Contents list available http://www.kinnaird.edu.pk/

Journal of Natural and Applied Sciences Pakistan

Journal homepage: http://jnasp.kinnaird.edu.pk/

EXPLORATION OF NEMATICIDAL PROPERTIES OF SEVERAL ORGANIC PRODUCTS AGAINST ROOT-KNOT NEMATODE (MELOIDOGYNE INCOGNITA) IN CAPSICUM ANNUUM L.

Sarir Ahmad*[1-2], Hamid Rasheed[2], Bibi Safia[3], Ayesha Hameed[2], Ikram Ullah[4], Sumbal[2]
[1]Department of Plant Protection, The University of Agriculture Peshawar, Pakistan.
[2]Department of Entomology, Abdul Wali Khan University Mardan, Pakistan
[3]Department of Zoology, Islamia College University Peshawar, Pakistan
[4]Department of Biochemistry, Abdul Wali Khan University Mardan, Pakistan

Articl Info

*Corresponding Author
Email: Sarirplants@gmail.com

Abstract

Root-knot nematode (*Meloidogyne incognita*) is a serious endophytic microbe of numerous vegetables globally. It attacks a wide range of agricultural and ornamental plants. *An in-planta* study was initiated to check the efficacy of essential oils (neem, castor), plant extracts (ginger, garlic), and furadon as control positive and control negative only received simple distilled water. Treatments were laid out in a Completely Randomized (CR) Design. All the treatments were replicated five times. All tested treatments had nematicidal effects on the nematode population as well as these treatments also improved the health of plants. Neem oil and garlic extract were highly significant treatments in improving plant growth parameters. Least egg masses were recorded in plants supplemented with neem oil (4.5) followed by garlic extract and furadon 6.8 and 7.6 respectively. The least galls were observed in plants supplemented with Neem oil (4.0) followed by garlic extract (4.4) and then followed by furadon (4.8) at the *P=0.05* level of significance. Neem oil and garlic extract showed statistical significance over other treatments and control as well. It is there for, concluded, and recommended that neem oil and garlic-based natural products should be used as an alternative to petrochemicals to tackle the problems of root-knot nematode.

Keywords

Meloidogyne Incognita, Organic Nematicides, Garlic, Neem

1. Introduction

Chili (*Capsicum annuum* L.) is important member of the family *Solanaceae* (Ochoa *et al*., 2001). Chilies plants are treated as tender seasonal annuals outside their native habitat. Chilies originate from South America (Baloch *et al.,* 2008). They are also produced in China, Spain, the United States, Turkey, Mexico, Indonesia, India, and Pakistan (Panella & Calatayud, 2018). It is an essential ingredient for food preparation all over the world. The wholesome price of chilies is superb. Chilies square measure wealthy in ascorbic acid, and pro-vitamin A. Yellow and particularly inexperienced chilies (which square measure primarily unripe fruit) contain a significantly lower quantity of each substance. Additionally, Chilies square measure a decent supply of most B vitamins, and B vitamin especially. They are extensively high in Iron and metal. Their high ascorbic acid content can even greatly increase the uptake of non-heme iron from different ingredients in an exceeding meal, like beans and grains (Kim *et al.,* 2019). They are additionally made in Pakistan on an oversized scale, and plenty is earned from its business. Chili crop includes a tremendous export potential thanks to its demand within the international market. Red chilies area unit exported to Egypt, Dubai, Bangladesh, Oman, Yemen, Qatar, Mexico, Kuwait, Iran, Bahrain, Canada, U.S.A, metropolis and Central American country whereas the foremost of chilies area unit exported to land, Saudi Arab and UAE. Pakistan has the potential to extend its share in these markets (Arain, 2019). Many factors hinder the overall production of chilies in Pakistan. These include biotic and abiotic factors. The crop growing of chilies, particularly in the tropics, is restricted by pests and diseases. Insect pests play an important in lowering the production of chilies. One of the major factors of its lower production is the root-knot nematode (Thiyagarajan and Kuppusamy, 2014). Nematode belongs to the class *Secernentea* and Family *Meloidogynidae,* and the most important member of this family is the *Meloidogyne* species, its infestations add up a major constrain to the world's overall production of chilies (Changkwian *et al.,* 2019). Root-Knot nematode (RKN) in ornamental and vegetables is a serious threat in Pakistan as well as has global impacts. and all over the world. They cause damage to plants both by lowering the quality and overall yield reduction. Most important species of RKN present in Asia are *M. arenaria, M. javanica, M. hapla*, and *M .incognita* with *M. incognita*. However, the most destructive is *M. incognita* and *M. javanica* (Kihika *et al*., 2017). These species are the most prevalent and damaging species of vegetables in the warm sandy soils of Pakistan (Khan *et al*., 2019). These species are the most important nematode pathogenic spread over large areas in both tropical and subtropical cultivating production lands globally. The nematode is an obligate phyto-parasite infecting the roots of approximately 2000 plant species belonging to different families (Le *et al.,* 2019). Its development consists of four phases, the first phase is present inside the egg, Second phase juveniles (J2) are infective and considered free-living. It constantly pushes its stylet into the younger root tips, this process is assisted by the release of enzymatic juices

like pectinases and celluloses before it invades the roots for colonization (Mejias *et al*., 2019). The management practices include a few options like crop rotation and systemic nematicides but keeping in view, the cost of chemical control and its ill effects on the environment, alternatively scientists are constantly working on finding alternatives to these Petro expensive chemicals. Nowadays, trends have now shifted to botanicals having nematicidal properties.

2. Materials and Methods

An *in-planta* experiment was carried out in spring 2018. Essential oils were obtained from a local market, while botanicals were collected from the local gardens. The treatments used in the experiment are listed in table 1

Table 1: List of treatments used for evaluation against Root-knot nematode

Sr.	Common Name	Botanical name/Active ingredient	Type
1	Neem oil	*Azadirachta indica*	Essential oil
2	Castor oil	*Ricinus communis*	Essential oil
3	Ginger	*Zingiber officinale*	Extract
4	Garlic	*Allium sativum*	Extract
5	Carbofuran	Furadon	Nematicide
6	Simple Distilled water	-	-

2.1 Plant material; (nursery raising)

Chili seedlings (*cv. Gola Peshawari*) were bought from the local market and were sown in plastic pots (height 8cm width 4cm). Two weeks, after transplantation nematodes 200 J2 and 50 egg masses were applied to plants through the soil drench method under aseptic conditions through micropipette (Naz *et al*., 2015).

2.2 Plant Extract Preparation

The garlic pearls and ginger rhizomes were bought from the local store. These were chopped into small pieces and dried at room temperature. These pieces were grinded in an electric grinder to a fine powder. Ten g of the powder sample was mixed in 50 ml distilled water to make an aqueous extract. The suspension was filtered through Whatman's papeNo.1. The solutions were stored at 4^{o}C for further use (Naz *et al*., 2013).

2.3 Nematode inoculum

Initial root-knot nematode culture was obtained from infected galled roots of chilies (Jaban, Malakand division, Pakistan). The culture was initiated from a single egg mass on chilies sown in an earthen pot (1000g soil capacity) at 22-24^{o}C. This pure culture was further used in the experiment (Samaliev *et al*., 2017).

2.4 Nematode Extraction from galled roots

The field-collected roots having high galls due to RKN attack were firstly cleaned with water gently to remove any dust and debris and then chopped into small parts (1–5cm). This suspension having nematode different life stages was allowed to go through sieves of different sizes (75 and 26µm) to get the desired stage of nematode. As the eggs and J2 are smaller in size than adults so, eggs and j2 will settle on a smaller sieve size (26 µm) while the adults

will settle on a 75 µm sieve. The 26 µm sieve containing J2 and eggs was rinsed with water many times (Kepenekci *et al*., 2016). The eggs collected in 26 µm sieve size were stored in saline solution to avoid hatching for further utilization in the experiment (Soliman *et al*., 2019).

2.5 Nematodes and plant data parameters

The plants were allowed to grow for 45-50 days and the plants were removed from pots. As RKN approximately completes its lifecycle in 40 days. After uprooting, data were recorded on different parameters like root and shoot weight and lengths (fresh). Data on the root-knot nematode parameters like (galls and their index and egg masses (10 gram/soil) were also recorded. The galls per root system were subjected to a galling score index (0 till 5, where 0 means none galls and 5 means infinite galls) (Naz *et al*., 2015).

2.6 Statistical Analysis

Data of the experimental parameters were analyzed by using Statistix (Software, USA) (Campbell, 1990). Analysis of Variance was determined LSD for means for plant growth and nematode reproduction parameters were compared using Fisher's protected (LSD) test.

3. Results

Results of the *In-planta* trial indicate that Chile plants, amended with different essential oils, plant extracts, and control positive at standard dose, significantly (*P=0.05*) inhibited the nematode parameters, and promoted different plant growth parameters. The highest galls per root system were observed in the untreated control (32.7) which received simple distal water, followed by ginger by having 10.7 galls, and then followed by castor oil by having 7.2 galls. The lower galls per root system treated with neem oil, garlic extract, and furadon were 4.0, 4.1, and 4.8 respectively. However, no statistical difference was found among neem oil, garlic extract, and control positive furadon. All the treatments were statistically significant at *P=0.05* over the control negative (table 2).

Table 2: Effect of plant extracts and oils on number of galls in Chili per root system inoculated with Root-knot nematode

Treatments	Galls per root system	Galling Index
Neem oil	4.0 d[a]	2
Castor oil	7.2 c	2
Ginger	10.7 b	3
Garlic oil	4.1 d	2
Furadon	4.8 d	2
Control	32.7 a	4
Lsd	1.0	

Each value is the mean of five replications. [a]Means followed by the same letters do not differ significantly by the LSD test (P = 0.05)

Data in Figure 1 suggests that a maximum of egg masses were found in the control negative having 32.7 egg masses per 10 gram of root followed by ginger extract having 13.7 egg masses and then followed by castor oil having 10.4 egg masses. No statistically significant difference was found in garlic extract and furadon as egg masses were 6.8 and 7.6 per 10 gram of roots respectively. The best treatment was neem oil which had a lowered number (4.5) number of egg masses. All the treatments showed statistical significance over the control negative.

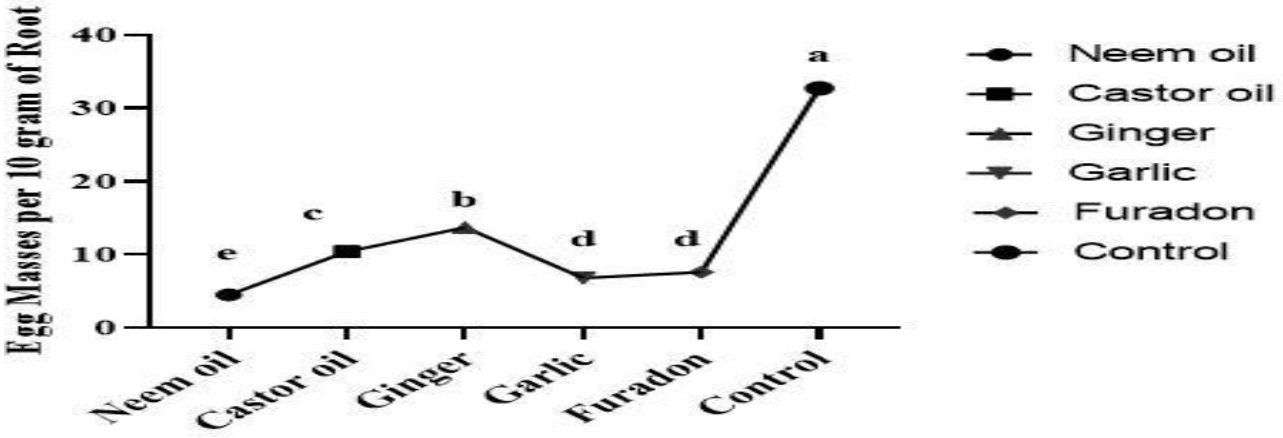

Figure 1: Number of Egg masses per 10 gram of roots treated with extracts and essential oils inoculated with *Meloidogyne incognita* at 200J2's

The fresh root weight increased significantly due to the presence of galls at all treatments. The highest gain in root weight was recorded in garlic, furadon, and untreated control (4.7g respectively), then followed by neem oil having 4.4g fresh root weight. However, no statistical difference was observed among garlic, furadon, untreated control, and neem oil at *P=0.05* level of significance. The least treatment was castor oil which had only 3.7g of fresh root weight as shown in Figure 2.

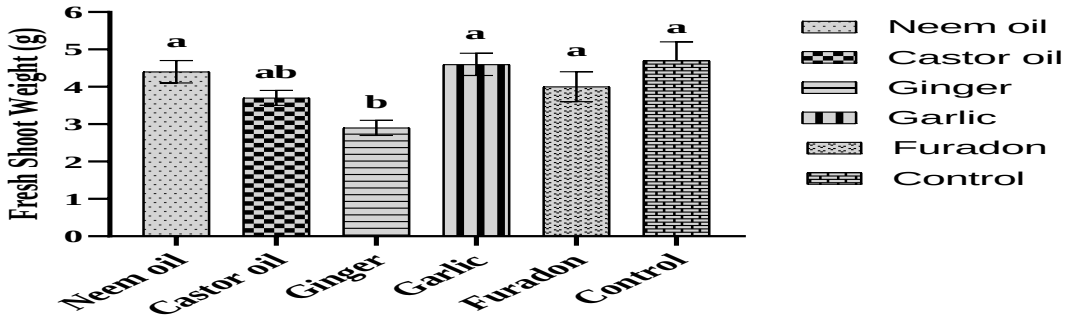

Figure 2: Fresh root weight in plants treated with extracts and essential oils inoculated with *Meloidogyne incognita* at 200 J2's

The highest raise in fresh shoot weight was achieved with neem oil and garlic extract 8.5g and 8.4g respectively followed by furadon 7.4 g and then followed by castor oil 7g at a 0.05% level of significance. Minimum fresh shoot weight was observed in the control negative by having 4.2g of fresh shoot weight as shown in figure 2. All the treatments were statistically significant over untreated control as shown in figure 3.

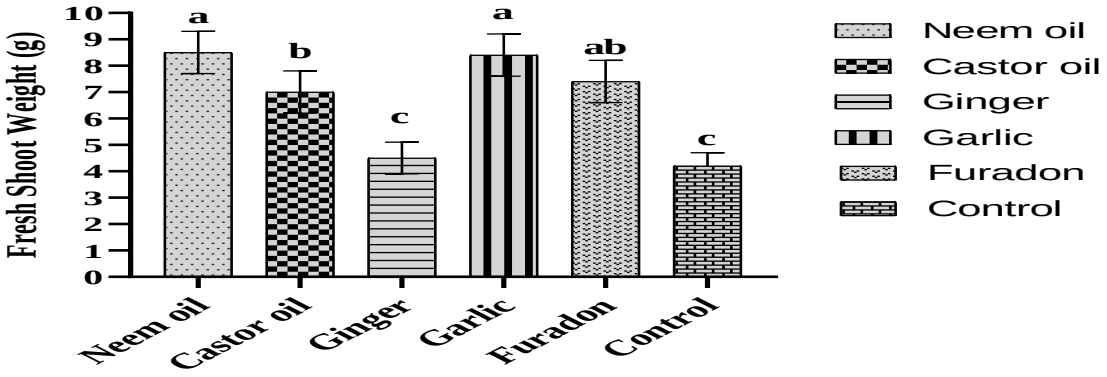

Figure 3: Fresh shoot weight in plants treated with extracts and essential oils

Figure 3: Fresh shoot weight in plants treated with extracts and essential oils inoculated with *Meloidogyne incognita* at 200j2's A maximum raise in shoot length was achieved with garlic extract by having 34.4cm length followed by neem oil (29.1cm), castor oil (27.8cm), furadon (27.3cm), and then followed by ginger (23cm) at *P=0.05%* significance. The least shoot length was observed in the control negative (15cm) as shown in Figure 4.

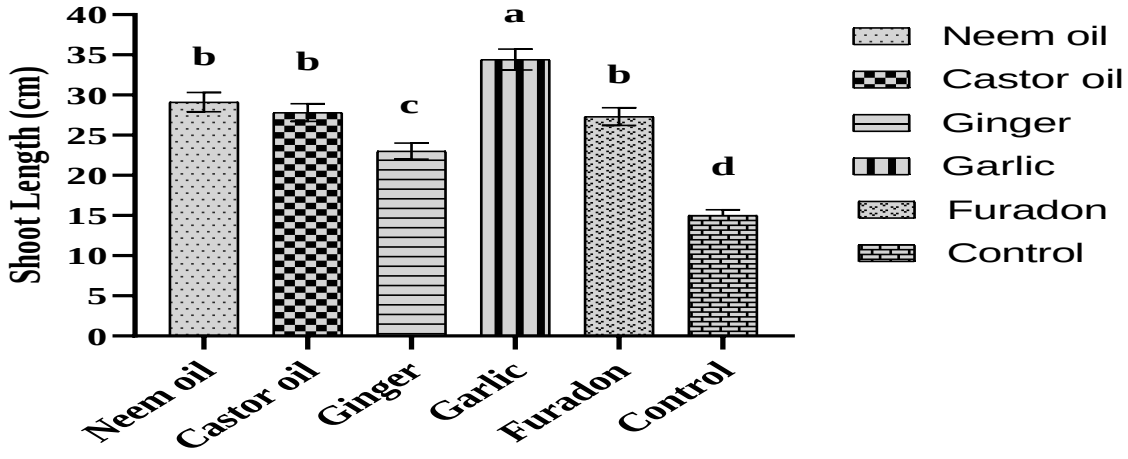

Figure 4: Shoot length observed in plants treated with extracts and essential oils inoculated with *Meloidogyne incognita* at 200 J2's

The maximum root length was achieved with garlic extracts (17cm) followed by furadan (14cm) and neem oil (13cm). All the treatments showed a significant difference over control negative (6cm) at *P=0.05*% as shown in figure 5. The different life stages and galled roots are presented in figure 6.

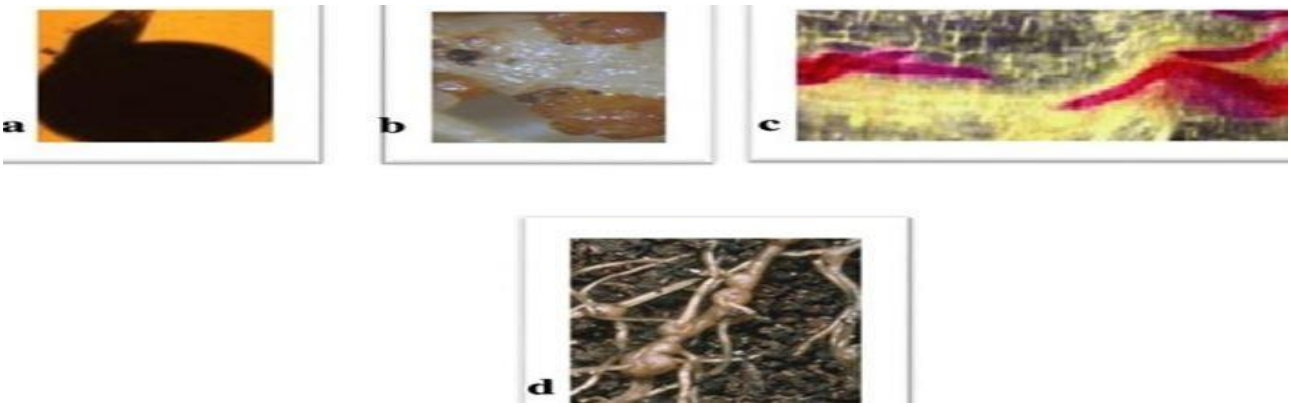

Figure 5: Root-Knot Nematode different stages

and galled roots. A, adult female, B. Egg Masses, C, J2 infective stage at root tissues, D. Galled roots of Chili in soil.

4. Discussion

The use of plant-based products as organic nematicides has been reputed as efficient, economically feasible, and environmentally friendly by several researchers (Chitwood, 2002; Naz *et al*., 2015). Bearing in mind the significance of organic nematicides a study was initiated at the pot level under greenhouse conditions in the spring of 2018. The study revealed the outstanding efficacy of several plant products against *M. incognita* on chili plants. A variety of treatments were used which effectively control the *M. incognita* on chili plants, and raised the plant parameters significantly. The study revealed that the galls per root system on chili plants and the number of egg masses were statistically reduced by the application of various preparations of organic nematicides. Under Greenhouse conditions, the application of different essential oils/ extracts exhibited maximum diminution in the number of galls per root system and terms of the Galling index in comparison to the untreated control. The most efficient treatment was neem oil has a positive effect on galls per root system and egg masses per 10g of roots. Neem oil also increased plant growth parameters. It could be because of the presence of bioactive constituents in neem oil. Our results conform with those of Hussein *et al*. (2016) and Abbassy *et al*. (2017). They significantly raised plant growth parameters and lowered nematode growth indices. It could be possibly due to the effect of neem oil in the roots of chili which made the root environment so unfavorable for the root-knot nematodes to the harbor. Neem oil contains Triterpenes (limonoids) are present in neem and most biologically important is *azadirachtin* which is widely used against pests including RKN and its J2s (Abbassy *et al*., 2017).

Garlic extract also reduced the overall nematode reproduction and galls per root system. Egg masses were also lowered as compared to untreated control. Current results conform with those of Osman. (2005) ascribed the nematode reduction to garlic cloves containing pyruvic acid, and ammonia. Also, El-Nagdi *et al*. (2014) suggested that garlic extract also contains biochemical substances that are considered highly toxic to nematodes. Garlic extract also has directly affected nematode reproduction and interrupted their movement, food utilization, and normal functioning (Mashama 2010). Castor oil also reduced the overall nematode factors and directly raised the plant growth parameters. Audi *et al*. (2005) reported that the nematicidal properties of castor could be due to the toxic constituent present in castor (*Ricin*). *Ricin* is supposed to be *toxalbumin* and is considered as phytotoxic as well as has nematicidal properties. Our results conform with those of Adomako and Kwoseh. (2013); Amer-Zareen and Naveed. (2003) and Adegbite and Adesiyan. (2005) who achieved similar findings against root-knot nematode by applying an aqueous extract of ginger and castor oil.

5. Conclusions

By looking into the current performance of plant extracts against RKN it is concluded that plant extracts are a direct and cheap source of nematicides of root-knot nematodes. The most influential and effective extracts of garlic, ginger, and essential oil neem provide immediate control of RKN. Current findings are in favor of controlling RKN without the use of commercially available nematicides which pollute the natural ecosystem and thus have many health issues for humans and other biotas. These may be a replacement for the synthetic dangerous and expensive chemicals which pose great environmental hazards.

References

Abbassy MA, Abdel-rasoul MA, Nassar AM and Soliman BS, 2017. Nematicidal activity of silver nanoparticles of botanical products against root-knot nematode, *Meloidogyne incognita*. Arch. Phytopathol. Pfl. 50: 909-926.

Adegbite AA and Adesiyan SO, 2005. Root extracts of plants to control root-knot nematode on edible soybean. *W. J. A. S.* 1: 18-21.

Adomako J and Kwoseh CK, 2013. Effect of Castor Bean (*Ricinus communis* L.) Aqueous Extracts on the Performance of Root-Knot Nematodes (*Meloidogyne* spp.) on Tomato (*Solanum lycopersicum* L.). *J. Sci. Technol*. 33: 1-11.

Amer-zareen M and javed N, 2003. Nematicidal Activity of Ginger and its Effect on the Efficacy of *Pasteuria penetrans* for the Control of Root-Knot Nematodes on Tomato. *Asian J. Plant. Sci.* 2: 858-860.

Arain S. 2019. Scenario of Chilli Production and Hindrances Faced by the Growers of Sindh Province of Pakistan. *M. C. D. A.* 4: 436-442

Audi J, Belson M, Patel M, Schier J and Osterloh J, 2005. *Ricin* poisoning: a comprehensive review. *J. A. M. A.* 294: 2342-2351.

Baloch QB, Chachar QI and Tareen MN, 2008. Effect of foliar application of macro and micro nutrients on production of green

chilies (*Capsicum annuum* L.). *J. Agri Technol.* 42: 177-184.

Campbell CL and Madden LV. Introduction to plant disease epidemiology. John Wiley & Sons, (1990).

Changkwian AJ, Venkatesh JH, Lee JW, Han JK, Kwon MI, Siddique S, Abate GJ. Choi E, Kim Y, Seo and Kim YH, 2019. Physical Localization of the Root-Knot Nematode (*Meloidogyne incognita*) Resistance Locus Me7 in Pepper (*Capsicum annuum*). Front. *Plant. Sci.* 10, p.886.

Chitwood DJ, 2002. Phytochemical based strategies for nematode control. Annu. Rev. *Phytopatho*, 40: 221-249.

El-Nagdi, WMA, Youssef MMA and Dawood MG, 2014. Efficacy of garlic clove and oil aqueous extracts against *Meloidogyne incognita* infecting eggplant. *Pak. J. Nematol.* 32: 223-228.

Gomez KA and Gomez AA, 1984. Statistical procedures for agricultural research," John Wiley & Sons.

Hussain MA, Mukhtar T and Kayani M Z, 2016. Reproduction of *Meloidogyne incognita* on resistant and susceptible okra cultivars. Pak. *J. Agric. Sci.* 53: 371-375.

Kepenekci ID, Erdogus and Erdogan P, 2016. Effects of some plant extracts on root-knot nematodes in vitro and in vivo conditions. Turk. *Entomol*. 40: 3-14.

Khan MT, Mukhtar AT and Saeed M, 2019. Resistance or Susceptibility of Eight Aubergine Cultivars to *Meloidogyne javanica*. Pak. *J. Zool.* 51: 2187-2192.

Kihika R, Murungi IK, Coyne D, Hassanali AP, Teal E and Torto B, 2017. Parasitic nematode *Meloidogyne incognita* interactions with different *Capsicum annum* cultivars reveal the chemical constituents modulating root herbivory. *Sci. Rep*. 7: p.2903.

Kim EH, lee SY, Baek DY, Park SY, Lee SG, Ryu TH, Lee SK, Kang HJ, Kwon OH, Kil M and Oh SW, 2019. A comparison of the nutrient composition and statistical profile in red pepper fruits (*Capsicums annuum* L.) based on genetic and environmental factors. *Appl. Biol. Chem.* 62:p.48.

Le TML, Nguyen TD, Nguyen HT, Liebanas G, Nguyen TAD and Trinh QP, 2019. A new root-knot nematode, *Meloidogyne moensi* n. sp.(*Nematoda: Meloidogynidae*), parasitizing Robusta coffee from Western Highlands, Vietnam. *Helminthologia.* 56:pp.229-246.

Masamha B, Gadzirayi CT and Mukutirwa I, 2010. Efficacy of *Allium Sativum* (Garlic) in Controlling Nematode Parasites in Sheep. *Int. J. Appl. Res. Vet. Med*. 8: 161-169.

Mejias J, Truong NM, Abad P, Favery B and Quentin M, 2019. Plant proteins and processes targeted by parasitic nematode effectors. *Front. Plant. Sci.,* 10: 1-10

Naz I, Palomares-Rius JE, Khan SM, Ali S, Ahmad M and Ali A, 2015. Control of Southern root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood on tomato

using green manure of *Fumaria parviflora* Lam (*Fumariaceae*). *Crop. Prot*. 67: 121-129.

Naz I, Palomares-rius JE, Blok V, Khan MR, Ali S and Ali S, 2013. *In vitro* and *in planta* nematicidal activity of *Fumaria parviflora* (*Fumariaceae*) against the southern root-knot nematode *Meloidogyne incognita. Plant. Pathol*. 62: 943-952.

Ochoa-Alejo N and Ramirez-malagon R, 2001. *In vitro* chili pepper biotechnology. *In Vitro Cell Deve Biol. Plant.,* 37: 701-729.

Osman HA, El-Gindi AY, Ameen HH, Youssef and lashein AM, 2005. Evaluation of the nematicidal effects of smashed garlic, sincocin and nemaless on the root-knot nematode, Meloidogyne incognita infecting cowpea plants. *Bulletin of the National Research Centre*. 30: 297-305.

Penella C and Calatayud A. 2018. Pepper Crop under Climate Change: Grafting as an Environmental Friendly Strategy. *Climate Resilient Agriculture: Strategies and Perspectives.* p.129.

Samaliev HD, Markova M, Nikolova and Baicheva O, 2017. Management of root-knot nematode *Meloidogyne hapla* on strawberry plant with some plant extracts. Российский паразитологический журнал. 4: 42

Soliman GM, Ameen HH, Abdel-Aziz SM and El-Sayed GM. 2019. In vitro evaluation of some isolated bacteria against the plant parasite nematode *Meloidogyne incognita*. *Bulletin of the National Research Centre.* 43:p.171.

Thiyagarajan SS and Kuppusamy H, 2014. Biological control of root knot nematodes in chillies through *Pseudomonas fluorescens's* antagonistic mechanism. *Plant. Sci*., 2: 152-158.