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Use of plant extracts to control activities of the invasive giant African land snail *Achatina fulica*

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Abstract

Giant African land snails (Achatina fulica; GALS) are a highly invasive herbivore and pose serious threats to native species and that could disrupt ecosystems. Various botanical extracts have been used as molluscicides to control mollusks for pest management. We aimed to identify the effects of neem (Azadirachta indica) and titepati (Artemisia vulgaris) to reduce GALS activities and survival using solutions of fresh neem leaf and titepati leaf, bark, and root. We found a fast movement in the GALS only in the extraction groups while the lethargic movement was lower in mulching method. A significant association of control groups with different extraction groups along with mulching treatment ($p<0.05$) however, no differences were observed among different extractions at 2.5% and 5% concentrations. Among the treatments, Neem leaf at 5% concentration was found to be effective as it helped immobilize the samples after ten days whilst other treatments required minimum 14 days to be effective. We recommend further studies of titepati and neem as control agents to reduce crop and vegetable damage from GALS.

Keywords: Giant African land snail, invasion, molluscicidal effects, neem, titepati

Introduction

Biological invasions are a major driver of ecosystem change and have increased rapidly as species adapt to new habitats and increase in abundance (Ehrenfeld 2010, Simberloff *et al*. 2013). Invasive species are harmful to native species as they displace native plant

species through habitat encroachment (Callaway *et al*. 2005). Currently, the rate of increase in populations of invasive species is rapid (Simberloff *et al*. 2013), and they are considered a major driver to loss of native and threatened species, human economy and accelerating the human health problem (Lockwood *et al*. 2013, Oduor 2013, Basaula *et al*. 2021 & 2022).

Overall 282 invasive species (flora: 218; fauna: 64) occur in Nepal (Siwakoti & Shrestha 2014, Budha 2015, Joshi *et al.* 2017). Among faunal species, the giant African land snail (*Achatina fulica;* GALS) is one of the most destructive invasive species in Nepal (Budha 2015) and was categorized as one of the World's Worst Invasive Alien Species in 2000 (Lowe *et al.* 2000). The GALS is exported as a food and as pest from East Africa (Fontanilla *et al*. 2014). The increased rate of distribution and risk associated with GALS in Nepal has escalated due to rapid human population growth and related development including roads (Adhikari *et al*. 2020). In Nepal, GALS are distributed from low land and mid-mountain regions (Adhikari *et al*. 2020).

Due to damage caused by GALS, their control is of great importance. To reduce adverse effects of GALS in agricultural fields, people kill the snails and their eggs using manually collection (Yu *et al*. 2002); however, this method is ineffective. Pesticide use to control GALS is rapidly increasing globally, but the effects of pesticides can be more detrimental than the GALS (Khare *et al*. 2019). Naturally occurring compounds from plants have been used as molluscides (Abera 2003) and could reduce potential adverse environmental effects. For example, the natural plant products of neem (*Azadirachta indica*) and titepati (*Artemisia vulgaris)* are biodegradable, environment friendly, and considered highly effective as molluscicides and may be effective as a GALS repellent (Raut *et al*. 2014).

Neem belongs to the family Meliaceae and is an evergreen tree found mostly in tropical (Raguraman & Singh 1999), and subtropical regions of Africa, Australia, and Latin America (Nisbet 2000). Neem contains numerous biologically active compounds including Salannin, nimbidin, azadiradione and epiazadiradione (Singh *et al*. 2004,Nisbet 2000) and has been used in ethnomedicine to control agricultural pests (Ley *et al.* 1993, Bansal *et al*. 2010, Paul *et al*. 2011). In Nepal, titepati leaf extract is used to treat numerous human ailments (Bassett *et al*. 1978, Tamang 2003, Hussain & Hore 2007, Khan *et al*. 2015). Titepati contains numerous flavonoids, terpenoids and saponins which developed oxidative stress to the organisms (Wagner & Elmadfa, 2003, Xie *et al*. 2008). Titepati also has been used as an insecticide (Hwang *et al*. 1985, Wang *et al*. 2006). Neem and titepati have been used as molluscicides (Abera 2003) to control GALS; however, their effectiveness is poorly known. As GALS distribution in Nepal

(Adhikari *et al*. 2020) and corresponding agricultural damage (Budha & Naggs 2008) have increased, we aimed to identify the effectiveness of neem and titepati plant extract to control GALS.

Materials and methods

Sample preparation

We conducted the experiments in Sanopalate-4 Bharabise (27.920884 N and 85.733491 E), Sindhupalchowk Nepal, from 31 August to 4 October 2019. Invasive Giant African Land Snails is widespread in Bharabise and it damages the crop, therefore, we performed this research in this area to minimize the agricultural damages from this species. We collected fresh leaves and stems of neem and titepati for this research. We chopped leaves and small stems and stored them in a shaded area to prevent desiccation before use, and these materials were used for mulching. We extracted neem and titepati products (leaves, stem and root) following Raut *et al.* (2014) in which each sample specimen was ground to a fine powder and prepared 2.5% and 5% concentrated treatment solutions.

Plant samples were first dried at room temperature, cut into small pieces, and milled to powder. Then we took 376 gm of neem leaf powder in a conical flask and added 1500 ml of 100% ethanol, and left it until the powder was fully wet the covered with aluminum foil. The solution was agitated and left it for 72 hrs at room temperature. Due to sample limitation, we did not prepare solutions for the bark and root of neem plants. After 72 hrs, the filtrate was removed from each sample and evaporated the solvent using a rotatory evaporator. The extract was kept in a Falcon tube and stored at 4°C for 4 days. From the crude extract of each sample, we prepared a 2.5% and 5% treatment solution. For example, we prepared a 2.5% concentrated solution of neem leaf extract using 2.5 gm of neem leaf extract with 97.5 ml of distilled water. We applied the same procedure to titepati extracts to make the treatments from leaf, stem bark, and root. Altogether we prepared eight solutions for this research.

Experiments

We established 11 framed boxes (50- x 30- x 45-cm) with netting and used one for each of the eight titepati and neem extracts, two for mulched samples, and one as an untreated control at Sanopalate Bharabise. We filled each box with loamy soil potentially the GALS burrow underground and hide as occurred in natural condition. We kept three GALS in each netted box and provided food (e.g., cabbage, cauliflower) and sprayed water. We left these GALS for one week to ensure whether they are in healthy. We found all GALS were healthy based on their movement and consumed food materials.

After the confirmation of their good health, we sprayed 10 ml of each of the 8 extract solution onto the body surfaces of the three snails in their respective enclosures and periphery of the snail. We recorded the snail's activities such as lethargic, immobile, and fast movements every 20 minutes before and after treatment, from 7.00 to 18.00 hrs.

Similarly, we used neem and titepati mulch separately on the soil surface into the enclosure. We recorded whether snails moved away from the mulch or were immobile, or had no apparent response. Specifically, we determined the time required to move > 10 cm from the mulch, and time to become immobile. We also recorded number of eggs and time taken to death of snails during this research.

Data analyses

We used one-way ANOVA with Tukey's HSD for multiple comparisons to assess the relationship among treatments (extraction and mulching) with control and within extraction treatments at 2.5% and 5% concentrations. We used multiple linear regression to compare mean daily distances moved by snails exposed to extraction, mulching, and control. All analyses were performed in R Program (R Core Team, 2020).

Results

Land snails exhibited different movement pattern across treatments (Figure 1-2). Fast movement of land snails were observed when treated with the extraction of neem leaf, titepati parts-leaf, root, bark- each at 2.5% and 5% concentrations whereas no fast movements were observed in control and mulching treatments (Figure 1). Immobility was observed for longer when snails was exposed to titepati leaf at 2.5% concentration compared to neem leaf at 2.5% (Figure 1), however no differences was observed when comparing the efficiency of neem leaf of 2.5% concentration with titepati leaf of 2.5% concentration (Tukey HSD P = 0.99, Table 1). The two treatments methods (extractions or mulching) with the control groups of land snail showed significant association (S.No 1.1-1.8, Table 1) while no association within the extraction treatment methods at same or different concentrations (S.No 1.9-1.36, Table 1).

Less time was spent on fast movement compared to immobile and lethargic movement, using 5% concentration each of neem leaf and titepati leaf (Figure 1), with no variation across activities between these two treatments (Tukey HSD P = 0.99, Table 1). Time spent among movement activities by land snails using titepati bark of 2.5% and 5% concentration was similar (Figure 1). Lethargic movement using neem leaf of 2.5% and 5% high compared to using titepati bark of different concentrations (Figure 1). Titepati root of 5% concentration resulted in snails exhibiting greater lethargic movements.

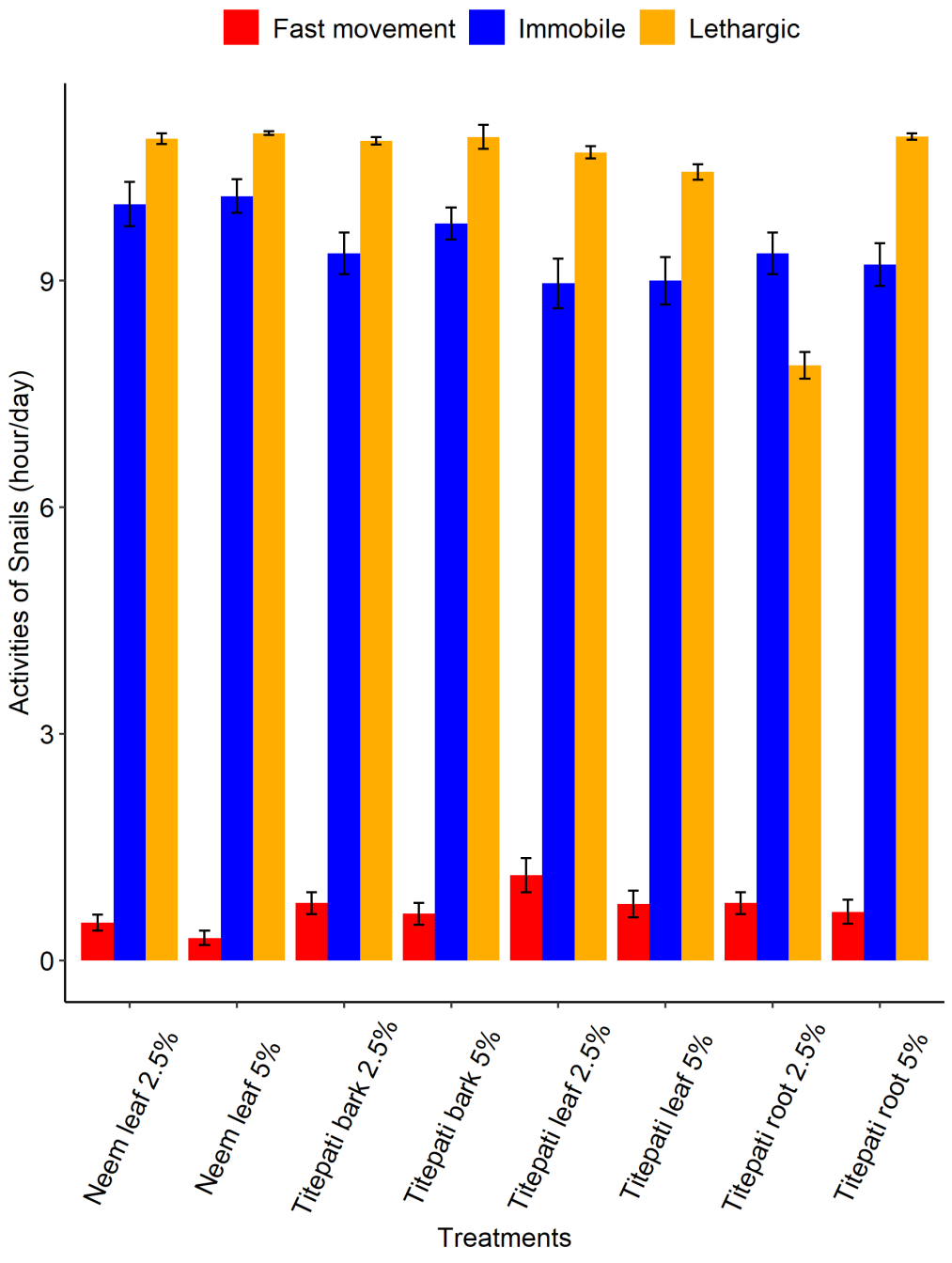

Figure 1: Daily time spent by Giant African Land Snails on different treatments

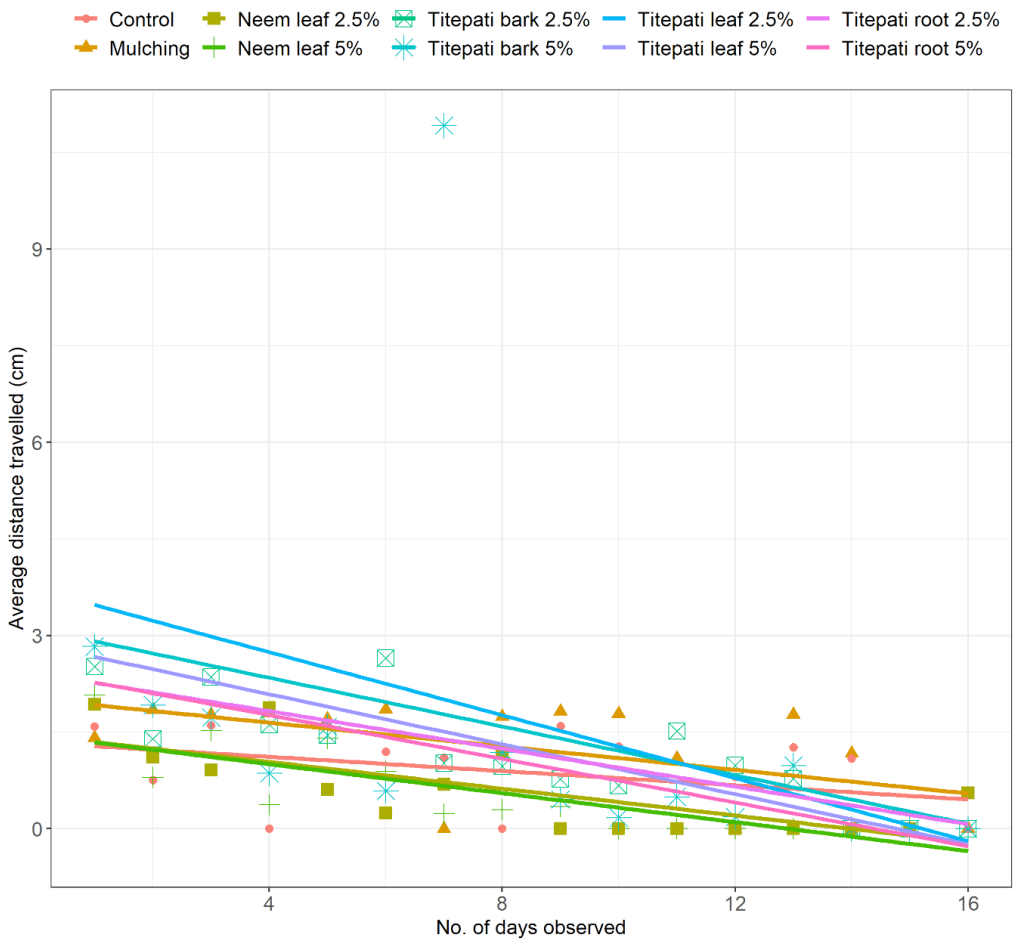

Figure 2: Average daily distance moved by giant African land snails

Average distance travelled decreased with increasing the number of days surveyed while average distance travelled was positively associated with extraction (Figure 2). Compared to starting days, from the day 14 to day 16 average distance travelled by snails was decreased. Among treatments, land snails were found immobilized after ten days when using 5% neem leaf while remaining treatments required longer durations for the land snails to immobilized. Average distance travelled in different treatment system was not varied ($R^2 = 0.0808$, df = 9, F = 1.466, p = 0.165; Figure 2), however found positively associated with all treatments except Neem treatments.

Table 1: Comparison the efficiency of different treatments (extraction and mulching) with control

S.No	Treatments	Difference	Lower	Upper	p
1	**Extractions**				
1.1	Neem leaf 2.5%-Control	-3.43	-6.53	-0.32	**0.020**
1.2	Neem leaf 5%-Control	-3.44	-6.54	0.33	**0.020**
1.3	Titepati bark 2.5%-Control	-3.57	-6.67	-0.46	**0.010**
1.4	Titepati bark 5%-Control	-3.47	-6.57	-0.36	**0.020**
1.5	Titepati leaf 2.5%-Control	-3.63	-6.74	-0.53	**0.010**
1.6	Titepati leaf 5%-Control	-3.83	-6.94	-0.73	**0.004**
1.7	Titepati root 2.5%-Control	-4.56	-7.66	-1.45	**0.0002**
1.8	Titepati root 5%-Control	-3.64	-6.74	-0.53	**0.009**
1.9	Neem leaf 5%-Neem leaf 2.5%	-0.007	-2.78	2.77	1.000
1.10	Titepati bark 2.5%-Neem leaf 2.5	-0.14	-2.92	2.64	1.000
1.11	Titepati bark 5%-Neem leaf 2.5%	-0.04	-2.82	2.74	1.000
1.12	Titepati leaf 2.5%-Neem leaf 2.5%	-0.20	-2.98	2.58	0.990
1.13	Titepati leaf 5%-Neem leaf 2.5%	-0.40	3.18	2.38	0.990
1.14	Titepati root 2.5%-Neem leaf 2.5%	-1.13	-3.91	1.65	0.940
1.15	Titepati root 5%-Neem leaf 2.5%	-0.21	-2.99	2.57	0.990
1.16	Titepati bark 2.5%-Neem leaf 5%	-0.13	-2.91	2.64	1.000
1.17	Titepati bark 5%-Neem leaf 5%	-0.03	-2.81	2.75	1.000
1.18	Titepati leaf 2.5%-Neem leaf 5%	-0.19	-2.97	2.58	0.990
1.19	Titepati leaf 5%-Neem leaf 5%	-0.39	-3.17	2.38	0.990
1.20	Titepati root 2.5%-Neem leaf 5%	-1.12	-3.89	1.65	0.940
1.21	Titepati root 5%-Neem leaf 5%	-0.20	-2.98	2.58	0.990
1.22	Titepati bark 5%-Titepati bark 2.5%	0.10	-2.68	2.88	1.000
1.23	Titepati leaf 2.5%-Titepati bark 2.5%	-0.06	-2.84	2.72	1.000
1.24	Titepati leaf 5%-Titepati bark 2.5%	-0.26	-3.04	2.52	0.990
1.25	Titepati root 2.5%-Titepati bark 2.5%	-0.99	-3.77	1.79	0.970
1.26	Titepati root 5%-Titepati bark 2.5%	-0.07	-2.85	2.71	1.000
1.27	Titepati leaf 2.5%-Titepati bark 5%	-0.16	-2.94	2.61	1.000
1.28	Titepati leaf 5%-Titepati bark 5%	-0.36	-3.14	2.41	0.99
1.29	Titepati root 2.5%-Titepati bark 5	-1.09	-3.87	1.69	0.95
1.30	Titepati root 5%-Titepati bark 5%	-0.17	-2.95	2.67	0.99
1.31	Titepati leaf 5%-Titepati leaf 2.5%	-0.20	-2.98	2.58	0.99
1.32	Titepati root 2.5%-Titepati leaf 2.5%	-0.93	-3.71	1.85	0.98
1.33	Titepati root 5%-Titepati leaf 2.5%	-0.007	-2.78	2.77	1
1.34	Titepati root 2.5%-Titepati leaf 5%	-0.73	-3.51	2.05	0.99
1.35	Titepati root 5%-Titepati leaf 5%	0.19	-2.58	2.97	0.99
1.36	Titepati root 5%-Titepati root 2.5%	0.92	-1.86	3.69	0.99
2	**Mulching**				
2.1	Mulching-Control	-5.07	-6.74	-3.39	**0.0001**

Discussion

Neem and titepati plant extracts were effective in reducing activity of GALS. Fast movement was observed while spraying the neem and titepati plant extraction on GALS whereas only immobile and lethargic movements were observed in control and mulching. This difference may be due to the presence of molluscicidal effects of compounds found in the extracts of these plants (Jerkovic *et al*. 2003; Ploomi *et al*. 2009, Khdier 2012). Mulching was not effective compared to other treatments, however GALS exhibited repellent movements after several days. This delay may be a consequence of organic materials of neem and titepati plants and exposure of chemical compounds on the surface.

Spraying the neem leaf extraction on GALS resulted in their separating of their body from the shell and rapid movements for several minutes before becoming lethargic, we suggest due to molluscicidal effects (Ufele *et al*. 2013) which appear more pronounced with the 5% concentration. Their effects were noticed after 6-8 days of treatments at which time the stopped feeding and became desiccated. The GALS failed to lay eggs and died within 15 days of the experiment using 2.5% and 5% concentration of the neem leaf extract. A similar experiment was conducted with giant West African snails (*Archachatina marginata)* and air-breathing land snails (*Limicolaria aurora*) (Ebenso 2003), where neem extracts were also effective to control their activities. Though the active compounds in Neem are unknown. Ploomi *et al*. (2009) suggested azadirachtin, nimbidin, nimbidol, sodium nimbinate in neem may be involved.

A similar type of activity pattern including fast movement during extract spray time and then the GALS becomes lethargic was also noticed after spraying the titepati extract. It might be due to the presence of flavonoid, glycoside constituent, and high amount of caffeic acid derivatives such as hydroxybenzoic acid derivatives (Khdier 2012), benzaldehyde, camphor, Artemisia ketone (Khdier 2012), benzaldehyde, camphor, Artemisia ketone (Jerkovic *et al.* 2003), Artemisia alcohol, acetate in titepati plants (Khangholil & Rezaeinodehi 2008), which act for antimicrobial and antimalarial activities (https://malariaworld.org/blog/artemisia-ketone-phytosterols-and-lipid-metabolism). The higher the concentration the more effectiveness of plant extract was found in this study. For example, a 5% concentrated solution of titepati leaf was more effective than a 2.5% concentrated solution of titepati leaf on the activities of snails. The molluscicidal activities mostly varied according to the derived extract solution from different parts of the same plant and also snail's species (Khdier 2012). In this study, the activities of snails were mostly observed on titepati leaf extracts than titepati bark and titepati root. Titepati bark and titepati root had a similar molluscicidal effect on GALS but less effective than leaf. The GALS exhibits the faster movement on Titepati

leaf extract solution which was quite higher than using titepati bark and titepati root. But titepati extract did not control the egg laying process of GALS. However, all eggs failed to undergo further developmental process.

The lethargic movement was slightly higher in titepati bark and titepati root and was also a bit similar to the neem leaf extract solution. This might be due to the various monoterpenoids such as terpinen-4-ol, α- terpinene, β-pinene, linalool, borneol found in fresh leaves of titepatii extract (Hwang *et al*. 1985). The presence of monoterpenoids, hydrocarbon monoterpenes, and other active compounds in plant had higher toxic effect on snails and have significant molluscicidal activity (Pereira *et al*. 2020). Not only the snails, these monoterpenoids also repel the blood-sucking insect pest (Hwang *et al*. 1985). Titepati extract of fresh leaves is also used to control activities of potato tuber moth (Giri *et al*. 2013) and also to control red pumpkin beetle in summer squash (Neupane 1993). The extract's effectiveness varied according the parts such as leaf, bark and roots on species specific. Neem bark extraction is comparatively more effective than the leaf to control the activities of snails mainly *L. acuminate due to the presence of higher* toxic effects than neem leaf. Whereas neem leaf has a higher toxic effect than neem bark and more effective against air-breathing fresh water snails (*Indoplanorbis exustus)* (Singh *et al*. 2003). *S*imilarly, essential oil of neem (24h/LC50-18.35mg/L) has a less toxic effect on *C. deodara (24h/LC50- 5.82mg/L) than in L. acuminate (Rao & Singh 2001). Generally, the root has higher toxic effects than other parts in some plants, for example, essential oil from the rhizomes of Zingiber officinale* has negative effects on the reproduction of *L. acuminate*. It might be due to the presence of α-zingiberene, geranial, nerolidol, gingerol which are found in ginger (Barros Gomes *et al*. 2019).

The effectiveness of the plant extract treatments may also vary with the size/weight of the snails and concentration of the plant extract. Greater concentrations of plant extracts appeared to result in greater lethargic activities. Similar results also were observed using titepati root, bark, and leaf extracts. Ufele *et al*. (2013) suggested a 100% concentrated solution of neem leaf extract had less longevity of effectiveness than 70% and 50% concentrations; suggesting molluscicidal activities may also depend on the concentration of the plant extract.

Conclusions

Neem and titepati plant extracts markedly reduce GALS activities. Increased concentrations of extracts increased mortality of eggs and individual GALS. Titepati leaves, bark and root and neem leaves appeared to have molluscicidal effects against GALS by repelling the snails from the sources. Neem and titepati plant have various biological active compounds, therefore, these can be used as mulluscicides. Botanical

pesticides are safer for handling by humans and are biodegradable. However, the effects of these molluscicides on other species are unknown and we recommend additional research to test whether these effects are detrimental to other species as well as the efficacy of these plant extracts under natural conditions.

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