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Limacide (moluscocide) action of L-ascorbic acid

Donyo Ganchev

Agricultural University – Plovdiv, Bulgaria
Corresponding author: d_ganchev@au-plovdiv.bg

Abstract

The aim of the present study was to assess L-ascorbic acid (Vitamin C) as potential limacid (molluscicide). The experiments were conducted in the Agricultural University-Plovdiv, Bulgaria. As test organisms were used some of the most common slug species in Bulgaria: *Arion hortensis*, *Limax maximus* and *Deroceras agreste*. The collected from apple orchard fields slugs were placed in plastic boxes (10 slugs per box) and sprayed with water solutions of vitamin C (L-ascorbic acid) at different concentrations. Five repetitions were conducted per test variant. The control variant was sprayed only with distilled water. The mortally rate was calculated by formula of Abbot. A Dose–response modeling and Dose– response Curve was done by R language of Statistical Computing (drc package). The results show that L-ascorbic acid at a concentration of 2.5-3% can kill slugs approximately 20-30 minutes after treatment. The ANOVA analysis shows that there were no significant differences ($p>0.05$) between effectiveness of L-ascorbic acid towards different slug species. L-ascorbic acid can be effective, relatively cheap, healthy and environmentally friendly alternative of commercial and dangerous molluscicides

Keywords: *Arion*, *Deroceras*, limacid, *Limax*, molluscicide, vitamin C

INTRODUCTION

L-ascorbic acid or Vitamin C is water soluble acid with chemical formula $C_6H_8O_6$ and molar mass = 176.13 g/mol. The acid is odorless white crystal dust. The log p of Vitamin C is -1.85 and LD50 = 11900 ppm, there is no data for significant health effects (The American Society of Health-System Pharmacists, 2016). The substance was discovered in 1912 and was the first vitamin to be chemically produced (Squires, 2011). L-ascorbic acid is important for the functioning of several enzymes and is important for immune system and has an antioxidant activity (The American Society of Health-System Pharmacists, 2016; Monsen, 2000). Although Vitamin C is a typical medical substance during recent years there is increasing studies of its action in the area of pest management. Just like salicylic acid, L–ascorbic acid participates and play important role of System Activated Resistance and respectively – Induced System resistance in plants (Khan, et al., 2011; Kumari et al., 2010; Hussain et al., 2015; Sun al., 2010). Other investigations show strongly stimulation action of Vitamin C onto plant growth and development and their resistance to adverse abiotic conditions (Ibrahim et al., 2013; Mohammed 2013; Walker et al., 2010). A fertilizer consisted from chelating essential minerals with a vitamin C was created and shows excellent efficacy that increases growth and harvest of lettuce (*Lactuca sativa* L.) solving the existing problems of chemical fertilizers such as sedimentation and lack of mineral absorptive in nutrient solution systems (Dudaš et al., 2016; Chae et al., 2018). A commercial extract of humic acids and kelp containing thiamine and L-ascorbic acid was tested as a biostimulant on marigold (*Tagetes patula*) with excellent results on promoting growth and production (Russo et al., 1993).

So the L-ascorbic acid now is a very popular plant simulator or fertilizer providing natural, cheap and efficacy way of plant growth promotion and yields (El-Badawy et al., 2018; Jasim & Hariz, 2023), including reducing environmental stress on the plants due to different abiotic and biotic conditions (Shinohara et al., 1980; Gallie, 2013; Noreen et al., 2021).

Unlike salicylic acid, L-ascorbic acid is water soluble and can be combined effectively with different pesticidal substances (synthetic or natural) and fertilizers (Li et al., 2014; Hafez & Gharib, 2016). In 2013 European Commission approve Vitamin C as basic substance for vegetation treatment of potatoes and tomatoes against *Phytophthora infestans* and *Botrytis* spp, and additionally as treatment for bulbs of decorative plants against soil pathogens (EU, 2013). In this study the possible limacidal (molluscicidal) action of L-ascorbic acid is tested against three slug species: *Arion hortensis, Limax maximus* and *Deroceras agreste* gathered from apple orchard fields. Slugs are an important crop and ornamental plant pest throughout temperate regions in all sectors of the growing industry. Their pest status is set to increase as environmental considerations such as using reduced tillage and over-winter green crops, along with legislative changes to pesticide usage and more extreme weather patterns favor population growth (Howlett, 2012). Slugs successfully adapted to these conditions and have become established in many areas as pests of field and horticultural crops. They can damage first-season tree seedlings. In addition to their importance as crop pests, slugs have been shown to act as vectors of helmin damage sites of domestic and wild mammals and birds, including the nematodes (Boycott, 1934; South & South, 1992; Honek et al., 2013). The main form of crop protection employed by farmers are pellets containing the active ingredient, metaldehyde which chemical is extremely poising for humans and mammals and especially dogs, with annually cases of stay dogs killings by metaldehyde. Even more during rainfall events or with poor application, metaldehyde can leach into the water system, thus preventing or limiting it from entering the water system is a high priority (Dolder, 2003; de Silva, 2022; Castle et al., 2017). The pesticidal action of vitamin C is established in some other studies against fire ant, *Solenopsis invicta* (Ning et.al., 2020), the acid can express strong antifungal action against *Monilia fructigena*, *Alternaria solani* and *Venturia inaequalis* in the conducted in vitro trials with these plant pathogens (Ganchev, 2022) or to had antitumoral activity (Martins et al., 2010). The search of new, safe and natural molluscicides is very important in order to replace the metaldehyde application in pest management.

In the present study the potential limacide action of L-ascorbic acid (Vitamin C) was investigated as possible safe molluscicide.

MATERIALS AND METHODS

Slugs from the following species *Arion hortensis, Limax maximus* and *Deroceras agreste* were used as test organisms. The slugs were placed in plastic boxes (10 slugs per box), five repetitions per test variant, and were sprayed with distilled water solutions of vitamin C at different concentrations. The control variant was sprayed only with distilled water. The mortality rate was calculated by the formula of Abbot (Fleming & Retnakaran, 1985), and Dose – Response Modeling and Dose – Response Curve was done by R language of Statistical Computing (R Core Team, 2015), drc package. ANOVA analysis was conducted also with R language (Ritz & Streibig, 2005).

RESULTS AND DISCUSSION

On the figure bellow is presented the Dose – Response Curve of limacide action of L-ascorbic acid against *Arion hortensis*.

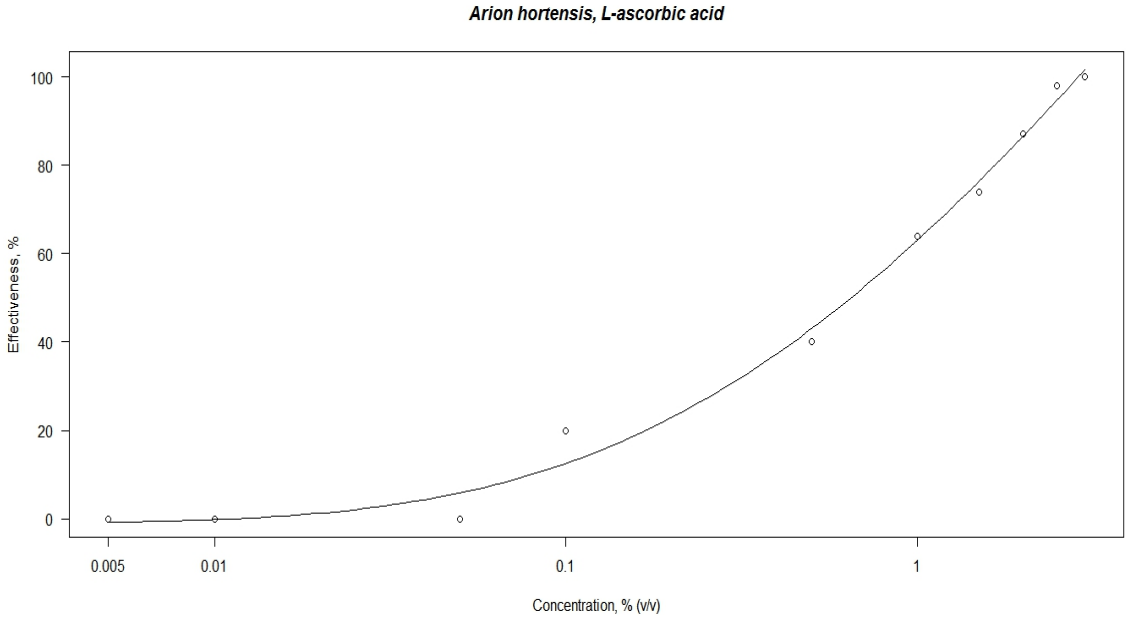

Figure 1. Dose – Response Curve of vitamin C towards *Arion hortensis*

The obtained toxicological indexes are:

- NOAEL = 0.015 %
- LOAEL = 0.22 %
- LC50 = 0.75 %
- LC90 = 2.4 %

The next figure represents Dose – Response Curve of vitamin C towards *Limax maximus.*

The obtained toxicological indexes are:

- NOAEL = 0.02 %
- LOAEL = 0.15 %
- LC50 = 0.6 %
- LC90 = 2.45 %

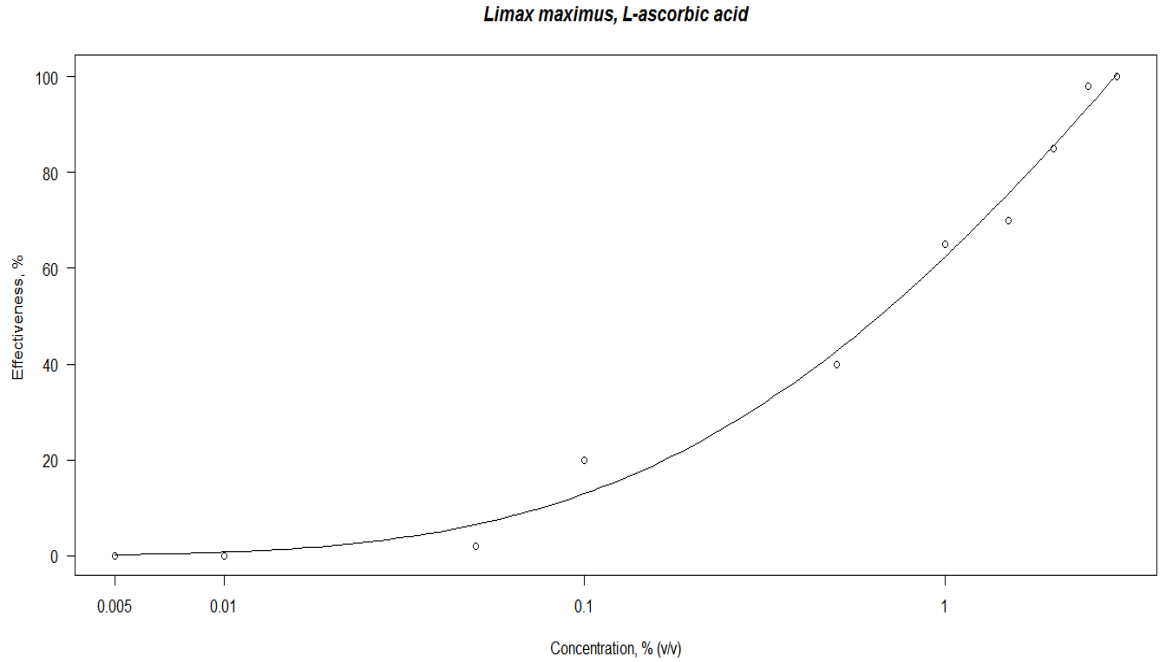

Figure 2. Dose – Response Curve of vitamin C towards *Limax maximus*

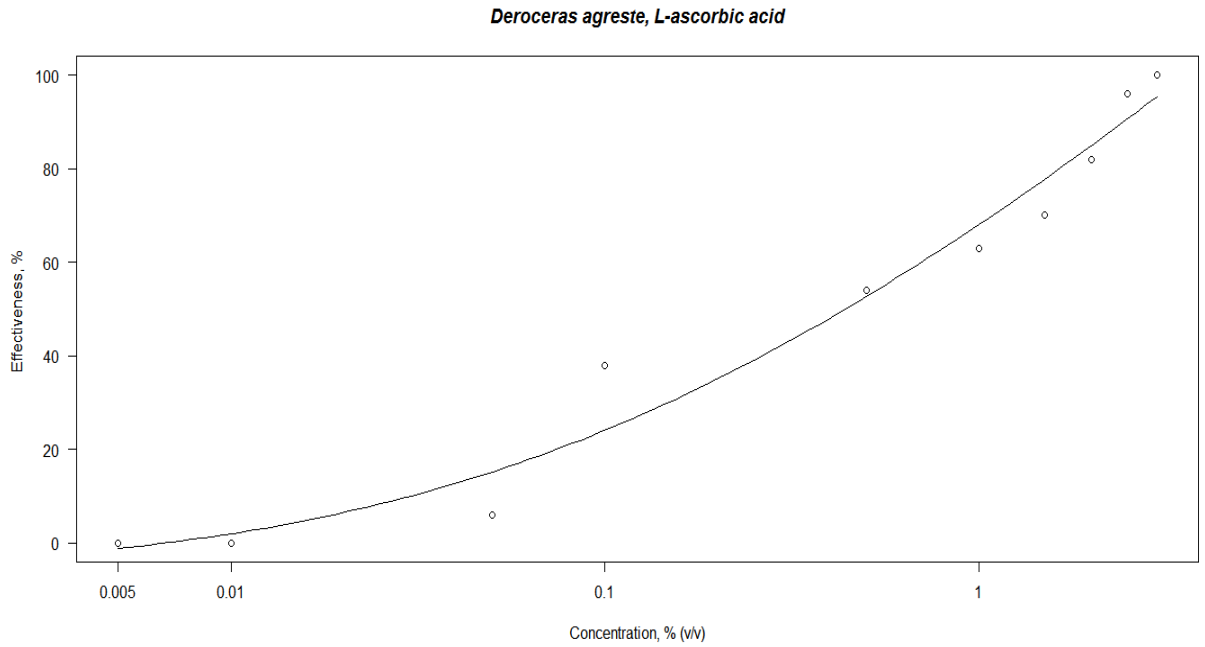

Figure. 3. Dose – Response Curve of vitamin C towards *Deroceras agreste*

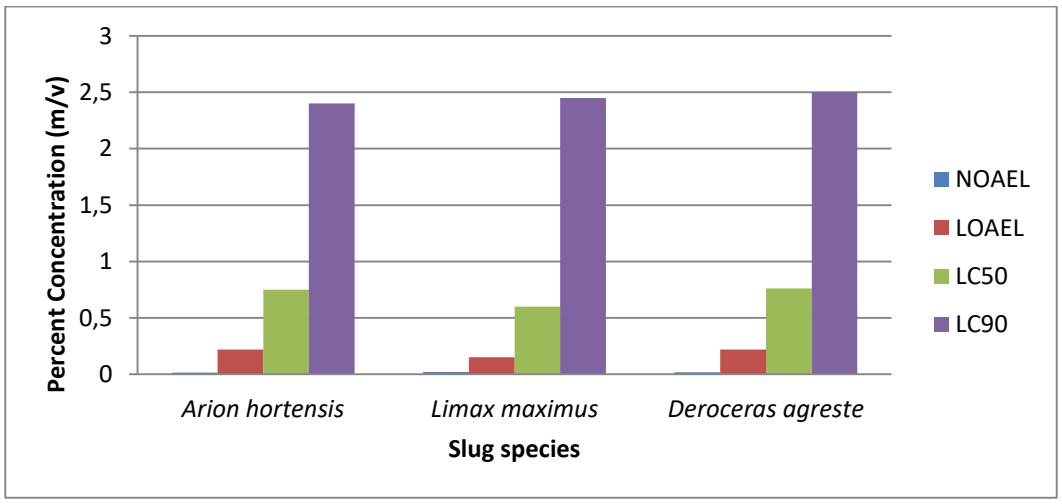

Figure. 4. Toxicological indexes of L-ascorbic acid against tested slug species

In the Figure 3 is presented Dose – Response Curve of vitamin C against *Deroceras agreste*

The obtained toxicological indexes are:

- NOAEL = 0.018 %
- LOAEL = 0.22 %
- LC50 = 0.76 %
- LC90 = 2.5 %

In the the Figure 4 are summarized obtained toxicological indexes from conducted trials.

The results show that L-ascorbic acid at 2.5-3% concentrations can completely kill the slugs approximately 20-30 minutes after treatment. There were no significant differences ($p>0.05$) between effectiveness of the substance towards different slug species which proves the strong limacide action of the tested acid due to the fact that all slug species were affected.

CONCLUSION

The conducted trials reveal the surprisingly new action of the most popular vitamin in the world. The fact that substance is freely available, relatively cheap and completely water soluble plus fact that at these concentrations will not cause any harmful effects on humans or environment, plus ISR action of the acid, made this very applicable as molluscicides. Other studies cited in this article also reveal the strong action of this acid as insecticide, fungicide, ISR promoter or even plant fertilizer.

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