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EFFICACY OF SOME SEEDS OF FAMILY APIACEAE AGAINST ROOT KNOT NEMATODE, *MELOIDOGYNE JAVANICA* (TREUB) CHITWOOD

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ABSTRACT

Root knot nematodes, *Meloidogyne* species cause severe losses to economically important crops. Synthetic nematicides put some major threats to humans and environment, that's why there is a need to find some environmentally safe control measures against root knot nematodes. Efficacy of seeds of some plants belonging to family Apiaceae viz., *Trachyspermum ammi* (L.) Sprague ex Turrill, *Coriandrum sativum* L., *Foeniculum vulgare* Mill., *Anethum graveolens* L. and *Cuminum cyminum* L. were evaluated against Root knot nematodes, *Meloidogyne javanica* (Treub) Chitwood, *in vitro*. Nematode eggs and juveniles were exposed for 24, 48, 72 and 96 h to crude aqueous extract of seeds powder for hatching and mortality test. Significant results were obtained in egg hatching and juvenile mortality test in all treatments and time durations. While *T. ammi, C. sativum, A. graveolens* were found to be hundred percent effective in killing juveniles and preventing egg hatching *in vitro*. Drenching and seed treatment by aqueous extracts of these seeds on okra and cowpea crops also exhibited significant enhancement in growth by all treatments. Aqueous extracts of *Trachyspermum ammi* and *Anethum graveolens* seeds were found to have best effects on growth parameters of okra and cowpea plants. The plants treated with aqueous extract of *Foeniculum vulgare* Mill., *C. cyminum* L. and *C. sativum* L. seeds had least root-knots.

Key-words: Root-knot nematodes, seed extract, Apiaceae, okra, cowpea.

INTRODUCTION

Most commonly found species of root knot nematodes are *Meloidogyne incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* Chitwood *a*nd *M. Hapla* Chitwood. Faster reproduction of *Meloidogyne* makes them difficult to control. They complete their life cycle in 20 to 30 days in warmer regions. A single female can easily lay many hundred eggs (Udo *et al*., 2008). Warmer regions are more prone to infection of root knot nematodes. Major difference in amount of crop production is observed due to root knot nematodes they make plant wilt by living inside the roots, blocking and damaging the water supply of plants and mean-while absorbing the plant nutrients and food material. All physiological processes are disturbed leading to less production rate (Adegbite and Adesiyan, 2001; Oyedunmade *et al*., 1992; Eisenback and Triantaphyllous, 1991). Very large host range of root knot nematodes makes it more available and easy to survive in fields for longer durations because crop rotation doesn't make any difference in control of root knot nematodes (Brodie *et al.*, 1993). Nematicides, fungicides and their combination with insecticides, were found to be effective against root-knot nematodes (Wang *et al*. 2004). It is important to control crop diseases to make less economic loss and to make the country more independent in food and plant derived materials. Plant driven pesticides is used by many scientists as a good alternative of synthetic pesticides (Olowe, 1992; Adesiyan *et al.*, 1990; Mangala and Mauria, 2006). Recently, the control of plant parasitic nematodes by using nematicides has declined internationally because of the inherent toxicity of many existing synthetic pesticides to non-target organisms and their persistence in the environment. One of possible alternatives is the utilization of pesticides from plant origin, known as botanical pesticides (Javed *et al*., 2006). Many locally available plants are known to possess insecticidal properties and used as a treatment for humans and animals. The biological activity of many plants has also been tested against root knot nematodes (Goswami and Vijayalakshmi, 1983; 1986; Jain and Hasan, 1984). Different plant parts are being tested to identify the sources of nematicidal substances. However seeds have received relatively lesser attention so far. Insecticidal properties of essential oils from family Apiaceae have been shown to possess strong nematicidal activity. These essential oils could be good alternative of chemical and synthetic nematicides (Ebadullahi, 2013). Strong nematicidal potential of essential oils of nine aromatic plants of family Apiaceae were evaluated in control of root knot nematodes (Amin, 2005). Seeds of *Coriandrum sativum* L. also shown to own insecticidal activity (Khani and Tahere Rahdari, 2012). Many scientists have used plants and their extracts in control of many fungi and bacteria (Beye, 1978; Renu *et* al., 1980; Ononuju and Okoye, 2003). More or less all crop plants are prone to infection of root knot nematodes so they are potential threat to world agriculture (Adekunle and Akinlua, 2007). Many plants have been found to be effective against root knot nematodes (Chitwood, 2002). Dried plant parts and elixir has been used in control of nematodes recently (Radwan *et al*. 2006; El-Rokiek and El-Nagdi 2011). Khurma and Mangotra (1996) and Khurma and Singh (1997) has tested the activity of seeds extracts in resistance of root knot nematodes and found promising results. Seeds are still not tested largely in this regard so there is much more to explore the nematicidal potential of seeds. Considering the nematicidal potential of different plant seeds and essential oils present work is based upon the screening of some plant seeds of family Apiaceae.

MATERIALS AND METHODS

IN VITRO HATCHING AND MORTALITY TESTS:

Preparation of Aqueous Extract:

Seeds of *Trachyspermum ammi* (L.) Sprague ex Turrill, *Coriandrum sativum* L., *Foeniculum vulgare* Mill., *Anethum graveolens* L. and *Cuminum cyminum* L. were washed thoroughly. The dried seeds were then powdered in an electric grinder and 10g of powdered seeds was soaked in 100mL of sterilized distilled water for 24 h. The solution was strained through muslin cloth and filtered through Whatman filter paper no.1. This standard solution, taken as 100% seed extract was used for further studies.

Hatching Test

Eggs of *Meloidogyne javanica* obtained from roots of eggplant (*Solanum melongena* L) were collected by the method of Hussey and Barker (1973). A suspension of eggs in distilled water was prepared, 2 mL egg suspension and 2 mL aqueous extract of seeds was transferred on glass cavity blocks, 2.5cm diameter, and kept at room temperature. Each treatment was replicated thrice. Egg suspension in sterile water served as control. After 24, 48, 72 and 96 h, number of hatched eggs were counted. The toxicity of seeds extract was judged by mean percentage of the hatched eggs.

Mortality Test

Two mL suspension of freshly hatched juveniles (40-50 juveniles/mL) and two mL of seeds extract was added to each glass cavity block to assess juvenile mortality. Each treatment was simulated thrice. Juvenile suspension in sterile water served as control. The glass cavity block without aqueous seed extract served as control. After 24, 48, 72 and 96 h, number of juveniles killed was counted. Toxicity of seeds extract was assessed by percentage of dead nematodes. Nematodes were considered dead if they didn't move when investigated with a fine needle (Cayrol *et al*, 1989).

GREEN HOUSE EXPERIMENT

Soil used for the experiment was obtained from experimental plots of Department of Botany, University of Karachi. The soil was sandy loam and basic in reaction (pH ranged from 7.5 – 8.1) with maximum water holding capacity (MWHC) of 40% as determined by the method of Keen and Raczkowski (1921). The total nitrogen as determined by the method of Mackenzie and Wallace (1954) was 0.08-0.10%. The pots were placed in a greenhouse with natural sunlight, in a randomized design and there were three replicates per treatment. For soil drenching, 15 ml aqueous seed extract of *Trachyspermum ammi, Coriandrum sativum, Foeniculum vulgare, Anethum graveolens* and *Cuminum cyminum* were drenched in the pots. Soil treated with sterilized distilled water served as control. For seed treatment Okra and cowpea seeds were soaked in aqueous seeds extract. After 30 minutes of treatment 5 seeds sown in each pot. Seeds and with sterilized distilled water served as control. Ten days after seedling emergence, the soil in each pot was inoculated with ±2000 juveniles of *M. javanica* by pouring the nematode suspension into holes made around the roots of each plant. Observations were recorded after 8 weeks of nematode inoculation by determining the number of galls per root system and recording the growth of crop plants.

ANALYSIS OF DATA

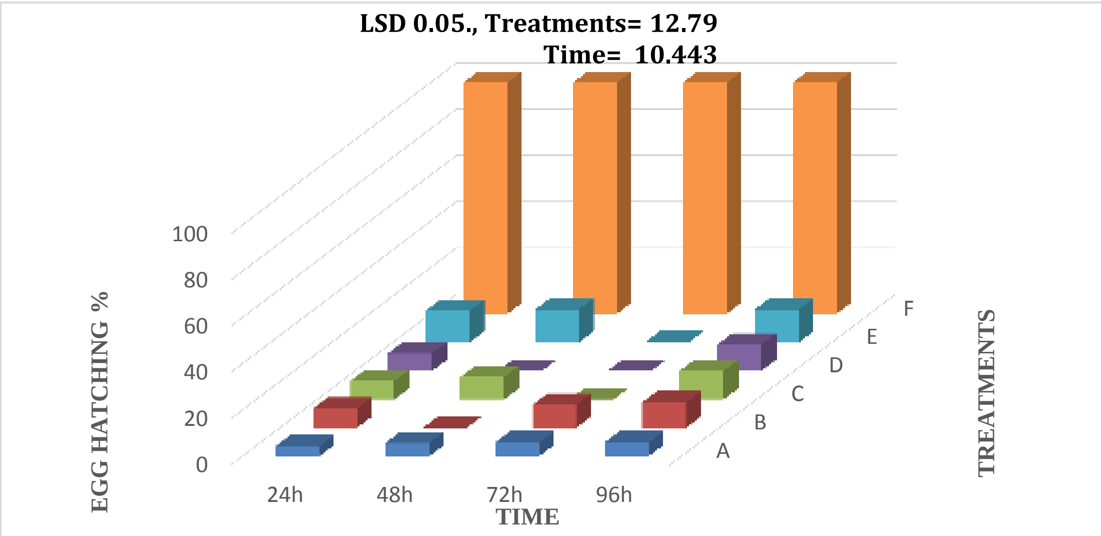
Data were analyzed for descriptive statistics and analysis of variance (ANOVA) / factorial analysis of variance (FANOVA) were performed. The follow up included calculation of Least Significant Difference (LSD). For comparison of the treatment means Duncan's Multiple Range Test (DMRT) was performed (Gomez and Gomez, 1984).

RESULTS

Aqueous extract of some seeds of family Apiaceae showed promising results in control of egg hatching and juvenile mortality of root knot nematodes. All seed treatments and time duration significantly controlled eggs hatching and juvenile mortality. Delayed hatching of eggs was observed with the treatments. Least egg hatching was found in treatment with *T. ammi* ($P<0.001$) (Fig. 1). In juvenile mortality test treatments of *T. ammi*, *C. sativum* and *A. graveolens* showed 100% mortality just after an interval of 24 h. Mortality was found to increase in treatment with time in case of F. *vulgare* and *C. cyminum* (Fig. 2).

Okra plants showed significant effect of drenching with aqueous seed extracts of seeds on all growth parameters and decrease in knot count in root system ($P<0.001$). Maximum increase in shoot length was found by the treatment of *F. vulgare* ($P<0.001$). Highest root length and root weight appeared in treatment of *C. cyminum* followed by *C.*

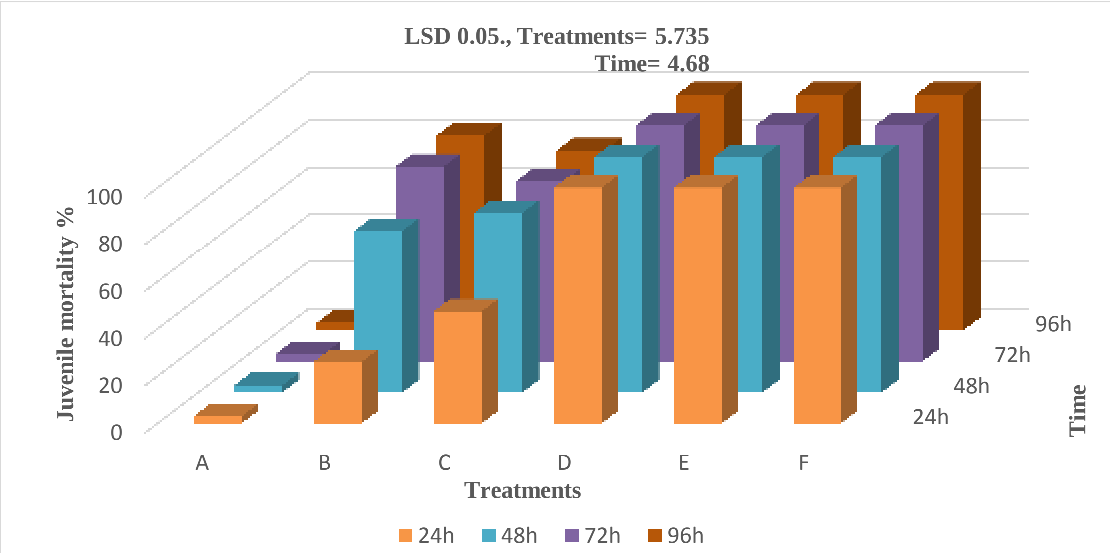
sativum and *A. graveolens* (P<0.001). Highest shoot weight was found in plants treated with aqueous seed extract of *T. ammi* and C. sativum. The largest Leaf area was found in treatment by *T. ammi* followed by *C. cyminum* and *F. vulgare* (Table 1). All seed treatments significantly reduced number of knots as compare to control plants. Least number of knot count observed in treatments by *C. cyminum* followed by *T. ammi*, *A. graveolens*, *C. sativum* and *F. vulgare*. Treatment of okra seeds with aqueous seed extracts had significant effect on shoot length, Shoot weight, root weight, leaf area and number of knots. Most increase in shoot length was found by the treatment of *F. vulgare* followed by *C. Sativum* (P<0.01). Highest root length appeared in treatment of *A. graveolens*. Highest shoot weight was found in plants treated by aqueous seed extract of *C. sativum* followed by *A. graveolens* (P<0.001). Largest Leaf area was found in treatment by *C. cyminum* followed by *T. ammi and C. sativum* (P<0.001). All seed treatments significantly reduced number of knots as compare to control plants. Least number of knot count observed in treatments by *C. sativum* (Table 2).



A= Control, B= C. cyminum, C= F. vulgare, D= T. ammi, E= C. sativum F= A. graveolens

Egg Hatching: $F_{treatment}$ = 47.3574 (p< 0.001), Ft_{ime} = 0.631 (NS), $F_{treatment\ x\ time}$ = 0.6363 (NS).

Fig. 1. Effects of Extracts of some seeds of Family Apiaceae on egg hatching of *M. javanica*.



A= Control, B= C. cyminum, C= F. vulgare, D= T. ammi, E= C. sativum F= A. graveolens

Juvenile Mortality: $F_{treatment}$ = 352.395 (p< 0.001), F_{time} = 17.25 (p < 0.001), $F_{treatment\ x\ time}$ = 7.98 (p < 0.001).

Fig. 2. Effect of aqueous extract of some seeds of family Apiaceae on Juvenile mortality of *Meloidogyne javanica.*

Table. 1. Effect of drenching of some seeds of family Apiaceae on okra plants.

Soil Drenching						
Treatments	Shoot Length	Root Length	Shoot Weight	Root Weight	Leaf area (cm²)	Number of Knots per Plant
Control	17.47 ± 0.4	6.35 ± 0.97	1 ± 0.09	0.25 ± 0.02	4.97 ± 0.90	71.67 ± 5.06
Trachyspermum ammi	24.8 ± 0.92	9.9 ± 0.95	1.6 ± 0.06	0.4 ± 0.03	19.80 ± 3.03	19 ± 1.06
Coriandrum sativum	25.2 ± 1.27	6.77 ± 0.39	2.45 ± 0.09	0.19 ± 0.01	20.69 ± 1.23	21.67 ± 2.56
Foeniculum vulgare	22.07 ± 0.51	9.6 ± 0.38	2.28 ± 0.06	0.28 ± 0.01	13.57 ± 1.72	25.17 ± 2.19
Anethum graveolens	24.17 ± 0.75	8.43 ± 0.55	1.97 ± 0.06	0.17 ±0.01	17.19 ± 1.06	32.33 ± 1.26
Cuminum cyminum	26.5 ± 0.51	9.63 ± 0.34	2.03 ± 0.09	0.25 ± 0.01	7.06 ± 0.5	21.83 ± 1.94
LSD 0.05	1.116	1.892	0.228	0.048	4.705	7.773

Table. 2. Effect of Seed treatment of some seeds of family Apiaceae on okra plants.

Seed Treatment						
Treatments	Shoot Length (cm)	Root Length (cm)	Shoot Weight (g)	Root Weight (g)	Leaf area (cm²)	Number of Knots per Plant
Control	28.8 ± 0	6.35 ± 0.97	1.0 ± 0.09	0.25 ± 0.02	4.97 ± 0.09	71.67 ± 5.06
Trachyspermum ammi	18.7 ± 2.23	5.83 ± 0.64	1.35 ± 0.17	0.35 ± 0.06	19.31 ± 1.54	24 ± 1.75
Coriandrum sativum	18.4 ± 0.72	6.89 ± 0.44	2.38 ± 0.14	0.48 ± 0.03	18.31 ± 1.17	1 ± 0.54
Foeniculum vulgare	15.4 ± 0.29	4.8 ± 0.22	1.03 ± 0.09	0.28 ±0.04	14.67 ± 1.36	27.67± 3.94
Anethum graveolens	21.18 ± 0.65	7 ± 0.53	1.45 ± 0.07	0.3 ± 0.03	17.06 ± 0.56	6 ± 1.41
Cuminum cyminum	21.87 ± 0.99	6.35 ± 0.54	1.47 ± 0.14	0.62 ± 0.08	7.17 ± 0.25	2 ± 0.68
LSD 0.05	3.123	1.7397	0.350	0.139	3.074	8.076

Table. 3. Effect of drenching of some seeds of family Apiaceae on Cowpea plants.

Soil Drenching						
Treatments	Shoot Length (cm)	Root Length (cm)	Shoot Weight (g)	Root Weight (g)	Leaf area (cm²)	Number of Knots per Plant
Control	11.83 ± 0.79	19.83 ± 0.48	1.85 ± 2.09	0.42 ± 0.09	7.09 ± 1.57	30 ± 3.39
Trachyspermum ammi	84.33 ± 5.52	10.67 ± 0.86	4.8 ± 0.60	0.37 ± 0.08	15.21 ± 0.93	30.33 ±1.36
Coriandrum sativum	122.67 ± 15.93	9.5 ± 0.76	3.75 ± 0.111	0.22 ± 0.04	22.52 ± 1.84	20.5 ± 1.76
Foeniculum vulgare	80.33 ± 9.64	11 ± 1.34	2.73 ± 0.43	0.45 ± 0.12	14.46 ± 0.8	11 ± 1
Anethum graveolens	86.33 ± 7.72	10 ± 2.08	3.21 ± 0.35	0.28 ± 0.03	10.65 ± 0.62	32.67± 1.12
Cuminum cyminum	106.83 ± 0.91	9.5 ± 0.76	2.5 ± 0.25	0.27 ± 0.05	23.27 ± 0.82	22.83 ± 1.4
LSD 0.05	30.760	3.906	1.056	0.214	3.422	5.126

Table. 4. Effect of Seed treatment of some seeds of family Apiaceae on Cowpea plants.

Seed Treatment						
Treatments	Shoot Length (cm)	Root Length (cm)	Shoot Weight (g)	Root Weight (g)	Leaf area (cm²)	Number of Knots per Plant
Control	11.83 ± 0.79	19.83 ± 0.48	1.85 ± 2.09	0.42 ± 0.09	7.09 ± 1.57	30 ± 3.39
Trachyspermum ammi	49.67 ± 2.8	9.08 ± 0.88	3.37 ± 0.33	0.43 ± 0.07	13.96 ± 1.86	26.17 ± 1.70
Coriandrum sativum	42.42± 2.18	8.17 ± 0.98	3.45 ± 0.34	0.18 ± 0.02	9.7 ± 2.16	9.17 ± 0.94
Foeniculum vulgare	68.42 ± 4.74	15.67 ± 2.36	5.6 ± 0.46	0.87 ±0.07	15.68 ± 0.87	2 ± 0.77
Anethum graveolens	52 ± 2.38	14.67 ± 2.16	4.73 ± 0.89	0.53 ± 0.09	17.19 ± 1.31	22.8 ± 1.83
Cuminum cyminum	68.25 ± 6.53	8.17 ± 0.49	2.77 ± 0.16	0.45 ± 0.04	14.62 ± 1.42	16 ± 1.59
LSD 0.05	3.123	1.739	0.350	0.139	4.582	5.499

Cowpea plants also showed significant effect of Drenching by aqueous seeds extracts. Maximum increase in shoot length was found in treatment with *C. Sativum* followed by *C. cyminum*. Maximum shoot weight was found in plants treated by aqueous seed extract of *T. ammi* followed by C. sativum. Largest Leaf area was found in treatment by *A. graveolens* followed by *T. ammi and C. cyminum* (P<0.001). Least number of knot count observed in treatments by *C. sativum* followed by *C. cyminum* followed by *T. ammi* and *A. graveolens* (P<0.001) (Table 3). Cowpea Seed treatments by aqueous extract had significant effect on all growth parameters and in control of knot count. Most increase in shoot length was found by the treatment of *C. cyminum* followed by *F. vulgare* and *A. graveolens* (P<0.01). Highest shoot weight was found in plants treated by aqueous seed extract of *F. vulgare* followed by A. graveolens. Root weight increased maximally with treatment with *F. vulgare*. Largest Leaf area was found in treatment by *F. vulgare* followed by *C. sativum* and *A. graveolens*. Least number of knot count was observed in the treatment with *F. vulgare* (Table 4).

DISCUSSION

Present study on seeds of some plants of family Apiaceae showed some promising results. Soil drenching on okra plants showed significant enhancement in all growth parameters and reduction in knots significantly by *T. ammi, C. cyminum, C. sativum* (P<0.001). Seed treatment on okra plants showed significant increase in shoot length, shoot weight, root weight and leaf area and reduced knot count significantly by *A. graveolens*, *C. sativum* and *C. cyminum*. (P<0.001) Drenching on cowpea showed significant increase in shoot length, root length, shoot weight, leaf area and knots decrease by *C. sativum, T. ammi* and *F. vulgare* with significance level of 0.001. Seed treatment on cowpea significantly enhanced all growth parameters and reduced knots by treatment of *A. graveolens,* and *F. vulgare* significantly. Treatments by *Foeniculum vulgare, C. cyminum* and *C. sativum* exhibited least number of knots. Comparison of both experiments indicated that drenching and seed treatment had significant differences in growth parameters of crops. In okra plants drenching has found to be more effective while in cowpea seed treatment was more affective. Ibrahim *et al*. (2007) reported that fenugreek caused great reduction in root galls and egg masses of *M. incognita.* Olaniyi *et al*. (2005) proposed that some chemical constituents from the plant materials could have deleterious influence on the growth and development of root knot nematodes and this ability may be exploited in a manner beneficial for root knot susceptible crops. Only small-scale work has been done on nematicidal activity of seeds. Some work was performed by Khurma and Khurma on leguminous seeds. Those seeds showed high nematicidal activity. Haq (2010) also reported the activity of some indigenous plant seeds against root knot nematodes. There is still much, to explore in this regard. Application of plants water extract directly on the field without the use of toxic organic solvents could be of interest in connection of the crop protection. The appeal of the use of plant water extracts is the budget efficiency for their production and potential use in developing countries and environmental friendliness.

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