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BAIT FORMULATIONS OF MOLLUSCICIDES AND THEIR EFFECTS ON BIOCHEMICAL CHANGES IN THE OVOTESTIS OF SNAIL *Lymnaea acuminata* (MOLLUSCA; GASTROPODA:LYMNAEIDAE)

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SUMMARY

The effect of sub-lethal feeding of bait formulations containing molluscicidal component of *Ferula asafoetida* (ferulic acid, umbelliferone), *Syzygium aromaticum* (eugenol) and *Carum carvi* (limonene) on biochemical changes in the ovotestis of snail *Lymnaea acuminata* were studied. Bait formulations feeding to *L. acuminata* were studied in clear glass aquaria having diameter of 30 cm. Baits were prepared from different binary combinations of attractant amino acid (valine, aspartic acid, lysine and alanine 10 mM) in 100 mL of 2% agar solution + sub-lethal (20% and 60% of 24h LC_{50}) doses of different molluscicides (ferulic acid, umbelliferone, eugenol and limonene). These baits caused maximum significant reduction in free amino acid, protein, DNA, RNA levels i.e. 41.37, 23.56, 48.36 and 14.29% of control in the ovotestis of the snail, respectively. Discontinuation of feeding after treatment of 60% of 96h LC_{50} of molluscicide containing bait for next 72h caused a significant recovery in free amino acid, protein, DNA and RNA levels in the ovotestis of *L. acuminata.*

KEYWORDS: Bait formulation; Molluscicides; Amino acids; *Lymnaea acuminata*; Biochemical changes.

INTRODUCTION

Fasciola hepatica and *F. gigantica* are the causative agent of endemic fascioliasis in different part of world[14]. This disease belongs to the plant-borne trematods zoonoses. The definite host is very broad and includes many herbivorous mammals, including humans. Bovine fascioliasis is very common in the eastern region of Uttar Pradesh, India[19]. One way to reduce the incidence of fascioliasis is to de-link the life cycle of fluke, by destroying the intermediate host snails[4,7,8,10]. The use of a combination of a feeding attractant and toxicant in the bait formulation is a good tool for pest management and has toxicological and ecological advantages over the release of molluscicides directly in the water[1,9,16,24]. It is therefore important to identify strong attractant and effective molluscicides for preparing bait formulations. Snails, like other gastropod molluscs, use chemical clues to locate food sources[2,3,6,17,25,26,27]. The freshwater snails inhabit an environment containing macrophytes algae and bacteria[23]. These aquatic organisms release different types of chemicals, such as carbohydrates and amino acids, into the surrounding water[5,12,22,23] which acts as attractant for snails. The aim of the present study is to evaluate the effect of sub-lethal feeding molluscicides (ferulic acid, umbelliferone, eugenol and limonene) in bait formulations with attractant amino acid (valine, aspartic acid, lysine and alanine) on different biochemical changes (free amino acid, protein and nucleic acid) in the ovotestis of *Lymnaea acuminata*, a known vector of fascioliasis. Withdrawal experiments were also performed to study the reversibility of the effect on the snails.

MATERIALS AND METHODS

Test animals: The adult snails (2.25 ± 0.20 cm in length) snails were collected locally from lakes and low lying submerged fields in Gorakhpur State of Uttar Pradesh in India. The snails were acclimatized for 72 hours in dechlorinated tap water at 25 ± 1 ^{0}C. The pH of the water was 7.2-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.5-7.3 mg/L, 5.2-6.3 mg/L and 102.0-106.0 mg/L, respectively.

Pure compounds: Agar-agar, amino acids (valine, aspartic acid, lysine and alanine), different active component (Molluscicides: eugenol, ferulic acid, umbelliferone and limonene) were used in bait formulation. The pure active component ferulic acid (4-Hydroxy-3 methoxycinnamic), umbelliferone (7-Hydroxy coumarin; 7-hydroxy-2H-1-benzopyran-2-one), eugenol (2-Methoxy-4-(2-propenyl) phenol) and limonene ((R)-4-Isopropenyl-1-methyl-1-cyclohexene): were purchased from Sigma chemical Co. (USA).

Preparation of bait formulations with molluscicides: Bait formulations containing binary combination of different amino acids (valine, aspartic acid, lysine and alanine 10 mM) and sub-lethal (20% and 60% of 24h and 96h LC_{50}) molluscicides were prepared in 100 mL of 2% agar solution by the method of MADSEN[13]. Concentrations of amino acids were based on the earlier reports of TIWARI & SINGH[25,26]. These solutions were spread at a uniform thickness of 5 mm. After

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cooling, the bait containing sub-lethal molluscicides were cut out with a corer measuring 5 mm in diameter. Six replicates were prepared for each concentration. Control aquaria were left untreated. After 24h of bait feeding the snails were washed with water and the ovotestis was removed from the snail and used for the measurement of biochemical changes. Different biochemical changes viz. free amino acid, protein, DNA and RNA were measured by feeding snails as well as a control group of snails.

In a withdrawal experiment free amino acid, protein, DNA and RNA level in the ovotestis of snail were measured in withdrawn snails after 96h feeding to 60% of 96h LC_{50} of bait feeding for next 72h to fresh water.

Assay apparatus and procedure: The bioassay was performed by the method by TIWARI & SINGH[25,26]. The bioassay chamber consists of a clean glass aquarium having a diameter of 30 cm. Each aquarium was divided into four concentric zones with diameters of 13, 18, 24 and 30 cm: Central zone (zone 3), Middle zone (zone 2 and 1) and Outer zone (zone 0). A small annular elevation of 9 mm height and 2.4 cm diameter was made in the centre of aquarium (Zone 3). Zone 0 had an area of 254 cm^2 on the periphery of aquarium. The aquaria were then filled with 500 mL of dechlorinated tap water to a height of 8 mm and maintained at 25 ± 1 ^{0}C. At the start of the assay ten individually marked snails were placed on the circumference of zone 0. The distance between two snails was 66 mm. Simultaneously, one of the prepared bait of different active component (molluscicides) was added on the small annular elevation in the center (Zone 3). Six sets of experiments have been designed with ten snails in each replicate. Snails were fed with sub-lethal i.e. 20% and 60% of 24h LC_{50} and 96h LC_{50} of the ferulic acid, umbelliferone, eugenol and limonene containing bait formulations. After 24h/96h of feeding changes in the levels of protein, the total free amino acid, nucleic acid (DNA/RNA) in ovotestis of snails was measured. These changes were also studied in ovotestis of *L. acuminata* withdrawn from 96h feeding for next 72h.

Biochemical estimations

Estimation of protein and free amino acids: Protein estimations (µg/mg) were made according to the method of LOWRY *et al.*[11] using bovine serum albumin as a standard. Ten percent trichloroacetic acid (TCA: w/v) was used to prepare homogenates of tissue. Total free amino acid (µg/mg) estimations were made according to the method of SPICE[21].

Nucleic Acids: Estimation of DNA and RNA (µg/mg) were prepared by the method of SCHNEIDER[15] using diphenylamine and orcinol. Homogenates (1 mg/mL,w/v) of ovotestis were prepared in 10% TCA at 90 ^{0}C and centrifuged at 5000g. Supernatants were used for the DNA and RNA estimations.

Statistical analysis: Each result was six times replicate estimation (measurement in six different pools of ovotestis). The values were expressed as mean ± SE. Student's t-test was applied to determine the significant ($p < 0.05$) difference between treated and control animals[20].

RESULTS

There was a significant ($p < 0.05$) decrease in protein levels in the ovotestis of snail *L. acuminata* fed to 20% and 60% of 24h and 96h LC_{50} of ferulic acid, umbelliferone, eugenol and limonene (Table 1). Maximum reduction (23.56 µg/mg of control) in protein levels was observed in the ovotestis of *L. acuminata* fed to 60% of 96h LC_{50} of eugenol (Table 1). Significant ($p < 0.05$) recovery in protein level was observed in the ovotestis of *L. acuminata* 96h, when feeding was discontinued for the next 72h.

Sub-lethal feeding to 20% and 60% of 24h LC_{50} and 96h LC_{50} of eugenol, ferulic acid, umbelliferone, and limonene caused a significant decrease in the total free amino acid levels in the ovotestis of the snail *L. acuminata* (Table 2). Maximum decrease (41.37% of control) in the total free amino acid was observed in the ovotestis of the snails fed to 60% of 96h LC_{50} of ferulic acid (Table 2). There was a significant ($p < 0.05$) recovery in the amino acid level in the ovotestis of withdrawn snails.

Significant decrease ($p < 0.05$) in DNA and RNA levels were observed in the ovotestis of *L. acuminata* fed to 20% and 60% of 24h LC_{50} and 96h LC_{50} of eugenol, ferulic acid, umbelliferone, and limonene (Tables 3,4). Maximum reduction in RNA (14.29% of control) and DNA (48.36% of control) levels were observed in the ovotestis of the snail exposed to 60% of 96h LC_{50} of limonene and umbelliferone, respectively (Tables 3,4). Significant ($p < 0.05$) recovery in RNA, DNA levels were observed in the ovotestis of withdrawn snails.

DISCUSSION

It is evident from the results section that active molluscicidal components of *Ferula asafoetida* (ferulic acid, umbelliferone), *Syzygium aromaticum* (eugenol) and *Carum carvi* (limonene) in bait formulations were more effective in killing the *L. acuminata.* Earlier, it had been reported that direct release of ferulic acid, umbelliferone, eugenol and limonene in aquarium water have significant molluscicidal activity against *L. acuminata*[7,10]. The present study clearly demonstrates that when these active molluscicidal components in bait formulations were fed to snails, it also acts as potent molluscicides. Mode of entry of molluscicide into the snail's body is through the digestive system as it was used as bait. In an earlier study it was through the body surface when molluscicides were released directly in water. Although the entry of molluscicide inside the body is different, both methods are equally effective in killing the snails. Snails fed with a sub-lethal dose i.e. 20% and 60% of 24h and 96h LC_{50} of different molluscicides inside snail attractant pellets, caused a significant change in free amino acid, protein, nucleic acid (DNA and RNA) in the ovotestis of snail *L. acuminata.* The reduction in protein levels may be due to the direct interference of the active molluscicidal component. KUMAR *et al*.[10] reported that there was a depletion of amino acids and reduction of protein and nucleic acid level in the ovotestis of *L. acuminata* when directly released in the aquarium. Due to depletion of free amino acids, there is a significant decrease in the levels of protein. The reduction in levels of proteins in the ovotestis of the treated snail may be due to the reduction synthesis of RNA, along with DNA[10,18].

It can be concluded from the above study the reduction of free amino acid, protein and nucleic acid in the ovotestis of snail *L. acuminata* fed to bait containing active molluscicidal component could control the reproductive capacity of the snails even at sublethal doses. An added advantage of using the plant derived active components in baits is demonstrated by significant recovery in biochemical parameters in ovotestis of snails after discontinuation of feeding. It indicates that if there will be any environmental toxicity, it would be short term.

Table 1

Effect of sublethal exposure (20% and 60% of 24h LC_{50} and 96h LC_{50}) of bait formulations with active molluscicidal component (eugenol, ferulic acid, umbelliferone, and limonene) on the level of proteins (µg/mg) in the ovotestis of the snail *L. acuminata*

Treatment	24h LC_{50}		96h LC_{50}		Withdrawal
	20%	60%	20%	60%	60% (96h LC_{50})
Vali+Aspa+Eug	23.62±0.65 * (25.19)	22.85±0.70 * (24.37)	23.11±0.96 * (24.64)	22.85±0.56 * (24.37)	23.85±0.96 + (24.21)
Vali+Aspa+Fer	38.62±0.36 * (41.19)	35.96±0.81 * (38.35)	37.56±0.36 * (40.05)	33.96±0.81 * (36.22)	38.92±0.38 + (41.14)
Vali+Aspa+Umb	26.96±0.31 * (28.75)	25.75±0.85 * (27.46)	26.66±0.57 * (28.43)	24.96±0.86 * (26.62)	26.96±0.55 + (28.49)
Vali+Aspa+Lim	33.96±0.77 * (36.22)	31.80±0.13 * (33.91)	30.65±0.30 * (32.68)	29.08±0.17 * (31.01)	32.75±0.66 + (34.61)
Lysi+Vali+Eug	24.21±0.70 * (25.52)	23.96±0.80 * (25.26)	24.11±0.18 * (25.41)	23.06±0.88 * (24.33)	25.96±0.46 + (27.69)
Lysi+Vali+Fer	35.16±0.63 * (37.06)	34.66±0.85 * (36.54)	34.96±0.81 * (36.85)	31.75±0.62 * (33.47)	34.75±0.31 + (37.06)
Lysi+Vali+Umb	26.72±0.80 * (28.17)	25.10±0.55 * (26.46)	25.02±0.19 * (26.37)	24.03±0.17 * (25.33)	26.11±0.72 + (27.85)
Lysi+Vali+Lim	36.55±0.26 * (38.53)	25.75±0.23 * (27.14)	35.33±0.87 * (37.24)	24.96±0.85 * (26.31)	28.96±0.21 + (30.89)
Lysi+Ala+Eug	24.62±0.76 * (26.54)	23.11±0.96 * (24.91)	23.85±0.36 * (25.71)	21.86±0.62 * (23.56)	23.98±0.12 + (25.55)
Lysi+Ala+Fer	35.96±0.66 * (38.76)	31.82±0.75 * (34.30)	34.96±0.70 * (37.68)	33.96±0.62 * (36.61)	34.81±0.38 + (37.09)
Lysi+Ala+Umb	25.63±0.26 * (27.63)	24.98±0.88 * (26.92)	25.11±0.73 * (27.06)	23.75±0.66 * (25.60)	25.03±0.82 + (26.67)
Lysi+Ala+Lim	34.76±0.63 * (37.47)	33.96±0.33 * (36.61)	33.05±0.72 * (35.62)	30.85±0.62 * (33.25)	33.87±0.26 + (36.08)
Ala+Vali+Eug	37.62±0.73 * (40.08)	36.15±0.28 * (38.51)	36.70±0.88 * (39.10)	34.66±0.96 * (36.92)	35.78±0.32 + (38.16)
Ala+Vali+Fer	37.68±0.96 * (40.14)	35.99±0.38 * (38.34)	35.12±0.72 * (37.41)	34.76±0.88 * (37.03)	36.36±0.89 + (38.78)
Ala+Vali+Umb	25.86±0.33 * (27.55)	24.72±0.23 * (26.33)	24.11±0.67 * (25.68)	23.98±0.23 * (25.54)	24.72±0.66 + (26.36)
Ala+Vali+Lim	30.62±0.69 * (32.62)	28.12±0.76 * (29.95)	30.10±0.82 * (32.06)	27.69±0.21 * (29.50)	30.02±0.96 + (23.02)
Control (Agar)	94.63±0.63 (100)				90.62±0.73 (100)
Control (a) Vali+Aspa	93.76±0.82 (100)				94.60±0.81 (100)
Control (b) Lysi+Vali	94.85±0.68 (100)				93.15±0.63 (100)
Control (c) Lysi+Ala	93.76±0.88 (100)				93.85±0.62 (100)
Control (d) Ala+Vali	93.86±0.31 (100)				93.75±0.60 (100)

Each value is mean ± SE of six replicates. Value in parenthesis is per cent change with control taken as 100%. Concentration (w/v) has been expressed as final concentration in aquarium water. (*) Significant ($p < 0.05$) when 't' test was applied in between treated and control group and (+) in between 60% of 96h LC_{50} and withdrawal group. Vali = valine, Aspa = aspartic acid, Lys = lysine, Ala = alanine, Eug = eugenol, Feb = ferulic acid, Umb = umbelliferone, Lim = limonene.

Table 2

Effect of sublethal exposure (20% and 60% of 24h LC_{50} and 96h LC_{50}) of bait formulations with active molluscicidal component (eugenol, ferulic acid, umbelliferone, and limonene) on the level of amino acid (µg/mg) in the ovotestis of the snail *L. acuminata*

Treatment	24h LC_{50}		96h LC_{50}		Withdrawal
	20%	60%	20%	60%	60% (96h LC_{50})
Vali+Aspa+Eug	15.18±0.67* (48.84)	14.66±0.75* (47.16)	15.15±0.63* (48.74)	13.39±0.55* (43.08)	14.75±0.29+ (47.45)
Vali+Aspa+Fer	14.68±0.32* (47.23)	14.12±0.13* (45.43)	13.99±0.25* (45.01)	12.86±0.36* (41.37)	13.75±0.55+ (44.24)
Vali+Aspa+Umb	16.62±0.83* (53.47)	15.72±0.63* (50.57)	15.85±0.70* (50.99)	14.36±0.55* (46.20)	15.86±0.13+ (51.02)
Vali+Aspa+Lim	15.50±0.96* (49.87)	14.99±0.83* (48.23)	15.63±0.96* (50.28)	14.85±0.63* (47.77)	16.11±0.85+ 51.83)
Lysi+Vali+Eug	16.66±0.75* (53.48)	15.32±0.32* (49.18)	16.38±0.31* (52.58)	15.96±0.81* (51.23)	16.89±0.36+ (53.90)
Lysi+Vali+Fer	15.75±0.81* (50.56)	14.92±0.66* (47.89)	15.75±0.98* (50.56)	14.73±0.58* (47.28)	15.96±0.31+ (56.94)
Lysi+Vali+Umb	16.82±0.55* (53.99)	15.93±0.72* (51.13)	15.90±0.45* (510.4)	14.82±0.48* (47.57)	16.31±0.82+ (52.05)
Lysi+Vali+Lim	15.85±0.66* (50.88)	15.70±0.71* (50.40)	14.62±0.35* (46.93)	14.60±0.70* (46.86)	15.68±0.41+ (50.04)
Lysi+Ala+Eug	16.66±0.40* (53.43)	15.99±0.85* (51.28)	16.82±0.82* (53.94)	15.71±0.38* (50.38)	16.92±0.80+ (56.19)
Lysi+Ala+Fer	14.75±0.98* (47.30)	14.78±0.71* (47.40)	15.33±0.62* (49.16)	14.63±0.89* (46.92)	15.96±0.89+ (53.00)
Lysi+Ala+Umb	16.66±0.75* (53.43)	15.96±0.38* (51.18)	16.30±0.77* (52.27)	15.96±0.80* (51.18)	16.76±0.32+ (55.66)
Lysi+Ala+Lim	15.87±0.96* (50.89)	15.12±0.66* (48.49)	15.82±0.60* (50.73)	14.12±0.89* (45.28)	15.66±0.59+ (52.00)
Ala+Vali+Eug	14.66±0.70* (47.00)	14.12±0.68* (45.27)	14.75±0.51* (47.29)	13.48±0.76* (43.21)	14.96±0.66+ (49.58)
Ala+Vali+Fer	15.63±0.61* (51.11)	15.87±0.36* (5088)	15.11±0.42* (48.44)	14.96±0.82* (47.96)	15.86±0.21+ (52.56)
Ala+Vali+Umb	15.76±0.96* (50.52)	14.85±0.96* (47.61)	15.60±0.36* (50.01)	13.33±0.82* (42.72)	15.87±0.85+ (52.60)
Ala+Vali+Lim	14.62±0.36* (46.87)	15.96±0.81* (51.17)	15.07±0.87* (48.31)	14.88±0.62* (47.70)	16.82±0.36+ (55.75)
Control (Agar)	30.12±0.15 (100)				28.33±0.13 (100)
Control (a) Vali+Aspa	31.08±0.11 (100)				31.33±0.18 (100)
Control (b) Lysi+Vali	31.15±0.06 (100)				31.15±0.12 (100)
Control (c) Lysi+Ala	31.18±0.18 (100)				30.11±0.11 (100)
Control (d) Ala+Vali	31.19±0.66 (100)				30.17±0.07 (100)

Each value is mean ± SE of six replicates. Value in parenthesis is per cent change with control taken as 100%. Concentration (w/v) has been expressed as final concentration in aquarium water. (*) Significant ($p < 0.05$) when 't' test was applied in between treated and control group and (+) in between 60% of 96h LC_{50} and withdrawal group. Vali = valine, Aspa = aspartic acid, Lysi = lysine, Ala = alanine, Eug = eugenol, Feb = ferulic acid, Umb = umbelliferone, Lim = limonene.

Table 3

Effect of sublethal exposure (20% and 60% of 24h LC_{50} and 96h LC_{50}) of bait formulations with active molluscicidal component eugenol, ferulic acid, umbelliferone, and limonene on the level of RNA (µg/mg) in the ovotestis of the snail *L. acuminata*

Treatment	24h LC_{50}		96h LC_{50}		Withdrawal
	20%	60%	20%	60%	60% (96h LC_{50})
Vali+Aspa+Eug	13.66±0.72 * (24.44)	11.87±0.96 * (21.23)	12.63±0.82 * (22.59)	10.75±0.82 * (19.23)	12.85±0.76 + (23.42)
Vali+Aspa+Fer	10.75±0.22 * (19.23)	9.85±0.65 * (17.62)	9.99±0.83 * (17.87)	8.96±0.33 * (16.03)	10.12±0.82 + (18.44)
Vali+Aspa+Umb	10.66±0.39 * (19.07)	9.66±0.72 * (17.28)	9.12±0.70 * (16.31)	8.23±0.62 * (14.72)	9.98±0.62 + (18.19)
Vali+Aspa+Lim	11.82±0.76 * (21.14)	9.88±0.32 * (17.67)	10.87±0.14 * (19.44)	9.66±0.92 * (17.28)	10.79±0.28 + (19.66)
Lysi+Vali+Eug	12.38±0.69 * (21.86)	11.72±0.63 * (20.69)	11.98±0.33 * (21.15)	10.33±0.41 * (18.24)	11.86±0.73 + (20.90)
Lysi+Vali+Fer	11.61±0.77 * (20.50)	10.63±0.71 * (18.77)	10.60±0.34 * (18.71)	9.86±0.72 * (17.09)	10.99±0.89 + (19.37)
Lysi+Vali+Umb	9.86±0.56 * (17.41)	8.24±0.69 * (14.55)	9.80±0.77 * (17.30)	8.11±0.52 * (14.32)	9.83±0.73 + (17.33)
Lysi+Vali+Lim	10.26±0.11 * (18.11)	9.66±0.32 * (17.05)	10.12±0.69 * (17.87)	9.10±0.94 * (16.06)	10.70±0.98 + (18.86)
Lysi+Ala+Eug	12.76±0.48 * (22.48)	10.62±0.55 * (18.71)	11.96±0.72 * (21.07)	10.13±0.58 * (17.84)	11.96±0.16 + (22.90)
Lysi+Ala+Fer	11.19±0.38 * (19.71)	10.62±0.52 * (18.71)	10.72±0.96 * (18.88)	9.66±0.92 * (17.01)	10.82±0.72 + (19.92)
Lysi+Ala+Umb	10.33±0.26 * (18.19)	9.70±0.58 * (17.08)	9.66±0.31 * (17.01)	8.69±0.38 * (15.31)	9.62±0.12 + (17.71)
Lysi+Ala+Lim	10.99±0.55 * (19.36)	9.86±0.39 * (17.37)	9.82±0.33 * (17.30)	8.83±0.72 * (15.55)	9.26±0.73 + (17.05)
Ala+Vali+Eug	12.92±0.37 * (22.75)	10.24±0.71 * (18.03)	11.62±0.11 * (20.46)	9.67±0.38 * (17.02)	10.75±0.23 + (19.32)
Ala+Vali+Fer	11.32±0.82 * (19.93)	10.31±0.23 * (18.15)	10.11±0.73 * (17.80)	9.22±0.83 * (16.23)	10.96±0.76 + (19.70)
Ala+Vali+Umb	10.12±0.32 * (17.82)	8.13±0.81 * (16.07)	9.66±0.51 * (17.01)	8.36±0.82 * (14.72)	9.62±0.82 + (17.29)
Ala+Vali+Lim	10.11±0.62 * (17.80)	9.08±0.66 * (15.98)	9.08±0.67 * (15.98)	8.12±0.89 * (14.29)	9.65±0.23 + (17.34)
Control (Agar)	57.66 ±0.88 (100)				56.63±0.78 (100)
Control (a) Vali+Aspa	55.89±0.62 (100)				54.86±0.13 (100)
Control (b) Lysi+Vali	56.63±0.96 (100)				56.72±0.82 (100)
Control (c) Lysi+Ala	56.76±0.72 (100)				54.31±0.96 (100)
Control (d) Ala+Vali	56.79±0.92 (100)				55.62±0.85 (100)

Each value is mean ± SE of six replicates. Value in parenthesis is per cent change with control taken as 100%. Concentration (w/v) has been expressed as final concentration in aquarium water. (*) Significant ($p < 0.05$) when 't' test was applied in between treated and control group and (+) in between 60% of 96h LC_{50} and withdrawal group. Vali = valine, Aspa = aspartic acid, Lysi = lysine, Ala = alanine, Eug = eugenol, Feb = ferulic acid, Umb = umbelliferone, Lim = limonene.

Table 4

Effect of sublethal exposure (20% and 60% of 24h LC_{50} and 96h LC_{50}) of bait formulations with active molluscicidal component (eugenol, ferulic acid, umbelliferone, and limonene) on the level of DNA (µg/mg) in the ovotestis of the snail *L. acuminata*

Treatment	24h LC_{50}		96h LC_{50}		Withdrawal
	20%	60%	20%	60%	60% (96h LC_{50})
Vali+Aspa+Fer	33.96±0.42 * (55.38)	32.11±0.62 * (52.36)	33.82±0.56 * (55.18)	30.31±0.98 * (49.42)	32.98±0.26 + (56.27)
Vali+Aspa+Umb	35.82±0.75 * (58.41)	34.61±0.87 * (56.44)	35.18±0.63 * (57.37)	33.75±0.82 * (55.03)	34.50±0.76 + (58.87)
Vali+Aspa+Lim	35.96±0.38 * (60.27)	34.72±0.72 * (56.62)	34.85±0.76 * (56.83)	33.98±0.51 * (55.41)	35.96±0.32 + (61.36)
Lysi+Vali+Eug	43.98±0.27 * (72.47)	40.69±0.81 * (67.05)	42.76±0.22 * (70.46)	40.11±0.62 * (66.10)	42.75±0.96 + (75.05)
Lysi+Vali+Fer	31.76±0.96 * (52.34)	30.22±0.70 * (49.80)	30.66±0.50 * (50.52)	29.86±0.63 * (49.20)	31.10±0.62 + (54.59)
Lysi+Vali+Umb	34.96±0.67 * (57.61)	33.25±0.86 * (54.79)	34.12±0.36 * (56.22)	32.75±0.96 * (53.97)	33.75±0.66 + (59.25)
Lysi+Vali+Lim	36.69±0.72 * (60.46)	34.88±0.79 * (57.48)	36.10±0.27 * (59.49)	34.50±0.69 * (56.85)	35.99±0.61 + (63.18)
Lysi+Ala+Eug	41.69±0.78 * (66.50)	40.05±0.59 * (63.88)	40.96±0.21 * (65.33)	38.25±0.72 * (61.01)	40.85±0.76 + (65.92)
Lysi+Ala+Fer	33.75±0.39 * (53.83)	31.85±0.66 * (50.80)	33.12±0.78 * (52.83)	30.62±0.38 * (48.84)	32.67±0.68 + (52.72)
Lysi+Ala+Umb	34.96±0.71 * (55.76)	32.90±0.38 * (52.48)	33.66±0.81 * (53.69)	30,32±0.88 * (48.36)	33.77±0.24 + (54.50)
Lysi+Ala+Lim	35.68±0.55 * (57.20)	33.62±0.72 * (53.62)	32.98±0.89 * (52.60)	30.79±0.96 * (49.11)	34.24±0.82 + (55.26)
Ala+Vali+Eug	42.85±0.25 * (70.50)	41.66±0.78 * (68.54)	40.62±0.56 * (66.83)	38.75±0.39 * (63.75)	40.85±0.96 + (68.27)
Ala+Vali+Fer	32.72±0.62 * (53.83)	30.66±0.96 * (50.44)	31.85±0.14 * (52.40)	29.72±0.39 * (48.89)	31.96±0.44 + (53.41)
Ala+Vali+Umb	35.62±0.72 * (58.60)	33.88±0.79 * (55.74)	33.79±0.85 * (55.59)	30.62±0.72 * (50.37)	32.85±0.62 + (54.90)
Ala+Vali+Lim	35.86±0.82 * (58.99)	33.87±0.62 * (55.72)	34.85±0.77 * (57.33)	33.28±0.11 * (54.75)	34.66±0.73 + (57.93)
Control (Agar)	62.66 ±0.96 (100)				60.75±0.23 (100)
Control (a) Vali+Aspa	61.32±0.62 (100)				58.60±0.62 (100)
Control (b) Lysi+Vali	60.68±0.83 (100)				56.96±0.38 (100)
Control (c) Lysi+Ala	62.69±0.82 (100)				61.96±0.73 (100)
Control (d) Ala+Vali	60.78±0.98 (100)				59.83±0.86 (100)

Each value is mean ± SE of six replicates. Value in parentheses is per cent change with control taken as 100%. Concentration (w/v) has been expressed as final concentration in aquarium water. (*) Significant ($p < 0.05$) when 't' test was applied in between treated and control group and (+) in between 60% of 96h LC_{50} and withdrawal group. Vali = valine, Aspa = aspartic acid, Lysi = lysine, Ala = alanine, Eug = eugenol, Feb = ferulic acid, Umb = umbelliferone, Lim = limonene.

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RESUMO

Formulações de iscas de moluscicidas e seus efeitos sobre as alterações bioquímicas no ovoteste do caramujo *Lymnaea acuminata* (Mollusca;Gastropoda:Lymnaeidae)

Foi estudado o efeito subletal das iscas usadas para alimentação contendo componentes moluscicidas de *Ferula asafoetida* (ácido ferúlico, umbeliferone), *Syzygium aromaticum* (eugenol) e *Carum carvi* (limonene) nas alterações bioquímicas do ovoteste do caramujo *Lymnaea acuminata.* A formulação das iscas usadas para alimentar *L. acuminata* foi estudada em aquários de vidros transparentes de diâmetro de 30 cm. As iscas foram preparadas por combinações diferentes binárias de aminoácidos (valina, ácido aspártico, lisina e alanina 10 mM) em 100 mL de solução de agar a 2% + doses subletais (20% e 60% durante 24 horas LC_{50}) de diferentes moluscicidas (ácido ferúlico, umbeliferone, eugenol e limonene). Estas iscas causaram redução significante máxima em aminoácidos livres, proteínas, níveis de DNA e RNA isto é 41,37%, 23,56%, 48,36% e 14,29% de controle no ovoteste do caramujo, respectivamente. Discontinuação da alimentação depois do tratamento de 60% de 96 horas de LC_{50} do moluscicida contendo a isca para as subsequentes 72 horas causou significante recuperação dos níveis de aminoácidos livres, proteína, DNA e RNA no ovoteste da *L. acuminata*.

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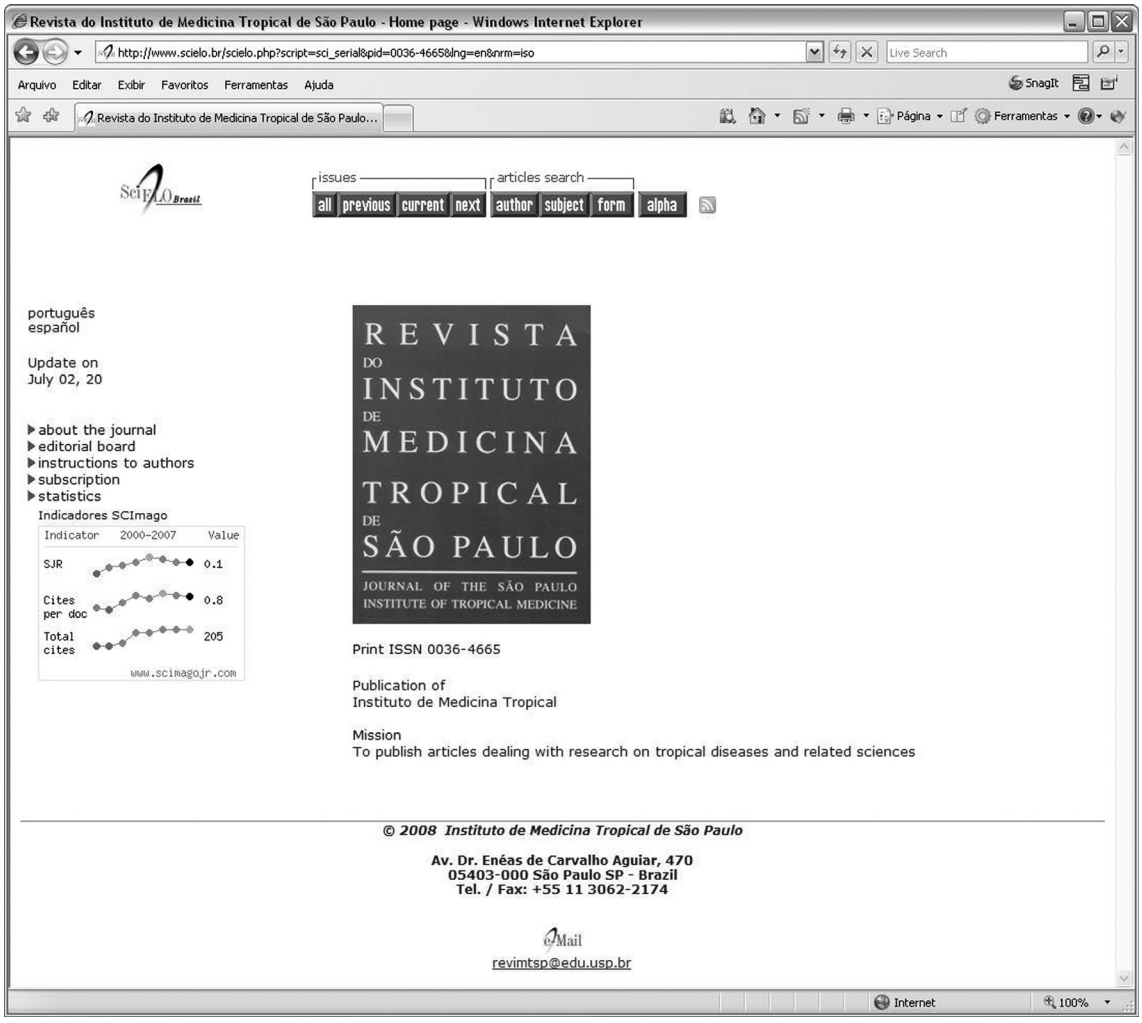

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