NEMATICIDAL ACTIVITY OF *Asphodelus tenuifolius* ON THE MANAGEMENT OF *Meloidogyne javanica* IN TOMATOES UNDER LABORATORY AND GREENHOUSE CONDITIONS

Rifat Ali[1]*, Ishrat Naz[2], Aisha Khan[3], Muhammad Numan[3], Ijaz Ahmad[2], Mehran Khan[4] and Shahab Khan[2]

DOI 10.28941/pjwsr.v28i4.1080

ABSTRACT

The nematicidal effect of *Asphodelus tenuifolius* (onion weed) extracts on root-knot nematodes, *Meloidogyne javanica* was evaluated under *in vitro*, and *in vivo* conditions. Methanolic crude extract of *A. tenuifolius* was fractionated into *n*-hexane and acetonitrile fractions. Results showed that both of the crude fractions significantly ($P \leq 0.05$) suppressed egg hatching and increased the $J_{2'}$s mortality *in vitro*. Among the different concentrations used, the highest concentration of 500 ppm was found more effective, resulting in maximum egg hatch inhibition (47.33%) and $J_{2'}$s mortality (93.14%) in the *n*-hexane crude fraction. Under greenhouse experiments, the application of *A. tenuifolius* significantly inhibited the number of galls, galling index, eggs per egg mass and egg mass per inch of the root system of tomato plants. Plant height, root length, fresh and dry root weight and dry shoot weight of tomato plants were significantly increased when *A. tenuifolius* @ 20g kg^{-1} of the potting mixture was used under un-inoculated conditions. No phytotoxicity of *A. tenuifolius* was observed on tomato plants.

Keywords: *Asphodelus tenuifolius,* Egg hatching inhibition, Juvenile mortality, *Meloidogyne javanica* and Tomato.

Citation: Ali. R., I. Naz, A. Khan, M. Numan, I. Ahmad, M. Khan and S. Khan. 2022. Nematicidal activity of *Asphodelus tenuifolius* on the management of *Meloidogyne javanica* in tomatoes under laboratory and greenhouse conditions. Pak. J. Weed. Sc. Res., 28(4): 465-477.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most important crop in the world next to potato with approximately 182.3 million tons of tomato fruits produced on 4.85 million ha each year (Hassan *et al*., 2020). Tomato suffers huge qualitative and quantitative losses due to biological stresses present in the ecosystem. Among the various pests and diseases affecting tomato, the most prevalent and destructive among the phyto-nematodes of tomato are *Meloidogyne* spp., which causes major economic losses on vegetables, especially in tropical and subtropical areas (Hassan *et al*., 2020). Root-knot nematodes can cause major destruction and yield losses. In subtropical and tropical regions of the world, 13-38% of crop losses have been reported (Netscher and Sikora, 1990 & Fuller et al., 2008). Root-knot nematodes, *Meloidogyne* spp., alone, causes losses of up to 125 billion U.S. dollars annually (Moens *et al.,* 2009). These nematodes affect the development of the plants resulting in poor growth, low yield of the crop, lower quality, and can easily break the resistance developed by the host plant and make the plants susceptible to other pathogens (Manzanilla- Lopez, 2004). In Pakistan, 40% of losses caused by

[1] *Directorate General Agricultural Research, Peshawar-Pakistan.*
[2] *Department of Plant Pathology, The University of Agriculture, Peshawar-Pakistan.*
[3] *Directorate of Soil & Plant Nutrition, Agriculture Research Institute, Peshawar-Pakistan.*
[4] *Ministry of National Food Security & Research, Department of Plant Protection, Karachi-Pakistan.*
* *Corresponding author's email: rifatali644@yahoo.com*

Meloidogyne spp., have been reported (Anwar and McKenry 2012).

Different management strategies such as managing PPNs through cultural practices, for example, crop rotation of susceptible host plants with non-hosts, use of nematode-resistant transgenic plants, biological control methods through the use of organic amendments to the soil, by applying wastes of the agricultural products or botanicals and synthetic nematicides have been used (Khattak, 2008). Many organic waste materials have shown nematicidal properties (Kimpinski *et al.,* 2003). Controlling nematodes by nematicides is very expensive and it also affects the agroecosystem, therefore alternate management strategies should be explored which are eco-friendly, cheap and easily available to the growers (Chitwood, 2002). Crops and weeds may exhibit biochemical substances or phytochemicals that have been reported to suppress the activity of nematodes in soil or within plant roots (Naz *et al.,* 2013). About 51 families of plant species have been reported to contain nematicidal compounds (Deng, 2022). Researchers have studied the effect of aqueous extracts of botanicals on the nematicidal activity of root-knot nematodes and found the inhibitory effect of seed extracts of *Melothria purpusilla*, *Jatropha curcas* and *Lantana camara* (Joymati *et al.,* 1998). In one study, the aqueous extracts of the fresh flower of *Verbesina enceliodes* showed the strongest egg hatch inhibition and juvenile mortality (Oka, 2012).

Similarly, the application of green manuring has been reported to be effective against plant parasitic nematodes in a greenhouse study (Mojtahedi *et al.,* 1991). For instance, the incorporation of *Crotalaria spectabilis* residue in soil resulted in reduced galling by root-knot nematode in tomatoes (Villar and Zavaleta, 1990), while similar findings were recorded by other researchers for controlling *M. incognita* on okra (Wang *et al.,* 2007). Some weeds could be used as green manure or in powder form to increase the organic content of soil and control root diseases. The objective of this study is to evaluate the effect of *A. tenuifolius* against root-knot nematodes, *M. javanica* on tomato plants *in vitro* and *in vivo*.

MATERIALS AND METHODS

Collection of root-knot nematode and collection and drying of *Asphodelus tenuifolius*

Galled roots of diseased tomato plants were collected from different infested tomato fields of Dargai in the Malakand division. Samples were packed in polythene bags, labeled and brought to the Department of Plant Pathology laboratory for further studies. The samples were then stored in a refrigerator at 4 °C. Onion weed viz., *A. tenuifolius* was collected from Dargai area of Malakand Division, stored in a paper bag, labeled and brought to the Department of Plant Pathology Laboratory, The University of Agriculture Peshawar. For further activities, the plant samples were then dried in shade for two months.

Identification and culturing of root-knot nematode

Galls were crushed with a sharp needle in a petri dish containing tap water and mature females were picked and placed in a petri dish with tap water. For the removal of body contents from the female root-knot nematode, the neck portion of the adult female was cut off with the help of a sharp needle. About 10-12 cuticles were placed in a drop of 45 % lactic acid on a plastic petri dish. Similarly, 5-10 cuticles were collected at the drop and allowed to stand for 24 hours. The posterior portions of the females were cut with a sharp surgical needle and the perennial pattern morphology was observed under a stereo microscope (Eisenback *et al.,* 1981). After identification, root-knot nematode, *Meloidogyne javanica* was cultured and maintained on a succulent and susceptible variety of tomatoes (Riogrande) in pots in the greenhouse. For each root zone of a 21-day-old tomato seedling, a single egg mass of *M. javanica* was inoculated in pots containing pasteurized soil. Inoculation was done with the help of a pipette under aseptic conditions. This mass pure culture was sub-cultured by inoculating new tomato seedlings of the same cultivar with

10-15 egg masses, obtained from the pure culture.

Laboratory assay

The shade-dried, plants (*Asphodelus tenuifolius*) were milled to fine powder particles by Thomas-Wiley laboratory mill using a 0.5 mm grinder and 100 g powder was soaked in 500 ml methanol in a 2 L Erlenmeyer flask for one week at room temperature. The solution was passed through a muslin cloth, and filtered through Whatman filter paper No. 1. Extraction was repeated five times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using a rotary evaporator under reduced pressure at 45 °C (Naz *et al.,* 2013). The aqueous crude extract was then placed in an oven at 40 °C for about 48 hours to remove the water. The resulting dried mass was then converted to powder, transferred to a fully dried glass vial and stored in the refrigerator at 4 °C until used. The residues obtained were termed crude extracts. These crude extracts were then fractioned for further study. A portion of crude methanol extract (1.0 g) was fractioned into non-polar and polar fractions using reagent-grade *n*-hexane (10.0 ml) and HPLC-grade acetonitrile (10.0 ml). The solutions were then transferred into a separating funnel (1.0 L) and the yield of *n*-hexane and acetonitrile extracts from plant material was separated, measured and dried. Dried fractions were then stored at 4 °C in the refrigerator in oven-dried bottles for use in the nematicidal bioassays against eggs and juvenile *M. javanica*. Different concentrations of 100, 250, and 500 ppm were prepared from acetonitrile and *n*-hexane crude fractions (Naz et al., 2013). Negative control was treated with Dimethyl Sulfoxide (5% DMSO) only and positive control was treated with Cadusafos® (nematicide) + 5% DMSO along with a lower concentration of fractioned crude extract i.e., 100 ppm. Data on egg hatch inhibition were recorded for up to eight days and J_2s mortality was recorded after 72 hours of incubation.

Hatchability test

Triplicates of each concentration of *n*-hexane and acetonitrile fractions of crude extract of *A. tenuifolius* except negative control were kept in a nematode counting dish. In positive control, the nematicide Cadusafos (Rugby®) with the addition of DMSO 5% and crude of lower concentration 100 ppm was used for the comparison and only 5% aqueous DMSO was used as a negative control. All the treatments were assigned according to a completely randomized design (CRD). A total of 50 eggs of *M. javanica* were placed in each block of nematode counting dish and incubated at 27±2 °C in the incubator. Un-hatched eggs were counted every day under a stereo microscope. Percent egg hatches inhibition was calculated in each cavity block using the following formula (Percent egg hatches inhibition = Total no. of unhatched eggs/total no. of eggs in each block x 100) (Naz *et al.,* 2013).

Juvenile Mortality

Similarly, a triplicate of each concentration of *n*-hexane and acetonitrile fractions of crude extract of *A. tenuifolius* except negative control was kept in each nematode counting dish. In positive control, the nematicide Cadusafos (Rugby®) with the addition of DMSO 5% and crude extract of lower concentration of 100 ppm was used for the comparison and only 5% aqueous DMSO was used as a negative control. All the treatments were assigned according to a completely randomized design (CRD). A total of 30 juveniles of *M. javanica* were placed in a block of nematode counting dish and incubated at 27±2 °C in the incubator. The total number of dead J_2s was counted after 1, 3, 6, 12, 24, 36, 48 and 72 hours of incubation under the stereo microscope. Mortality of J_2s was confirmed by touching the juvenile of *M. javanica* with a fine needle to find whether it is dead or alive (Rizvi and Shameel, 2006). Mortality was determined by the following formulae (Percent J_2s mortality = Total no. of dead J_2s/Total no. of J_2s in each block x 100) (Naz et al., 2013).

Greenhouse Experiments

Inoculum preparation

Galled roots were dipped in a plastic bucket containing tap water to remove adhering soil particles. The washed knots on the roots of the tomato plants were cut into small pieces with the help of a scissor and ground in an electrical grinder for 1 minute in sodium hypochlorite (NaOCl) solution (Hussey and Barker, 1973). The suspension containing nematode eggs was collected in the plastic beaker after passing the suspension through 500, 400, 375, 250, 100, 63, 36 and 25µm mesh sizes. The eggs were collected in distilled water and counted in a counting dish as eggs per one ml (200 eggs ml^{-1}) under the stereo microscope. This procedure was repeated three to four times and an average number of eggs was recorded and stored in 1% saline solution at 4 °C in the refrigerator (Khattak, 2008**).**

Tomato germplasms (cv. Riogrande) were obtained from Agriculture Research Institute (ARI) Tarnab, Peshawar. The potting mixture was prepared from soil, sand and compost in a balanced ratio (1:1:1 v/v). The potting mixture was pasteurized at 60-80 °C for 1 hour in heat-resistant plastic bags. The pasteurized soil was then allowed to cool at room temperature and was spread on a tray for 3 days before inoculation, to facilitate the release of harmful gases. The nursery was raised from the obtained tomato germplasms in the pasteurized potting mixture. Tomato germplasms were nursed separately in plastic bowls filled with potting mixture and were left for about 21 days to germinate, grow and transplanted into clay pots (6 inches). The pots were watered enough and left overnight. A proper drainage system was provided to prevent water stagnation in each pot 21 days post-germination, uniform and healthy seedlings were transplanted into pots. The plants were allowed for one week to get established.

Inoculation procedure

The tomato plants except for control treatments were inoculated with *M. javanica* eggs in 10 ml water suspension (200 $eggs^{-1}ml$) by pipetting a few holes 1.5 inches deep into the rhizosphere of the plants. The holes were then covered sterilized soil medium and watered in a lesser amount. About 2000 eggs were inoculated to each plant 10 days after transplantation when the plants were well-established.

In green manuring, the whole onion weed (*A. tenuifolius*) plant was washed with tap water and chopped finely into small pieces with a sterilized sharp scalpel. Different treatments from the chopped whole plant were applied to tomato plants in pots at a rate of 10, 20 and 30 g per pot. The control pots received no chopped plant materials. Green manure was applied to clay pots (6 inches) in the greenhouse 21 days before transplanting of tomato seedlings.

Asphodelus tenuifolius collected from different tomato fields of Dargai in Malakand division was applied in three parts, i.e., shoots (aboveground part), roots (below-ground part) and whole plant. Each part was air dried under shade at room temperature for about two months and milled by the Thomas-Wiley laboratory mill separately into 2.0 mm fine powder particles. The powder obtained from different parts of the *A. tenuifolius* was stored in polyethylene bags for further use. Desired quantity of powder obtained from each part of the *A. tenuifolius* was applied to the pots in different doses of 10, 20 and 30g. The dry powder particles were applied after 10 days of inoculation to pots containing tomato plants in the greenhouse. Each treatment was replicated five times in a complete randomized design (CRD).

Data collection

The experiment was terminated 50 days after inoculation. Each plant was uprooted, and the roots were washed carefully with tap water. Plant growth parameters, and nematode assessment were collected.

Plant growth assessment

Plants were gently uprooted and the root system separated from the shoot system at the first basal node. The root systems were carefully and thoroughly washed before taking them, plant height (cm), root length (cm), fresh and dry shoot weights of root and shoot(g)..

Nematode Assessment

The root systems were carefully and thoroughly washed before taking their, number of galls per plant root system, number of eggs per egg mass, the number of egg masses per inch of the root system, and galling index. The galling index was obtained by using the scale described by Sasser (1984).

Statistical Analysis

A completely randomized design (CRD) with two factors was used in the experiment having five replications in two factors. Means were calculated and all the recorded data were subjected to analysis using Analysis of Variance (ANOVA). Fisher's Least Significant Differences (LSD) test was used for mean separation (Steel *et al.,* 1997).

RESULTS

Laboratory Studies

Egg hatching and juvenile mortality

Results in Fig. 1 (panel, a) showed that acetonitrile crude fraction from *A. tenuifolius is* more effective in controlling root-knot nematode, *M. javanica*. It was noted that analysis of time interval showed that the highest percent J_2s mortality of root-knot nematode was 100% after 48 hours of incubation. There were found significant in different concentrations and on the overall basis, analysis of concentrations showed that the maximum percentage of larval mortality was observed at 500 ppm (85.43%) of larvae found whereas the lowest J_2s mortality was recorded in negative control at 1 h of incubation.

At the same time, results in Fig. 1 (panel, b) showed that *n*-hexane fraction crude from *A.* had an effective effect on root-knot nematodes, *M. Javanica*, it caused 100% mortality of J_2s after 48 hours of incubation. It was observed that significant differences were found in different concentrations of the crude extract and on the overall basis, analysis of concentrations showed that maximum percent J_2s mortality was observed at 500 ppm (93.14%). The lowest larval mortality was found after 1 h of incubation.

Results in Fig. 1 (panel, c) revealed that acetonitrile fraction of *A. tenuifolius* crude extract more effectively inhibits the egg hatching of *M. javanica*. The maximum percent hatch inhibition was 80.66% after 8 days. At the same time, the maximum percent egg hatch inhibition was (45.33%) with a concentration of 500 ppm.

However, analysis of the time interval in Fig. 1 (panel, d) showed that maximum hatch inhibition of *M. javanica* was observed on the 8^{th} day (77.33%) of incubation. As well, it increases in the concentrations of the extract significantly increased the percent hatch inhibition. Maximum percent egg hatch inhibition was observed at 500 ppm (47.33%).

Greenhouse studies

Application of different parts of *A. tenuifolius* had a significant effect ($P < 0.001$) on nematode multiplication and plant growth parameters when applied at 0, 10, 20 and 30 g kg^{-1} soil) (Table. 1). The application of *A. tenuifolius* significantly enhanced tomato height (cm). There was a linear increase in the plant height was noted with increasing doses (Table. 1). As regards the application methods, green manure application of *A. tenuifolius* was found more effective in increasing tomato plant height. The stimulative response of *A. tenuifolius* on plant height of tomato was also obvious under un-inoculated conditions and statistically similar results were obtained with root, shoot, whole plant powder and green manure of *A. tenuifolius* (Table. 1).

Also, the study indicated that the root length (cm) was significantly increased with the increasing doses of *Asphodelus tenuifolius*. A linear increase in the root length with doses was noted. As regards the application methods, root powdered application of *A. tenuifolius* was found more effective in increasing tomato root length. The stimulative response of *A. tenuifolius* on the root length of the tomato was also obvious under un-inoculated conditions and statistically similar results were obtained with root, shoot, whole plant powder and green manuring of *A. tenuifolius* (Table 1). Fresh

root weight (g), dry shoot weight (g), fresh shoot weight and dry shoot weight (g) of tomato were significantly increased with the application of *A. tenuifolius*. A linear increase in the fresh root weight with doses was noted (Table 1). In control treatment, where no inoculum and no *A. tenuifolius* were added, the fresh root weight of infected tomatoes was found minimum.

As regards the application methods, the green manure application of *A. tenuifolius* was found more effective in increasing the fresh root weight of tomatoes.

Results in (Table 1) revealed the number of galls per plant root of tomato, eggs per egg mass, egg mass per inch root system and galling index was significantly decreased by the application of *A. tenuifolius*. A linear decrease with increasing doses was noted. The minimum number of galls, eggs per egg mass, egg mass per inch root system and galling index were observed with the highest dose of *A. tenuifolius*. No gall per plant root, eggs per egg mass, egg mass per inch root system and the galling index was found in control treatments where no inoculums of root-knot nematode and no *A. tenuifolius* were added and no statistical difference was observed. As regards the application methods, green manure application of *A. tenuifolius* was found more effective in decreasing the number of galls per plant root of tomato, eggs per egg mass, egg mass per inch root system and galling index (Table 1). Stimulative response of *A. tenuifolius* on galls per plant root, eggs per egg mass and galling index of tomato was also obvious under un-inoculated conditions and statistically similar results were obtained with root, shoot, whole plant powder and green manuring of *A. tenuifolius* (Table 1).

Table 1. Effect of different parts of *A. tenuifolius* on nematicidal properties of root- knot nematode, *M. javanica* and plant growth parameters of tomato in greenhouse conditions.

Plant parts and doses (g/plant)	Growth parameters						Nematode parameters			
	Plant height (cm)	Root length (cm)	Fresh root weight (g)	Fresh shoot weight (g)	Dry root weight (g)	Dry shoot weight (g)	Number of galls per plant	GI [a]	Eggs per egg mass	Egg mass per inch of roots
Roots										
10	72.80	19.00	12.91	27.96	5.48	9.88	77.00	4.00	284.00	9.00
20	9.60	23.80	13.91	27.02	4.48	8.88	60.60	4.00	264.00	6.20
30	80.20	26.00	14.74	26.62	4.48	7.94	38.80	3.80	253.80	4.40
c^1	78.20	13.80	8.94	31.52	2.74	9.04	0.00	0.00	0.00	0.00
c^2	85.00	29.20	13.87	37.86	3.92	11.96	0.00	0.00	0.00	0.00
c^3	67.40	10.20	17.98	20.52	7.22	3.54	87.60	4.00	299.00	10.00
Shoots										
10	77.20	15.80	10.13	31.20	6.26	9.30	70.00	4.00	282.00	7.20
20	81.60	17.40	11.15	29.84	3.70	8.02	64.80	4.00	259.40	5.80
30	86.60	19.40	12.22	29.50	5.58	7.08	41.60	3.80	249.80	4.00
c^1	78.20	13.80	8.94	31.52	2.74	9.04	0.00	0.00	0.00	0.00
c^2	90.60	24.60	13.85	38.68	4.22	11.02	0.00	0.00	0.00	0.00
c^3	67.40	10.20	17.98	20.52	7.22	3.54	87.60	4.00	299.00	10.00
Whole										
10	81.00	18.60	10.42	34.90	6.12	9.98	57.80	4.00	277.80	6.60
20	85.20	15.40	13.35	33.72	4.90	8.86	48.00	4.00	249.80	5.40
30	89.60	22.40	15.82	33.24	4.60	7.60	32.00	4.00	241.40	4.40
c^1	78.20	13.80	8.94	31.52	2.74	9.04	0.00	0.00	0.00	0.00
c^2	93.60	27.80	13.80	39.38	4.70	10.92	0.00	0.00	0.00	0.00
c^3	67.40	10.20	17.98	20.52	7.22	3.54	87.60	4.00	299.00	10.00

Green manure										
10	83.40	17.40	14.82	38.20	7.04	10.92	79.00	4.00	273.00	9.20
20	87.20	21.20	15.58	37.92	6.66	10.00	61.00	4.00	245.60	8.60
30	92.60	23.60	16.38	37.40	6.30	9.18	30.40	3.40	234.60	7.40
c^1	78.20	13.80	8.94	31.52	2.74	9.04	0.00	0.00	0.00	0.00
c^2	94.60	27.80	14.16	37.54	5.10	13.90	0.00	0.00	0.00	0.00
c^3	67.40	10.20	17.98	20.52	7.22	3.54	87.60	4.00	299.00	10.00
P for interactions [P]										
Plant parts	1.996	0.760	0.431	0.717	0.268	0.255	1.925	0.087	2.497	0.268
Doses	1.629	0.620	0.351	0.878	0.328	0.312	2.358	0.107	3.036	0.329
Plant parts x doses	3.992	1.520	0.862	1.757	0.656	0.625	4.716	0.214	6.072	0.658

Data are average of 5 replicates.

[a] Galling index: 0 = no gall on roots; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; 5 = more than 100 galls per root according to (Sasser, 1984) .

[b] P for interaction between individual factors

c^1 Control containing no RKN + no *Asphodelus tenuifolius*

c^2 Control containing only *Asphodelus tenuifolius* @ 20g,

c^3 Control containing only RKN and no other applications

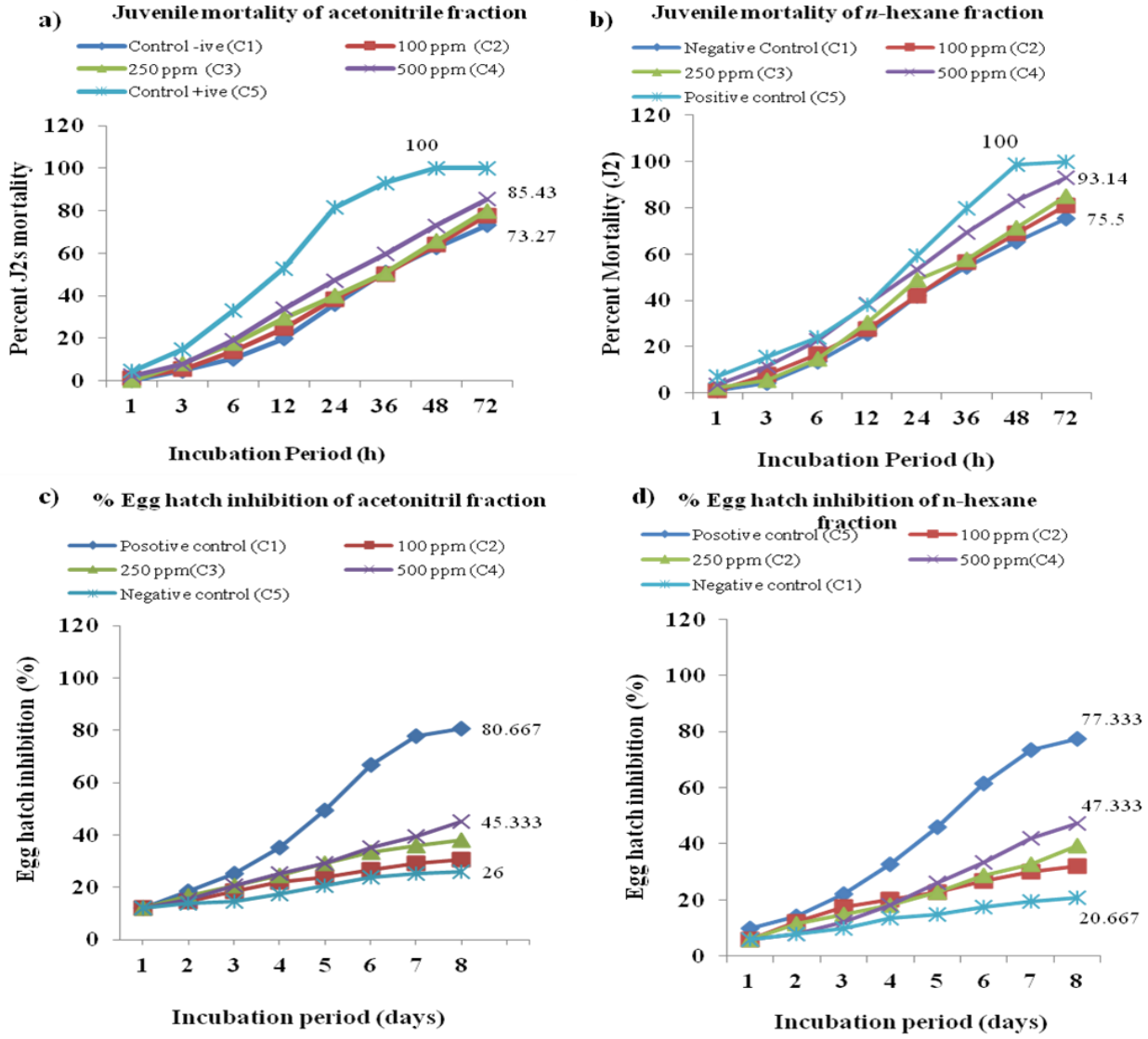

Figure 1. Juvenile mortality and egg hatch inhibition of *M. javanica* obtained at different concentrations of crude acetonitrile and *n*-hexane fractions of *A. tunifolius* at 27°C; panels (a and b) represents J_2s mortality obtained at 72 hrs of incubation at three different concentrations of crude extracts; panel (c and d) represents egg hatch inhibition of *M. javanica* obtained at different incubation period (1-8 days) at three different concentrations of crude extracts. Positive and negative controls are also shown in figure 1.

DISCUSSION

Many plants have been known for their medicinal and antimicrobial properties against plant-parasitic nematodes, many years ago, approximately, 2400 plant species are known to possess biologically active compounds that control various plant pests and pathogens effectively (Abdel-Baset and Abdel-Monaim, 2020). In the present study, *A. tenuifolius* weed plants were collected from Dargai, Malakand division to test the nematicidal effect of its different parts against root-knot nematodes, *M. javanica* in the laboratory as well as in greenhouse conditions. In this study, the highest nematicidal activity against *M. javanica* was achieved with a 500 ppm concentration of *A. tenuifolius* extract resulting in maximum egg hatch inhibition and J_2s mortality. These results are in agreement with Ajayi (1990) who stated that 100% egg hatch inhibition and larval mortality of root-knot nematodes were achieved with the application of bitter leaf extracts. Similarly, *Stoechospermum polypodioides* extracts showed strong nematicidal activities against juveniles of root-knot nematodes (Sulatana *et al*., 2000). Elbadri *et al.,* (2008) used n-hexane and methanol extract from leaves and seeds of *Solenostemma argel*, *Ziziphus spnachristi*, *Aregimone mexicano* and *Azadirachta indica* and found an increase in mortality of root-knot nematode. Moreover, Chitwood (2002) found that 90% of juvenile mortality within 24 hours by the application of flavones-C-glycoside and lantanoside from the extract of *Fumaria parviflora.* The same trend with (Abdel-Baset and Abdel-Monaim,2020) found that the ethyl alcohol extract of leaves *Eugenia jambolana* completely inhibited the egg hatching of *M. javanica*, and caused 100% mortality after 48 hours. The present study suggests that the efficacy of onion weed powder increased when the plant doses were gradually increased, which might be due to the level of concentration of toxic chemicals present in the plant materials (Naz *et al.,* 2013).

Various doses of *A. tenuifolius* such as 10, 20 and 30 g pot^{-1} were used. The highest dose, i.e., 30g pot^{-1} of green manure significantly increased plant growth parameters such as plant height, root length, fresh and dry shoot weight, etc. These results are in agreement with (Abdel-Baset and Abdel-Monaim, 2020) who reported that soybean plants treated with ethyl alcohol leaf extract of *E. jambolana* exhibited the highest shoot and root weights and lengths in general, this could be explained by the assumption that the plant extracts substantially reduced the population and damage of *M. javanica* to the infected plant and enhanced plant growth due to their fertilizing ability.

Also, our results revealed the number of galls per plant root of tomato, eggs per egg mass, egg mass per inch root system, and a galling index of *M. javanica* was significantly decreased by the application of *A. tenuifolius* as well, there was a linear decrease with increasing doses was noted. That reduction in galls, galling index and egg masses could be due to the decomposition of onion weed (*A. tenuifolius*), and the release of secondary metabolites such as glycosides, flavonoids, alkaloids, saponins, steroids, and phenols nematicidal to the root-knot nematodes (Naz *et al.,* 2013).

The galling index of the root-knot nematode was significantly reduced when *A. tenuifolius* was used as green manure. This finding is supported by the results of Abid *et al.,* (1997) who reported that soil amendment with neem extract reduced the galling index of root-knot nematode. Amin and Youssef (1999) reported that as compared to the dry powder application of *A. tenuifolius,* green manure gave fruitful results. Our results showed very promising suppressive effects of the green chopped amendments of the *A. tenuifolius* whole plant to the population of root-knot nematode when applied 21 days before tomato nursery transplanting. The application of *A. tenuifolius* as green manure stimulated plant growth. Early incorporation of green amendments of *A. tenuifolius* before seedling transplantation possibly resulted in the early release of secondary chemical substances into the potting mixture that inhibited the hatching of *M. javanica* eggs (Naz *et al.,* 2015). These compounds could interfere with and suppress root-knot nematode eggs and second-stage active juveniles. The fact that *A. tenuifolius* is a non-host for the attack of root-knot nematode.

Additionally, early decomposition of the green manure also results in the release of beneficial nutrients to the plant rhizosphere. According to Braddy and Weil (1999), green manure also has the capability of water holding and ion adsorption capacity.

Crude extract of *A. tenuifolius* possesses nematicidal properties and has reduced egg hatching and enhanced increased juvenile mortality using a crude concentration of 500 ppm. Thin layer chromatography showed that the *n*-hexane fraction of *A. tenuifolius* crude extract contains eight bioactive compounds than the acetonitrile fraction. The 30 g kg^{-1} potting mixture of dried powder and green manure of all parts of *A. tenuifolius* was more effective in managing *M. javanica* and enhancing plant growth. Green manure of *A. tenuifolius* applied before transplanting tomato seedlings was very effective in increasing tomato plant growth and reducing the root-knot nematode population.

Further biochemical studies are required to identify bioactive chemicals and their compositions. Studies are also required to test *in vitro* and *in vivo* efficacy of plants used as powders and green manures against other nematodes, bacterial, viral and fungal diseases.

REFERENCES CITED

Abdel-Basat, S.H. and M.F. Abdel-Monaim. 2020. Nematicidal Potential of some Natural Botanical Extracts in Biocontrolling *Meloidogyne javanica* on Soybean under Laboratory and Greenhouse Conditions. Egypt. J. Agronematol., 19: 1-18.

Abid, M., M.I. Choudhry, M.A. Maqbool, and A.U. Rahman. 1997. Preliminary screening of some plants and their nematicidal activity against *Meloidogyne javanica.* Nematol Mediterr., 25:155–7.

Ajayi, V.A. 1990. Comparison of Nematicidal Potential of Vernonia amygdalina (Bitter leaf) and carbofuran (Furadan) on the growth and yield of root-knot infested soybean *Glycine max* LM Sc. Nigeria: Department of Agric. Biology, University of Ibadan, 283.

Amin, A.W. and M.M. Youssef. 1997. Efficiency of certain plant leaves for controlling *Meloidogyne javanica* and *Rotylenchus reniformis* infecting sunflower in Egypt. Int. J. Nematol., 7:198-200.

Anwar, S.A. and M.V. McKenry. 2012. Incidence and population density of plant-parasitic nematodes infecting vegetable crops and associated yield losses in Punjab, Pakistan. Pak. J. Zool., 44:327-333.

Braddy, N. and R. Weil. 1999. Elements of the nature and properties of soil spp. Upper Saddle River. Prentice-Hall, Inc, New Jersey.Chitwood, D.J. 2002. Phytochemicals-based strategies for nematode control. Annual Rev Phytopathol., 40:221–249.

Deng, X., X. Wang, and G. Li. 2022. Nematicidal Effects of Volatile Organic Compounds from Microorganisms and Plants on Plant-Parasitic Nematodes. Microorganisms. 12; 10:1201. https://doi.org/10.3390/microorganisms10061201.

Eisenback, J.D., H. Hirschmann, J.N. Sasser, and A.C. Triantaphyllou. 1981. A guide to the four most common species of root knot nematodes (Meloidogyne spp.) with a pictorial key Plant Pathology (pp. 1-51). Department of Plant Pathology and Genetics North Carolina State University and The United States Agency for International Development.

Elbadri, G.A., D.W. Lee, J.C. Park, H.B. Yu, and H.Y. Choo. 2008. Evaluation of various plant extracts for their nematicidal efficacies against juveniles of *Meloidogyne incognita*. J. Asia-Pac.Entomol.11: 99–102.

Fuller, V.L., C.J. Lilley and P.E. Urwin. 2008. Nematode resistance. New Phytol. 180: 27–44.

Hassan, S., A.A. AL-Gendy, S.H. Abdel-Baset, S.M. AbdEl-kareem, S.I. Massoud, and M.Y. Abdallah. 2020. Biofumigation potentials of some brassica crops to control root-knot nematodes *Meloidogyne* spp., on tomato under field conditions. J. Plant Pathol. Microbiol., 11: 1-8.

Hussey, R.S and R.R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant. Dis. Rep., 57:1025-1028.

Joymati, L., C. Dhanachand and L.S. Devi. 1998. Effect of plant extracts on *Meloidogyne incognita*. Ind. J. Nematol., 28:225-230.

Khattak, B. 2008. Biological management of root-knot nematode, *Meloidogyne javanica* (Treub) with *Trichoderma harzianum* Rifai in tomato. Khyber Pakhtunkhwa Agricultural (KPK) University, Peshawar, Pakistan: PhD Thesis. pp. 157.

Kimpinski, J., C.E. Gallant, R. Henry, J.A. Macleod, J.B. Sanderson and A.V. Sturz. 2003. Effect of compost and manure soil amendments on nematodes and on yields of potato and barley. J. Nematol., 35:289–293.

Manzanilla-Lopez, R.K. and J. Bridge. 2004. Plant diseases caused by nematodes. CABI Publishers Beijing, China.

Moens, M., R. Perry, and J. Starr. 2009 *Meloidogyne* species- a diverse group of novel and important plant parasites. Pp. 483 *in* R. N. Perry, M. Moens and J. L. Starr, eds. Root-knot nematodes, 1. Wallingford, UK.

Mojtahedi, H., G.S. Santo, A.N. Hang and J.H. Wilson. 1991. Suppression of root-knot nematode populations with selected rapeseed cultivars as green manure. J. Nematol., 23:170–174.

Naz, I., J.E. Palomares-Rius, Saifullah, V. Blok, M.R. Khan and S. Ali. 2013. *In* vitro and *In* planta nematicidal activity of *Fumaria parviflora* (Fumariaceae) against th southern root-knot nematode *Meloidogyne incognita*. Plant Path., 6:94-99.

Naz, I., Saifullah, J.E. Palomares-Rius, V.S. Blok, M. Khan, S. Ali and A. Baig. 2015. Sustainable management of the southern root-knot nematode, *Meloidogyne incognita* (kofoid and white) chitwood, by means of amendments of *Fumaria parviflora.* Int. J. Agric. Biol., 17:289-296.

Netscher, C. and R.A. Sikora. 1990. Nematode parasites of vegetables. p. 237-283. *In*: Plant-parasitic nematode in subtropical and tropical agriculture. (Eds. M Luc, RA Sikora and J bridge), CAB International, Wallingford, Oxon, UK.

Oka, Y. 2012. Nematicidal activity of *Verbesina encelioides* against the root-knot nematode *Meloidogyne javanica* and effects on plant growth. Plant Soil., 3:311–322.

Oka, Y., B. Ben-Danielb and Y. Cohen. 2012. Nematicidal activity of the leaf powder and extracts of *Myrtus communis* against the root-knot nematode *Meloidogyne javanica*. Plant. Pathol., 61:1012–1020.

Rizvi, M.A and M. Shameel. 2006. *In* vitro nematicidal activities of seaweed extracts from Karachi coast. Pak. J. Bot., 38:1245-1248**.**

Sasser, J.N. 1984. An advance treatise on *Meloidogyne,* Biology and Control, North Carolina State University, Graphics. 1st Edition. Pp 15-25.

Steel, R.G.D, J.H. Torrie and D.A Pickey. 1997. Principles and Procedure of Statistics. A Biometric Approach 3rd Ed. McGraw Hill Book Co. Inc. New York. Pp. 480.

Sultana, V.S., Ehteshamul-Haque, J. Ara and V.U. Ahmad. 2000. Utilization of seaweeds for the control of root diseases of tomato. *In*: Proceedings of National ONR Symposium on Arabian Sea as a Resource of Biological Diversity. (Ed.): V.U. Ahmad. HEJRIC, University of Karachi. p. 193-206.

Villar, E.M.J and M.E. Zavaleta. 1990. Effecto de *Crotalaria longirostrata.* Hook y Arnott sobre nematodes agalladores (*Meloidogyne* spp.) revista. Mex. de. Fitopatol., 8:166–172.

Wang, Q.R, Y. Li, Z.A. Handoo and W. Klassen. 2007. Influence of cover crops in rotation on populations of soil nematodes. Nematropica., 37:79–92.