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Molluscicidal Activity of *Cannabis sativa*, *Acacia nilotica* and *Tinospora cordifolia* Extracts Against Vector Snail *Lymnaea acuminata*

Nilay Vishal Singh and Vinay Kumar Singh
Department of Zoology, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, Uttar Pradesh 273009, India

ABSTRACT

Background and Objective: Fresh water snails of family lymnaeidae serve as vectors for parasitic trematode *Fasciola hepatica* which causes a serious zoonotic disease fascioliasis. The study aimed to investigate the possibility of indigenous plants *Cannabis sativa*, *Acacia nilotica* and *Tinospora cordifolia* to serve as natural molluscicides to control the population of vector snails. **Materials and Methods:** Aqueous extracts were prepared from freshly collected aerial parts of these plants. Healthy and acclimatized snails were exposed against different concentrations of the plant extracts continuously up to 96 hrs to analyse the toxicity of these extracts. **Results:** Mortality of snails after exposure of different combinations of the extracts indicates that the extracts from indigenous plants *C. sativa*, *A. nilotica* and *T. cordifolia* shows very promising molluscicidal activity against *Lymnaea acuminata*. Leaves of *Cannabis sativa* showed highest toxicity. **Conclusion:** This study obviously indicates that these indigenous plant extracts can be used as a potent natural molluscicides.

KEYWORDS

Fascioliasis, *Fasciola hepatica*, *Lymnaea acuminata*, molluscicide, *Cannabis*, *Acacia*, *Tinospora*

INTRODUCTION

Fascioliasis is an important zoonotic disease caused by two trematodes, *Fasciola hepatica* and *Fasciola gigantica*[1-3]. These parasitic trematodes are of considerable veterinarian and medical importance. Fascioliasis has recently proved to be an important public health problem, with human cases reported in 91 countries and the global burden of fascioliasis estimated to be 2.7 million[4,5]. Population in the developing countries are likely to be most affected by facioliasis as the COVID-19 pandemic impacts their health symptoms and efforts to control Fasciola decline[6-8]. Yattoo *et al.*[9] reported two cases of human fascioliasis from Kashmir Valley in India.

Species of freshwater snails from the family Lymnaeidae are well known for their role as the intermediate host for *Fasciola* species. The most important host for *F. hepatica* is *Lymnaea acuminata* (Fig. 1 and 2) and *Lymnaea rufescence* in Indian subcontinent[8-12] and *Lymnaea trunculata* in Europe, Asia, Africa and North America[8,13,14]. The control of fascioliasis depends on the understanding of the ecology, biology and distribution of the intermediate host snails. Controlling the population of the host snails below the threshold level can be a rapid and effective means of reducing fascioliasis[3,14].

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Fig. 1: *Lymnaea acuminata*

Fig. 2: *Lymnaea acuminata* (Dead)

Various synthetic molluscicides have been widely used for effective control of harmful snails and such molluscicides includes copper sulphate, trifenmorph, Bayluscide, sodium pentachlorophenate, copper pentachlorophenate and niclosamide[15,16]. Significant success has been achieved by the use of these synthetic chemicals for the elimination of harmful snails. Nonetheless, the use of these synthetic molluscicides for controlling snails causes serious environmental pollution[17].

There are large numbers of pharmacological effects of *C. sativa*, *A. nilotica* and *T. cordifolia* have been reported that *C. sativa* shows anti-oxidant activity[18,19], larvicidal activity against mosquito larvae[20] and anti-microbial activity[21,22]. Revathi *et al.*[23] reported anti-cancer, anti-microbial and anti-oxidant properties of *A. nilotica*. Anti-cancer, anti-viral infection, inflammation, immunomodulatory role and neurological and anti-diabetes properties of *T. cordifolia* have already been reported[24].

The search indigenous of plants with molluscicidal activity is considered more sustainable than the use of synthetic molluscicides. The present study aimed to evaluate the molluscicidal activity of *Cannabis sativa*, *Acacia nilotica* and *Tinospora cordifolia* to explore the full potential of these indigenous plants as potent molluscicides.

MATERIALS AND METHODS

The experiment was carried out in the Malacology Laboratory, Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur in August to November, 2022.

Collection of plant materials: Aerial parts of *Cannabis sativa* (leaf, stem), *Acacia nilotica* (leaf, bark) and *Tinospora cordifolia* (leaf, stem) were collected freshly from local areas and identified by the Department of Botany, DDU Gorakhpur University, Gorakhpur, India.

Animal collection: Adult *Lymnaea acuminata* (1.35±0.2 cm in length) were collected from freshwater ponds of Gorakhpur District, Uttar Pradesh, India. The snails were acclimatized for 72 hrs in laboratory conditions. Ten snails were then allocated to each of the groups and immersed in 3 L untreated dechlorinated tap water at 23±1°C. The pH, free carbon dioxide, dissolved oxygen and bicarbonate alkalinity were 7.1-7.3, 5.2-6.3, 6.6-7.3 and 102-104 mg L^{-1}, respectively. The dead snails were removed from the aquaria to avoid any contamination.

Plant extract preparation: The extraction of plant parts was performed by the method of Singh and Agarwal[25] with some modifications. Freshly collected leaves and stems of *C. sativa* were washed thoroughly, then finely cut into small pieces and then separately mixed with water (50 g leaves/stem+50 mL water) then filtered with Whatman No. 1 filter paper. The filtered extract thus obtained was evaporated under a vacuum. The residues thus obtained were used for the determination of toxicity. The same procedure was performed with *A. nilotica* (leaf, bark) and *T. cordifolia* (leaf, stem) extraction.

Molluscicidal activity test: Molluscicidal evaluation of plant extracts was performed according to WHO guidelines as modified by Singh and Agarwal[25]. Ten uninfected snails were kept in a glass aquarium containing 3 L deionized water. In each setup, snails were prevented from crawling out of the aquaria employing a fine mesh placed above the water surface. Snails were challenged for 96 hrs with various concentrations of different plant part extracts singly and in various binary combinations (Table1). Six replicates were set up for each concentration. Control experiments were performed with deionized water without any treatment (negative control). These snails were neither fed nor disturbed during the exposure period. Mortality of snails was recorded at an interval of 24 up to 96 hrs. Death of the snails was determined and confirmed by lack of reaction to irritation of foot with needle prove to elicit typical withdrawal movements and contraction of their body in the shell (Fig. 2).

Statistical analysis ($p<0.05$): The LC_{50} values, Lower Confidence Limits (LCL), Upper Confidence Limits (UCL), t-ratio heterogeneity factor, slope values and g-values were calculated by polo software program (PoLo Plus LeOra software version 2.0).

Ethical consideration: All applicable protocols of institutional, national or international guidelines for the care and use of animals were followed during this experiment.

RESULTS

The toxicity of extracts of *C. sativa* (leaf, stem), *A. nilotica* (leaf, bark) and *T. cordifolia* (leaf, stem) against *Lymnaea acuminata* was time and concentration-dependent. The LC_{50} determined after 24 hrs treatment was highest in *C. sativa* leaf extract (304.31 mg L^{-1}) (Table 2). *Cannabis sativa* stem extract was more toxic (24 hrs LC_{50} = 404.39 mg L^{-1}) than aerial part extracts of *A. nilotica* and *T. cordifolia* (Table 2). There was a significant ($p<0.05$) negative correlation between the LC_{50} and the exposure period. The order of 24 hrs toxicity of different plant parts extracts singly against *L. acuminata* were *C. sativa* leaf>*C. sativa* stem>*A. nilotica* leaf>*T. cordifolia* leaf>*A. nilotica* bark>*T. cordifolia* stem (Table 2).

The binary combination (1:1) of *C. sativa* leaf+*A. nilotica* leaf was more toxic (24 hrs LC_{50} = 174.76 mg L^{-1}) than other combinations in the 1:1 ratio (Table 3) and their single treatments. The order of toxicity of different binary combinations (1:1) against *L. acuminata* was *C. sativa* leaf+*A. nilotica* leaf>*C. sativa* stem+*A. nilotica* bark>*T. cordifolia* leaf+*C. sativa* leaf>*T. cordifolia* leaf+*A. nilotica* leaf>*T. cordifolia* stem+*C. sativa* stem>*T. cordifolia* stem+*A. nilotica* bark (Table 3).

Table 1: Concentration of different plant products (crude extracts) used for toxicity determination against *Lymnaea acuminata*

Binary combinations	Molluscicide	Concentration (mg L^{-1})
	Cannabis sativa leaf	150, 200, 250, 300
	Cannabis sativa stem	250, 300, 350, 400
	Acacia nilotica leaf	200, 300, 400, 500
	Acacia nilotica bark	350, 450, 550, 650
	Tinospora cordifolia leaf	300, 400, 500, 600
	Tinospora cordifolia stem	450, 550, 650, 750
1:1	C.S. Leaf+A.N. leaf	110, 130, 150, 170
	C.S. Stem+A.N. bark	125, 150, 175, 200
	T.C. Leaf+C.S. leaf	100, 150, 200, 250
	T.C. Stem+C.S. stem	125, 175, 225, 275
	T.C. Leaf+A.N. leaf	125, 175, 225, 275
	T.C. Stem+A.N. bark	100, 175, 250, 325
1:5	C.S. Leaf+A.N. leaf	30, 50, 70, 90
	C.S. Stem+A.N. bark	70, 90, 110, 130
	T.C. Leaf+C.S. leaf	30, 50, 70, 90
	T.C. Stem+C.S. stem	50, 70, 90, 110
	T.C. Leaf+A.N. leaf	30, 50, 70, 90
	T.C. Stem+A.N. bark	50, 70, 90, 110
5:1	C.S. Leaf+A.N. leaf	10, 30, 50, 70
	C.S. Stem+A.N. bark	30, 50, 70, 90
	T.C. Leaf+C.S. leaf	50, 70, 90, 110
	T.C. Stem+C.S. stem	90, 110, 130, 150
	T.C. Leaf+A.N. leaf	50, 70, 90, 110
	T.C. Stem+A.N. bark	90, 110, 130, 150

C.S. leaf: *Cannabis sativa* leaf, C.S. stem: *Cannabis sativa* stem, A.N. Leaf: *Acacia nilotica* leaf, A.N. bark: *Acacia nilotica* bark, T.C. leaf: *Tinospora cordifolia* leaf and T.C. stem: *Tinospora cordifolia* stem

Table 2: Toxicity of crude extract (aqueous) of plant parts of *C. sativa*, *A. nilotica* and *T. cordifolia* against snail *Lymnaea acuminata*

			Limits				
Treatment	Exposure period	Effective dose (mg L^{-1})	LCL	UCL	Slope value	't' ratio	Heterogeneity factor
C. sativa leaf	24 hrs	LC_{50} = 304.31	271.84	375.92	4.33±0.86	5.03	0.24
	48 hrs	LC_{50} = 248.92	226.48	283.91	4.05±0.78	5.18	0.22
	72 hrs	LC_{50} = 197.54	172.66	218.64	3.73±0.76	4.91	0.25
	96 hrs	LC_{50} = 160.96	135.00	178.48	4.51±0.82	5.48	0.43
C. sativa stem	24 hrs	LC_{50} = 404.39	373.82	469.31	6.31±1.26	5.01	0.23
	48 hrs	LC_{50} = 350.89	328.72	384.67	5.86±1.14	5.10	0.23
	72 hrs	LC_{50} = 304.80	278.24	327.73	5.26±1.11	4.71	0.17
	96 hrs	LC_{50} = 264.00	273.29	281.80	7.02±1.23	5.69	0.37
A. nilotica leaf	24 hrs	LC_{50} = 535.98	463.48	704.34	3.63±0.70	5.16	0.24
	48 hrs	LC_{50} = 429.67	378.26	524.62	3.14±0.60	5.17	0.27
	72 hrs	LC_{50} = 323.46	285.36	364.42	3.33±0.58	5.57	0.53
	96 hrs	LC_{50} = 247.89	208.51	278.40	3.62±61	5.93	0.67
A. nilotica bark	24 hrs	LC_{50} = 675.75	607.06	833.45	4.81±0.98	4.91	0.02
	48 hrs	LC_{50} = 547.54	504.19	613.08	4.64±0.87	5.29	0.24
	72 hrs	LC_{50} = 453.52	407.75	494.14	4.45±0.85	5.18	0.38
	96 hrs	LC_{50} = 375.73	315.32	414.60	4.57±0.90	5.08	0.55
T. cordifolia leaf	24 hrs	LC_{50} =593.41	583.69	700.31	4.97±0.90	5.50	0.22
	48 hrs	LC_{50} =487.88	444.18	552.55	4.06±0.77	5.52	0.27
	72 hrs	LC_{50} =385.22	335.75	425.22	3.85±0.76	5.04	0.26
	96 hrs	LC_{50} =321.59	277.69	325.62	5.20±0.86	6.05	0.41
T. cordifolia stem	24 hrs	LC_{50} = 772.62	706.98	919.00	5.81±1.18	4.92	0.19
	48 hrs	LC_{50} = 651.25	606.67	719.66	5.39±1.05	5.10	0.19
	72 hrs	LC_{50} =552.65	504.35	593.78	5.30±1.03	5.10	0.19
	96 hrs	LC_{50} = 484.06	436.51	517.17	6.81±1.14	5.95	0.55

Mortality was determined every 24 up to 96 hrs, each set of experiment was replicate six times, *C. sativa*: *Cannabis sativa*, *A. nilotica*: *Acacia nilotica*, *T. cordifolia*: *Tinospora cordifolia*, LCL: Lower confidence limit, UCL: Upper confidence limit, Significant negative regression ($p<0.05$) was observed between exposure time and LC_{50} of treatments

Table 3: Toxicity of binary combinations (1:1) of crude extract (aqueous) of plant parts of *C. sativa*, *A. nilotica* and *T. cordifolia* against snail *Lymnaea acuminata*

			Limits				
Treatment	Exposure period	Effective dose (mg L^{-1})	LCL	UCL	Slope value	't' ratio	Heterogeneity factor
C. sativa leaf+	24 hrs	LC_{50} = 174.76	159.50	215.30	5.40±1.12	4.18	0.18
A. nilotica leaf	48 hrs	LC_{50} = 148.01	137.86	163.79	5.30±1.2	4.39	0.16
	72 hrs	LC_{50} = 121.81	103.64	132.28	4.66±1.1	3.89	0.31
	96 hrs	LC_{50} = 112.26	100.69	119.66	7.79±1.37	5.67	0.34
C. sativa stem+	24 hrs	LC_{50} = 193.53	178.60	225.74	5.40±1.18	4.57	0.29
A. nilotica bark	48 hrs	LC_{50} = 165.74	154.22	180.17	5.30±1.2	4.80	0.17
	72 hrs	LC_{50} = 141.06	127.20	150.82	4.66±1.1	5.18	0.15
	96 hrs	LC_{50} = 129.38	116.92	137.76	7.77±1.37	6.00	0.40
T. cordifolia leaf+	24 hrs	LC_{50} = 281.41	233.35	427.35	2.83±0.64	4.37	0.18
C. sativa leaf	48 hrs	LC_{50} = 226.43	190.61	322.13	2.32±0.58	3.98	0.14
	72 hrs	LC_{50} = 153.80	126.07	180.72	2.40±0.56	4.25	0.14
	96 hrs	LC_{50} = 114.90	93.20	130.70	3.50±0.61	5.69	0.49
T. cordifolia stem+	24 hrs	LC_{50} = 324.50	270.50	502.53	3.21±0.76	4.20	0.24
C. sativa stem	48 hrs	LC_{50} = 234.66	206.30	289.24	3.02±0.67	4.46	0.16
	72 hrs	LC_{50} = 153.64	116.90	177.67	2.65±0.65	4.02	0.30
	96 hrs	LC_{50} = 127.72	103.01	144.51	4.18±0.74	5.62	0.42
T. cordifolia leaf+	24 hrs	LC_{50} = 308.03	258.10	471.88	3.00±0.73	4.09	0.21
A. nilotica leaf	48 hrs	LC_{50} = 237.76	209.54	294.33	3.07±0.68	4.51	0.20
	72 hrs	LC_{50} = 169.35	142.80	191.28	3.10±0.66	4.67	0.25
	96 hrs	LC_{50} = 134.98	113.26	150.44	4.45±0.74	5.96	0.37
T. cordifolia stem+	24 hrs	LC_{50} = 390.27	304.10	695.39	2.14±0.56	4.24	0.19
A. nilotica bark	48 hrs	LC_{50} = 229.25	191.29	291.68	2.09±0.45	4.64	0.16
	72 hrs	LC_{50} =147.22	113.80	174.61	2.27±0.44	5.08	0.31
	96 hrs	LC_{50} = 117.68	88.00	140.08	2.65±0.47	5.60	0.42

Mortality was determined every 24 up to 96 hrs, each set of experiment was replicate six times, *C. sativa*: *Cannabis sativa*, *A. nilotica*: *Acacia nilotica*, *T. cordifolia*: *Tinospora cordifolia*, LCL: Lower confidence limit, UCL: Upper confidence limit, Significant negative regression ($p<0.05$) was observed between exposure time and LC_{50} of treatments

The binary combination (1:5) of *C. sativa* leaf+*A. nilotica* leaf was found to be more toxic (24 hrs LC_{50} = 82.32 mg L^{-1}) than other combinations (Table 4). The order of toxicity of different binary combinations (1:5) against *L. acuminata* was *C. sativa* leaf+*A. nilotica* leaf>*T. cordifolia* leaf+*A. nilotica* leaf>*T. cordifolia* leaf+*C. sativa* leaf>*T. cordifolia* stem+*A. nilotica* bark>*T. cordifolia* stem+*C. sativa* stem>*C. sativa* stem+*A. nilotica* bark (Table 4).

The binary combination (5:1) of *C. sativa* leaf+*A. nilotica* leaf was more toxic (24 hrs LC_{50} = 82.32 mg L^{-1}) than other combinations (Table 5). The order of toxicity of different binary combinations (5:1) against *L. acuminata* was *C. sativa* leaf+*A. nilotica* leaf>*C. sativa* stem+*A. nilotica* bark>*T. cordifolia* leaf+*A. nilotica* leaf>*T. cordifolia* leaf+*C. sativa* leaf>*T. cordifolia* stem+*A. nilotica* bark>*T. cordifolia* stem+*C. sativa* stem (Table 5).

It is evident from the results that extracts of *C. sativa* (leaf, stem), *A. nilotica* (leaf, bark) and *T. cordifolia* (leaf, stem) are toxic against the snail *Lymnaea acuminata*. The toxicity of *C. sativa* leaf is highest in comparison to the stem and other parts. Binary combinations of extracts of plant pats show increased toxicity than they are used separately.

The slope values given in Tables 2-5 were steep. Based on each of the six replicates the separate estimates of LC values were found to be within 95% confidence limit of LC_{50}. The t-ratio was higher than 1.96 and the heterogeneity factor was less than 1.0. The g-value was less than 0.5 at all the probability levels (90, 95 and 99). There was a significant negative regression ($p<0.05$) between exposure time and LC_{50} of the exposures (Table 2-5).

Table 4: Toxicity of binary combinations (1:5) of crude extract (aqueous) of plant parts of *C. sativa*, *A. nilotica* and *T. cordifolia* against snail *Lymnaea acuminata*

			Limits				
Treatment	Exposure period	Effective dose (mg L^{-1})	LCL	UCL	Slope value	't' ratio	Heterogeneity factor
C. sativa leaf+	24 hrs	LC_{50} = 82.32	69.53	110.88	4.49±0.51	4.86	0.38
A. nilotica leaf	48 hrs	LC_{50} = 56.97	46.87	69.89	2.09±0.47	4.42	0.21
	72 hrs	LC_{50} = 36.67	25.85	44.29	2.24±0.48	4.64	0.20
	96 hrs	LC_{50} = 31.70	24.29	37.21	3.23±0.54	5.96	0.42
C. sativa Stem+	24 hrs	LC_{50} = 128.45	115.92	155.92	4.51±0.93	4.85	0.15
A. nilotica bark	48 hrs	LC_{50} = 104.87	96.96	115.29	4.89±0.87	5.57	0.13
	72 hrs	LC_{50} = 86.29	75.26	94.70	4.08±0.85	4.79	0.21
	96 hrs	LC_{50} = 71.75	61.33	78.65	5.51±0.96	5.70	0.43
T. cordifolia leaf+	24 hrs	LC_{50} = 93.24	77.17	135.30	2.51±0.53	4.71	0.14
C. sativa leaf	48 hrs	LC_{50} = 63.34	55.07	74.96	2.74±0.49	5.35	0.14
	72 hrs	LC_{50} = 43.37	33.74	51.14	3.33±0.47	4.87	0.17
	96 hrs	LC_{50} = 33.65	26.31	39.20	3.19±0.52	6.02	0.46
T. cordifolia stem+	24 hrs	LC_{50} = 116.19	100.60	155.90	3.51±0.75	4.67	0.33
C. sativa stem	48 hrs	LC_{50} = 89.69	79.17	108.28	3.02±0.67	4.50	0.16
	72 hrs	LC_{50} = 64.40	52.47	73.24	2.95±0.66	4.45	0.33
	96 hrs	LC_{50} = 54.80	46.27	60.92	4.46±0.74	5.98	0.75
T. cordifolia leaf+	24 hrs	LC_{50} = 88.53	73.46	127.06	2.39±0.51	4.62	0.17
A. nilotica leaf	48 hrs	LC_{50} = 58.92	49.71	71.05	2.32±0.48	4.83	0.17
	72 hrs	LC_{50} = 42.65	32.01	50.93	2.16±0.47	4.56	0.23
	96 hrs	LC_{50} = 32.84	24.87	38.74	2.98±0.52	5.74	0.41
T. cordifolia stem+	24 hrs	LC_{50} = 114.54	98.12	160.29	3.11±72	4.33	0.13
A. nilotica bark	48 hrs	LC_{50} = 85.52	73.95	105.43	2.60±0.65	3.95	0.25
	72 hrs	LC_{50} = 62.68	50.34	71.43	2.95±0.66	4.44	0.23
	96 hrs	LC_{50} = 54.94	46.24	61.17	4.36±0.73	5.93	0.29

Mortality was determined every 24 up to 96 hrs, each set of experiment was replicate six times, *C. sativa*: *Cannabis sativa*, *A. nilotica*: *Acacia nilotica*, *T. cordifolia*: *Tinospora cordifolia*, LCL: Lower confidence limit, UCL: Upper confidence limit, Significant negative regression ($p<0.05$) was observed between exposure time and LC_{50} of treatments

Table 5: Toxicity of binary combinations (5:1) of crude extract (aqueous) of plant parts of *C. sativa*, *A. nilotica* and *T. cordifolia* against snail *Lymnaea acuminata*

			Limits				
Treatment	Exposure period	Effective dose (mg L^{-1})	LCL	UCL	Slope value	't' ratio	Heterogeneity factor
C. sativa leaf+	24 hrs	LC_{50} = 84.43	63.03	146.81	1.76±0.34	5.05	0.35
A. nilotica leaf	48 hrs	LC_{50} = 50.59	38.77	74.84	1.45±0.28	5.08	0.17
	72 hrs	LC_{50} = 21.88	14.58	29.01	1.35±0.26	5.08	0.24
	96 hrs	LC_{50} =13.65	8.96	17.87	1.77±0.28	6.23	0.43
C. sativa stem+	24 hrs	LC_{50} = 89.57	74.15	129.72	3.29±0.52	4.60	0.28
A. nilotica bark	48 hrs	LC_{50} = 64.03	53.99	80.03	2.22±0.48	4.61	0.17
	72 hrs	LC_{50} = 44.95	35.63	52.82	2.36±0.47	4.93	0.27
	96 hrs	LC_{50} = 37.05	30.10	42.51	3.22±0.51	6.22	0.39
T. cordifolia leaf+	24 hrs	LC_{50} = 119.25	104.08	155.39	3.99±0.80	4.95	0.20
C. sativa leaf	48 hrs	LC_{50} = 90.66	80.95	107.00	3.38±0.68	4.93	0.18
	72 hrs	LC_{50} = 66.03	56.67	73.64	3.51±0.67	5.21	0.18
	96 hrs	LC_{50} = 54.42	45.84	60.57	4.45±0.74	6.00	0.31
T. cordifolia stem+	24 hrs	LC_{50} = 162.24	144.24	215.24	4.63±1.12	4.10	0.11
C. sativa stem	48 hrs	LC_{50} = 131.28	121.99	146.16	5.21±1.05	4.95	0.14
	72 hrs	LC_{50} = 100.37	85.39	109.14	4.61±1.03	4.46	0.17
	96 hrs	LC_{50} = 93.57	83.51	100.32	6.97±1.18	5.91	0.39
T. cordifolia leaf+	24 hrs	LC_{50} = 113.90	99.97	146.24	3.84±0.77	4.98	0.12
A. nilotica leaf	48 hrs	LC_{50} = 92.26	82.24	109.89	3.35±0.68	4.87	0.11
	72 hrs	LC_{50} = 64.60	54.48	72.44	3.35±0.67	5.00	0.19
	96 hrs	LC_{50} = 52.98	44.00	9.27	4.39±0.75	5.85	0.40
T. cordifolia stem+	24 hrs	LC_{50} = 150.60	135.84	189.70	4.45±0.80	4.12	0.20
A. nilotica bark	48 hrs	LC_{50} = 120.21	108.69	134.32	4.05±1.01	4.00	0.16
	72 hrs	LC_{50} = 101.69	89.89	109.75	5.24±1.05	4.99	0.33
	96 hrs	LC_{50} = 91.73	80.40	99.04	6.56±0.16	5.63	0.45

Mortality was determined every 24 up to 96 hrs, each set of experiment was replicate six times, *C. sativa*: *Cannabis sativa*, *A. nilotica*: *Acacia nilotica*, *T. cordifolia*: *Tinospora cordifolia*, LCL: Lower confidence limit, UCL: Upper confidence limit, Significant negative regression ($p<0.05$) was observed between exposure time and LC_{50} of treatments

DISCUSSION

The results shown in Table 2 indicate that the extracts from indigenous plants *C. sativa*, *A. nilotica* and *T. cordifolia* shows very promising molluscicidal activity against *Lymnaea acuminata* that act as intermediate host of human and livestock disease fascioliasis. The highest toxicity was shown by extracts from leaves of *Cannabis sativa*. It is very obvious from this study that when used in different binary combinations, these plant extracts show enhanced toxicity against *L. acuminata*.

It has been reported that *Cannabis sativa* contains about 104 cannabinoids and 140 members of terpenes[26]. Mantzoukas *et al.*[27] reported the larvicidal activity of cannabidiol oil from *C. sativa*. It seems that cannabinoids present in *Cannabis sativa* caused the mortality of *L. acuminata*. The bark extract of *Acacia nilotica* contains terpenoids, tannins, alkaloids, saponins and glycosides[28] and saponins have been reported as potent molluscicides[29]. The chemical components of *Tinospora cordifolia* include terpenoids (tinosporide, tinosporaside and ecdysterone), alkaloids (tinosporine and magnoflorine), lignans, steroids, giloin and tinosponon[30] and terpenoids are found to be potent larvicidal agents[31]. It seems that terpenoids present in *T. cordifolia* extracts caused the mortality of snail *L. acuminata*.

The toxicity of the binary combination (1:5) of *C. sativa* leaf+*A. nilotica* leaf extract is 3.69 and 6.51 times higher than *C. sativa* leaf and *A. nilotica* leaf, respectively. The toxicity of binary combination (1:5) of *C. sativa* stem+*A. nilotica* bark is 3.14 and 5.26 times higher than *C. sativa* stem and *A. nilotica* bark, respectively used alone. The toxicity of *T. cordifolia* leaf+*C. sativa* leaf (1:5) is 6.36 and 4.33 folds higher than *T. cordifolia* leaf and *C. sativa* leaf, respectively. A binary combination (1:5) of *T. cordifolia* stem+*C. sativa* stem is 6.64 and 3.48 times more toxic than *T. cordifolia* stem and *C. sativa* stem, respectively. Similarly, the binary combination (1:5) of *T. cordifolia* leaf+*A. nilotica* leaf is 6.70 and 6.05 times more toxic than the *T. cordifolia* leaf and *A. nilotica* leaf, respectively. Likewise, the toxicity of binary combination (1:5) of *T. cordifolia* stem+*A. nilotica* bark is 6.74 and 5.89 times higher than *T. cordifolia* stem and *A. nilotica* bark, respectively.

The steep slope values indicate that an increase in concentrations causes increased mortality in the snails. The t-ratio value greater than 1.96 indicates that the regression is significant, the value of heterogeneity factor less than 1.0 indicates that replicate lines would fall within 95% confidence limit and thus the model fitsthe data adequately.

Synthetic molluscicides are costly and cause adverse effects on the environment. The use of plant molluscicides not only may eliminate the burden of expensive synthetic molluscicides, but also could prevent the environment. The present study shows that *C. sativa*, *A. nilotica* and *T. cordifolia* may be used as potent molluscicides. Further, investigations are required to elucidate the mode of action in the snail body, if used as molluscicides.

CONCLUSION

The indigenous plant extracts were found to be effective and efficient in killing the vector snails after time and concentration dependent treatment. The experimental results revealed that plant based molluscicides are a better substitute for the harmful synthetic molluscicides. Further investigations are required to reveal the phytoconstituent and its mode of action responsible for the toxicity.

SIGNIFICANCE STATEMENT

Use of molluscicides to control population of the vector snails is an approach to control fascioliasis. Synthetic molluscicides are costly and cause adverse effects on the environment. This study discovered the potentiality of indigenous plants as cost-effective and eco-friendly molluscicides.

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