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RESEARCH ARTICLE

MOLLUSCICIDAL PROPERTIES OF THREE PLANT EXTRACTS WITH REFERENCE TO THE INTESTINAL ENZYME ALKALINE PHOSPHATASE ON THE SLUG *LAEVICAULIS ALTE* (FERRUSAC) (MOLLUSCA: GASTROPODA)

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ABSTRACT

Laevicaulis alte is one of the important pulmonate pests infesting the vegetable crops. Mostly they feed on vegetation available in and around their habitat. According to the malacologist point of view the slug *Laevicaulis alte* is not inferior to any other insect in damaging the agricultural crops. The controlling of this particular slug pest is important to minimize the infestation on agricultural land. For the past two decades, malacologists recommend the use of inorganic synthetic compounds such as copper sulphate, calcium sulphate, calcium cyanide etc. to control the molluscan species. But, all these compounds may also affect non–target organisms, soil texture and other organisms which are living in the soil. Moreover these compounds are carcinogenic, highly non-degradable in nature and in residual concentrations may cause skin rashes in human beings. In the recent years, malacologists are severely attempting environmentally safe plant based phyto - compounds to control various types of terrestrial molluscan pests. Hence, the present study has been initiated to evaluate the efficacy of some specific plant extracts as molluscicides in controlling the slug *Laevicaulis alte* by studying their impact on intestine process.

INTRODUCTION

The pulmonate which forms one of the subclasses of Gastropoda contributes largely towards the abundance of molluscan species. Some pulmonates were reported as pests of agricultural field in world wide. These terrestrial slugs commonly eat the vegetation available in their habitat (Runham and Hunter,1979).The slug have long been regarded worldwide as severe pests of agricultural land, horticultural production thereby attacking a vast array of crops (South, 1992; Godan, 1983). Snails and slugs are among the most bothersome pests in many gardens and landscapes. The high cost of imported synthetic compounds, along with increasing concern over the possible build-up of snail resistance to these compounds and their toxicity to non-target organisms, has given new impetus to the study of plant molluscicides (Kloos and McCullough, 1987). Several plants, such as *Tetraplura tetraptera* (Adewunmi, 1991), the well studied *Phytolacca dodecandra* (Lemma, 1970) and *Swartzia madagascariensis* (Sarda *et al*., 1986) have already been identified as potentially useful in control of the intermediate hosts of schistosomes. Many countries search for safe and low-cost molluscicides by using the naturally occurring plants that can be applied effectively in different habitats, and a large number of plant products with molluscicidal activity have been identified (Preetee and Singh, 2008; Adedotun and Alexander, 2008). The use of plants with molluscicidal properties appears to be a simple and inexpensive alternative to chemical molluscicides (Perrett and Whitfield, 1996), a simple, inexpensive and safe alternative (Tantawy *et al*., 2004; Bakry and Hamdi, 2007; Al-Daihan, 2010; Singh and Singh, 2010). *Laevicaulis alte* in its natural environment seems to thrive mainly on the green foliage where a variety of tender vegetables are available around their home range. The slug generally prefers vegetable material with plenty of water content. *Laevicaulis alte* is considered as one of the serious pests of agriculture lands. Many plant based substances were reported to be modulators of enzymes secreted by the slugs. Alkaline Phosphatase (AKP) is primarily found in the intestinal epithelium of animals and its major function is to provide phosphate ions from mononucleotide and ribonucleo-proteins for a variety of metabolic processes. AKP is involved in the transphosphorylation reaction and the midgut has the highest AKP activity as compared to other tissues (Sakharov *et al*., 1989).

Insecticidal properties of few plants have been reported earlier. The plant *Melia azedarach* has long been recognised as an insecticidal and medicinal plant all over the world (Awadh Ali *et al*., 2001; Kahn *et al*., 2001; Chistokhodova *et al*., 2002). The ethanol extract of *Sphaeranthus amaranthoides* is well known for its medicinal value for the treatment of blood disorder, filariasis, fever and piles (Kirtikar and Basu, 1971;

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Swarnalatha *et al*., 2009) *Sarcostemma brevistigma* Wight (Asclepiadaceae), commonly known as *soma* (Sanskrit) and *somlata* (Hindi), is a leafless, trailing shrub. A fraction of this plant extract has been reported to have antiallergic and antiinflammatory activities (Oberai, 1985; Saraf, 1988). So there is an impetus on research towards the use of plant based products as alternative environmental friendly compounds with minimal side effects, to control the molluscs and which are more effective and readily available(Jaiswal and Sing, 2009; Upadhyay and Sing, 2011; Sing *et al*., 2012). Hence the present study is initiated to investigate the effect of *Melia azedarach, Sphaeranthus amaranthoids* and *Sarcostemma brevistigma* leaf extracts on the digestive enzymes (Alkaline Phosphatase) of slug *Laevicaulis alte* and thereby to assess their potential molluscicidal properties.

MATERIALS AND METHODS

The slugs *L. alte* is collected from Thiruppalai in Madurai district particularly from shady moist places and also from the green lawns. The slugs were collected randomly from different places of the collection spot in order to get a good representation of different weight groups. The collected slugs were temporarily stocked in a perforated polythene bag and were brought to the laboratory, Department of Zoology, Raja Doraisingam Government Arts College, Sivagangai. There, the collected slugs were housed in normal wooden cages (40" x 22"), strewn with wet humus at the base as a medium in order to ease up the mobility of creeping animal. The top of the cages were covered with glass plates to facilitate easy observation. The animal in cages were maintained at low temperature and placed at a corner to avoid the radiation effects of sun light. In each cage, only ten slugs were maintained in order to avoid overcrowding. All the experimental cages were cleaned every day to prevent any infection due to deposition of excreta and also to remove the leftover food stuff. In order to prevent the fungal and bacterial growth, the humus medium in each cage was replaced twice in a week. The slugs showing signs of weakness and lethargy were removed periodically during pre-experiment stage. The plant leaves of *Melia azedarach, Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* were collected from the backyard of houses in and around Sivagangai. The mature slugs alone were considered for experimental purpose. The mature slugs were divided into seven groups with 10 slugs in each group. Of which one group is designated as control group, which does not receive any plant extract. The remaining six groups are considered as treatment groups. The six treatment groups were split into three groups comprising of doublets, and every doublets receive sub lethal dose I and II formulated from the plant extracts of *Melia azedarach , Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* respectively. The treatment regimen were divided into two, the sub lethal dose I and sub lethal dose II. In the sub lethal dose I , 10ppm concentration of *Melia azedarach* ., 1ppm concentration of *Sphaeranthus amaranthoides* and 30ppm concentration of *Sarcostemma brevistigma* were used for treatment. In sub lethal dose II, 5ppm concentration of *Melia azedarach* 0.5ppm concentration of *Sphaeranthus amaranthoides* and 15ppm concentration of *Sarcostemma brevistigma* were used for treatment.

All the six treated along with control group were housed in separate cages. The intestine of the control and the treated animals were dissected out for enzyme biochemical analysis. The experiment was conducted for 30 days and the animals were sacrificed for intestinal biochemical analysis of intestinal enzymes on 1st, 3rd, 7th, 15th and 30th day. The soxhlation method followed for the present study was that of Shiping Fang *et al.* (1999).

RESULTS

The slug *Laevicaulis alte* injected with 10ppm (sub lethal dose-I) of *Melia azedarach*, 1ppm of *Sphaeranthus amaranthoides* and 30ppm of *Sarcostemma brevistigma* leaves extracts separately for five alternative days, the hepatopancrease were analysed for their alkaline phosphatase activities at different time intervals such as 1st, 3rd,7th,15th and 30th day of experiment. The mean values calculated from the AKP activities obtained from both control and treated slugs at 1st, 3rd, 7th, 15th and 30th day of experiments were presented in Table 1.

Table 1. Alkaline phosphatase activities (µmol/min^{-1}/mg protein) in the Intestine of sub lethal dose-I concentrations of *Melia azedarach, Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* extracts treated slug *Laevicaulis alte*

Days of exposure	Name of the plants		
	Melia azedarach	*Sphaeranthus amaranthoides*	*Sarcostemma brevistigma*
Control	0.40± 0.023	0.43±0.025	0.38±0.026
1st day	0.34±0.018	0.30±0.027	0.22±0.012
3rd day	0.28±0.009	0.35±0.016	0.17±0.014
7th day	0.36±0.014	0.45±0.012	0.12±0.006
15th day	0.42±0.019	0.48±0.015	0.28±0.011
30th day	0.53±0.032	0.52±0.013	0.45±0.028

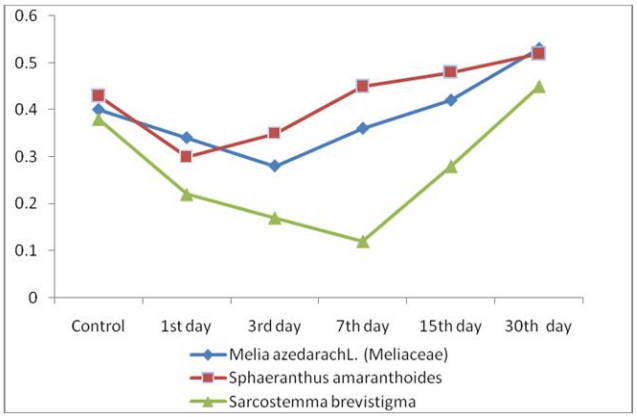

Figure 1. Alkaline phosphatase activities (µmol/min^{-1}/mg protein) in the Intestine of sub lethal dose-I concentrations of *Melia azedarach, Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* extracts *treated* slug *Laevicaulis alte*

The graphical representation of the results was shown in Figure 1. Slugs which are treated with 10ppm of *Melia azedarach*, the AKP level observed was 0.34±0.018 µmol/min^{-1}/mg proteins in 1st day, which was 53.12% percent lower than the control slugs. But, both the 3rd and 7th day observations indicated that AKP values increased to certain extent than the result obtained on the1st day, however, their level is just below to the control

level. This increasing trend continues upto 30th day of experiment. The increased level of AKP at 15th and 30th day of observation was noted as 0.42±0.019 µmol/min^{-1}/mg proteins and 0.53±0.032 µmol/min^{-1}/mg proteins. The percentage over control reduction of mean AKP for 3rd ,7th ,15th and 30th day observation was noted as 53.12%, 34.37%, 12.5%, -9.38% and -18.75% respectively.

The slug *L. alte* treated with *Sphaeranthus amaranthoides*, shows 0.30±0.027µmol/min^{-1}/mg proteins of AKP in their intestine at 1st day observation which is 40% higher than the respective control slugs. But from the 3rd day onwards, the AKP levels were increased and attain the maximum at the 15th day of observation. The maximum level of AKP obtained at 15th day of observation is just above the control values. After 15 days the AKP level declined towards the control level. The AKP levels observed at 3rd, 7th, 15th and 30th day observation were recorded as 0.35±0.16 µmol/min^{-1}/mg proteins, 0.45±0.012 µmol/min^{-1}/mg proteins 0.48±0.015 µmol/min^{-1}/mg proteins and 50.25±0.015 µmol/min^{-1}/mg proteins respectively. The percentage over control reduction of mean AKP for 3rd, 7th, 15th and 30th day observation was noted as 40.0%, 10.0%, -6.67%, 23.34% and 0% respectively.

The AKP in *Sarcostemma brevistigma* leaves extract treated slug intestine was noted as 0.22±0.012 µmol/min^{-1}/mg proteins, 0.17±0.014 µmol/min^{-1}/mg proteins, 0.12±0.06, µmol/min^{-1}/mg proteins, 0.28±0.011 µmol/min^{-1}/mg proteins and 0.45±0.028 µmol/min^{-1}/mg proteins at 3rd, 7th, 15th and 30th day of observation respectively. The AKP activities show some irregular fluctuations throughout the experiment period. The declining trends of AKP activities compared with their respective control noted at the 1st and 3rd day's observation were again raised at 7th, 15th and 30th day of experiment and reached to the maximum level at 30th day of observation. The maximum level of AKP observed at 30th day of experiment is only feebly higher than the control slug. The percentage over control decline of mean AKP at observed days calculated as 54.83%, 41.93%, 32.25% and -3.23% respectively.

In the sub lethal dose - II treatment, the slugs were treated with 5ppm of *Melia azedarach,* 0.5ppm of *Sphaeranthus amaranthoides* and 15ppm of *Sarcostemma brevistigma* for five alternate days and the alkaline phosphatase activities were estimated in the both control and treated slug intestine on the 1st, 3rd, 7th, 15th and 30th day of experiment. The mean values calculated for both control and treated slugs were presented in Table 2. Figure 2 shows the mean values of alkaline phosphatise during the study period.

Table 2. Alkaline phosphatase activities (µmol/min^{-1}/mg protein) in the intestine of sub lethal dose-II concentrations of *Melia azedarach, Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* extracts treated slug *Laevicaulis alte.*

Days of exposure	Name of the plants		
	Melia azedarach	*Sphaeranthus amaranthoides*	*Sarcostemma brevistigma*
Control	0.47 ± 0.031	0.45±0.038	0.49 ±0.042
1st day	0.21±0.027	0.23 ±0.025	0.22±0.031
3rd day	0.25 ±0.021	0.25±0.021	0.31 ±0.028
7th day	0.22 ±0.020	0.21±0.018	0.28±0.021
15th day	0.19±0.014	0.18±0.012	0.25±0.022
30th day	0.13±0.009	0.16±0.013	0.21±0.018

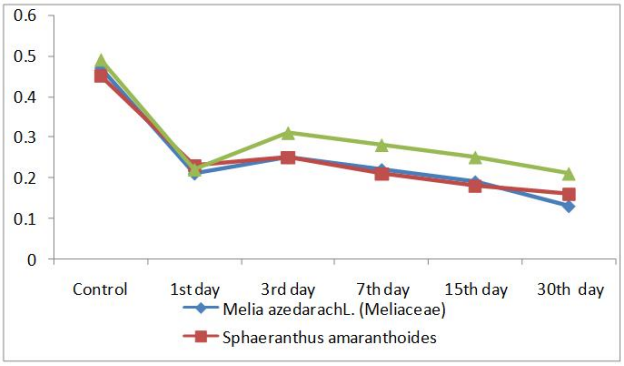

Figure 2. Alkaline phosphatase activities (µmol/min^{-1}/mg protein) in the intestine of sub lethal dose-II concentrations of *Melia azedarach, Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* extracts *treated* slug *Laevicaulis alte*

The AKP levels for the intestine of (sub lethal dose-1) 5ppm of *Melia azedarach*, 0.5ppm of *Sphaeranthus amaranthoides* and 15ppm of *Sarcostemma brevistigma* treated slugs were noted as 0.21±0.027 µmol/min^{-1}/mg proteins, 0.23 ±0.025 µmol/min^{-1}/mg proteins and0.22±0.031 µmol/min^{-1}/mg proteins respectively at the 1st days of observation. The AKP activities in all three plants extract treated animals exhibited low alkaline phosphatise activity on the 1st day observation when compared to control. But on the third day, the AKP values for all three treated shows a slight elevation which were noted as 0.25 ±0.021 µmol/min^{-1}/mg proteins, 0.25±0.021 µmol/min^{-1}/mg proteins and .31 ±0.028 µmol/min^{-1}/mg proteins respectively. From 7th day onwards, the AKP values again declined to certain extent in all the three treated slugs and this trend continues upto 30th day of observation. The declining level of AKP for all three plant extract in treated slugs were recorded as 0.22 ±0.020 µmol/min^{-1}/mg proteins, 0.19±0.14.µmol/min^{-1}/mg proteins and 0.13±0.009 µmol/min^{-1}/mg proteins; 0.21±0.018, 0.18±0.012 and 0.16±0.013; 0.28±0.021, 0.25±0.022 and0.21±0.018 at 7th, 15th and 30th day observation respectively. The percentage reduction of AKP level when compared to control showed an increasing 55.31%, 46.80%, 53.19%, 57.14% and 69.38% for 5ppm of *Melia azedarach* treated slugs, 48.88%, 44 .44%, 53.33%, 60.0% and 64.44% for 0.5ppm of *Sphaeranthus amaranthoides* treated slugs and 55.10%,36.73%,42.85%,48.97% and 57.14% for 15ppm of *Sarcostemma brevistigma* treated slugs on the respective 1st, 3rd, 7th, 15th and 30th day of observation.

DISCUSSION

In the present study, the shell less terrestrial slug *Laevicaulis alte* cause great economic loss worldwide; however, there is no specific compound available to control these pests without being harmful to the non-target organisms. The research on plants molluscicides have increased in recent years. This is due to its advantages of being more readily available, less expensive, more aligned to self reliant control strategy and less polluting than the synthetics (Webbe and Ducan, 1978; Kloos and McCullough, 1981; Hostettmann and Marston, 1987; Kuo, 1987; McCullough, 1992). In many countries search for safe and low cost molluscicides by using the naturally occurring plants that can be applied effectively in different habitats, and

a large number of plant products with molluscicidal activity have been identified (Preetee and Sing, 2008; Adedotun and Alexander, 2008). Hence in the present study, plants such as *Melia azedarach, Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* screened for molluscicidal activities and their impact on alkaline phosphataes activities in the intestine of slug *Laevicaulis alte* were investigated. In the plant extract of *Melia azedarach, Sphaeranthus amaranthoides* and Sarcostemma *brevistigma* treated intestine, the alkaline phosphataes activities were observed as decreasing trends for 1st, 3rd and 7th days of treatment. Thereafter, the AKP activity showed a gradual increment both in 15th and 30th days of treatment. Even though, the level of AKP in the 15th and 30th day of treatment is not higher than that of the control slugs. From the above results, it is evidenced that the phyto-compounds present in all three plants such as *M.azedarach, S.amaranthoides* and *S. brevistigma* have some effective molluscicidal properties.

In the present study, the enzyme alkaline phosphatase activities was analyzed to test the molluscicidal activities of *Melia azedarach, Sphaeranthus amaranthoides* and *Sarcostemma brevistigma* that showed decreasing trends in all plant extract treated slug *Laevicaulis alte* from the 1st day of experiment to the 15th day of experiment. Overall activity of AKP decreased due to increasing of plant extract concentrations so that there were significant differences among control and three treated groups. These findings coincided with other reports of plant extract treated to control insects. For example, Senthil Nathan (2006) showed that treatment of rice plants with *Melia azedarach Juss* extracts decreased the activity level of AKP in *Cnaphalcrocis medinalis* (Guenee). These authors reported that *Spodoptera litura* feeding (Lepidoptera: Noctuidae) *on Ricinus communis L*. treated with *azadirachtin* decreases the amount of this enzyme in the midgut (Senthil Nathan and Kalaivani, 2005).

Al-Sharkawy *et al.* (1996) reported that the alkaline phosphatase of *B. alexandrina* digestive gland decreased after exposure to *Ammi majus*. Atlam (2000) stated that *B. alexandrina* haemocytes after acute exposure to *E. peplus* showed lower activity of both acid and alkaline phosphatase. ElMehaEawy and Rizk (2000) also showed a decrease in alkaline phosphatase activity and increase in acid phosphatase after long-term exposure of *B. alexandrina* to diazinon .In the present study the declining trend was observed from the 3rd day, which continued till the 15th day of experiment. The above mentioned decline results shows some concordance with Singh *et al.* (1992) who found that, the latex of some *Euphorbiales* reduces the acid and alkaline phosphatases in the nervous tissue of *Lymnaea acuminata*.

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