

Research Article

Management of Root-Knot Nematode *Meloidogyne javanica* through Homeopathic Medicines

Asma Hanif* and Shahnaz Dawar

Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Abstract | The study was carried out to screen the vermicultural activity of various homeopathic medicines at 100, 75 and 50% v/v concentrations of different potencies including mother tincture (Q), 200C and 30C against root-knot nematode *Meloidogyne javanica* using hatching and mortality test at different time intervals. Notable results were noticed by *Kent-20* and *Santonine-43* at all tested concentrations by reducing the hatching and complete mortality of *M. javanica* after 96 hours, while *Cina* (Q) showed greater reduction in hatching and mortality at 100% concentration followed by minimum hatching of eggs when used at 75 and 50% concentrations but in case of 200 and 30 (C) potency, only 100% concentration showed maximum effect after 72 and 96 hours followed by 75% concentration which had slight effect, whereas 50% concentration found to fail in killing the second stage of juveniles. Other homeopathic drugs used in the experiment showed no significant nematocidal effect against *M. incognita*. *Kent-20* and *Santonine-43* at three different concentrations of 50, 100 and 75% was highly effective when used as seed treatment of okra, sunflower, mung bean and mash beans followed by drenching the soil with these concentrations of both homeopathic drugs separately, also resulted in increasing the weight and height of tested crops and reducing the nematode infection on roots plant.

Received | July 23, 2021; **Accepted** | November 26, 2021; **Published** | December 07, 2021

***Correspondence** | Asma Hanif, Department of Botany, University of Karachi, Karachi-75270, Pakistan; **Email:** asma.hanif@uok.edu.pk

Citation | Hanif, A. and Dawar, S., 2021. Management of root-knot nematode *Meloidogyne javanica* through homeopathic medicines. *Pakistan Journal of Nematology*, 39(2): 73-81.

DOI | <https://dx.doi.org/10.17582/journal.pjn/2021.39.2.73.81>

Keywords | Homeopathic medicines, Plant parasitic nematode, *Vitro* and *vivo* experiments

Introduction

Plant parasitic nematodes are major concern for crop production throughout the world (Askary and Haider, 2010; Abd-Elgawad and Askary, 2015). They reduce the quality and quantity of the crop and on an average worldwide crop loss of 12.6% (\$215.77 billion), due to these nematodes for only the top 20 life-sustaining crops based on the 2010–2013 production figures and prices has been estimated (Abd-Elgawad and Askary, 2015). Moreover, 14.45% (\$142.47 billion) was an average annual yield loss in the subsequent group of food or export crops. Among the plant parasitic nematodes, root-knot nematode,

Meloidogyne species are serious pathogen of several crops of agricultural importance (Ali and Askary, 2001; Jones *et al.*, 2013).

In Pakistan, *Meloidogyne* spp. infect wide range of host plants resulting in heavy economic losses (Zaki, 2000). Infectious second-stage juveniles (J_2) enters root tip of susceptible host produces strong interactive relationship with the host root and migrate intercellularly towards the cortex and inhabitant in the vascular bundles (Caillaud *et al.*, 2008) where they establish permanent feeding sites and produce J_3 and J_4 stages. They made multiple multinucleate giant cells due to hypertrophy and

hyperplasia that can be easily distinguished known as “knots or galls” on the roots (Ibrahim *et al.*, 2006; Askary, 2017). Due to the formation of galls, it caused blockage of xylem tissue, reduced the formation of nodules, interferes in nitrogen fixation and disrupt the photosynthetic activity of plants (Abdollahi and Ghazalbash, 2012). RKNs begin the parasitic process by secreting effector proteins through the stylet in the roots of host plant cells (Quentin *et al.*, 2013) which are essential for generating and continuing the feeding site (Williamson and Hussey, 1996). These secreted proteins affect the development, cell cycle, nitrogen degradation and disrupt the physiological and biochemical processes in host cell (Davis *et al.*, 2004; Akker and Birch, 2016).

Fastest management strategy of controlling *Meloidogyne* spp. in modern era usually achieved by the application of synthetic nematicides (Medina-Canales *et al.*, 2019); however due to toxicity and hazardous produce in the soil ecosystem (Kim *et al.*, 2018) replacement of chemicals by friendly measures have been developed, although pathologist have not been completely successful in attaining the similar levels of effectiveness (Desaeger *et al.*, 2017) which is imperative to note that results of *in vivo* and *in vitro* experiments from the similar treatment may differ due to additional factors such as soil pH, moisture content, degradation of nematicide active ingredients, soil structure, temperature or interaction with microorganisms (Xiang *et al.*, 2018; Bell *et al.*, 2019; Sikder and Vestergård, 2020). Therefore, present research carried out with the aim to explore the nematicidal effectiveness of homeopathic medicines in the management of *Meloidogyne javanica* under *vitro* and *vivo* conditions.

Materials and Methods

Extraction of eggs and juveniles

Infected egg plant roots showing characteristic of galls symptom due to *M. javanica* were obtained from the green house maintained at the Department of Botany were washed thoroughly with sterilized distilled water for twice and cut into small pieces and was put in a Nalgene wide mouth bottle in which three drops of sodium hypochlorite (1.0%) solution was added and placed in mechanical shaker for half an hour. The contents were poured into a 100-mesh sieve, fitted over a 400-mesh sieve were washed under running tap water (2 mins.). Residue obtained on a

400-mesh sieve was transferred into a beaker. Number of eggs and juveniles/ mL suspension was recorded in a counting dish (Hussey and Barker, 1973).

Nematicidal activity of homeopathic drugs using *in vitro* test

Using both hatching and mortality test of nematodes, Dr. Willmar Schwabe homeopathic drugs of mother tincture (30Q) includes; *Abroma augusta*, *Arnica montana*, *Artemisia vulgaris*, *Bellis perennis*, *Berberis vulgaris*, *Bryonia alba*, *Calendula officinalis*, *Calotropis gigantea*, *Clerodendron*, *Cina*, *Fagopyrum esculentum*, *Foeniculum vulgare*, *Hamamelis Virginia*, *Hedera helix*, *Inula*, *Jasminum officinale*, *Lamium album*, *Nux vomica*, *Opuntia*, *Psoralea corylifolia*, *Rosmarinus officinalis*, *Ruta graveolens*, *Salvia officinalis*, *Sulphur*, *Thuja occidentalis*, *Trifolium repens*, *Withania somnifera*, *Yucca filamentosa*, *Santonine-43* and *Kent-20* along with 30C and 200C potencies of all the above tested drugs including *Abies nigra*, *Calcarea carbonica*, *Caltha palustris*, *Carbo vegetabilis*, *Natrum muriaticum*, *Opium* and *Phosphorus* were used to detect the potent nematicidal activity against *M. javanica* (Cayrol *et al.*, 1989).

For hatching and mortality test, one mL egg suspension (20-50 eggs/mL) and one mL hatched J₂ suspension (20-45 juveniles/mL) were taken separately, one mL of tested homeopathic drugs (Q, 30C and 200C) with different concentrations (100, 75 and 50% v/v) were poured in cavity blocks having lids, respectively. Cavity block with sterilized distilled water and absolute alcohol taken as control and kept at room temperature. Each treatment was replicated thrice and after 0, 24, 48, 72 and 96 hours of exposure, numbers of hatching larvae and dead juveniles were counted under stereo microscope and expressed as percentage of the total nematode incubated.

Nematicidal activity of homeopathic drugs using *in vivo* test

Sandy loam soil was used for pot experiment containing sand (76%), clay (9%) and silt (15%) was determined by Gee and Bauder (1986) method having ≥ 7.2 pH (Brady, 1990) and organic matter present in the soil was 1.2% (Sparks, 1996). Tested seeds were treated with *Santonine-43* and *Kent-20* at three concentrations (100, 75 and 50% v/v) separately and dried aseptically. Different concentrations of treated seeds and soil drenched (20mL) with both homeopathic drugs separately and five tested seeds were sown in the 300g of soil. Untreated seeds and

without drenched soil served as control and each treatment replicated thrice. The whole experiment was conducted on okra, sunflower, mung bean and mash bean crops. Pots were kept under natural sunlight and after seedling emergence $\approx 2500 J_2$ of *M. javanica* were inoculated by making holes to the nearby host roots and uprooted tested plants after eight weeks of nematode inoculum addition and determine growth parameters and root knot nematode infection.

Results of data was estimated by three-way analysis of ANOVA by using Duncan's multiple range as proposed by Sokal and Rohlf (1995).

Results and Discussion

In hatching test under *vitro* condition, mother tinctures of *A. augusta*, *B. perennis*, *B. vulgaris*, *F. esculentum*, *H. virginia*, *Inula*, *L. album* and *N. vomica* was least effective against *M. javanica* eggs at 100 and 75% and also minimum controlled of *M. javanica* juvenile at 48, 72 and 96 hours ($P < 0.001$) was observed, while complete hatching of eggs was observed at 50% concentration and failed to kill the J_2 nematode. *A. vulgaris*, *T. repens* and *Y. filamentosa* at 100% concentration produced smallest nematicidal effect at 96 hours but failed at both concentrations both in hatching and mortality tests. When pure concentration ($P < 0.001$) of *A. montana*, *C. officinalis*, *C. gigantea*, *Clerodendron*, *H. helix*, *J. officinale*, *P. corylifolia*, *R. officinalis*, *R. graveolens*, *T. occidentalis* and *W. somnifera* were used, they exhibit maximum hatching and mortality of *M. javanica* at 96 hours. However, mother tincture of *Cina* resulted in better reduction in hatching followed by 30C and 200C potencies recorded in all three concentrations. *Cina* (30Q) showed greater effect at 100 and 75% concentrations ($P < 0.05$) which caused the death mortality of juveniles at 96 hours followed by 50%. Using *Cina* at 200C potency at 100% found greater mortality of juvenile at 96 hours but when 30C potency was used, only 100% showed slightest effect after 72 and 96 hours followed by 75%, while 50% failed in killing the nematode juveniles. When mother tincture of *B. alba*, *F. vulgare*, *Opuntia* and *Sulphur* were used at different concentrations showed complete hatching and juveniles of *M. javanica* were fully emerge out after 96 hours indicating no nematicidal activity present in it. Rest of the homeopathic drugs failed in killing the larvae and unable to stop the emerging larvae from the eggs of *M. javanica* but also

the sterilized water and absolute alcohol (control) showed same results. Mother tincture of *Kent-20* and *Santonine-43* when used at different concentrations gave pronounced effect in reducing the hatching of eggs but also noticed highest mortality of *M. javanica* after 96 hours (Table 1) selected as the best nematicidal medicine from all the tested drugs. *In vitro* studies revealed that at 50% concentration, cell free filtrate of all tested bacterial isolates (BT-10, BT-14, BT-16 and BT-64) caused remarkable juveniles' mortality of *Meloidogyne javanica* after 24 hours as compared to untreated control (Khan *et al.*, 2010). Many researchers worked on aqueous extracts of medicinal plant leaves proving the nematicidal effect against *Meloidogyne* sp. by stopping the hatching of egg-masses and enhanced the mortality of juveniles with respect to exposure of time under laboratory conditions (Ibrahim *et al.*, 2006; Dawar *et al.*, 2007; Sultana *et al.*, 2011; Latif *et al.*, 2014; El-Baha, 2017; Neeraj *et al.*, 2017).

In vivo experiment, highest shoot weight and height of mung bean plants showed by *Santonine-43* at all concentrations when drenched in soil. Growth parameters such as shoot weight, root length, root weight and number of nodules increased at 100% concentration when *Kent-20* was used in both seed treatment and soil drenching methods. However, highest shoot length was recorded at 75% followed by 50% concentration observed in the interaction between the drug, concentration and method. When mother tincture of *Santonine-43* drenched in soil it significantly ($P < 0.001$) reduced the galls and egg masses on the roots of mung bean plant followed by *Kent-20* (Figure 1). Significant interaction between drugs and concentration on growth parameters ($P < 0.05$) and root knot infection ($P < 0.001$) were noticed. Reduction in the formation of galls and egg masses on mash bean roots significantly ($P < 0.001$) controlled by the mash bean seeds treated with pure *Kent-20* followed by 75% concentration. Moreover, both homeopathic drugs at 100% diminished the gall formation when drenched in soil. As compared to control when soil was drenched with 50% concentration of *Kent-20* and *Santonine-43* drugs respectively showed minimum effect against root knot infection. Significant ($P < 0.001$) effect on shoot/ root length and weight was caused by the drug and concentration interaction. 100% of *Santonine-43* and *Kent-20* showed highest growth parameters on mash bean plants (Figure 2).

Table 1: Effective results of homeopathic drugs against *M. javanica* at different time intervals under vitro condition.

Homeopathic drugs (Mother tincture-30Q)	Conc. (%)	Hatching (%)				Mortality (%)			
		Time (Hours)				Time (Hours)			
		24±SE	48±SE	72±SE	96±SE	24±SE	48±SE	72±SE	96±SE
Control (Sterilized water)	0	37.3±0.97	55.3±0.98	79.8±1.25	100±1.44	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Control (Absolutealcohol)	100	41.2±2.23	59.6±1.96	80.7±2.16	100±2.41	0.0±0.0	0.0±0.0	7.5±0.47	16.75±1.18
Arnica montana	100	38.9±1.72	62.2±1.85	77.8±1.69	89.5±1.41	0.0±0.0	0.0±0.0	0.0±0.0	9.62±0.72
Berberis vulgaris	100	28.9±1.24	46.0±1.44	60.0±1.25	80±1.26	0.0±0.0	0.0±0.0	0.0±0.0	9.5±0.71
	75	26.4±1.84	48.9±1.18	69.9±1.24	90.3±0.94	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Calendula officinalis	100	31.9±0.98	57.5±0.98	74.4±0.23	85.3±0.72	0.0±0.0	12.4±0.94	25.7±0.97	37.1±0.47
	75	41.7±0.97	59.6±1.44	76.1±1.44	90.4±0.71	0.0±0.0	0.0±0.0	12.8±0.72	35.0±0.46
Calotropis gigantea	100	22.4±0.94	40.3±1.85	55.2±1.38	78.3±1.25	0.0±0.0	0.0±0.0	0.0±0.0	36.0±1.69
Clerodendron	100	36.8±2.19	61.8±2.49	75.0±1.69	90.4±2.87	0.0±0.0	0.0±0.0	0.0±0.0	14.8±1.18
Cina	100	17.1±0.72	49.3±1.44	64.5±0.46	76.9±0.27	8.6±0.47	17.1±0.47	23.7±0.70	42.0±1.78
	75	23.7±0.94	50.2±0.98	68.4±0.97	80.2±0.71	0.0±0.0	18.5±0.72	26.5±0.94	34.4±0.72
	50	18.5±0.93	43.3±0.98	66.7±0.94	85.2±1.25	0.0±0.0	0.0±0.0	34.6±0.72	51.3±0.97
Fagopyrum esculentum	100	29.7±0.98	41.2±0.72	56.8±1.18	77.8±0.47	0.0±0.0	12.6±0.46	18.9±0.46	25.2±0.46
Hamamelis virginia	100	17.9±1.41	35.1±0.98	53.2±0.72	63.3±0.72	0.0±0.0	0.0±0.0	14.4±0.94	26.3±0.72
Inula	100	28.9±0.94	44.4±0.94	62.2±0.54	80.7±0.97	0.0±0.0	1.7±0.54	6.4±0.72	11.9±0.71
Jasminum officinale	100	31.0±1.69	54.1±1.89	71.4±0.62	83.8±0.97	0.0±0.0	0.0±0.0	15.2±0.47	25.5±1.18
Lamium album	100	19.2±1.44	44.8±1.44	61.6±0.95	74.1±1.41	0.0±0.0	0.0±0.0	0.0±0.0	19.6±0.72
Psoralea corylifolia	100	42.9±1.25	59.1±1.08	76.3±0.62	86.6±1.36	0.0±0.0	13.6±0.47	28.8±0.44	49.4±0.44
Rosmarinus officinalis	100	24.9±1.44	42.6±1.44	58.9±0.42	75.1±0.94	0.0±0.0	0.0±0.0	0.0±0.0	23.1±0.72
Salvia officinalis	100	26.2±1.18	49.9±1.78	71.6±2.62	87.2±1.24	0.0±0.0	0.0±0.0	0.0±0.0	8.0±0.54
Thuja occidentalis	100	25.3±1.65	47.5±1.25	68.5±2.12	84.8±1.08	0.0±0.0	9.3±0.72	19.8±0.44	34.8±0.72
Trifolium repens	100	29.9±1.51	54.1±1.78	73.7±0.98	91.6±0.54	0.0±0.0	0.0±0.0	6.3±0.40	15.7±0.47
Withania somnifera	100	31.2±0.97	51.5±1.65	68.9±0.47	82.6±0.94	0.0±0.0	14.4±0.94	25.9±0.41	37.5±0.46
	75	35.3±0.94	55.4±0.94	75.6±1.62	90.7±0.93	0.0±0.0	0.0±0.0	0.0±0.0	19.4±0.72
	50	29.5±0.94	53.8±0.98	79.5±0.94	91.6±0.72	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Kent-20	100	10.2±0.98	25.6±1.69	40.4±1.65	47.6±0.83	48.4±0.72	70.7±0.47	92.9±0.97	100±1.95
	75	25.3±0.72	39.9±1.51	49.6±1.41	59.2±0.56	37.0±0.71	55.4±0.45	75.8±0.42	94.2±1.18
	50	37.0±1.25	53.8±0.47	61.6±0.72	74.1±0.54	32.6±0.97	55.1±0.96	78.6±1.18	93.6±2.04
Santonine-43	100	12.0±2.19	27.6±1.18	41.5±0.81	51.3±0.72	37.7±0.98	72.2±2.59	91.6±2.04	100±2.12
	75	20.2±0.72	31.7±0.47	41.4±0.94	50.5±1.18	40.3±1.24	58.6±0.94	73.3±0.72	90.5±1.18
	50	28.9±0.98	38.5±0.94	46.2±0.93	55.6±1.18	22.4±0.94	51.1±0.27	77.6±1.44	94.9±1.51
Cina (200C)	100	26.5±0.94	54.2±0.72	85.8±0.98	100±1.18	0.0±0.0	29.9±1.18	41.2±0.72	53.2±0.47
	75	30.0±0.72	45.5±1.25	79.6±1.36	100±1.44	0.0±0.0	15.2±0.98	37.0±0.47	54.7±0.72
	50	41.9±0.71	64.2±1.41	86.5±1.51	100±1.43	0.0±0.0	0.0±0.0	27.1±0.72	45.8±0.73
Cina (30C)	100	44.3±0.72	86.7±0.72	93.8±0.47	100±0.27	0.0±0.0	0.0±0.0	33.9±0.72	55.9±1.18
	75	39.4±0.83	75.2±0.97	88.2±0.97	100±0.72	0.0±0.0	0.0±0.0	0.0±0.0	12.2±0.72
	50	42.8±0.94	80.8±0.98	93.3±0.94	100±1.24	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

LSD_{0.05} = (C)= 2.549, (P)= 14.865, (T)= 2.944

LSD_{0.05} = (C)= 1.151, (P)= 6.713, (T)= 1.329

Where; Conc. / (C): Concentrations; (P): Potency of drugs; (T): Time; SE: Standard Error.

Application of pure *Santonine* 43 was effective in improving sunflower height and weight ($P<0.001$; $P<0.05$) followed by 75% concentration. However, root length and weight were amplified due to sunflower seeds treated with *Kent-20* at 75 and 50% concentration. Both pure homeopathic drugs ($P<0.001$) gave noticeable reduction in the galls formation and egg masses recorded in seed treatment and soil drenching methods followed by 75 and 50%

concentrations (Figure 3). Better shoot/root length and weight of okra were achieved by *Kent-20* when drenched in soil (100%) followed by *Santonine-43*. Heavy root weight of okra was recorded when 75% concentration of *Santonine-43* drug drenched in soil. Okra seeds treated with 100% concentration of both homeopathic drugs decreased the galls and number of egg masses significantly ($P<0.001$) which results in the improvement of plant growth as compared to control

in which occurrence of infection were noticed due to formation of galls produced by *M. javanica* (Figure 4). The soil-inhabitant nematode can be controlled by a broad range of organic amendments such as biofertilizers, crop residues or byproducts of plants, composts, green or animal manures either applied “*in vitro*” and “*in vivo*” assays significantly decreased the population (Hu and Cao, 2008; Hu and Qi, 2010; McSorley, 2011; Mennan and Melakeberhan, 2010; Soheili and Saeedizadeh, 2017). Used of homeopathic drugs in the control of *Meloidogyne* spp., studied by various researchers proving positive nematicidal effects (Datta, 2006; Carneiro *et al.*, 2010; Carneiro, 2011). Present results showed that out of three concentrations used (100, 75 and 50%), tested seeds treated with 100% of *Kent-20* and *Santonine-43* found best in the suppressing of galls formation and reduced egg masses remarkably produced healthy crops (sunflower, okra, mung and mash beans) followed by the 75 and 50% concentrations.

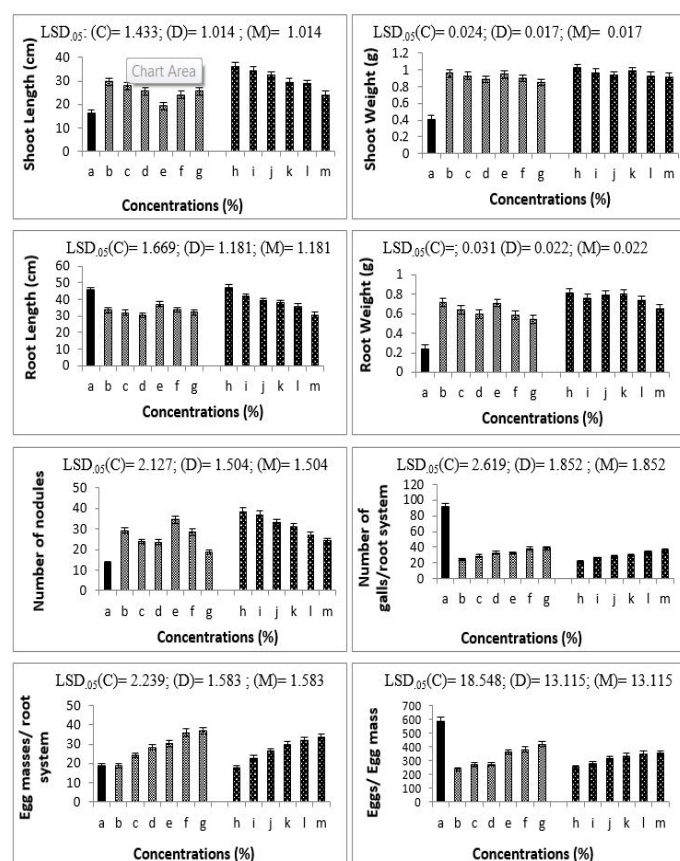


Figure 1: Application of homeopathic drugs against root knot infection on growth parameters of mung bean plants. Where; C: Concentrations; D: Drugs; M: Methods. (Sterilized water) a=Control; Seed treatment: b= *Kent-20* @100%, c= *Kent-20* @ 75%, d= *Kent-20*@ 50%, e=*Santonine-43* @ 100%, f= *Santonine-43* @ 75%, g= *Santonine-43* @ 50% v/v concentrations; Soil drenching: h= *Kent-20*@ 100%, i= *Kent-20*@ 75%, j= *Kent-20*@ 50%, k= *Santonine-43* @ 100%, l= *Santonine-43* @ 75%, m= *Santonine-43* @ 50% v/v concentrations.

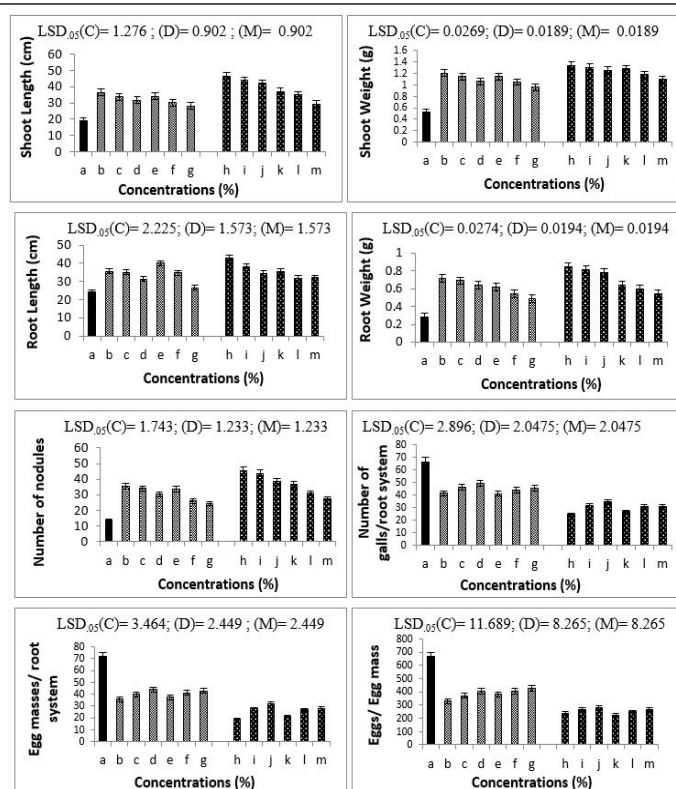


Figure 2: Application of homeopathic drugs against root knot infection on growth parameters of mash bean plants. Where; C: Concentrations; D: Drugs; M: Methods. (Sterilized water) a=Control; Seed treatment: b= *Kent-20* @100%, c= *Kent-20* @ 75%, d= *Kent-20*@ 50%, e=*Santonine-43* @ 100%, f= *Santonine-43* @ 75%, g= *Santonine-43* @ 50% v/v concentrations; Soil drenching: h= *Kent-20*@ 100%, i= *Kent-20*@ 75%, j= *Kent-20*@ 50%, k= *Santonine-43* @ 100%, l= *Santonine-43* @ 75%, m= *Santonine-43* @ 50% v/v concentrations.

Throughout the world, nematologist have been putting efforts into developing new environmentally benign strategies in the management of *Meloidogyne* spp. having nematicidal activity such as application of beneficial microbes (bacteria/fungi) essential oils, plant extracts, green manure, oil seeds cake, mulching, use of natural drugs derived from plants active compounds *etc.* are some of the ecofriendly treatments that have been tested for their efficacy against root knot nematodes (Tiyagi *et al.*, 2002; Irshad *et al.*, 2006; Ogwulumba and Ugwuoke, 2011; Ghazalbash and Abdollahi, 2013; Sivasakthi *et al.*, 2014; Kokalis-Burelle *et al.*, 2016; Askary, 2012, 2020). Recently, homeopathic pellets showed positive results in killing the J₂ of *Meloidogyne javanica* on agricultural field. Soil amended with homeopathic pellets (*Kent-20* used at 75% v/w concentration) produced healthy seedlings of okra, sunflower, mung and mash bean as it suppressed the *M. javanica* infection as compared to control (non-amended with homeopathic pellets) which produced galls on roots and disrupt the growth of tested crops (Hanif and Dawar, 2019). Experimental

researches on the principle of homeopathy in plants were mostly performed by Mexico, India, Europe and Brazil recently (Marques *et al.*, 2011).

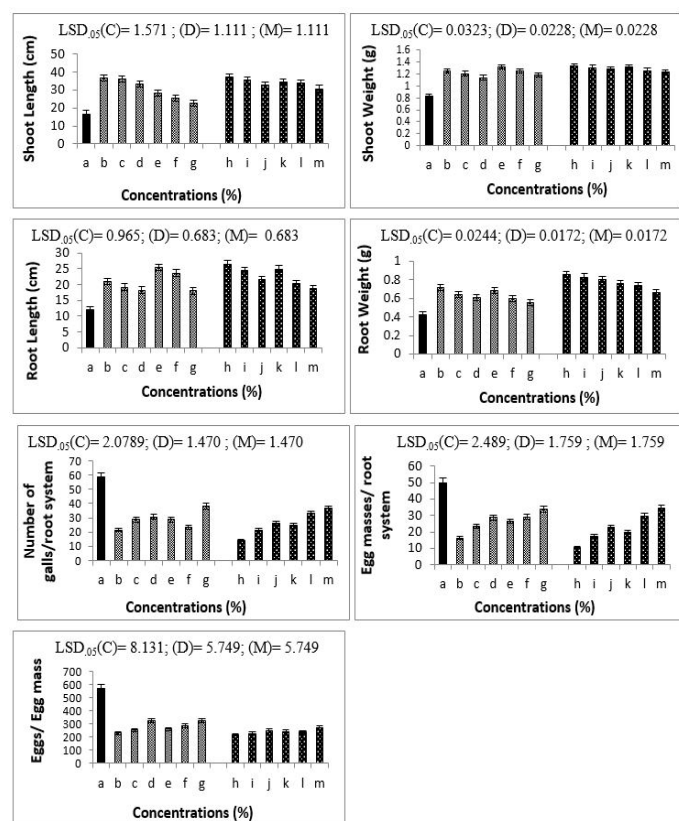


Figure 3: Application of homeopathic drugs against root knot infection on growth parameters of sunflower plants.

Where; C: Concentrations; D: Drugs; M: Methods. (Sterilized water) a=Control; Seed treatment: b= Kent-20 @100%, c= Kent-20 @ 75%, d= Kent-20@ 50%, e=Santonine-43 @ 100%, f= Santonine-43 @ 75%, g= Santonine-43 @ 50% v/v concentrations; Soil drenching: h= Kent-20@ 100%, i= Kent-20@ 75%, j= Kent-20@ 50%, k= Santonine-43 @ 100%, l= Santonine-43 @ 75%, m= Santonine-43 @ 50% v/v concentrations.

Homeopathic medicines in prescribing substances either highly diluted or potentized form provide potential technology for sustainable agriculture (Rossi *et al.*, 2004) due to controlling plant diseases (El-Mougy *et al.*, 2004) showed inexpensive, eco-friendly and used in little doses (Toledo *et al.*, 2011). Treated plants with homeopathy protect against root knot nematode attack and result in better crop yield (Hanif and Dawar, 2018).

Conclusions and Recommendations

Since vermicide homeopathic drug is still very little studied, there are many divergences about it. Homeopathic drugs (30Q, 200C and 30C potencies) were tested at different concentrations to check the hatching and mortality of *M. javanica*,

all drugs showed negative results except *Cina* (in all potencies) but not as excellent as compared to *Kent-20* and *Santonine-43* showed complete mortality of nematode at 96 hours exposure which found to be best vermicide medicine which was further checked in the *vivo* experiment and confirmed its efficacy on the leguminous and non-leguminous plants by actively reducing galls formation which was recorded in both seed treatment and soil drenching methods. Conclude that both the methods are effective in reducing the infestation of *M. javanica* on plant root. However, drenching the soil with homeopathic drugs are difficult on large scale in farmers' field. On the other hand, seed treatment is easy and cost-effective method which can be recommended for the crops grown in microplots.

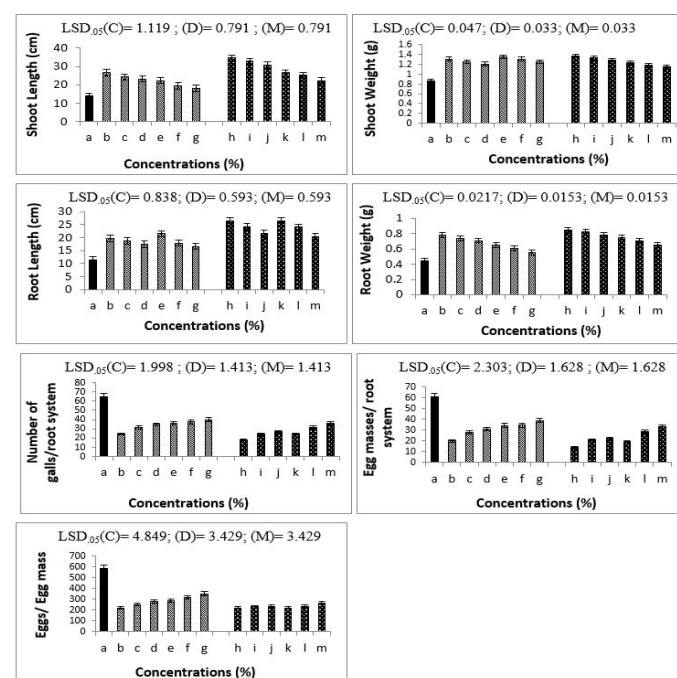


Figure 4: Application of homeopathic drugs against root knot infection on growth parameters of okra plants.

Where; C: Concentrations; D: Drugs; M: Methods. (Sterilized water) a= Control; Seed treatment: b= Kent-20 @100%, c= Kent-20 @ 75%, d= Kent-20@ 50%, e=Santonine-43 @ 100%, f= Santonine-43 @ 75%, g= Santonine-43 @ 50% v/v concentrations. Soil drenching: h= Kent-20@ 100%, i= Kent-20@ 75%, j= Kent-20@ 50%, k= Santonine-43 @ 100%, l= Santonine-43 @ 75%, m= Santonine-43 @ 50% v/v concentrations.

Novelty Statement

Seeds treated with homeopathic medicines found highly effective in controlling root knot nematode especially *Meloidogyne javanica* can be used as eco-friendly method in commercial scale.

Author's Contribution

Asma Hanif and Shahnaz Dawar: Contributed equally.

Conflict of interest

The authors have declared no conflict of interest.

References

- Abd-Elgawad, M.M.M. and Askary, T.H., 2015. Impact of phytonematodes on agriculture economy. In: Askary, T.H. and Martinelli, P.R.P. (eds.) biocontrol agents of phytonematodes. CAB International, Wallingford, UK. pp. 3-49. <https://doi.org/10.1079/9781780643755.0003>
- Abdollahi, M. and Ghazalbash, N., 2012. Evaluation of plant materials of two Iranian local plants, *Zataria multiflora* and *Ferulago angulata* on *Meloidogyne incognita*. 2nd International conference on agrochemicals protecting crops, health and natural environment-role of chemistry for sustainable agriculture. February 15-18, New Delhi, India.
- Akker, S.E.D. and Birch, P.R.J., 2016. Opening the effector protein toolbox for plant-parasitic cyst nematode interactions. *Mol. Plant*, 9: 1451-1453. <https://doi.org/10.1016/j.molp.2016.09.008>
- Ali, S.S. and Askary, T.H., 2001. Taxonomic status of phytonematodes associated with pulse crops. *Curr. Nematol.*, 12: 75-84.
- Askary, T.H. and Haidar, M.G., 2010. Plant parasitic nematodes associated with forest nurseries. *Indian J. Nematol.*, 40: 239-40.
- Askary, T.H., 2012. Management of root-knot nematode *M. javanica* in pigeonpea through seed treatment. *Indian J. Ecol.*, 39: 151-152.
- Askary, T.H., 2017. Diversity of plant parasitic nematodes in pulses. In: Plant diversity: Present situation and future scenario (eds. Abid A. Ansari, Sarvajeet S. Gill, Z.K. Abbas and M. Naeem). CAB International, Wallingford, UK. pp. 239-274. <https://doi.org/10.1079/9781780646947.0239>
- Askary, T.H., 2020. Management of root-knot nematode, *Meloidogyne hapla* infecting two solanaceous crops in Kashmir valley. *J. Entomol. Zool. Stud.*, 8: 1914-1917.
- Bell, C. A., Lilley, C.J., McCarthy, J., Atkinson, H.J. and Urwin, P.E., 2019. Plant-parasitic nematodes respond to root exudate signals with host-specific gene expression patterns. *PLoS Pathog.*, 15: 1-19. <https://doi.org/10.1371/journal.ppat.1007503>
- Brady, N.C., 1990. The Nature and Properties of Soils. 10th edition. Macmillan pub. Company. New York.
- Caillaud, M.C., Lecomte, P., Jammes, F., Quentin, M., Pagnotta, S., Andrio, E., Engler, J.A., Marfaing, N., Gounon, P., Abad, P. and Favery, B., 2008. MAP65-3 microtubule-associated protein is essential for nematode-induced giant cell ontogenesis in *Arabidopsis*. *Plant Cell*, 20: 423-437. <https://doi.org/10.1105/tpc.107.057422>
- Carneiro, S.M.T.P.G., 2011. Homeopathy in agriculture: Experimental results. (Ed.): Carneiro, S.M.T.P.G., Homeopathy, principles and use in agroecology, IAPAR, Londrina, pp. 135-170.
- Carneiro, S.M.T.P.G., Romano, E.D.B., Pignoni, E., Teixeira, M.Z., Vasconcelos, M.E.C. and Gomes, J.C., 2010. Effect of biotherapeutic of *Alternaria solani* on the early blight of tomato plant and the *In-Vitro* development of the fungus. *Int. J. High Dilut. Res.*, 9: 147-155.
- Cayrol, J.C., Djian, C. and Pijarowski, I., 1989. Studies on the nematocidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. *Rev. Nematol.*, 12: 331-336.
- Datta, S.C., 2006. Effect of *Cina* on root-knot disease of mulberry. *Homeopathy*, 95: 98-102. <https://doi.org/10.1016/j.homp.2006.01.005>
- Davis, E.L., Hussey, R.S. and Baum, T.J., 2004. Getting to the roots of parasitism by nematodes. *Trends Parasitol.*, 20: 134-141. <https://doi.org/10.1016/j.pt.2004.01.005>
- Dawar, S., Younus, S.M. and Zaki, M.J., 2007. Use of *Eucalyptus* sp. in the control of *Meloidogyne javanica* root-knot nematode. *Pak. J. Bot.*, 39: 2209-2214.
- Desaeger, J., Dickson, D.W. and Locascio, S.J., 2017. Methyl bromide alternatives for control of root-knot nematode (*Meloidogyne* spp.) in tomato production in Florida. *J. Nematol.*, 49: 140-149. <https://doi.org/10.21307/jofnem-2017-058>
- El-Baha, A.M., El-Sherbiny, A.A., Salem, M.Z.M., Sharrawy, N.M.M. and Mohamed, N.H., 2017. Toxicity of essential oils extracted from

- Corymbia citriodora* and *Eucalyptus camaldulensis* leaves against *Meloidogyne incognita* under laboratory conditions. Pak. J. Nematol., 35: 93-104. <https://doi.org/10.18681/pjn.v35.i01.p93-104>
- El-Mougy, N.S., Abd-El-Karem, F., Nadia, G.E. and Fotouh, Y.O., 2004. Application of fungicides alternatives for controlling cowpea root rot diseases under greenhouse and field conditions. Egypt. J. Phytopathol., 32: 23-35.
- Gee, G.W. and Bauder, J.M., 1986. Particle-size analysis. In: *Methods of Soil Analysis*, Part I, American Society of Agronomy, Madison, WI, USA. pp. 383-411. <https://doi.org/10.2136/sssabookser5.1.2ed.c15>
- Ghazalbash, N. and Abdollahi, M., 2013. Effects of two medicinal plants on some physiological changes in tomato, inoculated with *Meloidogyne javanica* (Treub) Chitwood and *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) Snyder and Hans. Pak. J. Nematol., 31: 21-37.
- Hanif, A. and Dawar, S., 2018. Application of homeopathic medicines in addition with synthetic chemicals in the control of root rot and root knot pathogens and on the growth of crop plants. Int. J. Biol. Biotech., 15: 285-300.
- Hanif, A. and Dawar, S., 2019. Effect of homeopathic nematicide pellets on plant-nematode interaction under controlled conditions. Pak. J. Bot., 51: 367-375. [https://doi.org/10.30848/PJB2019-1\(46\)](https://doi.org/10.30848/PJB2019-1(46))
- Hu, C. and Cao, Z.P., 2008. Nematode community structure under compost and chemical fertilizer management practice, in the north China plain. Exp. Agric., 44: 485-496. <https://doi.org/10.1017/S0014479708006716>
- Hu, C. and QI, Y., 2010. Effect of compost and chemical fertilizer on soil nematode community in a Chinese maize field. Eur. J. Soil Biol., 46: 230-236. <https://doi.org/10.1016/j.ejsobi.2010.04.002>
- Hussey, R.S. and Barker, K.R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Dis. Rep., 57: 1925-1928.
- Ibrahim, S. K., Traboulsi, A.F. and El-Haj, S., 2006. Effect of essential oils and plant extracts on hatching, migration and mortality of *Meloidogyne incognita*. Phytopathol. Medit., 45: 238-246.
- Irshad, L., Dawar, S., Zaki, M.J. and Ghaffar, A., 2006. Effect of nursery fertilizers on plant growth and in the control of *Meloidogyne javanica* root knot nematode on mung bean and okra plants. Pak. J. Bot., 38: 1301-1304.
- Jones, J. T., Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J., Jones, M.G.K., Taisei, K., Rosa, M.L., Juan, E.P.R., Wim, M.L.W. and Roland, N.P., 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Mol. Plant Pathol., 14: 946-961. <https://doi.org/10.1111/mpp.12057>
- Khan, M.Q., Abbasi, M.W., Zaki, M.J. and Khan, S.A., 2010. Evaluation of *Bacillus thuringiensis* isolates against root knot nematodes followed seed application in okra and mungbean. Pak. J. Bot., 42: 2903-2910.
- Kim, T.Y., Jang, J.Y., Yu, N.H., Chi, W.J., Bae, C.H., Yeo, J.H., Park, A.R., Hur, J.S., Park, H.W., Park, J.Y., Park, J.H., Lee, S.K. and Kim, J.C. 2018. Nematicidal activity of grammicin produced by *Xylaria grammica* KCTC 13121BP against *Meloidogyne incognita*. Pest Manage. Sci., 74: 384-391. <https://doi.org/10.1002/ps.4717>
- Kokalis-Burelle, N., Roskopf, E.N., Butler, D.M., Fennimore, S.A. and Holzinger, J., 2016. Evaluation of steam and soil solarization for *Meloidogyne arenaria* control in Florida floriculture crops. J. Nematol., 48: 183-192. <https://doi.org/10.21307/jofnem-2017-026>
- Latif, R., Abbasi, M.W., Zaki, M.J. and Khan, D., 2014. Nematicidal activity of bark of some tree species against root knot nematode *Meloidogyne javanica*. FUUAST J. Biol., 4: 247-251.
- Marques, R.M., Reis, B., Cavazin, A.C.T., Moreira, F.C., Buchoski, M., Silva, H.A., Lolis, M. and Bonato, C.M., 2011. Germination and vigor of seed of sorghum (*Sorghum bicolor* L. Moench) treated with *Arsenicum album*. Int. J. High Dilut. Res., 10: 239-244.
- Mcsorley, R., 2011. Overview of organic amendments for management of plant-parasitic nematodes, with case studies from Florida. J. Nematol., 43: 69-81.
- Medina-Canales, M.G., Terroba-Escalante, P., Manzanilla-López, R.H. and Tovar-Soto, A., 2019. Assessment of three strategies for the management of *Meloidogyne arenaria* on carrot in Mexico using *Pochonia chlamydosporia* var. *mexicana* under greenhouse conditions. Biocontr. Sci. Technol., 29: 671-685. <https://doi.org/10.1007/s10541-019-00000-0>

- doi.org/10.1080/09583157.2019.1582267
- Mennan, S. and Melakeberhan, H., 2010. Effects of biosolid amendment on populations of *Meloidogyne hapla* and soils with different textures and pHs. *Bioresour. Technol.*, 101: 7158-7164. <https://doi.org/10.1016/j.biortech.2010.04.023>
- Neeraj, N., Goel, S.R., Kumar, A., Singh, G. and Madan, V.K., 2017. Effect of plant extracts on hatching and mortality of root-knot nematode, *Meloidogyne incognita* larvae (*In-Vitro*). *Biosci. Biotech. Res. Asia*, 14: 467-471. <https://doi.org/10.13005/bbra/2466>
- Ogwulumba, S.I. and Ugwuoke, K.I., 2011. The effect of coloured plastic mulches on the control of root-knot nematode (*Meloidogyne javanica* Treub.) infections on some tomato (*Solanum lycopersicum*) cultivars. *Int. J. Plant Pathol.*, 2: 26-34. <https://doi.org/10.3923/ijpp.2011.26.34>
- Quentin, M., Abad, P., Favery, B., Rivas, S. and Reymond, P., 2013. Plant parasitic nematode effectors target host defense and nuclear functions to establish feeding cells. *Front. Plant Sci.*, 1: 312-257. <https://doi.org/10.3389/fpls.2013.00053>
- Rossi, F., Melo, P.C.T., Ambrosano, E.J., Guirado, N. and Mendes, P.C.D.A., 2004. Science of homeopathy in horticulture. *Hortic. Bras.*, 2: 1-8.
- Sikder, M.M. and Vestergård, M., 2020. Impacts of root metabolites on soil nematodes. *Front. Plant Sci.*, 10: 1-18. <https://doi.org/10.3389/fpls.2019.01792>
- Sivasakthi, S., Usharani, G. and Saranraj, P., 2014. Biological potentiality of plant growth promoting bacteria (PGPR) -*Pseudomonas fluorescens* and *Bacillus subtilis*: A review. *Afr. J. Agric. Res.*, 9: 1265-1277.
- Soheili, A. and Saeedizadeh, A., 2017. Suppression of brassicaceous tissue on *Meloidogyne javanica* in a rhizosphere. *Int. J. Agric. Biol.*, 19: 1012-1018. <https://doi.org/10.17957/IJAB/15.0400>
- Sokal, R.R. and Rohlf, F.J., 1995. *Biometry: The principles and practices of Statistics in biological research*. Freeman, New York, pp. 887.
- Sparks, D.L., 1996. *Methods of soil analysis, part 3, Chemical methods*. Soil Science Society of America (SSSA), Book series 5. <https://doi.org/10.2136/sssabookser5.3>
- Sultana, N., Akhter, M., Saleem, M. and Ali, Y., 2011. Nematicidal effect of *Acacia nilotica* and *Gymnema sylvestris* against second stage juveniles of *Meloidogyne incognita*. *J. Entomol. Nematol.*, 3: 25-29.
- Tiyagi, S.A., Khan, A.V. and Alam, M.M., 2002. Biodegradable effect of oil-seed cakes on plant parasitic nematodes and soil-inhabiting fungi infesting *Trigonella foenumgreacum* and *Phaseolus aureus*. *Indian J. Nematol.*, 32: 47-57.
- Toledo, M., Stangarlin, J. and Bonato, C., 2011. Homeopathy for the control of plant pathogens, *Physiology*, pp. 19-21.
- Williamson, V.M. and Hussey, R.S., 1996. Nematode pathogenesis and resistance in plants. *Plant Cell*, 8: 1735-1745. <https://doi.org/10.1105/tpc.8.10.1735>
- Xiang, N., Lawrence, K.S. and Donald, P.A., 2018. Biological control potential of plant growth-promoting rhizobacteria suppression of *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean: A review. *J. Phytopathol.*, 166: 449-458. <https://doi.org/10.1111/jph.12712>
- Zaki, M.J., 2000. *Bio-management of root-knot nematodes problem of vegetables*. DFID, UK Research Project Report. Department of Botany, University of Karachi, Karachi-75270.