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The Effect of Combination of Oleander Leaf Extract (*Nerium oleander* L.) With Neem Leaf (*Azadiracta indica* A. Juss) on Mortality Golden Snail (*Pomaceae canaliculata* L.)

Supriatno[1], Nur Asiah[1*], Asiah M D[1], Djufri[1], Safrida[1]

[1]Biology Education, Teaching and Education Faculty, Syiah Kuala University, Indonesia.

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Corresponding Author:
Mawardi
nurasiah.bio18@fkip.unsyiah.ac.id

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Abstract: The golden snail is a pest that causes crop failure in rice plants. Farmers control the golden snail by using pesticides, which can have a negative impact on the environment, so another alternative is needed that can control the golden snail, namely vegetable pesticides. This study aims to determine the effect of a combination of oleander leaf extract and neem leaves on the mortality of Keong Mas. The method used in this research is the experimental method with a factorial completely randomised design (CRD). The study consisted of 2 factors, namely factor N (Oleander leaf) and factor A (Azadiracta indica leaf), each of which had 4 levels, so there were 16 treatment combinations and 3 replications. There were 480 golden snails in the study. After 12 hours of observation, the N3A3 treatment tested by ANOVA showed significantly different results (fcount > ftable = 66.00 > 2.01) and caused 30 test animals to die with a mortality rate of 77%. The conclusion from this study is that the combination of oleander leaf extract with neem leaves greatly influences the mortality of the golden snail.

Keywords: Mortality; Neem; Oleander; The golden snail; Toxicity

Introduction

Indonesia, also known as Mega Biodiversity, is a nation with a high biodiversity. Biodiversity includes every facet of life. Because it is connected to the ecosystem in which humans live, biodiversity is considered as the cornerstone of human existence. The diversity of plants found in Indonesia has the potential to act as natural insecticides and molluscicides since they contain unique compounds that are harmful to certain organisms. Farmers in Indonesia still have difficulties controlling plant pests (OPT). One of the pests that causes the failure of rice yields in Indonesia is the golden snail.

Because of its flexibility, mobility, and high reproductive capacity, the golden snail (Pomacea canaliculata) is one of the rice pests that is challenging to manage. The reason the golden snail is referred to as a nuisance is that it consumes 10-day-old rice plants in rice fields, and the eggs that the golden snail lays cause the rice plants to perish (Maruni et al., 2022).

Submerged places are where the golden snail chooses to reside. Additionally, snails lay numerous eggs during one ovulation phase, which can result in a large number of golden snails and seriously harm rice crops by triggering crop failure (Arsi et al., 2022). Farmers generally eradicate golden snail pests by using pesticides. The use of pesticides that are carried out continuously and excessively will cause side effects on the environment and kill non-target organisms. because most pesticides contain toxic chemicals.

Based on the problems above, a farmer can switch from chemical pesticides, many of which are harmful to the environment, to plant-based pesticides (Osborne et al., 2022). The use of vegetable pesticides can be carried out on the basis of consideration and utilisation of the potential of Indonesian flora, which is commonly found around humans and contains substances that are anti-pest, and pests control policies that place more emphasis

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on approaches to ecosystem management while maintaining environmental sustainability.

By producing secondary metabolites including phenols, quinone flavonoids, terpenoids, alkaloids, and tannins, plants defend themselves against herbivores and microorganisms (Sayed et al., 2021). Botanical pesticides are pesticides derived from plant-based ingredients that are easily decomposed in nature, so they are safe for humans and do not pollute the environment (Tripathy et al., 2022).

Oleander, also known as Nerium oleander, is an evergreen perennial that is indigenous to the Mediterranean region and has a long history of harming both people and animals (Sykes et al., 2022). Oleander contains oleandrin and cardiac glycosides, which are insecticidal, rodenticidal, anti-microbial, and anti-feedant agents (Farkhondeh et al., 2020). All parts of the oleander plant are toxic to animals, humans, and insects. According to research, oleander is toxic and can cause a burning sensation in the mouth, as well as vomiting, diarrhea, and gastrointestinal effects (Dey et al., 2022).

Neem, or Azadirachta indica, is an evergreen tree in the Meliaceae family. There are reportedly many phytochemicals in its leaves (Patil et al., 2022). There are numerous phytochemicals with varying biological and pharmacological properties that may be extracted from the various parts of neem, including the leaves, bark, seeds, blossoms, fruits, and roots (Braga et al., 2021). The neem plant also has ingredients that are useful in preventing plant pests, namely the ingredients azadiractin, salanin, meliantriol, and nimbin (Dono et al., 2020). Besides that, Azadirachtin also have a functions as a pesticide, stomach poison, and contact poison (Putri et al., 2019)

A variety of botanical items have been studied for their insecticidal and animal repellant properties. The most readily available insecticidal and animal repellent products seem to be those made of neem. Products made from neem seem to be among the most readily available. Neem (Azadirachta indica), one of the most significant green plants, produces this natural pesticide in its leaves (Zatelli et al., 2022).

The combination of oleander leaf extract with neem leaves as a vegetable pesticide was carried out because these two plants have toxic substances that can have a pesticide effect on pests and contain pest repellents. The oleander ethanol extract is similar to neem leaf extract in that it has an effective exposure time for pest control. Toxicity can increase if a compound is added to another compound.

This research can be used as information and knowledge about molluscicides using a combination of oleander leaf extract and neem leaves for the community, especially farmers to control golden snail pests and as a reference for students in Invertebrate Zoology and Toxicology courses.

Method

The research was conducted in May - June 2022. This research was conducted in Beurawe village, Banda Aceh. The combination extract of oleander leaves with neem leaves was carried out using a rotary evaporator in the Pharmacy Laboratory, Faculty of Mathematics and Science (MIPA), Syiah Kuala University.

The object in this study was the male golden snail with a shell size of 2 - 3 cm in diameter with a total of 480. The golden snail was taken from a rice field area in Barabung Village, Darussalam District, Aceh Province.

Result and Discussion

Mortality Rate of The Golden Snail at 12 Hours of Observation

The results of the study giving a combination of oleander leaf extract with neem leaves on the mortality of the golden snail had an effect on each treatment. The effect of giving this extract can be observed after 12 hours of treatment.

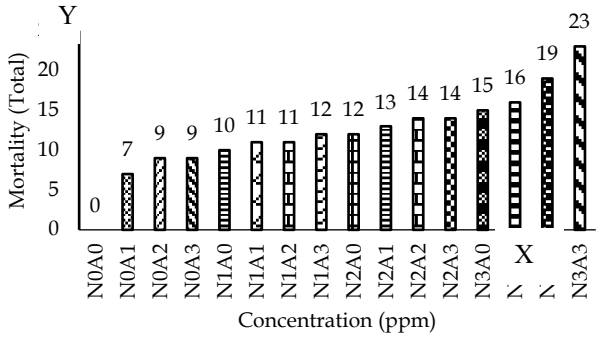

Figure 1. Percentage of Golden Snail Mortality based on the Toxicity Level of a Combination of Oleander Leaf Extract and Neem Leaf

Mortality of the golden snail due to the administration of a combination of oleander leaf extract and neem leaves began to occur in the N0A1 treatment (extract N 0 ppm + A 750 ppm) by 23%, or as many as 7 golden snails. The extract combination with the highest mortality rate occurred in the N3A3 extract combination treatment (N 2,250 ppm + A 2,250 ppm), which was 77%.

Based on the observational data that has been collected, it shows that the higher the concentration of the combination of oleander leaf extract and neem leaves given, the greater the percentage of golden snail mortality. Conversely, the lower the concentration of the combination of extracts given, the smaller the percentage of golden snail mortality. To find out more about the effect of giving a combination of oleander leaf extract and neem leaves on the mortality of the golden snail, see Table 1.

Table 1. Analysis of Mortality Variants of the Golden Snail at 12 Hours of Treatment

SD	DF	SS	MS	F Count	F Table	
					0.05	0.01
Treatment Combination	15	9.95	0.66	66.00**	2.01	2.70
N	3	6.61	2.20	220.00**	2.92	4.51
A	3	1.70	0.57	57.00**	2.92	4.51
NA Interactions	9	1.64	0.18	18.00**	2.21	3.07
Error	32	0.36	0.01			
Total	47	10.31				

** Very Real and Different

According to Table 1, the results of statistical analysis using analysis of variance (Anava) for the combination of oleander leaf extract and neem leaves showed a highly significant difference in mortality of golden snails. So, the hypothesis in this study is accepted. The results of the study showing the effect of giving a combination of oleander leaf extract and neem leaves to the golden snail can be seen in F count > F table = 66.00 > 2.01 using a level of 0.05 so that further tests were carried out. Based on the results of the calculation of the coefficient of diversity, which was 4.78%, the follow-up test used was the Tukey's Honest Significant Difference Test (HSD). The results of the Honest Significant Difference test, which can be seen in Table 2.

Table 2. HSD Test Results Combined Factors NA

Treatment	Average	HSD + Average	Code
N0A0	1.07	1.35	a
N0A1	1.68	1.96	b
N0A2	1.87	2.15	c
N0A3	1.87	2.15	c
N1A0	1.95	2.23	c
N1A1	2.04	2.32	c
N1A2	2.04	2.32	c
N1A3	2.12	2.4	cd
N2A0	2.12	2.4	cd
N2A1	2.19	2.47	cd
N2A2	2.27	2.55	d
N2A3	2.27	2.55	d
N3A0	2.35	2.63	d
N3A1	2.41	2.69	de
N3A2	2.61	2.89	e
N3A3	2.86	3.14	f

Note: Numbers followed by letters and in the same column are not significantly different at the 0.05 test level.

The results of the combination of oleander leaf extract with neem leaves were also analyzed using probit regression analysis so that the LC50 value was known. The results of the probit analysis showed that the calculated concentration of the combination of oleander leaf extract and neem leaves that caused the death of 50% of the test animals was 9,388.9 ppm. The concentration that can be used is 4,500 ppm, a combination of oleander leaf extract and neem leaves, namely the treatment (N3A3).

Discussion

Combination of plants with various active ingredients can provide a synergistic impact and increase effectiveness (Rifda & Lisdiana, 2022). Research using a combination of oleander leaf extract and neem leaves showed results that greatly influenced the mortality of the golden snail. The results of the study on control observations (without extract concentration) did not show a mortality effect on golden snails. Observations 12 hours after treatment showed a significant effect. The combination treatment of N3A3 extract (2.250 ppm N extract plus 2.250 ppm A) was the most effective extract combination, causing the golden snail to die 77% at 12 hours of observation. The higher the concentration of the toxic extract used, the higher the mortality rate of the golden snail. The leaves contain high levels of active ingredients or compounds.

The results of the study showed that there were golden snails as test animals that died after being treated with a combination of oleander leaf extract and neem leaves. The dead golden snail has the characteristics of a mantle that has entered the inside of the shell; it looks wrinkled and pale and secretes mucus so that on the top surface of the water there is white foam. The golden snail, when it is still alive, is a greedy animal and will stick to its food or to the jar as a treatment container. The golden snail that died was then released from the plastic jar. The body of the golden snail goes in and out of the shell continuously until, in the end, it no longer comes out, with the mantle going into the deepest part of the shell and the golden snail meat coming out of the shell. Another feature is the absence of a response when given a stimulus.

Nerium oleander, a toxic plant, can be used to control a significant agricultural pest (Subakti et al., 2022). The effect of oleander leaf extract on golden snail mortality is due to the presence of toxic substances produced naturally by plants. Oleander leaf extract greatly affects the mortality of golden snails because oleander leaves contain active compounds, namely glycosides in the form of oleandrin and neriin compounds, which are active ingredients belonging to the alkaloid group. Oleander leaf extract has an appetite suppressant (antifeedant) that causes golden snails. Oleander also has a toxic power that inhibits respiration,

affects the nervous system, and can be used as a repellent (Salim et al., 2020). That oleander leaf extract can be used as a natural pesticide to control golden snail pests. Another toxic substance, namely strychnin, is an active ingredient found in oleander plants. Oleander leaves also contain polyphenolic compounds (as much as 61%) and cinnamic acid, which function as antioxidants. This is consistent with (Ahmed et al., 2023) assertion, which mentioned that the leaf phytochemical screen (which looked at alkaloids, terpenoids, saponins, glycosides, carbohydrates, and tannins) produced encouraging results. Nerium oleander's toxicity targets pests with a nervous, reproductive, or digestive system (Zaid et al., 2022).

The plant *Azadirachta indica* A. Juss (Meliaceae), sometimes known as neem from the Meliaceae family, has become the focus of intensive research because it contains abundant biological resources (Cesa et al., 2019). Neem was often used as a shade tree because of its lush green leaves. Neem has several substances that are toxic, so it can be used as a vegetable pesticide. Almost all parts of the neem plant have a bitter taste, have an antifeedant effect, can disrupt hormonal processes in insects, and can inhibit the reproductive system in insects (Thiyagu & Rajeswari, 2021). Some of them are azadirachtin, nimbidin, tannins (gallic acid, epicatechin, catechin, and epigallocatechin), limonoids, nimblodes, diterpenoids (margolonone, isomargolonone), dehydrosalanol, gedunin, and mahmoodin (Adusei, 2022). The substance that has the highest toxicity is azadirachtin. Due to the many chemical functions of neem as repellent, anti-feeding action and safety from non-target organisms, neem-based pesticides are widely used (Kala et al., 2019).

Mechanism of Toxicity Content in Oleander Leaves and Neem Leaves

The active chemicals contained in oleander leaves accumulate and will be toxic to the test animals. Toxicants will be distributed to all parts of the body's cells through the blood circulation system, which causes the body's circulation system to be disrupted. If the enzyme secretion is disturbed, the digestive process will also be disrupted, causing the golden snail to become deficient in energy and eventually die.

The oleandrin substance in oleander leaves can work as a stomach poison and inhibit eating power. The stomach poison contained in oleander leaf extract enters the golden snail's body and affects the snail's metabolism after treatment. Stomach poison will enter the middle channel, which will then be circulated with other fluids whose function is like blood (Nasir et al., 2021). The poison the snail carries will affect its nervous system and cause its death. Cardiac glycosides in oleander can inhibit Na+/K+ ATPase, which causes an increase in intracellular Ca2+ concentration and a subsequent positive inotropic effect. In addition, it can also have a direct effect on the sympathetic nervous system. Oleandrin is quickly absorbed, and once it reaches the heart, it directly damages the cardiomyocytes (Shridhar, 2022). The active ingredient in neem leaves, namely azadirachtin, contains a substance that also plays a role in inhibiting appetite because this substance will bind to proteins in the protein absorption process in the digestive system, which is needed by the golden snail for growth, so that protein absorption in digestion will be disrupted. Azadirachtin is also known to have an impact on the neurosecretory system of insects like prothoracycotopic hormone (PTTH) and allatostatin, two morphogenetic hormones such as peptides can be inhibited (Wulansari et al., 2022).

The results of observations 12 hours after treatment with oleander leaf extract at concentrations of 750 ppm, 1,500 ppm, and 2,250 ppm differed between treatments. The different concentrations between treatments caused the difference in the number of deaths at the start of the observation. Chemical compounds at low concentrations did not work optimally at concentrations of N0A1 and N1A0. The treatment with the highest concentrations, namely the N3A3 treatment, had the highest mortality. This shows that the combination of oleander leaf extract with neem leaves is effective for use as a natural pesticide.

Conclusion

The highest mortality rate of golden snails at 12 hours of observation was in treatment (N3A3). During the control treatment (without being given the extract), there was no death in the test animals. The results showed that the combination of oleander leaf extract and neem leaves had an effect on the mortality of golden snails. So that the findings of this study can be used as an alternative method of controlling the golden snail pest. Using natural pesticides as pest control is the right choice because it reduces the impact of residues and poisoning on non-target pests. The use of pesticides derived from natural ingredients is more environmentally friendly, and the residue left behind is easier to remove (Dwi et al., 2020). Biopesticides can meet the required standards, namely inexpensive, specific targets, and biodegradable (Souto et al., 2021). Also, the use of plant insecticides is much safer for humans.

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Conflicts of Interest
There is no conflict interest of the authors.

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