MOLLUSCICIDAL EFFECT OF NEEM AND TITEPATI EXTRACT ON GIANT AFRICAN LAND SNAIL (*Achatina fulica*)

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A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with special paper Ecology and Environment.

SUBMITTED TO

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself and has not been submitted elsewhere for the award for any degree. All sources of information have been specifically acknowledged by reference to authors or institutions

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RECOMMENDATIONS

This is to recommend that the thesis entitled "Molluscicidal effect of neem and titepati extract on Giant African Land Snail (*Achatina fulica*)" has been carried out by Ms. Rubina Thapa Magar for the partial fulfillment of Master's Degree of Science in Zoology with special paper Ecology and Environment. This is her original work and has been carried out under my supervision. To the best of my knowledge, thesis work has not been submitted for any other degree in any institution.

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Abstract

Invasive Giant African Land Snails (GALS) are herbivorous, voracious feeders, pose serious threats to native species, and that could affect the ecosystem and is considered as a snail pest. The various botanical plant has been used as a source of molluscicides to control mollusks pest. Many plant species have chemical compounds and using these plants or their extracts can be used to control the activities or to kill the targeted pest species. This study aimed to identify the effects of Neem (*Azadirachta indica)* and Titepati (*Artemisia vulgaris)* on the survival activities of GALS. Differently concentrated solutions of fresh neem leaf and titepati leaf, bark, and root were used for the experimental design to observe the activities of GALS. The solution for each part was prepared from collected plant samples which were dried and extracted with the help of a rotatory evaporator at the laboratory. The findings of this experiment showed that there was a significant effect of extract solution treatment on the activities of GALS in comparison to control treatment ($p < 0.0001$), and also the activities of GALS were varied between control and mulching treatments ($p < 0.0001$). In addition, the higher concentrated solution (at 5%) of neem leaf was more effective ($p < 0.0001$) than at 2.5% concentrated solution to control the activities of GALS. Similar, effects were also noticed on the titepati extract on the activities of GALS. The severe effects of these extracts were noticed within two weeks of the experiment. Eggs were completely damaged and the two GALS died within the treatment experiments. Both titepati leaf, bark and root, and neem leaf have molluscicidal effects on the activities of GALS that repels the snails away from the sources and kill them. Therefore, based on the effectiveness of Neem and Titepati plant extracts from this study, I recommend using these plants and their products to control the potential distribution of GALS to control the crop and vegetable damages from GALS.

1. INTRODUCTION

1.1 Background

Biological invasion is a major driver for ecosystem changes due to non-native organism, which has grown rapidly to increase its population densities and adapt to new habitats (Ehrenfeld 2010). Species invasion becomes widespread in the new environment due to their clonal growth, environmental changes, and effective nutrient storage capacity (Yu et al. 2016). Invasive alien species can be found in all main taxonomic groups including microorganisms, viruses, fungi, ferns, higher plants, invertebrates, and vertebrates and alters the ecosystem (Meyerson and Mooney 2007). Invasive species are harmful to the survival of other native species because of long-lived and voracious with able to spread very rapidly across landscapes (Kindlmann et al. 2011) and reduce the number of native plant species by encroaching habitat (Callaway et al. 2005). Currently, the rate of increase in the population of invasive species is rapid (Simberloff et al. 2013). Invasive species are one of the major drivers to the loss of native species, human economy, and accelerating the human health problem (Lockwood et al. 2013, Oduor 2013). For example, Golden Apple Snail (*Pomacea canaliculata*) is one of the severe invasive pest species in the tropic and sub-tropic area, and it damages most of the paddy in a short interval of time (Halwart 1994, Salleh et al. 2012). Biological control is also used to keep the harmful snails at bay. According to (Aditya and Raut 2002) Spike-topped apple snails (*Pomacea bridgesii*) were applied as a biological control against fresh-water snails (*Indoplanorbis exustus*) via egg predation. However, due to its high resistance and reproductive rate of invasive species the biological control method was not properly effective (Dong et al. 2012, Ip and Qiu 2017).

Around 300,000 vascular plants were noticed to invade 10% of the world's habitat (Richardson et al. 2000). Altogether 282 alien species (flora: 218; fauna: 64) are recorded from Nepal (Siwakoti and Shrestha 2014, Budha 2015, Joshi et al. 2017). Among 64 alien faunal species, seven were a mammal, six birds, and 19 fish, 22 Arthropods, nine Mollusks and Platyhelminthes (Budha 2015). Among the faunal species, the Giant African Land Snail (GALS; *Achatina fulica*) is one of the most destructive invasive species for Nepal (Budha 2015). The GALS is categorized as one of the World's Worst Invasive Alien Species in 2000 (Lowe et al. 2000), poses threats to native biodiversity and humans (Budha 2015). The

increased rate of distribution and risk associated with this invasive species has been generally escalating due to rapid human population growth and related activities through environmental disturbances (Pimentel et al. 2001) and human infrastructure developments including road (Adhikari et al. 2020). In most of the countries, the GALS is exported as food sources and as a pet from East Africa (Fontanilla et al. 2014). As a result of human mobility, the GALS arrived in Nepal through the open space between Nepal and India (Budha and Naggs 2008) and was reported from low land and mid-mountain regions of Nepal (Adhikari et al. 2020).

Giant African Land Snails damage crops and native species massively (United States Department of Agriculture 2002), and transmit pathogens (Cowie et al. 2009, Moreira et al. 2013). Due to the wider and rapid potential distribution of GALS (Adhikari et al. 2020) and the damage they caused, the control of this species is necessary. To reduce the adverse effects of GALS on an agricultural field, people kill the snails and their eggs manually (Yu et al. 2002). However, these methods are not effective and environment friendly, because people kept the bulk mass of snails on the road to be killed by running vehicles. People used pesticides/insecticides to control the GALS and the process is rapidly increasing globally, however, the effects of these pesticides/insecticides on other species and human health are found more detrimental (Khare et al. 2019). A large number of chemical pesticides are used to minimize pests but eventually, they can destroy other beneficial organisms (Bernays and Chapman 2001). To avoid the potential effects of pesticides/insecticides, it should be better to use chemical compounds found in some plants. Some plants can be used as a natural chemical remedy in molluscicidal activity (Abera 2003) because there is no harmful effect on other animals and the environment as well. The natural plant products of Neem (*Azadirachta indica*) and Titepati (*Artemisia vulgaris),* which are biodegradable, environment-friendly, and highly effective (Raut et al. 2014) can be used to control the invasion of GALS.

Neem tree belongs to the family Meliaceae, is an evergreen plant and found mostly tropical (Raguraman and Singh 1999), and subtropical regions of Africa, Australia, and Latin America (Nisbet 2000). Neem plants are used for traditional medicine as a source of many therapeutic agents and are widely used in the Indian continental (Raut et al. 2014). Neem plant contains a high degree of biologically active compounds azadirachtin, salannin, nimbin,

nimbidin (Nisbet 2000, Abdel-Rahman 2017), azadiradione, epiazadiradione, and imonoids (Singh et al. 1996) which has been preferred as antipyretic and tremendous therapeutic potential (Ley et al. 1993, Bansal et al. 2010), agricultural and ethnomedicinal value (Paul et al. 2011). Neem leaves have antibacterial properties (Quelemes et al. 2015) used for controlling airborne bacterial contamination (Khan and Aslam 2008). Neem extract has antioxidant, antimalarial, and antifeedant (Khattak et al. 2006), anti-carcinogenic, anti-mutagenic, anti-inflammatory, antiulcer, and anti-diabetic properties (Talwar et al. 1997). In addition, the seed extract contains azadirachtin that controls the harmful insect pest (Abudulai and Shepard 2003) and is also used to control phytopathogenic and entomopathogenic fungi (Depieri et al. 2005). Boiled neem leaf water was also be used to cured skin problems, cleaning wounds, and swelling (Bojar and Holland 2004). Abudulai and Shepard 2003 Depieri et al. 2005

In Nepal, Titepati is famous for bitter leaves and is commonly known as mugwort or wormwood (Xie et al. 2008). This plant has great medicinal values and its leaf juice is used to be cured digestive problems, intestinal worms (Tamang 2003), nose bleeds, and neurological disorders (Hussain and Hore 2007). Titepati leaf has also been used for pain relief, fever, diuretic agent (Khan et al. 2015), and skin allergic treatment (Bassett et al. 1978). Titepati plants have a huge number of flavonoids, terpenoids, saponins, and polysaccharides (Xie et al. 2008). *Artemisia* species can be used to minimize the growth of grain beetle larvae, pest repellent, fumigant (Wang et al. 2006), blood-sucking insects mosquitos, and anopheles insect larvae (Hwang et al. 1985).

In Nepal, both the Neem and Titepati plants are used to control insect pests but no more practice is done to control harmful snail pests including GALS. In Nepal, GALS were rapidly spreading from low land areas to the mountain regions (Adhikari et al. 2020), and have become more destructive on the agricultural field (Budha and Naggs 2008). These two plants' chemicals can be used as molluscicides (Abera 2003) to control GALS. However, the detailed information on the effectiveness of molluscicides from the extract of Neem and Titepati is little known. Therefore, this study aimed to identify the effectiveness of Neem and Titepati plant extract to control the activities of GALS.

1.2 Objectives

The general objective of the proposed study was to determine the effectiveness of natural pesticides to control the invasive Giant African Land Snail.

The specific objectives are:

- To understand the effectiveness of Neem plant on Giant African Land Snail.
- To test the effectiveness of the Titepati plant on Giant African Land Snail.

1.3 Statement of problems

Giant African Land Snail is one of the highly destructive invasive faunal species in Nepal (Budha 2015). People are suffering from this species, and searching for chemical pesticides to control the species. However, the use of chemical pesticides potentially affects human health, native flora, and fauna, and damages the environment (Khare et al. 2019). As compared to chemical pesticides, plants and their products (natural plant products) are more effective to control the agriculture pests like GALS(Abera 2003). These products are environmentally friendly and do not affect human health. The utilization of natural plants helps to minimize the occurrence of pests and to improve crop production. Neem and Titepati plants have specific potential to control the activities of harmful pests, different fungus (Depieri et al. 2005, Raut et al. 2014), and insects (Abudulai and Shepard 2003). Different types of essential oil present in both Neem and Titepati plants are useful for soil burn fungi by the extraction process. However, little is known on the effectiveness of these plant products on GALS, therefore the proposed study aimed to provide the usefulness of plant products/extracts to control the rapid spreading of invasive GALS. The findings of this study can be used to develop the policy for the industrial production of plant extract to control the GALS.

1.4 Research limitations

Due to sample limitations, the concentration of Neem and Titepati plant extract was only 2.5% and 5%, respectively. During the laboratory procedure of plant extraction, the concentration of neem bark and root was not possible to be collected for this experiment. Because sample preparation and laboratory work took too long, the experiment was started after the monsoon.

2. LITERATURE REVIEW

The giant African land snail is one of the major crop pest (Raut and Baker 2002, Budha and Naggs 2008), primarily found in moist terrestrial habitats, a seasonal animal that can be found in greater numbers during the rainy season, particularly in habitats with green vegetation (Okafor 2009). GALS are polyphagous, voracious, and feed on a wide range of agricultural and kitchen garden crops (Budha and Naggs 2008). They are most commonly found attacking young plants (Kumar et al. 2018). Specially, snail damages plants by their slime, faeces, and feeding contaminated on harvested plants (South 1992). Snails have high rate of prolific fecundity compared with other livestock and are capable of continuous egg laying several times over a period after a single mating (Ebenso 2003). Snails that have reached maturity, or have a body weight of 110 g to 125 g, can begin laying eggs (Okan et al. 2008). Because of their strong reproductive potential of land snails, a single snail can quickly proliferate in the field, making population management challenging (Kumar 2020).

Snail control was measured using a variety of techniques, including mechanical control (Yu et al. 2002), biological control (Aditya and Raut 2002), bait formulation (Roda et al. 2018), and the application of plant derivatives (Singh et al. 1996, Ebenso 2003, Khdier 2012, Prabhakaran et al. 2017). Extracts from a range of plants have been shown to have molluscicidal effects on snails and was more effective than other control process. In the current situation, a researcher is concentrating on the utilization of plant-derived compounds as molluscicidal agents against hazardous snails like GALS. Plant derivatives with molluscicidal activity can be more effective and safer than synthetic applications (Singh et al. 1999, Mandefro et al. 2017). Due to the compound composition found in the plant, the molluscicidal action of essential oils of plant derivatives becomes difficult to resist for snail species (Salama et al. 2012).

2.1 Neem plant

The plant derivatives in the Neem plant, such as leaf, bark, cake, and neem oil, have a high poisonous effect on the freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus*, as well as higher repelling effects (Singh et al. 1996). This could be due to the presence of plant derivatives such as azadirachtin, nimbidin (Nisbet 2000), azadiradione, epiazadiradione, and imonoids (Singh et al. 1996). Furthermore, a higher concentration of Neem leaf extract

disrupts the feeding behavior of air-breathing land snails (*Limicolaria aurora*), as well as the Giant West African Snail (*Archachatina marginata*) and *Limicolaria aurora* (Ebenso 2003). (Abdel-Rahman 2017). Depending on the exposure procedure, the toxicity of a chemical molecule on plant extract (Ebenso 2003, Khdier 2012, Pereira et al. 2020).

The molluscicidal action of the binary and tertiary combinations of Neem plant extract significantly reduces the snail pest freshwater Snail (*Pomacea maculata*) (Prabhakaran et al. 2017). This type of experiment was carried out on *L. acuminate* in Bellyache Bush (*Jatropha gossypifolia*) and drastically lowered fertility, hatchability, and survival rate (Yadav and Singh 2014). Similarly, *Lymnaea acuminata* is poisoned by the essential oil of the Neem plant. However, a binary and tertiary combination of *A. indica*, *Embelia ribes*, piperonal, and MGK-264 is more molluscicidal than a single Neem plant extract (Rao and Singh 2001). The combination of azadirachtin from neem seed with acetone, ethanol, and methanol in various concentrations has a high harmful effect on Golden Apple Snail (*P. canaliculata*) mortality (Massaguni and Latip 2015). The neem-based NeemAzal-T/S, which includes 1% azadirachtin, Trifolio-M, and GmbH, has a high repelling ability against Land Snail (*Arianta arbustorum*), reducing the population of snails on agricultural fields when sprayed in natural conditions (Ploomi et al. 2009). (Singh et al. 2004) reported that a Poinsettia (*Euphorbia pulcherima*) and Asthma-plant (*E. hirta*) extract solution was highly effective in changing the behavior of freshwater snails. The extract solution of *E. pulcherima* and *E. hirta* kills *L. acuminata* and *Indoplanorbis exustus*. The Devils Tree (*Alstonia scholaris*) stem bark extract contains a high amount of anti-cholinesterase, which controls the *L. acuminate* (Singh and Singh 2003).

On insects that control the nymphs of the Cockroach (*Periplaneta americana*), neem plant extract has repellent, antifeedant, and growth inhibitory properties (Ukoroije and Bobmanuel 2019). Aqueous Neem leaf extract has a high efficiency in reducing the number of pod-sucking bugs on Cowpea (*Vigna unguiculata*) (Degri et al. 2013). According to (Khattak et al. 2006), neem oil and neem seed extract were highly toxic and had a high chronic effect on Whiteflies (*Trialeurodes vaporariorum*), Thrips (Thysanoptera), and Jassids/Treehoppers (*Eurymeloides bicincta*). The leaf and bark extract of the Neem plant (*A. indica*) has anti-bacterial, anti-microbial, and anti-malarial properties that inhibit the growth of both gram-

positive and gram-negative bacteria, *Vibrio cholera* and *Bacillus subtilis* (Raut et al. 2014), as well as MRSA biofilm, planktonic formation on water, and *Schistosoma mansoni worms* (Quelemes et al. 2015).

2.2 Titepati plant

Titepati leaf (*Artemisia vulgaris*) contains various monoterpenoid compounds which act as an anti-malarial activity that can repel the insect's pest, such as bloodsucking insect, mosquitos, and sand flies (Hwang et al. 1985). Similarly, essential oil of Titepati leaf has strong fumigant activity and had significantly decreased the larvae pupating and pupae of Red Flour Beetles (*Tribolium castaneum*) (Wang et al. 2006). Presence of (E)-β-farnesene, (Z)-en-yn-dicycloether, (Z)-β-ocimene component on essential oil of Mugwort (*Artemisia absinthium*) which was highly effective on the larva and controls these mosquitos vector such as *Anopheles stephensi, Anopheles subpictus, Aedes aegypti, Aedes albopictus, Culex quinquefasciatus,* and *Culex tritaeniorhynchus* (Govindarajan and Benelli 2016).

The Piper Fruit *(Piper guineense*) has traditional therapeutic qualities, is non-toxic to humans, and kills the intestinal parasite, according to (Ukwandu et al. 2011). Fresh-water snails such as *Biomphalaria pfeifferi* are caustic to piper's fruit extract, which also induces ovipositional consequences in the species. Thorn Tree (*Balanites aegyptiaca*) has high levels of toxic materials such as glycosides, steroids, and terpenoid, which reduce of survival rate *P. canaliculata* (Vijay 2010). On air-breathing snails *Subulina octona*, the Spanish needle (*Bidens pilosa*) plant extract displays molluscicidal and ovicidal characteristics, affecting the hatchability and survival of offspring hatched from exposed eggs, as well as a reduction in survival and growth in snails exposed (Souza et al. 2013) Similar types of the result were found on Argel (*Solenostemma argel*) against *B. Pfeifferi* (Ahmed et al. 2014). This is due to the presence of saponins, tannins, flavonoids, alkaloids, terpenoids on both the Spanish needle and Argel plant (Souza et al. 2013). *Psychotria microphylla* has oxidative stress and biochemical alterations in Giant African Land Snail that disrupt the antioxidant and pro-oxidant level and damage the macromolecules inside the tissue which kills the *A. fulica* (Atama et al. 2019). Zinger rhizomes (*Zingiber officinale*) extract affect the behavioral changes of freshwater snails (*Biomphalaria glabrata*) (Barros Gomes et al. 2019). Excessive loss of body fluid of the snail’s body was observed in dry dust and slurry form of silica and

tend to became inactivate and killed snails GALS (Selvi et al. 2015). The Java-plum (*Syzygium cumini*) plant, according to (Dias et al. 2013), is very good at reducing the number of air-breathing freshwater snails like *Biomphalaria glabrata*. This is owing to the high monoterpene content of *S. Cumini*. In a comparable study, similar results were achieved (Mandefro et al. 2017).

3. MATERIALS AND METHODS

3.1. Study area

Giant African Land Snail is widespread in the study area and is easily available in agricultural and forest land in both rural and urban areas. Therefore, the experimental setup for this study was carried out in agricultural land, Sanopalate-4 Bharabise, Sindhupalchowk Nepal (Figure 1). The experiment was conducted between 31 August to 4 October 2019 in the already constructed shed.

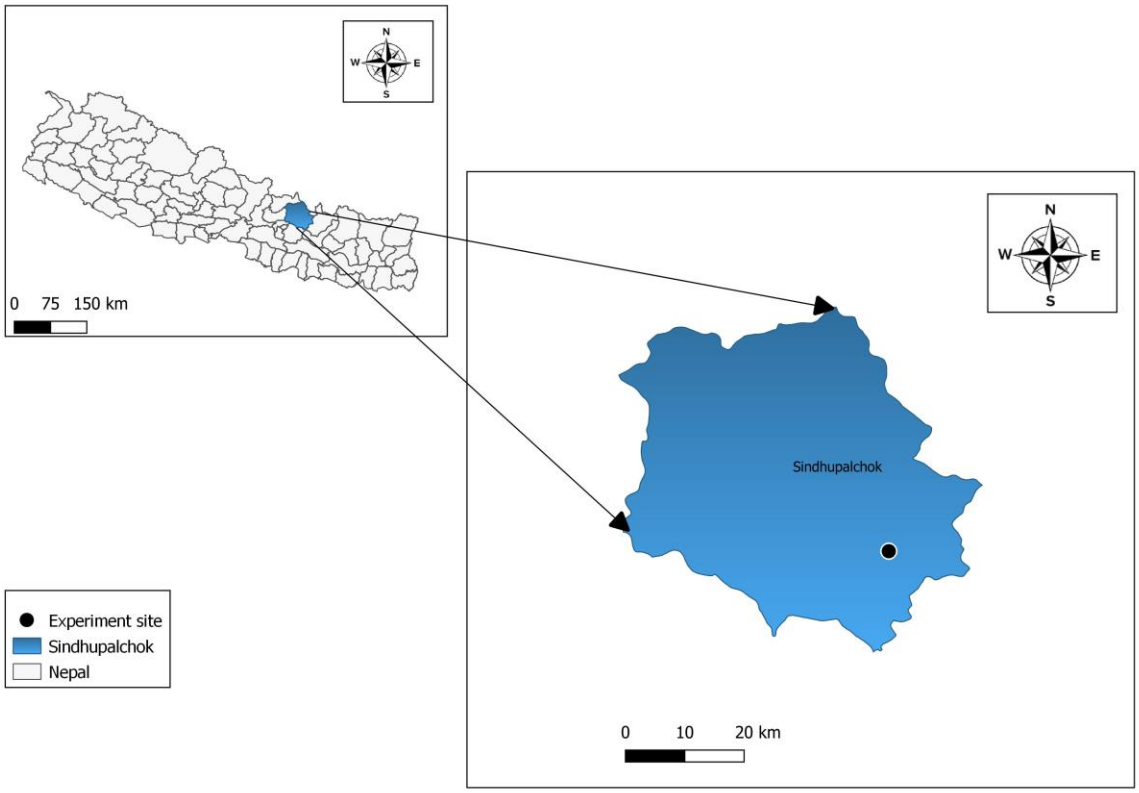

Figure 1 Experiment location at Sindhupalchowk, Nepal.

3.2 Sample preparation

Two main plant sample sources such as Neem and Titepati plant were used to test whether the content of these sources controls the activities of GALS. Two major different media were used for the experiment.

3.2.1 Mulching

The fresh leaf and stems of both plants were collected from the natural field. Leaves and a small stem of these plants were chopped and stored in a shaded area to prevent these from dry before use.

3.2.2 Solution

Leaf, stem, and root of neem and titepati plants were collected from the natural field separately. Two different concentrated (2.5% and 5%) solution for each plant source were prepared. Plant component were extracted by following the method of (Raut et al. 2014) in which each sample specimen was made dried and grind to a fine powder. The powder was used for preparing the treatment solution.

3.3 Laboratory work

The laboratory work was conducted at the Central Department of Zoology, TU, and Central Department of Biotechnology TU, Kirtipur, Kathmandu Nepal. In the laboratory work, the plant extraction method was followed by:

3.3.1 Dry method

The collected plant samples were dried at room temperature, cut into pieces, and further milled to a powder form. In a conical flask, 376 grams of neem leaf powder was added, along with 1500 ml of 100% ethanol, and covered by aluminum foil (Figure 2, a). The solution was shaken well and left it until the powder was fully wet for 72 hr at room temperature. The same procedure was applied for titepati (leaf, stem bark, and root) powder which was placed separately on the different conical flasks. The filtrate solution of each sample and kept it in new glass bottles separately. Quantity of neem leaf, titepati (leaf, bark and root) and volume of ethanol were taken differently.

3.3.2 Extract preparation

The solvent was evaporated from samples by using a rotatory evaporator to obtain the extract. The extract was kept on a falcon tube and stored at 4°C for 4 days. From the crude extract of each sample, 2.5% and 5% treatment solution was prepared.

Figure 2 Different types of method used: a- Dry Methods; b-Extraction Preparation; c-Control; d- Mulching

Calculation:

1. Weight of neem leaf powder = 376 gm

   Volume of 100% ethanol = 1500 ml

   Volume of neem leaf filtrate solution = 856 ml

   Weight of neem leaf extract = 35.857 gm

Weight of yield % (final weight %) = (weight of extract ÷ weight of plant powder) ×100%

= (35.857÷375.91) ×100%

= 9.53%

2. Weight of titepati leaf powder = 182.70 gm

Volume of 100% ethanol = 450 ml

Volume of titepati leaf filtrate solution = 272 ml

Weight of titepati leaf extract = 21.153 gm

Weight of yield % (final weight %) = (weight of extract ÷ weight of plant powder) ×100%

= (21.15 ÷ 182.70) ×100%

= 11.57%

3. Weight of titepati bark powder = 309.88 gm

Volume of 100% ethanol = 1500 ml

Volume of titepati bark filtrate solution = 810 ml

Weight of titepati bark extract = 19.871 gm

Weight of yield % (final weight %) = (weight of extract ÷ weight of plant powder) ×100%

= (19.871 ÷ 309.88) ×100%

= 6.412%

4. Weight of titepati root powder = 185.55 gm

Volume of 100% ethanol = 450 ml

Volume of titepati root filtrate solution = 275 ml

Weight of titepati root = 10.832 gm

Weight of yield % (final weight %) = (weight of extract ÷ weight of plant powder) ×100%

= (10.832 ÷ 185.55) ×100%

= 5.837%

3.3.3 Treatment preparation

Different parts of Neem and Titepati extract solution were prepared as percent solution. After 4 days, the crude extract was ready for preparing the treatment. 2.5% and 5% treatment

solutions were prepared for the experiment. For the 2.5% and 5% concentrated solution, 2.5 gm and 5 gm of Neem leaf extract were weighted and mixed into 97.5 ml and 95 ml of distilled water in neat and clean glass bottles. The same process was applied to Titepati's extract to make different treatments of leaf, bark, and root. The prepared solution was stored in the refrigerator for 4 days. Altogether eight treatments were prepared in laboratory condition**.**

3.4 Experiment

The experiment was set up in an already constructed shed at an agricultural field near to human settlement. Altogether 11 net box (50 X 30 X 45) cm^3 were used for the experiment. Out of 11 net boxes, 8 net boxes were used for the experiment of different concentrations of titepati and neem extract solution, 2 boxes for mulching samples, and 1 box for control treatments. Each box was filled with loamy soil to keep them in natural condition for the GALS under the ground (Figure 2, c). Three individuals of GALS were kept in each net box and provided abundant food (vegetable leaf: cabbage, cauliflower, etc.) and sprayed water. Each net box was covered by a transparent net that did not let them out of the box. These boxes with snails have left them for one week and noticed their health condition and their activities. After confirming the healthy condition of the GALS, the experiment was performed over on them.

3.4.1 Using treatment

Treatments of prepared extract solution were spread on GALS in a separate box once a day at 14.00 hrs. Usually, 10 ml of 5% neem extract solution was sprayed on the body surface of three snails in each of the separated boxes and periphery of the snail by maintaining a 20 cm distance away from the snail. A similar process was continued to the remaining neem leaf 2.5% concentrated solution and both 2.5% and 5% concentrated solution of titepati leaf, bark, and root. The snail's activities was observed at the interval of 20 minutes before and after spraying the treatment solution from 7.00 to 18.00 hrs. The activities such as lethargic, immobile, and fast movements were observed and were defined in Appendix 1 and 2. To extra behavior, distance covered by snails, number of egg deposition, and the possibility of egg fertility was observed during the treatment period.

3.4.2 Using mulching

Neem plants (leaf or stem) were placed on the soil into the net box. Healthy and mature snails were collected from the agricultural field and 3 healthy snails were kept on a net box (Figure 2, d). The movement of each snail has noticed whether the snail moves away from the mulching area, immobile, or no effect of neem mulching. However, the observation was focused on how long it takes to move a certain distance away from the source, how long it takes to become immobile and how long it takes to get death, how much time taken to destroy the laid egg. The same procedure was applied for titepati too.

3.4.3 Using control

In control boxes, sufficient food was provided regularly, and the activities of GALS were observed, their number of eggs, and larvae production were also noticed.

3.5. DATA ANALYSIS

The effects of different media i.e. mulching and extracts of neem and titepati on GALS' activities were compared using a two-way ANOVA test. We compared the efficiency of different treatments between the control group and different treatments, and also among the treatment groups. Besides, a multiple linear regression was calculated to understand the association of distance moved by the land snail during each survey day, using three methods (control, extraction, and mulching). All analyses were performed in R Program (R Core Team 2020).

4. RESULTS

4.1 Effects of Neem leaf on GALS

Different activities were observed by applying the neem and titepati extract solution on GALS and the activities were categorize as fast movement, immobile, and lethargic (Figure 3). Giant African Land Snails had spent more time in lethargic movement in all treatments (Figure 3). Both the neem leaf extraction at 2.5% and 5% concentrated solution had a high lethargic effect. Giant African Land Snails had spent less time in fast movement on neem leaf at 5% solution rather than on neem at 2.5% concentrated solution (Figure 3) and had a significant difference between the movements of GALS ($p < 0.0001$; Table 1). GALS mortality was proportional to the concentration of neem leaf extract solution. The death rate of snails increased when the concentration was raised. Small snails having 50 gm of weight died in a 2.5% concentrated neem leaf extract solution, whereas two snails died in a 5% concentrated solution having 50 gm and 75 gm of weight, respectively.

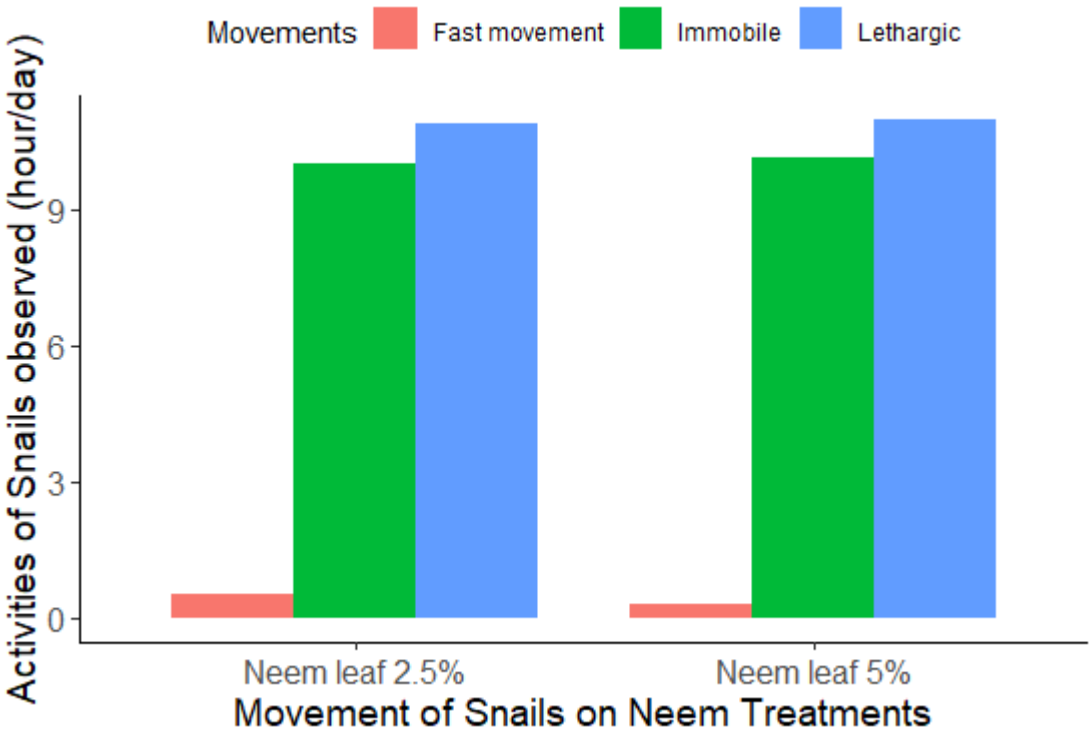

Figure 3 Movements of Giant African Land Snails on Treatments of Neem Leaf

4.2 Effects of Titepati plants on GALS

4.2.1 Effects of Titepati leaf on GALS

The GALS at 2.5% concentrated solution of titepati leaf showed a fast movement but for times than the titepati leaf at 5% concentrated solution (Figure 4). On the other hand, there are no changes in lethargic movement on both treatments (2.5% and 5% concentrated solution), however, a significant difference was found between the movements of GALS between these two concentrated solutions ($p < 0.0004$; Table 1).

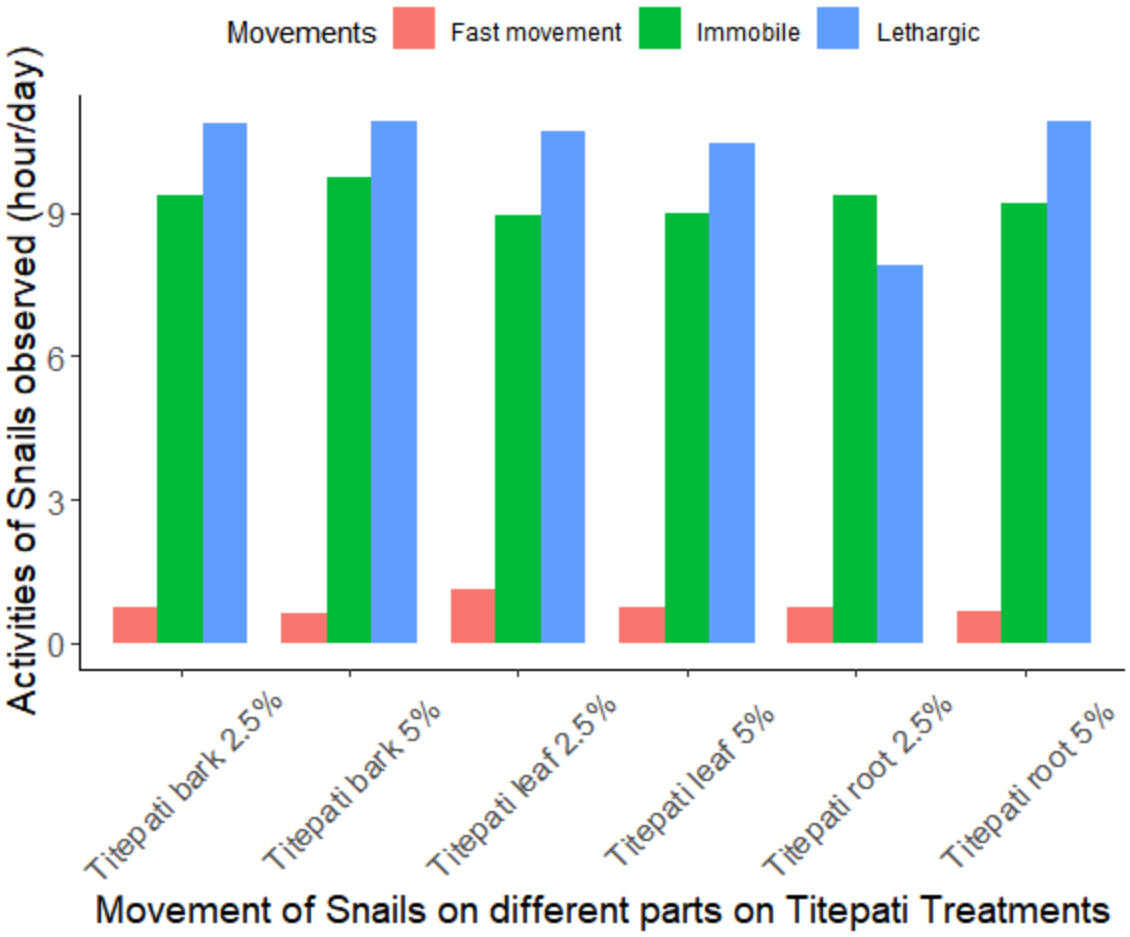

Figure 4 Movements of Giant African Land Snails on different Treatments of Titepati Plants

4.2.2 Effects of Titepati bark on GALS

Similar movement patterns were also observed in both titepati bark solutions i.e. lethargic and immobile activities were highly observed in titepati bark in both 2.5% and 5%

concentrated solution, and movement was controlled at 5% concentrated solution than at 2.5% solution ($p < 0.0001$, Figure 4; Table 1).

4.2.3 Effects of Titepati root on GALS

The fast movement or very few movement activities was quite similar on both 2.5% and 5% concentrated solution of titepati root (Figure 4). Lethargic movement was found higher on a 5% solution of titepati root extract than at 2.5% concentrated solution (Figure 4), and movement was controlled at 5% concentrated solution than at 2.5% solution ($p < 0.0001$; Table 1).

4.3 Effects of Control, Extraction, and Mulching on GALS

On the different events control, extraction, and mulching, a high variation in the movement activities of GALS was noticed. The fast movement was highly observed on the extraction solution (Figure 5). The high lethargic movement was observed in the presence of mulching while the less lethargic movement was observed in control and extraction experiments (Figure 5). The GALS spent a long time with immobile activity on control treatments rather than extraction and mulching treatments (Figure 5). A strong association of control groups with extraction groups was noticed ($p < 0.0001$; Table 1).

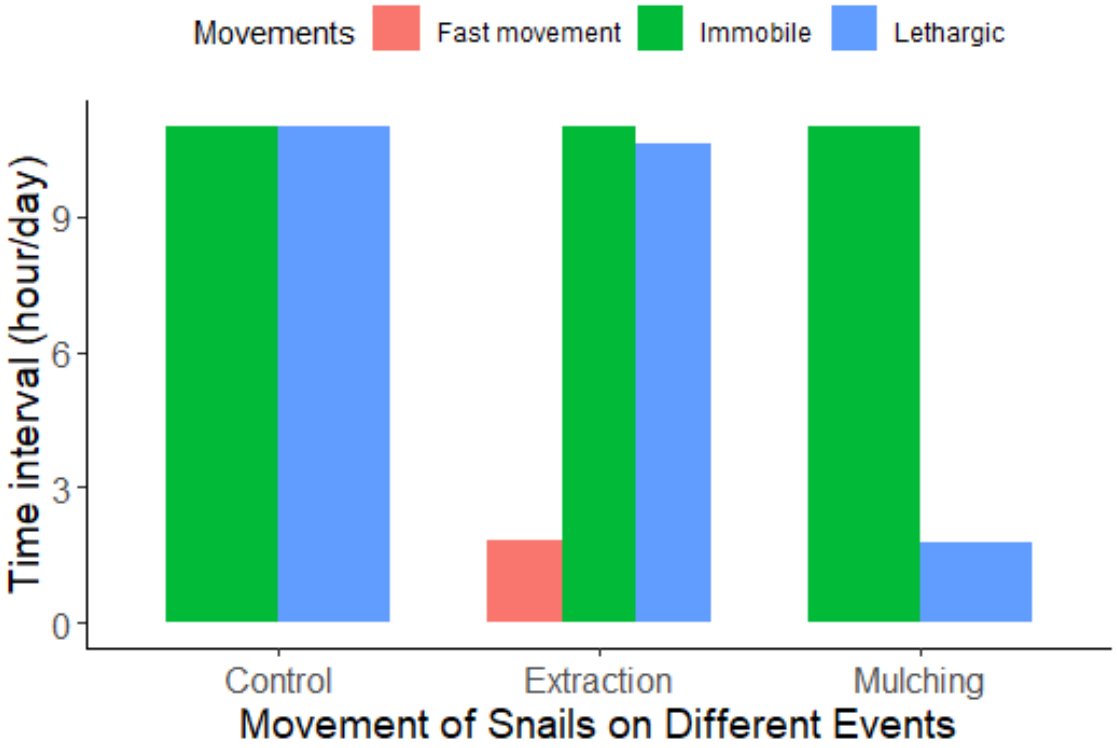

Figure 5 Activities of Giant African Land Snails on different treatment Events

4.4 Comparisons of GALS activities between Neem and Titepati extract

As compared to the neem and titepati's extract solution, the GALS had spent lesser time in fast movements on both neem and titepati treatments than immobile and lethargic movements, on using 5% concentrated solution between neem and titepati leaf (Figure 3-4). The immobile movement was observed for a quite long period when GALS were exposed to Titepati leaf at 2.5% concentrated solution compared to neem leaf at 2.5% concentrated solution (Figure 3-4; Table 2). Fast movements were quite similar on both the titepati bark and titepati root at 2.5% solution and higher on titepati leaf at 2.5% concentrated solution, but no differences between the 2.5% solution of titepati leaf, bark, and root.

4.5 Egg deposition by GALS

GALS on titepati leaf treatments decreased the number of eggs deposition in each egg deposition time on different days, and the eggs did not fertilize. The egg deposition of GALS is not controlled in the control treatment, and the number of egg laying processes was almost

the same from the start of the experiment. The egg hatchability of the GALS is affected by both the concentration of neem leaf extract that could not lays egg in the whole experiment.

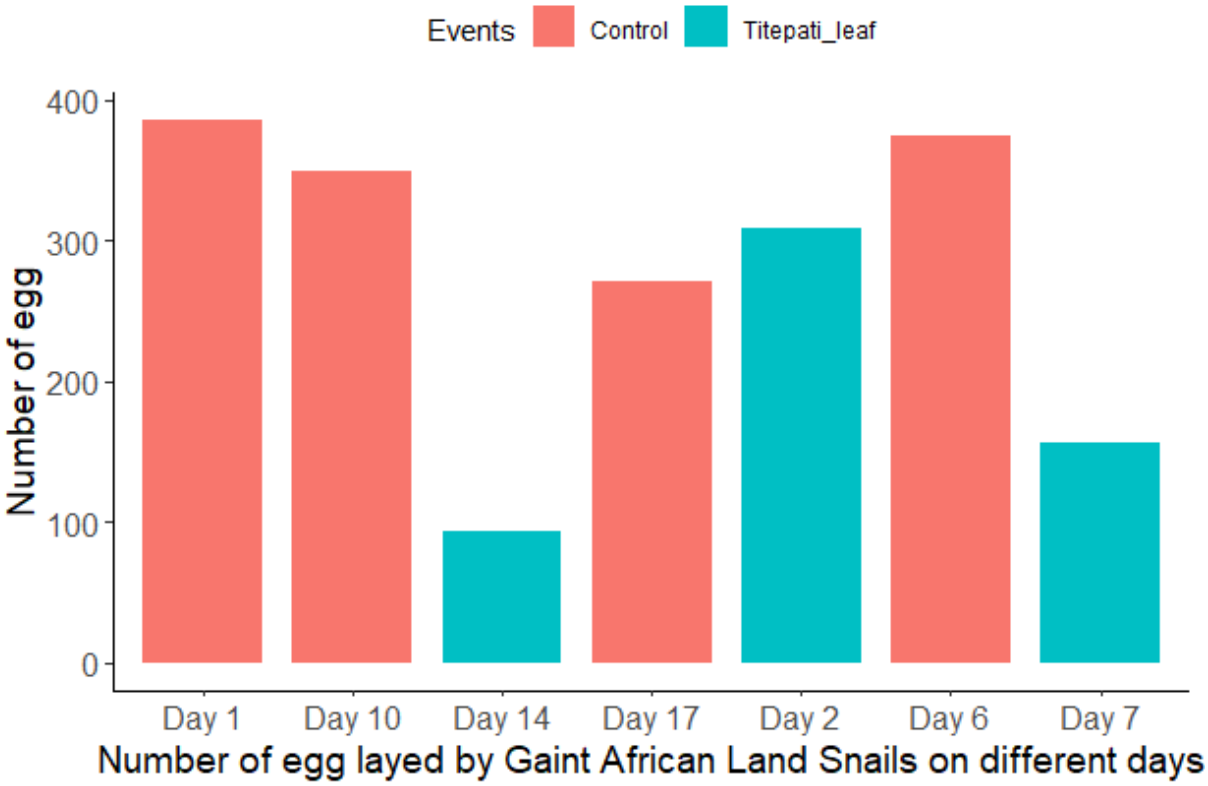

Figure 6 Egg laying process by Giant African Land Snails on different treatments

4.5 Distance moved by GALS

The average distance moved by GALS was decreased by increasing the number of days of treatments. The average distance traveled was highly associated with extraction ($p < 0.001$; Table 2). Compared to starting days, between day 14 to day 16 the average distance moved by the GALS showed a negative association ($p < 0.001$; Table 2, Figure 7).

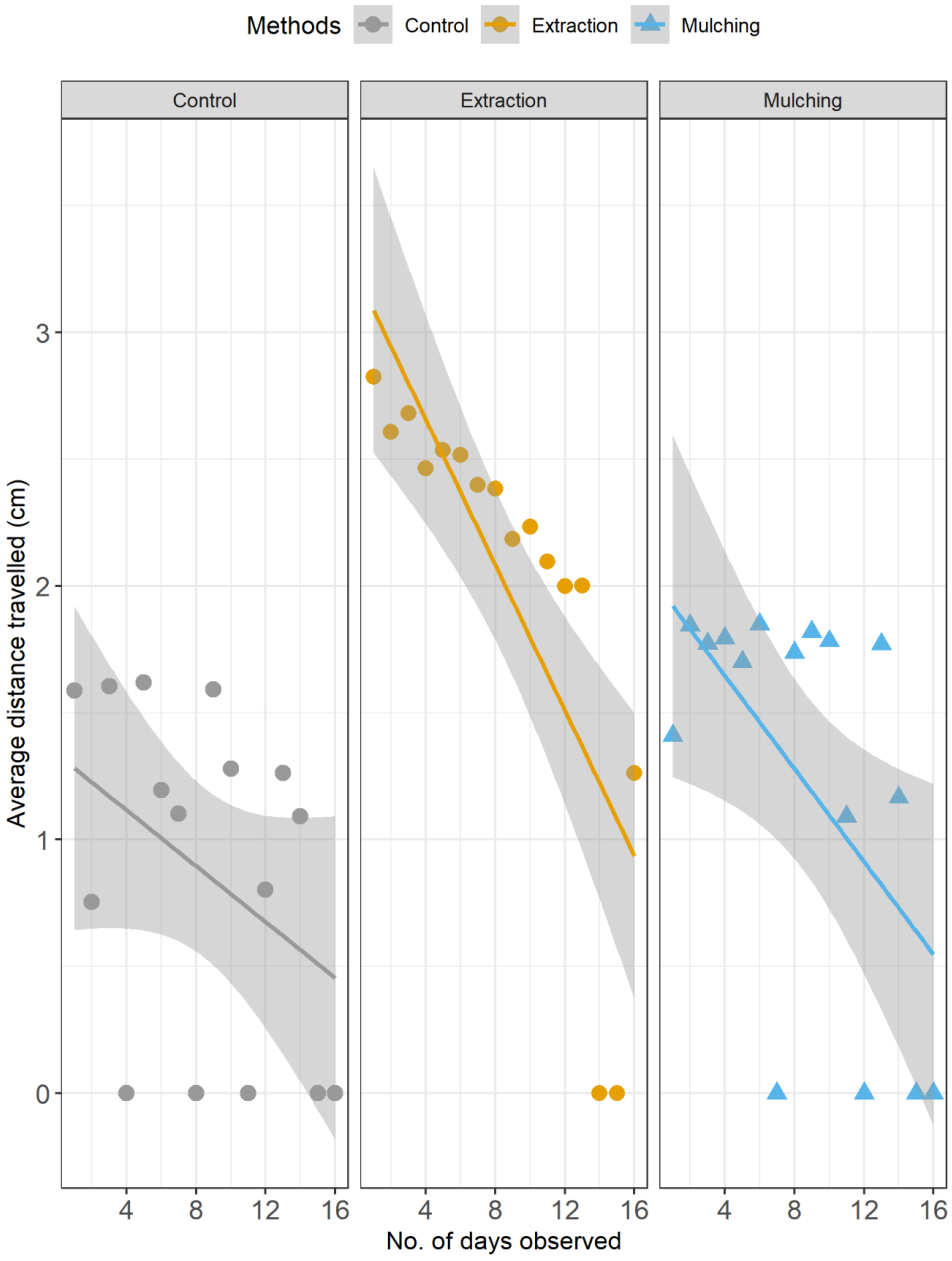

Figure 7 Average distance moved by Giant African Land Snails in each day

Table 1 Comparison of the efficiency of GALS at different treatments. The difference was compared at a 95% Confidence Interval, and the values in bold are indicated as significant. The values are in minutes.

Experiments	Activities	Df	Sum sq	Mean sq	f	P
Titepati root (2.5% and 5% solution)	Movements	2	99.55	49.78	29.929	**0.0323**
	Treatments	1	1.27	1.27	0.765	0.474
	Residues	2	3.33	1.33		
Titepati leaf (2.5% and 5% solution)	Movements	2	106.70	53.35	2329.067	**0.0004**
	Treatments	1	0.06	0.06	2.622	0.2468
	Residues	2	0.05	0.02		
Titepati bark (2.5% and 5% solution)	Movements	2	1965.5	982.7	1738.996	**< 0.0001**
	Treatments	7	0.2	0.2	0.437	0.511
	Residues	77	43.5	0.6		
Neem leaf (2.5% and 5% solution)	Movements	2	2185.0	1092.5	2340.169	**< 0.0001**
	Treatments	7	0.0	0.0	0.002	0.9620
	Residues	77	35.9	0.5		
Mulching, and Control	Movements	1	40206696	40206696	38.104	**< 0.0001**
	Treatments	1	93866	93866	0.0829	0.7670
	Residues	46	48538372	1055182		
Extraction and control	Movements	2	1.251e+09	6.254e+08	64.939	**< 0.0001**
	Treatments	7	1.782e+09	1.782e+09	185.043	**< 0.0001**
	Residues	77	7.416e+08	9.631e+06		
Leaf (neem, titepati 2.5%)	Movements	2	117.68	58.84	166.866	**0.0060**
	Treatments	1	0.06	0.06	0.172	0.7183
	Residuals	2	0.71	0.35		
Leaf (neem, titepati 5%)	Movements	2	124.33	62.16	198.904	**0.0050**
	Treatments	1	0.23	0.23	0.748	0.478
	Residuals	2	0.63	0.31		
Titepati (Leaf, bark, root) at 5%	Movements	2	178.59	89.30	1375.35	**< 0.0001**
	Treatments	2	0.2	0.1	1.524	0.32200
	Residuals	4	0.26	0.06		
Titepati (Leaf, bark, root) at 2.5%	Movements	2	149.75	74.87	75.32	**0.0006**
	Treatments	2	1.84	0.92	0.936	0.4641
	Residuals	4	3.94	0.99		

Table 2 A linear regression was performed to identify the association distance traveled by GALS on different mediums, such as Control, Extraction, and Mulching. The difference was compared at a 95% Confidence Interval, and the values in bold are indicated as significant.

Variables	**Estimate± S.E.**	**P**
Intercept	1.44 ± 0.36	**<0.001**
Day2	-0.20 ± 0.48	0.67
Day3	0.08 ± 0.48	0.87
Day4	-051 ± 0.48	0.28
Day5	0.01 ± 0.48	0.98
Day6	-0.09 ± 0.48	0.86
Day7	-0.77 ± 0.48	0.12
Day8	-0.57 ± 0.48	0.25
Day9	-0.08 ± 0.48	0.88
Day10	-0.18 ± 0.48	0.72
Day11	-0.88 ± 0.48	0.08
Day12	-1.01 ± 0.48	**0.04**
Day13	-0.26 ± 0.48	0.59
Day14	-1.19 ± 0.48	**0.02**
Day15	-1.94 ± 0.48	**<0.001**
Day16	-1.52 ± 0.48	**<0.005**
Extraction	1.14 ± 0.21	**<0.001**
Mulching	0.37 ± 0.21	0.09

5. DISCUSSION

Both neem and titepati plant extraction were effective in the activities of GALS. Their effectiveness was noticed in their movement, lethargic and immobile conditions on control, extraction, and mulching treatment on GALS. Fast movement or hyperactivity was observed while spraying the neem and titepati plant extraction on GALS whereas only immobile and lethargic movements were observed in control and mulching. It might 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). The mulching was not more effective promptly, however, the GALS showed the repellant activities after few days. It might be due to the decay of organic materials of neem and titepati plants and exposure to chemical compounds on the surface.

The fast movement was observed after spraying the neem leaf extraction solution on GALS for a few minutes. In the beginning, the GALS released the whole body part outside the shell and moved so fast away from the source for a few minutes then it became lethargic. Neem extract solution has a repellent effect on GALS may be due to molluscicidal effects. The immobile and higher lethargic movement were observed for most of the time rather than the fast-moving. It might be due to the higher toxic compounds present in the neem plants (Ufele et al. 2013). Therefore, the movement of GALS became slower or lethargic at 5% concentrated solution of neem leaf extract. Their effects were noticed after 6-8 days of treatments because from these days they fail to feed, and their body becomes dry. Finally, they failed to lay eggs and died within 15 days of the experiment on both 2.5% and 5% concentrations of the neem leaf extract. This type of experiment was also performed on Giant West African Snail (*Archachatina marginata)* and air-breathing land snails, such as *Limicolaria aurora* (Ebenso 2003). In these snails, the neem extracts were also effective to control their activities. It might be due to the presence of different active compounds azadirachtin, nimbidin, nimbidol, sodium nimbinate in the neem plant (Ploomi et al. 2009).

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 (Khdier 2012), benzaldehyde, camphor, artemisia

ketone (Jerkovic et al. 2003), Artemisia alcohol, acetate in Titepati (Khangholil and Rezaeinodehi 2008), which act for antimicrobial and antimalarial activities (Hwang et al. 1985, Lutgen 2013, Govindarajan and Benelli 2016). 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, Pereira et al. 2020). 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 the 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 the 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 monoterpenoid such as terpinen-4-ol, α- terpinene, β-pinene, linalool, borneol found in fresh leaves of titepati extract (Hwang et al. 1985). The presence of monoterpenoid, hydrocarbon monoterpenes, and other active compounds in the plant had a higher toxic effect on snails and have significant molluscicidal activity (Pereira et al. 2020). Not only the snails, but these monoterpenoid 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 to 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 freshwater snails, such as *Indoplanorbis exustus* (Singh et al. 1996). Similarly, 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 and Singh 2001). Generally, the root has higher toxic effects than other parts in some plants, for example, essential oil from the rhizomes of *Z. 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 plant extract also varies the size/weight of the snails and concentration of the plant extract. Increase the concentration of plant extract on the smaller weight of the snails, higher the lethargic activities of the snails were noticed. A 5% concentrated solution of neem leaf extract has taken less time in lethargic movement on 50 gm snails than 2.5% of neem leaf extract, while both 2.5% and 5% concentrated solution of neem leaf extract took longer time in lethargic movement on 150 gm snails than 50 gm of snails. A similar result was observed in titepati root, bark, and leaf extract too. According to (Ufele et al. 2013), a 100% concentrated solution of neem leaf extract has less longevity than 70% and 50% concentrated extract solution while decreasing the concentration of extracted solution has a longer life period of snails. Thus a molluscicidal activity also depends on the concentration of the plant extract.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The plant extracts Neem and Titepati have a strong influence on GALS activities and regulate movement. The increased mortality rate of eggs and individual GALS is caused by increasing the concentration of extract solution. Both titepati leaf, bark, and root, as well as neem leaf, contain molluscicidal properties that repel snails away from food sources. Plants like Neem and Titepati have a variety of physiologically active chemicals that can be utilized as molluscicides. Plant pesticides are easier to handle, are safer for human health, and are environmentally benign and biodegradable. As a result, future generations will be safer when using these plants and their products.

6.2 Recommendations

In this study, I found that the molluscicidal effects of both neem leaf and titepati leaf, bark, and root were more effective to control the movement activities of GALS. However, the effects of these molluscicides on other species in the field are unknown. Therefore, I recommend performing more researches to test whether these effects are detrimental to other species in the field.

In addition, this study was performed under the constructed shed so still I want to know the performance of these plants extract at a natural condition where we can face the extreme condition of precipitation.

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APPENDICES

Appendix 1 Response of Giant African Land Snails treated with different concentration solution of neem and titepati extract

Activities	**Description**
Immobile	No movement, body enclosed inside the shell
	Body covered with transparent layer to protect the body part
	Body covered with mucus outside the body part and shell
Crawling	Crawling on net and rod
Lethargic	Moved only tentacles and head part, not covered any distance
Fast movement	If the snails move rapidly and moved from the original positions

Appendix 2 Response of Giant African Land Snails treated with different concentration solution of neem and titepati extract. N (neem) and T (titepati) plant, n (number of GALS performing activities).

S.N	Extract	Day	n	Response	Died	n	Response	Died
				2.5%			5%	
3	T_leaf	1	3	feeding/moved inside the soil		3	feed normally	
		5	1	mucus secretion/2 stop crawling		3	feed normally, crawling	
		10	3	stop feeding/mucus secretion		3	stop feeding/mucus secretion	
		15	3	body covered with transparent layer		2	covered by transparent layer	

3	T_Bark	1	3	feed normally		3	feed normally	
		5	3	stop crawling		2	stop crawling	
		10	3	stop crawling and feeding		3	stop crawling	
		15	3	body covered by transparent layer		3	covered by transparent layer	
3	T_root	1	1	feeding and crawling		3	crawling	
		5	2	feeding, 1 stop crawling		3	stop crawling	
		10	3	stop crawling		3	stop feeding	
		15	3	covered by transparent layer		3	covered by transparent layer	
3	N_leaf	1	3	crawling		3	feed normally	
		5	2	mucus secretion		3	mucus secretion/stop feeding	
		10	3	mucus secretion		3	mucus secretion/stop feeding	
		15	2	body covered by transparent layer	1	1	body covered by transparent layer	2

Neem concentration	Snail Weight (gm)	Snail_dead
Neem_2.5%	50	1
Neem_2.5%	75	0
Neem_2.5%	150	0
Neem_5%	50	1
Neem_5%	75	1
Neem_5%	150	0

Photo Galleries

Photo plate 1

Photo plate 2

Photo plate 3