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Azadirachta indica as Natural Pesticide for *Pomacea canaliculata* Control: A Review

Nik Amalia Nasratiena M. Z.[1], Chia-Chay T.[1], Saiyidah Nafisah H.[2] and Wan Rozianoor M. H.[1,3*]

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia*

[2]*Faculty of Applied Science, Universiti Teknologi MARA Cawangan Perak Kampus Tapah, 35400 Tapah, Perak, Malaysia.*

[3]*Human Genetic and Biochemistry, Research Nexus of UiTM (ReNeU), Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.*

**Corresponding author*: *rozianoor@uitm.edu.my*

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ABSTRACT

Pomacea canaliculata, or golden apple snail, is known as a major rice pest in Malaysia. Since 2002, almost 20000 ha of rice-growing areas in Malaysia have been infested by *P. canaliculata.* The snail destroys the rice seedlings by consuming the soft stems and leaves of the paddy. Consequently, farmers use synthetic pesticides to control the invasion of *P. canaliculata*. However, farmers have concerned about the negative impact of synthetic pesticides on human health and the environment, as well as the high costs of the application. *Azadirachta indica* is one of the most well-known plants for natural pesticides that has played a vital role in pest management and is widely used in agriculture. The seed and leaf extracts of *A. indica* have been used as a biological molluscicide in controlling *P. canaliculata*. The most common active compounds in *A. indica* are azadirachtin, salannin, meliantriol, and nimbin. Previous studies established that the pesticidal properties of *A. indica* are derived from azadirachtin, which inhibits the acetylcholinesterase enzyme (AChE) activity of the pests. Most of the research only focused on *A. indica* extracts and essential oils as the lethal doses and time to achieve lethal effects. Therefore, this review provides information about the infestation of *P. canaliculata* and the effectiveness of *A. indica* as a biopesticide due to the inhibition of AChE, which is known as the primary resistance mechanism of pesticides in many pests.

Keywords: *Azadirachta indica, Pomacea canaliculata, azadirachtin, biopesticides, AChE.*

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INTRODUCTION

P. canaliculata is commonly known as the golden apple snail. *P. canaliculata* is a native snail to tropical and subtropical regions, then disperses from South America to Taiwan, Japan, China, and the Philippines [1]. In China, *P. canaliculata* was first introduced into Guangdong province in 1981 and became a severe pest of rice since 1984 [2]. Besides that, *P. canaliculata* causes direct damage to seeding rice in the Philippines, Thailand, and Vietnam [3]. The invasion of *P. canaliculata* leads to economic losses to aquatic crops in the Philippines, estimated to be up to USD 1200 million per annum. *P. canaliculata* was first detected in Malaysia in 1991 and found in Selangor, Kedah, and Perak [4].

P. canaliculata is listed as one of the world's 100 worst invasives because *P. canaliculata* has become a significant paddy pest in Southeast Asia regions such as Malaysia, Philippines, Thailand, and Vietnam [5]. Initially, local people commercialized the snails as food, but the snail was abandoned due to market value loss, and the snail has spread into agricultural areas, including paddy fields [4]. It altered the ecosystem of the paddy fields it invaded [6]. In paddy fields, young stems and leaves of rice seedlings will be consumed by *P. canaliculata*, which results in terrible damage to the rice-growing area [7].

Farmers prefer to use chemical molluscicides such as niclosamide and metaldehyde to solve the *P. canaliculata* invasion problem [8]. However, the application has adverse effects, especially on the farmer's health and ecosystem. The World Health Organization (WHO) estimates that 200,000 people are killed worldwide yearly due to pesticide poisoning [9]. According to the previous study, applying chemical molluscicides such as chlorpyrifos, copper sulfate, endosulfan, fentin acetate, and sodium salt of pentachlorophenol (NaPCP) causes severe water pollution. It is toxic to non-target organisms [4]. Therefore, natural pesticides from plants have been widely studied due to their unharmful to non-target organisms, biodegradability, cost-effectiveness, and prevention resistance against phytochemical mixtures [10].

More than 1400 plant species have been proven to have molluscicidal properties [11]. One of the plants is Neem, also known as *Azadirachta indica* (*A. indica*). *A. indica* has been used as a natural source of insecticides, pesticides, and agrochemicals [12]. Additionally, *A. indica* is well known for having eco-friendly characteristics [13]. The most common active compounds in *A. indica* that have pesticide characteristics are azadirachtin, meliantriol, nimbin, and salannin [14]. Azadirachtin plays an essential role as a feeding deterrent and causes the mortality of *P. canaliculata* [15].

A. indica has molluscicidal properties due to azadirachtin inhibiting AChE activity [16]. In the nervous system of pests, AChE is an important enzyme that terminates nerve impulses by catalyzing the hydrolysis of acetylcholine [17]. Besides, azadirachtin plays a role in the metabolism of pests by influencing specific functional proteins related to their growth and reproduction [18]. Azadirachtin also can cause inhibition of both cell division and protein synthesis [19]. Thus, a new advanced formula using *A. indica* could be utilized as biopesticides in agriculture, mainly to control the infestation of the *P. canaliculata* in Malaysia. The *A. indica* serves as a potential commercialized biopesticide product with low cost and security to the health of farmers and benefits the environmental safety.

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Pomacea canaliculata in Paddy Field

P. canaliculata, or golden apple snail, is a large freshwater snail with a shell length up to 60 mm [20]. The snail was identified based on its shell morphology, which is most visible in newly hatched juveniles. The shell of *P. canaliculata* is thin and has a smooth surface in yellowish or dark brown [20]. *P. canaliculata* has a high, pointed, dark spiral band [21]. Unlike other apple snail species, the umbilicus and channeled suture of *P. canaliculata* is broad and deep. The snail also has a rounded body, a whorl shoulder, and a not-pigmented inner pallial lip [21]. *P. canaliculata* eggs are identified by a richness of nutrients, a reddish or pinkish color, and aerial oviposition. The neurotoxicity of the eggs is due to the perivitelline fluid (PVF), which fills the space between the eggshell and the embryo and comprises carbohydrates, lipids, and proteins [22].

The population of *P. canaliculata* can expand rapidly due to its high fecundity and can survive in extreme environments [23]. The snail has a voracious appetite and can eat 7 to 24 young stems and leaves of rice seedlings per day. Moreover, a female *P. canaliculata* can produce a mass of eggs containing up to 500 eggs in a week, and the incubation period for the eggs to hatch only take 10 to 15 days with an 80 % hatchability rate [24]. *P. canaliculata* has both a brachial respiration system and a gill to withstand drought by aestivating in the soil for several months [25]. The ability to survive without water for long periods due to behavioral and physiological changes such as inactivity reduces metabolic rate and switching to anaerobic metabolism [26]. Besides that, adult of *P. canaliculata* can also survive for months without eating in pipelines, artificial channels, or water tanks [6]. Thus, these characteristics enable *P. canaliculata* to become a severe pest in many cultivated areas in Asia, especially in paddy fields.

Various approaches at different stages of cultivation have been taken to control the damage caused by *P. canaliculata*. Both masses of eggs and snails can be collected by hand or tools before destruction. Adult snails can be handpicked easily using attractants such as jackfruit, papaya, and tapioca leaves [27]. In the paddy field, the water level is adjusted to be shallower than the shell of the snails to reduce their movement [24]. *P. canaliculata* is more interested in young seedlings than matured paddy. Thus the farmers choose to transplant older seedlings which can reduce the damage to the paddy field by the snail [28]. Additionally, duck pasturing is applied in the paddy field as biological management and significantly impacts snail control [28]. Tobacco waste also can be applied as a molluscicide, but this option is the least effective because it cannot kill the *P. canaliculata* in the soil.

Thus, the farmers use chemical pesticides such as niclosamide and metaldehyde to destroy the snail in a short time [1]. However, the active ingredients are highly corrosive and poisonous, which directly causes many severe effects on farmers and other organisms in the paddy field [8]. Also, improper administration of chemical pesticides results in environmental pollution, such as soil, groundwater, and natural waterways [29]. Nowadays, the proper management to control and reduce the invasion of *P. canaliculata* to other new regions is still a gap of study. Extensive and inventive research on alternative plants to produce biopesticide has drawn attention among researchers.

Azadirachta indica

A. indica, or Neem tree, belongs to the mahogany family of *Meliaceae* [30]. *A. indica* is usually found in Bangladesh, India, Nepal, Pakistan, Thailand, Africa, America, and Australia [31]. In India, *A. indica* is

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locally known by many names such as "Divine Tree," "Life-giving tree," "Nature's Drugstore," "Village Pharmacy," and "Panacea for all diseases"[32]. Farmers called *A. indica* as Indian Neem (margosa tree) or Indian lilac, and latter the Persian lilac. According to Chandrawathani [33], *A. indica* is known as "Mambu" by the locals in Malaysia.

A. indica can grow well on calcareous and hardpan soil depths of 1.5 to 2.0 meters [14]. The tree also can be adapted in minimum rainfall countries [34]. Usually, *A. indica* has a long life span of more than 200 years [9]. The tree can grow from 12 to 24 meters with moderately thick and rough bark [35]. Based on a research by Ogbeuwa [9], the leaves of *A. indica* are compound, imparipinnate, and comprise up to 15 leaflets arranged in alternate pairs of terminal leaflets. Other than that, the tree produces abundant small white flowers with a sweet scent [9]. The fruit of *A. indica* is smooth, ellipsoidal drupe and turns yellow or greenish-yellow when ripe [9].

Primary chemical constituents of *A. indica* are limonoids and terpenes, which consist of azadirachtin, 3-deacetyl-3-cinnamolyazadirachtin, I-tigloyl-3-acetyl-II-methoxyazadirachtin, 22, 23-dihydro-23β-methoxyazadirachtin, nimbanal, 3-tigloylazadirachtol, 3-acetyl-salanno V, nimbidio V margocin and margocilin. In addition, *A. indica* consists of terpenoids such as visoazadirolide, 6 nimbocinolide, nibonolone, nimbonone, methylgrevillate, and margosinone [9]. These chemical constituents have been isolated from different parts of *A. indica*, including leaf, bark, root, and seed, which contain numerous functional biological activities [14].

Recent studies have shown that the azadirachtin compound of *A. indica* proved to have insecticidal, antifeedant, molluscicidal, and antiviral activities [12, 15, 32, 36]. Also, *A. indica* shows antibacterial and antifungal activity due to quercetin and ß-sitosterol [37]. The antioxidant activity of *A. indica* is derived from azadirachtin and nimbolide [32]. Besides that, both active compounds extracted from leaves and flowers of *A. indica* are responsible for anticancerous activity [38]. Nimbin, isolated from the bark and leaves of *A. indica*, has been known to have anti-inflammatory, antisecretory, and antiulcer activities [39]. Apart from that, the antimalarial activity of *A. indica* has also been confirmed by azadirachtin, salannin, and deacetylgedunin [40]. Thus, *A. indica* is a very advantageous plant and is considered a safe medicinal plant that modulates many biological activities without adverse effects [41].

A. indica as Pesticides

Almost all parts of the *A. indica* have been used for pesticide formulations, including the stem bark, root bark, leaves, flowers, fruits, seeds, and seed kernels. The pesticide formulations of *the A. indica* plant can either be in the form of powder, crude oil extracts, ethanol extracts, aqueous extracts, or commercial formulation [13]. Table 1 lists the *A. indica* shown to have pesticide effects on various pest types. The kernel extract of *A. indica* showed juvenile hormone mimic activity and inhibited larval development of the rice leaf folder *(Cnaphalocrocis medinalis)* [14].

The leaves and seeds are the preference of selected parts of *A. indica* in pesticide application due to their accessibility and abundance locally. The extract isolated from the seed of *A. indica* exhibited pesticide effects on *Helicoverpa zea* and *Leptocorisa acuta* [14]. Moreover, the seed extracts also can control the *Anopheles*, *Culex*, and reduction of *Aedes* [42]. Azadirachtin, one of the active compounds isolated from the seed of *A. indica* has been applied as a repellant, whereas saponins serve as an inhibitor

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for *Tribolium castaneum* [43]. In addition, the seed crude extracts significantly killed the *P. canaliculata* based on different solvents and concentrations. Methanol extract had the highest potency and had the lowest LC_{50} values (21.008mg/ml), followed by ethanol extract (43.726mg/ml), acetone extract (48.110mg/ml), and water extract (53.654mg/ml) [15]. The leaf extract of *A. indica* was found to be an effective repellent to kill *Arianta arbustorum* [44]. Apart from that, oil spray of leaves and kernel of *A. indica* showed mortality to *Lipaphis erysimi* [14]. However, there is limited study on quantifying the active constituent of *A. indica* that is responsible for the mortality of *P. canaliculata,* especially in determining the mechanism of molluscicidal activity by using the leave and seed*s* of *A. indica* extract [15].

Table 1: Part of *A. indica* as a pesticide and its effects

Part of *A. indica*	**Pest**	**Effects**	**Reference**
Kernel	*Cnaphalocrocis medinalis*	It can inhibit the larval development of *Cnaphalocrocis medinalis*.	[14]
Seed	*Helicoverpa zea*	It can reduce more than 60 % of *Helicoverpa zea*.	[14]
Seed	*Leptocorisa acuta*	Oil emulsion spray of *A.indica* is used to protect developing rice grains by *Leptocorisa acuta.*	[14]
Seed	Anopheles, Culex, Aedes and larval	It can reduce 98.1 % of *Anopheles*, 95.5 % of *Culex*, and 95.1 % of *Aedes* in 24 hours and 100 % larval control in 7 days.	[42]
Seed	*Tribolium castaneum*	Azadirachtin is found as a repellant of stored grain insects, while saponins as an inhibitor for the insects.	[43]
Seed	*P. canaliculata*	The mortality rate of *P. canaliculata* subjected to various concentrations and solvent extracts indicated that *A. indica* seed crude extracts significantly killed the snail.	[15]
Leaves	*Arianta arbustorum*	Azadirachtin is found as a repellent on herbivorous land snail *Arianta arbustorum.*	[44]
Leaves and kernel	*Lipaphis erysimi*	Oil spray of *A. indica* caused 100 % mortality of *Lipaphis erysimi*	[14]

Molluscicidal Activity of A. indica on Neurotransmission of Acetylcholine

One of the primary molecules controlling nerve impulses transmitted from a nerve cell or involuntary muscle is acetylcholine (ACh) [45]. The ACh is the first neurotransmitter found at the synaptic cleft, the space between two nerve cells [46]. The action of acetylcholine is mediated by nicotinic (nAChRs) and muscarinic acetylcholine receptors (mAChRs) [47]. AChE belongs to the α/β hydrolase fold protein superfamily, defined by structural homology, cholinesterases, carboxylesterases, and lipases [48]. AChE terminates cholinergic neurotransmission by catalyzing the hydrolysis of ACh into acetic acid and choline [47]. AChE plays a significant role in nerve impulse transmission in vertebrates and invertebrates [49].

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Pesticides typically obstruct the passage of impulses in a pest's nervous system [42]. Organophosphates and carbamates, two major classes of pesticides, are known to target AChE [50]. The AChE inhibition results in the accumulation of ACh at the nerve synapses and leads to the overstimulation of cholinergic receptors [47]. This process results in the acetylcholine receptor permanently opening and increasing nerve excitement [51]. AChE inhibitors disrupted the function of ACh in controlling the physiology of autonomic ganglia neuromuscular, parasympathetic, and sympathetic effector junctions [29]. The pest will then be paralysis, ataxia, lack of coordination in the neuromuscular system, and death [52]. The altered AChE is well known as the primary resistance mechanism of pesticides in many pests [53].

Numerous studies have investigated the ability of *A. indica* to inhibit AChE activity. Table 2 shows the mechanism of inhibition of AChE activity by the active compound of *A. indica*. The azadirachtin compound isolated from *A. indica* had shown inhibitory effects on AChE enzyme activity [16]. Besides, azadirachtin proved to inhibit the activity of AChE of *Nilaparvata lugens* and altered AChE of *Periplaneta americana* [17, 53], LC_{50} concentrations of azadirachtin significantly inhibited the activity of AChE compared with control. Other active compounds of *A. indica*, such as citral, ferulic acid, umbelliferone, azadirachtin, and allicin, can inhibit the activity of AChE, acid or alkaline phosphates, and ATPase of *Lymnaea acuminate* [54]. Besides that, saponins have a higher affinity for insect pests by inhibiting the AChE in the nervous system [55]. Therefore, AChE is used as a bio-indicator to evaluate pesticide exposure in eggs and tissues of *P. canaliculata* [56]. However, there is lack of studies to prove the AChE inhibition in *P. canaliculata* using *A. indica* that leads to molluscicidal activity.

Table 2: Mechanism of inhibition of AChE activity by *A. indica*

Active compound	**Mechanism of action**	**Target pest**	**References**
Azadirachtin	Inhibit the activity of AChE.	*Nilaparvata lugens*	[53]
	Alteration of AChE is discovered at 4 mg/L of azadirachtin.	*Periplaneta americana*	[17]
Citral, ferulic acid, umbelliferone, azadirachtin, and allicin	Inhibit the activity of AChE, acid/alkaline phosphates, and ATPase.	*Lymnaea acuminata*	[54]
Saponins	Inhibit the nervous system AChE.	*Tribolium castaneum*	[56]

Inhibition Mechanism of A. indica

The inhibition of AChE by *A. indica* has been proven to disrupt the functioning of the cholinergic nervous system. A previous study has discovered that inhibition of AChE will cause cell death and inflammation to occur in the cell. According to Chen [57], the accumulation of ACh at synapses by inhibiting AChE will lead to rapid dysfunction and excitotoxicity of cholinergic neurons due to the over-activation of mAChR. Then this will result in neuronal cell death and neural loss in cholinergic regions of the brain. Both azadirachtin and nimbolide are identified as the most potent *A. indica* limonoids that exhibit cytotoxic effects [58]. Both compounds affect various processes such as apoptosis, regulate cell survival and cell proliferation by influencing the Bcl-2 family proteins and caspases.

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Besides that, the inflammation occurs from the inhibition of AChE when the α7 nAChR leads to calcium influx and stops nuclear factor kappa B (NF-ƙB) stimulation [59]. NF-κB also plays a vital role in cell proliferation, differentiation, apoptosis, inflammation, stress response, and several signal transduction pathways [60]. Nuclear translocation of NF-κB with upregulation of IκB and p53 by azadirachtin and nimbolide induce apoptosis [58]. However, the molecular signaling pathways that lead to cell death or inflammation in *P. canaliculata* after exposure to *A. indica* extract are still undiscovered and understood.

CONCLUSION

The invasion of the *P. canaliculata* to the nature of the paddy field has led to crucial pest problems because they are very fast-growing and have high reproductivity. This results in a significant loss to the farmer and directly impacts the country's economy. Hence, farmers develop and employ many methods to control the invasion of snails. However, most farmers prefer chemical molluscicides, which have a rapid effect on controlling the snail but cause adverse effects on human health and the ecosystem. Therefore, *A. indica* is explored as a biopesticide for a better and safer sustainable solution. The signaling pathway of the inhibition mechanism of AChE by *A. indica* serves as a new advanced formula to control the invasion of *P. canaliculata* in the paddy field. A potential low-cost commercialized biopesticide product could be developed from *A. indica* to benefit farmers and further creates safety environment.

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AUTHOR'S CONTRIBUTION

All the authors have contributed in conceptualized the central research idea, providing the theoretical framework, and writing the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits or commercial or financial conflicts and declare the absence of conflicting interests with the funders.

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