}^{-1}$. Application of the formulated extract in the field showed that the formulated extract treatment had an effect on population density, the intensity of *S. frugiperda* larvae attacks and corn production had a significant effect and had no effect on the presence of natural enemies. Formulated *C. gigantea* leaf extract has the potential to be used as an environmentally friendly botanical insecticide compound against *S. frugiperda* larvae in corn plantations.

Conclusion: Non-invasive independent predictors for screening esophageal varices may decrease medical as well as financial burden, hence improving the management of cirrhotic patients. These predictors, however, need further work to validate reliability.

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Keywords: Attack intensity, *Calotropis gigantea*, formulation, population density, *Spodoptera frugiperda*

1. INTRODUCTION

Plants and insects experience reciprocal interactions primarily as a result of phytophagous insects consuming plant tissue. With the interaction of plants and herbivores, plants develop secondary compounds to protect themselves from attacks by pests and pathogens.

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Plants are a source of very diverse natural chemical compounds and bioactive compounds (Altaf et al., 2018). Plant chemical compounds are found and secreted from all above- and below-ground plant tissues, and they support each other in defense mechanisms against insects (Mundim et al., 2017).

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The large group of defensive chemical compounds known as secondary metabolites includes alkaloids, saponins, tannins, phenols and terpenes. Secondary metabolite compounds act as poisons and repellents or antinutrients against herbivores and as a promising source of biopesticides in pest management.

The use of secondary compounds in pest management is a very effective alternative. According to Campos et al. (2018) stated that secondary metabolite compounds have several mechanisms of action against target insects and have low toxicity against non-target organisms. Secondary compounds act as poisons, deterrents, repellents and reduce the nutritional value of food (Belete, 2018).

Toxic chemicals derived from secondary plant metabolites are used as botanical insecticides (Miresmailli & Isman, 2014). Several research results on the use of plant extracts have potential as botanical insecticides. Several plant families that are commercial botanical insecticides are Amaranthaceae, Asteraceae, Fabaceae, Meliaceae, Piperaceae, Salicaceae, Solanaceae (Velasques et al., 2017).

The *biduri* plant (*Calotropis gigantea* L.) belongs to the Asclepiadaceae family and contains main phytochemicals such as glycosides, alkaloids, flavonoids, tannins, kalotoxins, flavonoids, terpenes, and non-protein amino acids (Wang et al., 2008; Tripathi et al., 2013).

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Previous research results showed that *C. gigantea* root extract showed antifeedant activity against the nymphs of the desert locust *Schistocerca gregaria* (Wang et al., 2008). Apart from that, *C. gigantea* extract has repellent activity against *Tribolium castaneum* (Alam et al., 2009), mortality and larvicidal activity against *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* (Kovendan et al., 2012; Amelia et al., 2020), Adult *Culex quinquefasciatus* (Dhivya & Manimegalai, 2013), causes mortality against *T. castaneum* (Parvin et al., 2014; Habib & Karim, 2016), and *Paracoccus marginatus* (Sumathi et al., 2017), is larvicidal against *Helicoverpa armigera* (Prabu et al., 2012), *Schirpophagina notata* (Makmur et al., 2016), oviposition, deterrents and ovidical against *Paraeucosmetus pallicornis* (Sjam et al., 2017).

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From the results of this research, information was obtained that the *C. gigantea* plant has insecticidal capabilities. Thus, it is necessary to formulate the extract so that it can be produced commercially for use in the field so that it can reduce dependence on synthetic insecticides and, more importantly, can reduce the negative impacts caused by pest control activities.

This study aims to examine the effectiveness of the *C. gigantea* extract formulation as an environmentally friendly botanical pesticide to control the fall armyworm *Spodoptera frugiperda* on corn plants.

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2. MATERIAL AND METHODS

The research was conducted at the Plant Pest and Disease Laboratory at the Faculty of Agriculture, Tadulako University, and on corn plantations in Sigi Regency, from May 2023 to November 2023. The Research in the laboratory ~~is was~~ to obtain the LC₅₀ value from formulated *C. gigantea* leaf extract and field research is the application of formulated *C. gigantea* leaf extract to determine the ability of the extract to control *S. frugiperda*.

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2.1. Experimental Design

This research uses the T test which consists of treatment and control tests. Application of the extract in the field uses different plant tests as a treatment. The application was carried out on corn plants ~~aged of~~ 21 days after planting (DAP) to 49 DAP with an application interval of once every 7 days with an extract concentration of 5 ~~mg/~~ mg l⁻¹ and

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control. Observation variables include population density, intensity of armyworm attacks, natural enemies and corn production.

2.2. Preparation of Extraction Formulation

The *piduri* plant ~~is-was~~ cleaned and dried, then cut into small pieces using a blender until it becomes powder. Next, the plant powder is soaked in methanol, hexane, or diethyl ether. The soak is then filtered using a Buchner funnel and then evaporated using a rotary evaporator at low pressure to obtain a crude extract. After obtaining the crude extract, it ~~is was~~ formulated by adding carrier and emulsifier. The carrier materials used were zeolite flour, silica gel, and kaolin flour, while the emulsifier and adhesive were Triton x-114, Agristik, and Laytron 77L. Each carrier and emulsifier ~~is-was~~ added to the solution and then stirred until evenly distributed in a ratio of 1:10, 1:20 and 1:30, then the mixture is evaporated using a rotary evaporator until a perfect mixture of extract and carrier is obtained. Next, it ~~is-was~~ packaged in porous paper and each package contains 5 g in sachet form.

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2.3. Propagation of Test Insects

The test insects were obtained from farmers' plantations in Sidera village, Biromaru subdistrict, Sigi Regency, Central Sulawesi. A total of 20 larvae were collected from the field and reproduced in the Plant Pest Laboratory until they became imago. After becoming imago, they are released into a propagation box (100 ~~cm~~² x 60 ~~cm~~²) which has been given corn plants as a laying medium and fed with ten percent honey dripped on cotton which is hung above the propagation box. After the imago lay eggs and hatch, the larvae are harvested and placed in a plastic container (4 x 4 x 4 ~~cm~~³). One larva was placed in each container and fed with slices of young corn cob (baby corn) (figure 1). Every two days cleaning and changing food is carried out until the larvae become pupae. After becoming pupae, the imago is selected and released into the propagation box until uniform larvae (F2) are obtained as test insects.

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2.4. Preparation of Extraction Formulation

Making the extract formulation begins with preparing 20 kg of wet leaves of *Widuri C. gigantea* found in the Palu valley. Fresh *C. gigantea* leaves were cleaned and air-dried in the oven at 40°C for 3 days until dry. Next, the leaves are crushed using a blender until they become powder. The extraction used is the macerai method (Harborne, 1980). 350 g of *C. gigantea* leaf powder was macerated in a vessel with the addition of ethanol as solvent and stirred evenly and then stirred occasionally to avoid precipitation and to obtain maximum active ingredient extract. The marinade ~~is-was~~ closed and stored indoors to protect from direct light. Soaking is carried out for 48 hours, then filtered using a Buchner funnel lined with filter paper. After obtaining the filtrate, the solution is then separated from the solvent using a Heidolph brand vacuum rotary evaporator, WEIRTHEIM specifications, Cole-Parmer water bath type 7049-05 with a temperature of 40 - 180°C, rotating 160 - 280 rpm for three hours until 25 ml of extract is obtained, then added with 50 g of kaolin flour then rotated again until homogeneous, then air-dried for 2 hours and ground until it became flour.

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2.5. Biological Activity of Extract Formulation

The concentrations used in this study were 2, 1, 0.5, 0.25 ~~g/l~~^{l⁻¹} and control (distilled water). The third instar *Spodoptera S. frugiperda* larvae used were starved for 2 hours before the experiment. The feed used in the test was corn leaves formed into a rectangle measuring 3 x 3 ~~cm~~². In each treatment, 2 corn leaves were used which had been dipped in each concentration for 20 seconds, then air dried for 45 minutes. In each treatment, 10 larvae were used. Larvae were placed individually in petri dishes (diameter 9 cm, height 2 cm) which had been fed according to the treatment. Observations were made every 24, 48, and 72 hours after application.

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2.6. Extract Formulation Application

Application of the formulated extract using a concentration of 5 g and control. The application is carried out on corn plantings aged 3 WAP, with an application interval of once every 7 days for 5 applications. Observation variables included larval population density, percentage of *S. frugiperda* attacks, presence of natural enemies of the pest, and corn production.

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2.7. Data Analysis

The toxicity test used probit analysis (Finny, 1925) and the significant difference test used the T test.

3. RESULTS AND DISCUSSION

3.1. Toxicity of Extract Formulation Against *S. frugiperda*

The toxicity of *C. gigantea* leaf extract against *S. frugiperda* (Table 1) showed a probit analysis with an estimated LC_{50} of $0.522 \mu\text{g l}^{-1}$ with a confidence interval between $0.356 \mu\text{g l}^{-1}$ to $0.770 \mu\text{g l}^{-1}$. Based on probit analysis, it shows that increasing concentration is directly proportional to increasing mortality of *Spodoptera-S= frugiperda* larvae. In accordance with the research results of Nasir et al., (2022), increasing the treatment concentration of crude extract of *C. gigantea* leaves caused an increase in mortality of *Spodoptera-S=frugiperda* larvae.

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Table 1. Toxicity of *Galotropis-C. gigantea* extract formulations against third instar larvae of *Spodoptera-S.frugiperda* L.

Ekstrak	Volume	n	Slope (\pm SE)	LC_{50} (SK95%; $\mu\text{g l}^{-1}$)	df
<i>Galotropis-C. gigantea</i>	40	500	0.598 (\pm 0.116)	0,522 (0.356 - 0.770)	3

3.2. Density of *Spodoptera-S. frugiperda* Larvae in Corn Plantations

The average population of *S. frugiperda* larvae in each observation (Figure 1), shows that the formulated *C. gigantea* extract treatment had an effect on the population density of *S. frugiperda* larvae in corn plantings. The population density of *S. frugiperda* in the control was higher than in the treatment, namely ranging from 2.18 individuals to 2.89 individuals, while in the treatment it ranged from 1.4 individuals to 1.88 individuals. The low population density of *S. frugiperda* larvae shows that the formulated *C. gigantea* extract has the ability to control *S. frugiperda* larvae in the field.

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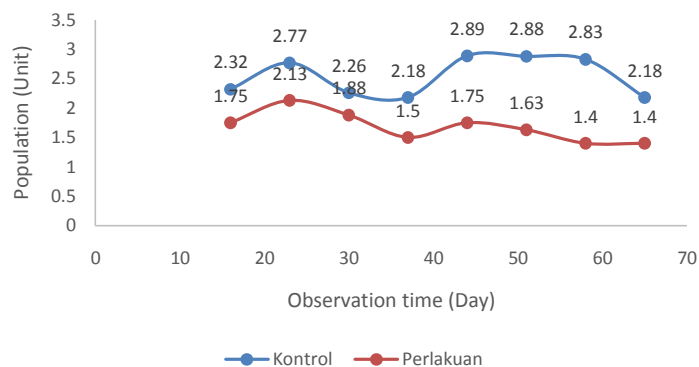


Figure 1. Density of *Spodoptera-S=frugiperda* larvae in corn plantations treated with *Calotropis-C. gigantea* leaf extract formulation

The population density of *S. frugiperda* larvae in corn plantations in the treatment is included in the low category, but it is very important to be aware of it because their presence is very dangerous because this species is able to survive well on corn plants in its new habitat, so it will threaten food plants, especially corn. *Spodoptera-S- frugiperda* is considered one of the most invasive and serious insect species that attacks corn plants.

3.3. Intensity of attacks by *Spodoptera-S. frugiperda* larvae

The intensity of *S. frugiperda* larvae attacks on plants treated with *C. gigantea* extract formulations. Each observation (Figure 1) shows that the attack intensity of *S. frugiperda* larvae ranged from 11.18% to 24.89%, while on controls it ranged from 21.18% up to 30.26%. Symptoms of attack by *S. frugiperda* larvae are found on the tops of corn plants, namely there is a coarse powder resembling sawdust which is brown in color. The larvae attack corn plants by making holes and eating leaf tissue from the edge to the inside, causing damage to the corn plants. According to CABI (2019), this pest attacks the growing point which can result in failure to form shoots or young leaves of the plant, apart from that it also attacks the cobs.

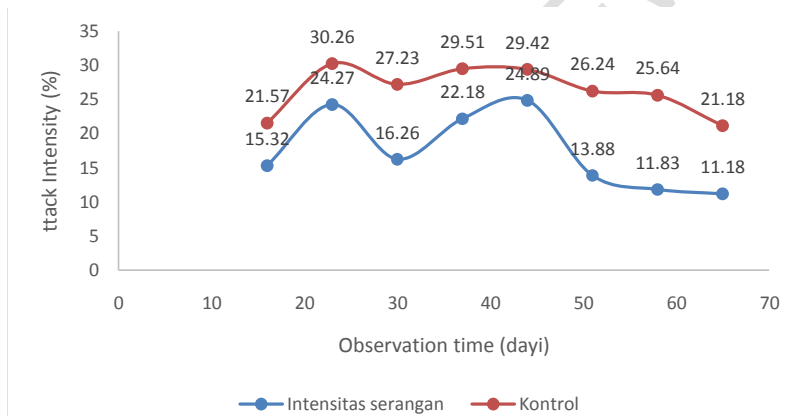


Figure 2. Intensity of attacks by *Spodoptera-S= frugiperda* larvae on corn plantations treated with *Calotropis gigantea* leaf extract formulation

Leaf damage due to attacks by *S. frugiperda* larvae causes damage to plants by eating the leaves. As a result, it can disrupt plant photosynthesis, damage plant growth and reproductive structures, or directly damage the cobs (Chimweta et al. 2019). There are symptoms of damage caused by *S. frugiperda*, in the early instars the larvae eat the leaves leaving some parts semi-transparent. Instars at advanced stages tend to enter and eat the leaf veins of corn, so that the typical symptoms of hollow leaves become more visible as the plant develops. According to Sisay et al. (2019), feeding preferences at plant growing points can inhibit plant growth and sometimes cause fatal damage. In older plants, late stage instars can damage corn cobs, thereby reducing the quality and yields obtained. The intensity of *S. frugiperda* larvae attacks in the treatment was lower than the control, indicating that the formulated *C. gigantea* extract was effective in controlling *S. frugiperda* larvae in corn plantations. The effectiveness of the formulated *C. gigantea* extract is greatly influenced by the compound content contained in the formulated extract. According to Sharma et al., (2016), all parts of the *C. gigantea* plant contain poison in the leaves, flowers,

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sap, stems and roots. *C. gigantea* leaf extract contains alkaloids, saponins, tannins, phenols, flavonoids and terpenoids (Khasanah et al., 2012 and Nasir et al., (2022). Alkaloids, saponins, glycosides, tannins, flavonoids, terpenoids are used for plant defense against herbivorous and has been reported to have insecticidal properties (Hikal et al., 2017). Toxic effects generally depend on dose, application time and individual characteristics such as site of action sensitivity and developmental stage (Matsuura and Fett-Neto, 2015).

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3.4. Natural Enemies

Based on the results of the identification of insects found in corn plantations, seven orders were obtained (Orthoptera, Coleoptera, Arachnida, Coleoptera, Hymenoptera, Homoptera, Diptera and Lepidoptera) consisting of sixteen families (gyllinae, copiphorinae, arcididae, gryllidae, Tetigonidae, geotrupinae, coccinellidae, lycocidae, salticidae, chrysomelidae, apidae, formicidae, delpacidae, tephritidae, tachinidae, and noctuidae). The arthropods found in corn plants treated with the *C. gigantea* extract formulation and the control were two families of tetigonidae, coccinellidae, lycosidae, Salticidae, Formicidae, and Tachinidae which act as predators.

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Table 2. Number of Orders, Families and Number of Arthropod Populations in Corn Plantings Treated with *Caloptropis-C. gigantea* L Extract Formulation.

No	OrdoOrder	Family	Number		Fungtions
			Control	Treatment	
1	Orthoptera	Gyllidae	8	27	Pest
		Copiphorinae	1	2	Pest
		Arcididae	7	8	Pest
		Gryllidae	2	2	Pest
		Tetigonidae	7	6	Predators
2	Coleoptera	Geotrupinae	5	6	Decomposer
		Coccinellidae	14	11	Predators
		Chrysomelidae	4	1	Pest
3	Arachnida	Lycosidae	15	12	Predator
		Salticidae	4	5	Predator
4	Hymenoptera	Apidae	33	25	Pollinator
		Formicidae	76	73	Predators
5	Homoptera	Delpacidae	33	26	Pest
6	Diptera	Tephritidae	1	2	Pest
		Lepidoptera	Tachinidae	6	6
7	Lepidoptera	Noctuidae	41	24	Pest
Emount	5	16	257	240	

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Based on Table 2, it shows that the treatment and control obtained the same natural enemies and acted as predators. This shows that the *C. gigantea* extract formulation treatment does not affect the presence of natural enemies that act as predators.

3.5. Corn Production

Corn plant production (Table 2) shows that the *C. gigantea* extract formulation treatment has a significant effect on corn plant yields. The high corn production in the treatment shows-showed that the formulated *C. gigantea* extract has the ability to control *S. frugiperda* on a field scale. This is in accordance with research by Nasir et al (2022), the crude extract of *C. gigantea* caused mortality of *S. frugiperda* in the field.

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Table 3. Effect of *Calotropis-C. gigantea* extract formulation on corn production

Treatment	Test					Total	Average	SD
	1	2	3	4	5			
Control	4.20	4.04	4.48	4.76	4.40	21.88	4.38 ^b	5,61
<i>Calotropis-C. gigantea</i>	6.48	6.68	6.36	7.12	7.52	34.16	6.83 ^a	6,83

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4. CONCLUSION

Formulated *C. gigantea* leaf extract is toxic to *S. frugiperda* and is effective in reducing population density, intensity of *S. frugiperda* attacks and increasing corn production and has no effect on the presence of arthropods which act as predators in corn plantings. Formulated leaf extract of *C. gigantea* has the potential as an environmentally friendly botanical insecticide in controlling the armyworm *S. frugiperda*.

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REFERENCES

1. Alam. M. A, M. R. Habib & F. Nikkon. 2009. Insecticidal Activity of Root Bark of *Calotropis gigantea* L. Against *Tribolium castaneum* (Herbst). *World Journal of Zoology*. 4 (2) : 90-95, 2009
2. Altaf, M.M., Ahmad Khan, M.S. and Ahmad, I. (2019) Diversity of Bioactive Compounds and Their Therapeutic Potential. *New Look to Phytomedicine*, Elsevier. p. 15–34. <https://doi.org/10.1016/B978-0-12-814619-4.00002-1>
3. Amelia KAR, Khaerunnisa, Haeruddin. 2020. Analisis Ekstrak Kulit Batang Tanaman Biduri Terhadap Kematian Jentik Nyamuk Aedes Aegypti. *Window of Health: Jurnal Kesehatan*, 3 (3):211-217
4. Begum. N., B. Sharma & R. S. Pandey. 2013. *Calotropis procera* and *Annona squamosa*: Potential Alternatives to Chemical Pesticides. *British Journal of Applied Science & Technology*. 3(2): 254-267
5. Belete, T. (2018) Defense Mechanisms of Plants to Insect Pests: From Morphological to Biochemical Approach. *Trends in Technical & Scientific Research*, 2, 1–9. <https://doi.org/10.19080/TTSR.2018.02.555584>
6. Campos, E.V.R., Proença, P.L.F., Oliveira, J.L., Bakshi, M., Abhilash, P.C. and Fraceto, L.F. (2019) Use of botanical insecticides for sustainable agriculture: Future perspectives. *Ecological Indicators*, 105, 483–95. <https://doi.org/10.1016/j.ecolind.2018.04.038>
7. Dhivya, R. and K. Manimegalai, 2013. Preliminary Phytochemical Screening and GC-MS profiling of ethanolic flower extract of *Calotropis gigantea* Linn. *J. of Pharmacognosy and Phytochemistry*. 2 (3) : 28-32.
8. Freitas. C. D. T., J. S. Oliveira, M. R. A. Miranda, N. M. R. Macedo, M. P. Sales, L. A. Villas-Boas & M. V. Ramos. 2017. Enzymatic Activities and Protein Profile of Latex From *Calotropis procera*. *Plant Physiology and Biochemistry*. 45; 781-789
9. Ghule. S. D, G. Vidyasagar, P. Sharma & A. P. Gunjal. 2014. CNS Activity of Leaves Extract of *Calotropis gigantea*. *Asian Pacific Journal of Tropical Disease*. 4(2): 902-905

10. Habib MR, Karim MR. 2016. Chemical characterization and Insecticidal activity of *Calotropis gigantea* L. Flower extract Against *Tribolium castaneum* (Herbst). Asian Pacific Journal of Tropical Disease. Vol. 6(12): 996-999.
11. Kovendan, K, K. Murugan, S.P. Shanthakumar, S. Vincent & J.S. Hwang. 2012. Larvicidal activity of *Morinda citrifolia* L. (Noni) (Family: Rubiaceae) leaf extract against *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*. *Parasitology Research*. 111(4) : 1481–1490
12. Kumar. P., E. Suresh. And S. Kalavathy. 2013. Review on a Potential herb *Calotropis gigantea*(L.) R. Br. Sch. Acad. Journal Pharm. 2:2:135-143
13. Khasanah N, E. Martono , Y A. Trisyono, and A. Wijonarko. 2021. Toxicity and Antifeedant Activity of *Calotropis gigantea* L. Leaf Extract Against *Plutellaxylostella*L. (Lepidoptera: Plutellidae). *International Journal of Design & Nature and Ecodynamics*. Vol. 16 (6)677-682
14. Makmur. A, A. H, S.Sjam, and A. Rosmana. 2016. Control of white stem borer *Schirpophagainnotata*Walker and earhead bug *Leptocorisatoratorius*Fabricius by using formulated *Calotropis gigantea* linn extract in rice field. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 7(5); 3012-3018.
15. Miresmailli, S. and Isman, M.B. (2014) Botanical insecticides inspired by plant–herbivore chemical interactions. *Trends in Plant Science*, 19, 29–35. <https://doi.org/10.1016/j.tplants.2013.10.002>
16. Mundim, F.M., Alborn, H.T., Vieira-Neto, E.H.M. and Bruna, E.M. (2017) A whole-plant perspective reveals unexpected impacts of above- and belowground herbivores on plant growth and defense. *Ecology*, 98, 70–8. <https://doi.org/10.1002/ecy.1619>
17. Nasir B.H, Khasanah N, and Idham. 2022. Insecticidal Activity of Leaf Extracts of *Calotropis gigantea* L, *Ageratum conyzoides*L, and *Vitex negundo* L. Against *Spodoptera frugiperda*J. E. Smith (Lepidoptera: Noctuidae). *International Journal of Design & Nature and Ecodynamics*. Vol. 17, (6) 899-905 <https://doi.org/10.18280/ijdne.170610>
18. Parvin. S., M. A. Kader, A. U. Chouduri, M. A. S. Rafshanjani, & M. E. Haque. 2014. Antibacterial, Antifungal and Insecticidal Activities of The N-Hexane And Ethyl-Acetate Fractions of Methanolic Extract of The Leaves of *Calotropis gigantea* Linn. *Journal of Pharmacognosy and Phytochemistry*. 2:5: 47-51
19. Prabhu, S., Priyadarshini, P., and A. Thangamalar, A. (2017). Study on larvicidal effect of different plant parts of milk weed plant (*Calotropis gigantea* R. Br.) against *Helicoverpa armigera*. *International Journal of Current Microbiology and Applied Sciences*, 6(10), 655–660.
20. Tafokou, R.B. I, 2010. *Calotropis gigantea* (L.) W.T.Aiton. [Internet] Record from PROTA4U. Schmelzer, G.H. & Gurib-Fakim, A. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. Protologue: Hort. kew. 2, 2: 78 (1811). <http://www.prota4u.org/search.asp>. Accessed 22 October 2017
21. Tripathi. P.K, S. Awasthi, S. Kanojiya, V. Tripathi & D.K. Mishra. 2013. Callus Culture And In Vitro Biosynthesis of Cardiac Glycosides from *Calotropis gigantea* (L.) Ait. *In Vitro Cell Dev. Journal Biol-Plant*. 13:49: 455–460
22. Supartha, I.W, I. W. Susila , A. A. A. S. Sunari , I. G. F. Mahaputra, I.K. W. Yudha, and P. A. Wiradana. 2021. Damage characteristics and distribution patterns of invasive pest, *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae) on maize crop in Bali, Indonesia. *Biodiversitas* ISSN: 1412-033X Volume 22, Number 6, Pages: 3378-3389