

## Effect of Plant Extracts on Egg Hatching and Juvenile Mortality of Reniform Nematode, *Rotylenchulus reniformis*

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### ABSTRACT

Experiment were study to exposed egg-masses and juveniles of *Rotylenchulus reniformis* to aqueous extracts from fresh leaves of *Withania somnifera*, *Ocimum tenuiflorum*, *Mentha arvensis*, *Lantana camara*, *Calotropis gigantea* plants leaves and Margozyme neem based product were used as hatching and mortality effect on eggs and juvenile of *R. reniformis* in vitro. Plant extracts viz., *Lantana camara*, *Calotropis gigantea* and *Withania somnifera* 20 and 30 per cent concentration were suppress the hatching eggs and increase juvenile mortality percentage of reniform nematode, *R. reniformis* in vitro.

**Key words:** *Rotylenchulus reniformis*, *Withania somnifera*, *Ocimum tenuiflorum*, *Mentha*

### INTRODUCTION

Nematodes are small unsegmented worms live in water, soil, plants and animals. Plant parasitic nematodes are mostly microscopic. It causes significant damage for almost all crops. The reniform nematode attacks over 140 species of more than 115 plant genera in 46 families<sup>3</sup>.

Generally, nematicides are recommended for the management of nematodes. But nematicides are highly toxic compounds that have very low LD 50 values. Most of the

nematicides are banned because of their harmful effect on human and environment. Moreover, in organic farming there should be alternative nematode control strategies, as chemical nematicides cannot be recommended. Therefore, it has become an important issue to find alternative control strategies.

Many plants are known to have nematicidal properties which may be utilized as organic amendments or bio-pesticides.

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Plant extracts on eggs and larvae in reducing the nematode population is promising in the crop protection against reniform nematode<sup>16</sup>. Plant extracts having the nematicidal properties and they not only reduce the nematode population but also enhance the plant growth<sup>2</sup>. Muhammad Shakeel Ahmad *et al*<sup>15</sup>, tested the water extracts of neem (*A. indica*), aak (*Calotropis procera*) and datura (*D. alba*) for their effects on larval mortality of citrus nematode (*Tylenchulus semipenetrans*). Neem gave the maximum larval mortality followed by aak and datura. Philippe Jourand *et al*<sup>8</sup>, found that Aqueous extracts from leaves of *Crotalaria virgulata* subsp. *grantiana* is nematostatic on 2<sup>nd</sup> stage juveniles of *Meloidogyne incognita* and protected susceptible tomato plants from *M. incognita* infestation.

Qamar *et al*<sup>10</sup>, isolated Lantanic acid, camaric acid and oleanolic acid from the methanolic extract of the aerial parts of *Lantana camara* through bio-assay guided fractionation. These compounds exhibited significant mortality against root-knot nematode *Meloidogyne incognita* at 0.5% concentration.

Olabi<sup>17</sup> reported that aqueous extracts from the roots of marigold, nitta and basil plant reduced root-knot nematode populations in the soil with corresponding increases in plant height, plant leaf and fruit yield. Khan *et al*<sup>4</sup>, examined the effect of ethanol extracts of *Azadirachta indica* (neem), *Withania somnifera* (ashwagandha), *Tagetes erecta* (marigold) and *Eucalyptus citriodora* (eucalyptus) against nematodes (*Meloidogyne incognita*) associated with papaya (*Carica papaya*).

The objective of present study is to evaluate extracts of different plants commonly available in *W. somnifera*, *O. tenuiflorum*, *M.*

*arvensis*, *L. camara*, *C. gigantea* for their nematicidal efficacy against reniform nematodes in cavity block.

## MATERIALS AND METHODS

### Culture of nematodes

Reniform nematode was selected as test organism. The culture of reniform nematode, *R. reniformis* is maintained on potted castor plants inside the cage house. The infected plants will be uprooted, carefully washed in running tap water and egg masses will be collected in to Petri dishes containing distilled water.

### Preparation of extraction

Healthy leaves of Ashwaganthan (*Withania somnifera*), Tulsi (*Ocimum tenuiflorum*), Mint (*Mentha arvensis*), Lentana (*Lantana camara*), Aak (*Calotropis gigantea*) plants were collected from herbal park and Margozyme neem based plant product from department of Nematology RCA, Udaipur (Raj). The collected plant parts were washed with sterile distilled water. 100 gm of clean fresh plant material was grind with 100 ml absolute alcohol. The mixture was allowed to stand for 48 hrs at room temperature and was subsequently filtered through filter paper. The solvent was completely evaporated from the extract at 70° C till it become a semi-solid material. These semi-solid materials become stock solution by adding distilled water and different concentrations of each plant extracts were prepared for experimentation.

### In vitro experiment

Hatching assay was done according to the method suggested by Saravanapriya *et al*<sup>11</sup>. The egg masses were kept in glass cavity blocks (2 egg mass/ cavity block) containing 3 ml of plant extracts of 5, 10, 20 and 30 per cent respectively concentrations. A distilled water control was maintained simultaneously.

Each treatment was replicated three times. Numbers of juveniles hatched after at every day up to 7 days *in vitro* were counted with the aid of inverted microscope at magnification 40X.

Mortality assay of plant extracts on *R. reniformis* juveniles was done according to the method given by Prasad and Suverna<sup>9</sup>. Freshly hatched second stage juveniles of *R. reniformis* were transferred to different cavity blocks (10 juveniles/ cavity block) containing respectively 5, 10, 20 and 30 per cent concentration of plant extracts (3 ml/ cavity block). Juveniles put in distilled water were treated as control. Three replicates of each treatment were maintained. Per cent juvenile mortality rate was counted at the intervals of at every day up to 7<sup>th</sup> days. All dead and alive juveniles were counted with the aid of inverted microscope at magnification 100 X. The mortality was ensured by touching the juvenile with a fine needle. The ratio of dead nematodes/number of total nematodes expressed the percentage mortality. The data were subjected to statistical analysis.

## RESULT AND DISCUSSION

### Effect on hatching

Data on effect of plant extracts on hatching of *R. reniformis* larvae are presented in Table-1. All plant extracts showed inhibitory effect on hatching of *R. reniformis* juvenile. The rate of hatching was directly proportionate to exposure period and inversely proportionate to concentration of extracts as it was decreased with increase in concentration. The highest rate of hatching was observed in 5 per cent while lowest rate at 30 per cent concentration in all plant extracts tested. Among extracts, leaves extract obtained from Lantana (*L. camara*) showed most inhibitory effect followed by aak (*C. gigantea*) and

Ashwaganthan (*W. somnifera*) respectively. Minimum hatching recorded with *L. camara* i.e. 1.67 per cent at 7<sup>th</sup> day followed by *C. gigantea* (2.78 per cent) and *W. somnifera* (3.89 per cent) at 7<sup>th</sup> day hrs. The maximum egg hatching i.e. 91.11 per cent at 7<sup>th</sup> day was recorded with untreated check.

### Effect on mortality

Effect of plant extracts on larval mortality of *R. reniformis* is presented in Table-2. Rate of mortality was directly proportional to exposure period and concentration of extracts. The highest mortality of larvae was observed at 7<sup>th</sup> day with 30 per cent concentration of all extracts of tested plants while lowest was observed at low concentration i.e.5 per cent. Mortality was nil in control i.e. 0.00 per cent at 7<sup>th</sup> day. All plant extracts were found mortal (to have nematicidal action) to juveniles of *R. reniformis*. Among plant extracts maximum mortality recorded with Lantana (*L. camara*) followed by Aak (*C. gigantea*) and Ashwaganthan (*W. somnifera*) respectively. Maximum mortality was recorded with *L. camara* i.e. 86.67 per cent at 30 per cent conc., followed by *C. gigantean* (80.00 per cent) at 30 per cent conc., *W. somnifera* (73.33 per cent) at 30 per cent conc., 7<sup>th</sup> day.

Similar findings were reported by Abdul Nazir Chandekal<sup>1</sup> who reported that aqueous extract of leaves of Lantana is more toxic against the 2<sup>nd</sup> stage juveniles of root knot nematode. Saravanapriya *et al*<sup>11</sup>., they were evaluated screened plant extracts and tested against the root-knot nematode, *Meloidogyne incognita*, for egg hatch. The seed extract of *Arecha catechu* recorded the highest inhibition rate at 0.1 per cent and *Calotropis gigantea* also caused 100 per cent inhibition at 10.0 per cent concentration.

**Table 1: Effect of different plant extracts on hatching of reniform nematode, *R. reniformis* larvae (in vitro)**

Treatments	Percent Hatching of Larvae up to Seven Day							
	T	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day	6 <sup>th</sup> day	7 <sup>th</sup> day
Aak leaves ( <i>Calotropis gigantea</i> ) at 5% conc.	T <sub>1</sub>	5.56 (13.60)	7.78 (16.17)	8.89 (17.24)	10.00 (18.40)	11.11 (19.39)	12.22 (20.40)	13.89 (21.83)
Aak leaves ( <i>Calotropis gigantea</i> ) at 10% conc.	T <sub>2</sub>	2.78 (9.49)	4.44 (12.12)	5.00 (12.80)	7.78 (16.04)	8.89 (17.24)	10.00 (18.40)	10.00 (18.40)
Aak leaves ( <i>Calotropis gigantea</i> ) at 20% conc.	T <sub>3</sub>	1.11 (4.95)	1.67 (5.98)	4.44 (11.77)	5.56 (13.60)	6.11 (14.28)	7.78 (16.04)	7.78 (16.04)
Aak leaves ( <i>Calotropis gigantea</i> ) at 30% conc.	T <sub>4</sub>	0.00 (0.00)	0.00 (0.00)	0.56 (2.47)	0.56 (2.47)	1.67 (5.98)	2.22 (8.45)	2.78 (9.49)
Ashwaganthan leaves ( <i>Withania somnifera</i> ) at 5% conc.	T <sub>5</sub>	6.11 (14.28)	8.33 (16.73)	9.44 (17.79)	10.56 (18.88)	11.67 (19.94)	11.67 (19.94)	14.44 (22.25)
Ashwaganthan leaves ( <i>Withania somnifera</i> ) at 10% conc.	T <sub>6</sub>	3.33 (10.29)	5.00 (12.80)	6.11 (14.09)	8.33 (16.73)	9.44 (17.88)	10.56 (18.95)	11.11 (19.43)
Ashwaganthan leaves ( <i>Withania somnifera</i> ) at 20% conc.	T <sub>7</sub>	1.67 (5.98)	2.22 (8.45)	5.00 (12.80)	6.11 (14.28)	7.22 (15.57)	8.33 (16.73)	8.33 (16.73)
Ashwaganthan leaves ( <i>Withania somnifera</i> ) at 30% conc.	T <sub>8</sub>	0.00 (0.00)	0.56 (2.47)	2.22 (8.45)	3.33 (10.29)	3.33 (10.29)	3.89 (11.32)	3.89 (11.32)
Lantana leaves ( <i>Lantana camara</i> ) at 5% conc.	T <sub>9</sub>	5.56 (13.41)	7.22 (15.49)	8.33 (16.56)	9.44 (17.84)	10.56 (18.88)	11.67 (19.94)	12.78 (20.91)
Lantana leaves ( <i>Lantana camara</i> ) at 10% conc.	T <sub>10</sub>	2.78 (9.49)	3.89 (11.32)	5.56 (13.60)	7.22 (15.49)	7.78 (16.04)	8.33 (16.73)	8.33 (16.73)
Lantana leaves ( <i>Lantana camara</i> ) at 20% conc.	T <sub>11</sub>	0.00 (0.00)	1.11 (4.95)	3.89 (11.32)	5.56 (13.41)	6.11 (14.21)	7.22 (15.49)	7.22 (15.49)
Lantana leaves ( <i>Lantana camara</i> ) at 30% conc.	T <sub>12</sub>	0.00 (0.00)	0.00 (0.00)	0.56 (2.47)	1.11 (4.95)	1.11 (4.95)	1.67 (7.42)	1.67 (7.42)
Mint leaves ( <i>Mentha arvensis</i> ) at 5% conc.	T <sub>13</sub>	6.67 (14.89)	8.89 (17.11)	10.00 (18.27)	11.11 (19.39)	12.22 (20.40)	13.33 (21.39)	14.44 (22.28)
Mint leaves ( <i>Mentha arvensis</i> ) at 10% conc.	T <sub>14</sub>	3.89 (10.97)	5.00 (12.80)	6.67 (14.89)	8.89 (17.24)	9.44 (17.79)	10.56 (18.91)	11.67 (19.94)
Mint leaves ( <i>Mentha arvensis</i> ) at 20% conc.	T <sub>15</sub>	1.11 (4.95)	1.67 (5.98)	4.44 (11.77)	5.56 (13.60)	6.11 (14.28)	7.78 (16.04)	7.78 (16.04)
Mint leaves ( <i>Mentha arvensis</i> ) at 30% conc.	T <sub>16</sub>	0.00 (0.00)	0.00 (0.00)	0.56 (2.47)	0.56 (2.47)	1.67 (5.98)	2.22 (8.45)	2.78 (9.49)
Tulsi leaves ( <i>Ocimum tenuiflorum</i> ) at 5% conc.	T <sub>17</sub>	6.67 (14.89)	9.44 (17.88)	10.56 (18.95)	11.11 (19.46)	12.78 (20.83)	13.89 (21.77)	15.56 (23.14)
Tulsi leaves ( <i>Ocimum tenuiflorum</i> ) at 10% conc.	T <sub>18</sub>	5.00 (12.80)	6.11 (14.09)	7.22 (15.44)	8.89 (17.24)	10.00 (18.40)	10.56 (18.88)	12.22 (20.40)
Tulsi leaves ( <i>Ocimum tenuiflorum</i> ) at 20% conc.	T <sub>19</sub>	2.22 (8.45)	2.78 (9.49)	5.00 (12.80)	6.67 (14.89)	7.78 (16.17)	8.33 (16.73)	8.89 (17.33)
Tulsi leaves ( <i>Ocimum tenuiflorum</i> ) at 30% conc.	T <sub>20</sub>	0.00 (0.00)	1.11 (4.95)	3.89 (11.32)	4.44 (12.12)	5.00 (12.80)	5.56 (13.60)	6.67 (14.89)
Margozyme (Neem based product) at 5% conc.	T <sub>21</sub>	7.78 (15.95)	9.44 (17.72)	11.11 (19.33)	12.22 (20.40)	13.89 (21.83)	15.00 (22.77)	16.67 (24.08)
Margozyme (Neem based product) at 10% conc.	T <sub>22</sub>	5.00 (12.80)	6.67 (14.89)	7.78 (16.17)	8.89 (17.24)	10.56 (18.88)	11.11 (19.33)	12.78 (20.88)
Margozyme (Neem based product) at 20% conc.	T <sub>23</sub>	2.22 (8.45)	2.78 (9.49)	5.56 (13.60)	6.67 (14.76)	8.33 (16.73)	8.89 (17.24)	8.89 (17.24)
Margozyme (Neem based product) at 30% conc.	T <sub>24</sub>	0.56 (2.47)	1.11 (4.95)	3.89 (11.32)	5.00 (12.80)	5.00 (12.80)	6.11 (14.28)	7.22 (15.57)
Untreated check	T <sub>25</sub>	10.56 (18.91)	23.89 (29.22)	27.22 (31.42)	38.89 (38.57)	54.44 (47.55)	85.56 (68.26)	91.11 (72.96)
SEm±		0.859 (1.503)	0.990 (1.648)	1.157 (1.485)	1.119 (1.292)	1.122 (1.298)	1.499 (1.331)	1.193 (1.144)
CD at 5%		2.43 (4.270)	2.81 (4.680)	3.28 (4.217)	3.17 (3.671)	3.18 (3.686)	4.25 (3.780)	3.39 (3.249)

Note: (i) Data are average value of three replication. (ii) Initial value: 60 larvae and egg mass (iii) Data in parenthesis are the angular transformed values of respective data.

**Table 2: Effect of different plant extracts on mortality of reniform nematode, *R. reniformis* larvae (in vitro)**

Treatment	Percent Mortality of Larvae up to Seven Day							
	T	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Aak leaves ( <i>Calotropis gigantea</i> ) at 5% conc.	T1	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	15.33 (23.05)	25.33 (30.22)	26.33 (30.87)	33.33 (35.26)
Aak leaves ( <i>Calotropis gigantea</i> ) at 10% conc.	T2	0.00 (0.00)	3.33 (10.52)	13.33 (21.41)	26.67 (31.09)	30.00 (33.21)	35.67 (36.67)	40.00 (39.23)
Aak leaves ( <i>Calotropis gigantea</i> ) at 20% conc.	T3	0.00 (0.00)	13.33 (21.41)	23.33 (28.88)	40.00 (39.23)	43.33 (41.17)	46.67 (43.09)	53.33 (46.91)
Aak leaves ( <i>Calotropis gigantea</i> ) at 30% conc.	T4	10.00 (0.00)	26.67 (31.09)	46.67 (43.09)	60.00 (50.77)	63.33 (52.74)	73.33 (58.93)	80.00 (63.47)
Ashwaganthan leaves ( <i>Withania somnifera</i> ) at 5% conc.	T5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.00 (18.43)	16.67 (24.09)	24.33 (29.55)	24.00 (33.21)
Ashwaganthan leaves ( <i>Withania somnifera</i> ) at 10% conc.	T6	0.00 (0.00)	3.33 (10.52)	6.67 (14.96)	24.67 (29.78)	28.00 (31.95)	34.67 (36.07)	38.00 (38.06)
Ashwaganthan leaves ( <i>Withania somnifera</i> ) at 20% conc.	T7	0.00 (0.00)	13.33 (21.42)	23.33 (28.88)	31.33 (34.04)	40.00 (39.23)	45.67 (42.52)	51.33 (45.76)
Ashwaganthan leaves ( <i>Withania somnifera</i> ) at 30% conc.	T8	10.00 (18.43)	23.33 (28.88)	46.67 (43.09)	53.33 (46.91)	56.67 (48.83)	66.67 (54.74)	73.33 (58.91)
Lantana leaves ( <i>Lantana camara</i> ) at 5% conc.	T9	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	16.67 (24.09)	26.33 (30.87)	28.33 (32.16)	35.00 (36.27)
Lantana leaves ( <i>Lantana camara</i> ) at 10% conc.	T10	3.33 (0.00)	6.67 (14.96)	16.67 (24.09)	26.67 (31.09)	33.33 (35.26)	36.67 (37.27)	43.33 (41.17)
Lantana leaves ( <i>Lantana camara</i> ) at 20% conc.	T11	3.33 (10.52)	16.67 (24.09)	26.67 (31.09)	43.33 (41.17)	46.67 (43.09)	50.00 (45.00)	56.67 (48.83)
Lantana leaves ( <i>Lantana camara</i> ) at 30% conc.	T12	13.33 (21.41)	30.00 (33.21)	50.00 (45.00)	66.67 (54.74)	80.00 (63.47)	86.67 (68.67)	86.67 (68.67)
Mint leaves ( <i>Mentha arvensis</i> ) at 5% conc.	T13	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	13.33 (21.41)	13.33 (21.41)	16.67 (24.09)	20.00 (26.56)
Mint leaves ( <i>Mentha arvensis</i> ) at 10% conc.	T14	0.00 (0.00)	0.00 (0.00)	3.33 (10.52)	16.67 (24.09)	23.33 (28.88)	26.67 (31.09)	33.33 (35.26)
Mint leaves ( <i>Mentha arvensis</i> ) at 20% conc.	T15	0.00 (0.00)	10.00 (18.43)	20.00 (26.56)	33.33 (35.26)	40.00 (39.23)	43.33 (41.17)	50.00 (45.00)
Mint leaves ( <i>Mentha arvensis</i> ) at 30% conc.	T16	6.67 (14.96)	20.00 (26.56)	40.00 (39.23)	50.00 (45.00)	53.33 (46.91)	60.00 (50.78)	63.33 (52.75)
Tulsi leaves ( <i>Ocimum tenuiflorum</i> ) at 5% conc.	T17	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.67 (14.96)	10.00 (18.43)	13.33 (21.41)	16.67 (24.09)
Tulsi leaves ( <i>Ocimum tenuiflorum</i> ) at 10% conc.	T18	0.00 (0.00)	0.00 (0.00)	3.33 (10.51)	14.00 (21.97)	22.67 (28.43)	24.00 (29.33)	31.33 (34.03)
Tulsi leaves ( <i>Ocimum tenuiflorum</i> ) at 20% conc.	T19	0.00 (0.00)	10.00 (18.43)	18.00 (25.10)	28.00 (31.94)	35.00 (36.27)	42.33 (40.59)	46.67 (43.09)
Tulsi leaves ( <i>Ocimum tenuiflorum</i> ) at 30% conc.	T20	3.33 (10.52)	20.00 (26.56)	33.33 (35.26)	50.00 (45.00)	50.00 (45.00)	56.67 (48.84)	60.33 (50.97)
Margozyme (Neem based product) at 5% conc.	T21	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.67 (14.96)	13.33 (21.41)	13.33 (21.41)
Margozyme (Neem based product) at 10% conc.	T22	0.00 (0.00)	0.00 (0.00)	3.33 (10.52)	12.00 (20.26)	20.67 (27.04)	22.67 (28.43)	30.00 (33.21)
Margozyme (Neem based product) at 20% conc.	T23	0.00 (0.00)	6.67 (14.96)	16.67 (24.09)	30.00 (33.20)	33.33 (35.26)	40.00 (39.23)	45.67 (42.51)
Margozyme (Neem based product) at 30% conc.	T24	3.33 (10.52)	16.67 (24.09)	26.67 (31.09)	46.67 (43.09)	50.00 (45.00)	50.00 (45.00)	56.67 (48.84)
Untreated check	T25	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEm+		0.086 (0.092)	0.293 (0.221)	0.528 (0.336)	0.796 (0.482)	0.893 (0.552)	1.001 (0.641)	1.104 (0.703)
CD at 5%		0.244 (0.260)	0.831 (0.628)	1.500 (0.955)	2.262 (1.368)	2.537 (1.568)	2.843 (1.821)	3.136 (1.998)

Note: (i) Data are average value of three replication (ii) Initial value: 10 larvae / egg mass and (iii) Data in parenthesis are the angular transformed values of respective data.

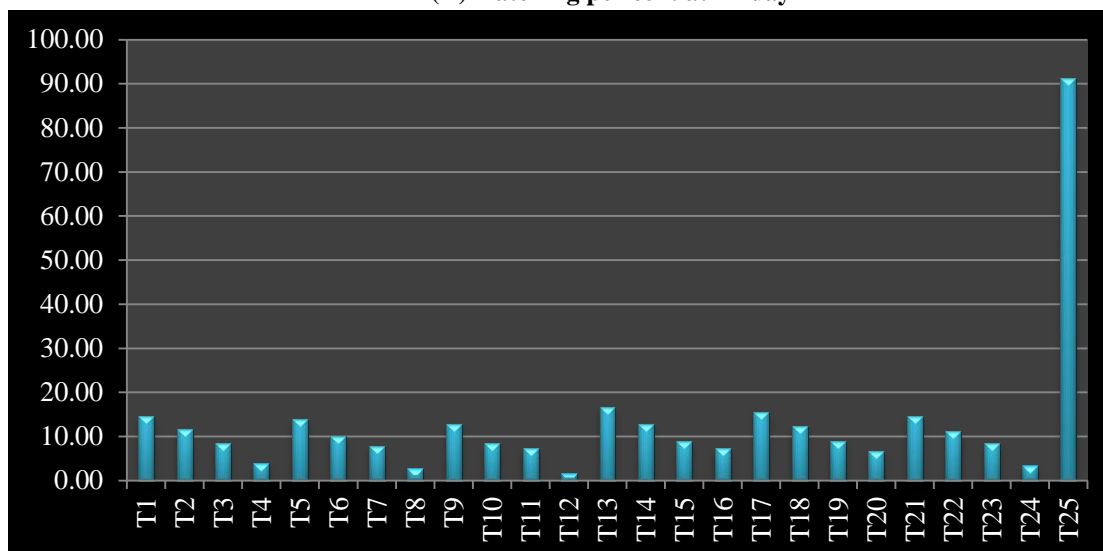
(A) Hatching per cent at 7<sup>th</sup> day

Fig. 1 Effect of different plant extracts on hatching and eggs masses of reniform nematode, *R. reniformis* in vitro

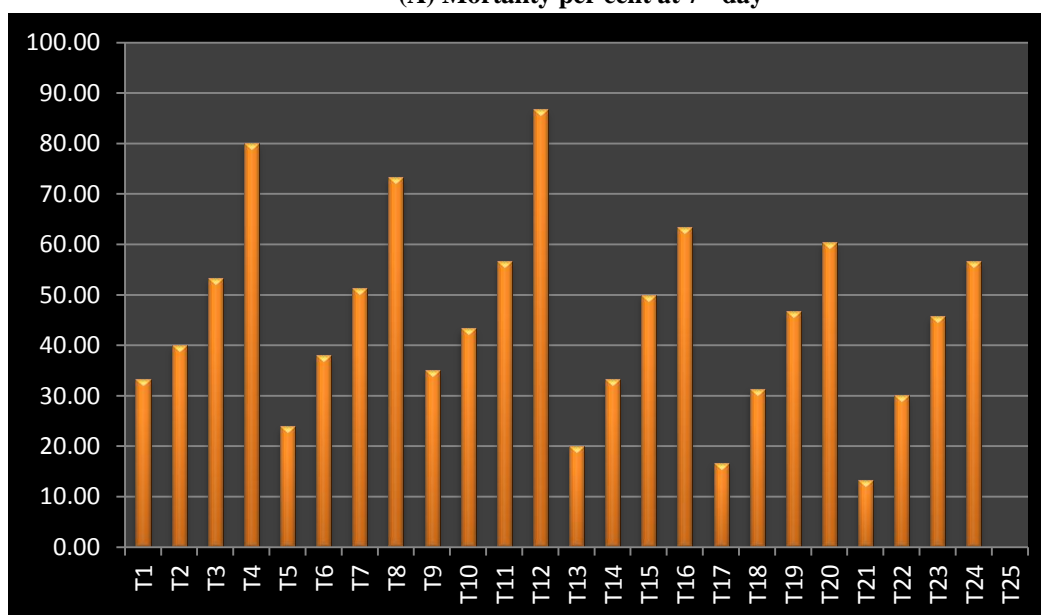
(A) Mortality per cent at 7<sup>th</sup> day

Fig. 2: Effect of different plant extracts on mortality of reniform nematode, *R. reniformis* larvae (in vitro)

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