Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env.Pharmacol. Life Sci., Vol 11[1] December 2021 : 112-117

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Potential Role of Aqueous Stem Extracts of *Achyranthes aspera* L. on Root-Knot Nematode (*Meloidogyne javanica*) Parasitism in Pomegranate

Magar Bharat [1*], Dengale Bhushan[2], Thorat Yogesh[3] and S.P. Giri
[1,2,4] Department of Botany and Research Centre, Padmashri Vikhe Patil College of Arts, Science and Commerce, Pravaranagar, Maharashtra, India413 713,
[3]ICAR-Indian Institute of Sugarcane Research, Biological Control Centre, Pravaranagar, Maharashtra, India-413712
Email: bharatmagar3112@gmail.com

ABSTRACT
Pomegranate (Punica granatum L.) is an economical and commercial fruit crop of tropical and subtropical India. Despite having production, the pomegranate growers unable to produce quality fruits as per the market demand because of abiotic and biotic stress factors. Insect-pests and disease incidence are one of the major hindrances for quality production. Root-knot nematodes (Meloidogyne spp.) parasitisms have aggravated the problem of pomegranate wilt in Ahmednagar district of Maharashtra. In the present study, soil and root sampling were conducted in infested pome orchards from Sangmaner tehsil for nematode isolation and identification. Root galls were dissected for female and egg mass isolation and the perineal pattern of the female have confirmed the nematode species (M. javanica). Use of locally available plant extracts as botanicals against nematodes would be cheap, nontoxic alternative available with farmers. Aghada (Achyranthes aspera) plant contains triterpenoid saponins which possess oleanolic acid as the aglycone which may have anti-nematode property. The succulent stem of Aghada was washed, ground in mortar pestle for fine powder formation and filtered through muslin cloth for an aqueous suspension. Different dilutions of stem extract (10%, 20%, 30%, 40% and 50%) were prepared. The results showed juvenile's mortality observed at 65%, 51.66%, 93.33%, 100% and 100% with each concentration, respectively within 24-120 hours of exposure. However, increasing the concentration of extract has increased juvenile mortality within less time. Our in vitro studies have illustrated the aqueous stem extract of Achyranthes aspera has antinematode properties against M. javanica in pomegranate.
KEY WORD: *Meliodo jawanica, Aghada, Nematode*

Received 11.06.2021 Revised 21.09.2021 Accepted 20.11.2021

INTRODUCTION

Pomegranate (*Punica granatum* L) is belonging to the family *Lythraceae.* It is mostly cultivated in the tropical and subtropical regions of the world *viz*., Afghanistan, Iran, Pakistan, Chine, U.A.E. and India [2]. India rank first position in pomegranate production all over the world which cultivated over an area of 2.62 lakh ha., and producing 30.36 lakh metric tons in 2018-19 [5]. The Maharashtra state is at top position in pomegranate production grown an area about 90,000 ha with the production of 9.45 lakh metric tons [3]. Nutritionally, the pome arils are rich in Vitamin C, E, A, K, iron, calcium and various antioxidants which helps medicinally to cure against several diseases like diabetes, cancer, stomach disorders, blood pressure, etc. Pome fruits are also the plentiful source of different phytochemicals *viz*., sterols, terpenoids, organic and fatty acids which improves the strength of bones, increases the hemoglobin content and boost the immune system [4].

The important diseases of pomegranate are bacterial blight, oily spot and Fusarium wilt and Nematode. Root-knot nematode (RKN) (*Meloidogyne* spp.) is considered to be an economically important pest of pomegranate cultivation worldwide and responsible for about 30% to 40% yield losses. The species of RKN (*M. incognita*, *M. javanica* and *M. Arenaria*) are observed to be associated with pomegranate orchards in Maharashtra. Crop rotation practice (with non-host crop) have been used for RKN management, but often yields ineffectual results due to vast host range of nematode. Various bio control agents such as endoparasitic fungi (*Paecilomyces lilacinus*, *Pochonia* spp.), parasitic bacteria (*Pasteuria penetrans*) and plant growth promoting bacteria (PGPR) are being used, but their field efficacy and stability is unsuccessful. Use of chemical nematicides have recommended so far to control the RKN at field

conditions, but these nematicides are costlier to farmers, have toxic effects of bio flora and fauna and environmentally harmful. Nematicidal uses have been restricted to specific areas of nematode infection or at nursery level. Therefore, there is need to search alternative nematode management practices that should be agronomically compatible, economically sound and ecologically green and harmless. There is a lot of scope to use botanicals (extracts of locally available plants) for nematode management as these botanicals are neamticidal or contains anti nematode properties.

Achyranthes aspera is the herbaceous plant commonly called Aghada, which belongs to family Amaranthaceae, distributed throughout the tropical world. Mostly aerial part is use for medicinal purposes [9]. The phytochemically *Achyranthus aspera* L. consists of saponin, tannin, coumarin, protein, amino acids, flavonoid, phenol, glycosides and alkaloids, anthocyanin [8]. *Achyranthes aspera* shows antioxidant and antibacterial activities [6]. Therefore, the present study has been undertaken to evaluate the extracts of *Achyranthes aspera* against root-knot nematodes infesting pomegranate.

MATERIAL AND METHODS

Soil and Root Sampling:

A random field survey was carried out in the search of root-knot nematode infested pomegranate orchards in the Nimgaonjali village (19˚34'18" N 74˚24'07" E) near Kolhar-Ghoti highway in the tehsil of Sangamner of Ahmednagar district in the state of Maharashtra (India) 413714. The popular pome variety (Bhagwa) is cultivated over an area of 1 acre and orchard was almost 8-9 years old. Withered and drooped pome trees were spotted in the orchard and soil sampling was done under such diseased trees. Approximately, 1 kg of soil was collected at three different directions of each tree and at a depth of 15 cm, 30 cm and 40 cm with 1 to 1.5-meter-wide away from tree trunk. Soil samples were gathered into polyethene bags, labeled properly and brought to laboratory. The nematode infested roots were also collected and put into test tubes. In the laboratory, the soil samples were stored in refrigerator at 4^0c while root samples were washed under tap water to remove adhered soil debris and stored in refrigerator at 4^0c.

Isolation of nematodes from soil samples:

The soil samples were taken out and mixed well to each other. Plant debris, sand stones, gravels and weed roots were removed from the soil samples. A composite 250 gm soil was taken for further soil processing. In plastic tub, soil sample was mixed with tap water where large soil lumps was crushed, mixed and stirred well, so that soil was uniformly mixed with water. Soil suspension was passed through 20-mesh size sieve and collected into another plastic tube. The collected soil suspension was then passed into 60-mesh size sieve and collected into another plastic tub. Finally, collected soil suspension was poured on to 325-mesh size sieve and the residue was collected into plastic beaker. Thereafter, a soft tissue paper was placed on the wire gauge supported by Petri dish and filled with water. Soil suspension collected in beaker was poured into wire gauge and kept for 24 hours at room temperature for nematode isolation. On next day, nematodes were seen under stereo microscope [10].

Identification and staining of the nematodes:

Staining of nematode induced root galls:

The nematode infested roots were washed thoroughly under tap to dislodge soil particles attached to the roots. Roots along with galls were immersed into 4% sodium hypochlorite solution for 5 minutes for clearing the root and washed repetitively under tap water to remove the chemical residue. A stock solution of the acid fuchsin stain was prepared with 3.5 g of acid fuchsin powder into 250 ml of glacial acetic acid and 750 ml of distilled water and stored in glass bottle. Approximately, 20 ml of freshly prepared stain stock solution was taken into glass beaker and heated to boil. Washed roots along with galls were wrapped into muslin cloth and immersed into boiled acid fuchsin stain solution and kept for 5-6 minutes. The roots are then put on blotting paper to remove an extra stain and placed in glycerine dish acidified with 1% HCl. Next day, the stained nematodes and females were seen under stereomicroscope.

Identification of root-knot nematode species by perineal pattern:

The females were dissected from the infected pomegranate roots and placed in glycerine for observation. A sharp cut was made on the posterior portion of female with blade and cleared for perineal area. A temporary mount of perineal part of female was prepared and observed under compound microscope for species identification [11].

Preparation of aqueous stem extract:

The stem of *Achyranthus aspera* was collected and washed thoroughly in distilled water and cut in to small pieces. The stem was shade dried at room temperature. Dried stem pieces were uniformly grinded using mechanical grinder to make fine powder [7]. Take 5 gm powders add into 80 ml distilled water mixing properly. Thereafter, the suspension was filtered through double layered muslin cloth and the

filtrate was again filter Whatman filter paper 1 and 0. The supernatant was carefully transferred to glass bottle and stores as stock solution of nodal part extract of stem and used for further experiments (Plant extract stored at 4°C temperature in refrigerator.) [1]

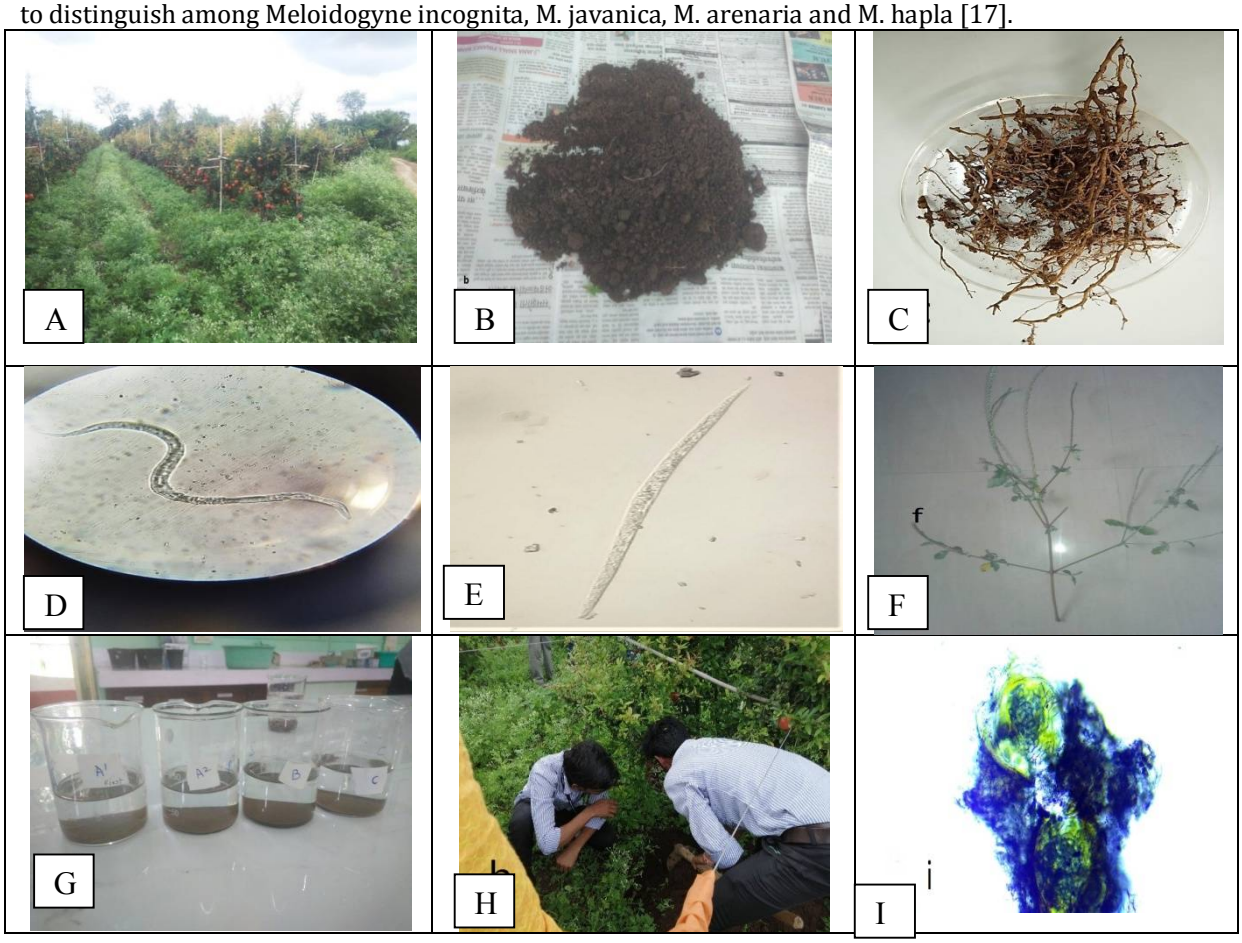
Bio efficacy studies of aqueous nodal part extract of Aghada stem against root-knot nematode:

The bio efficacy experiment was conducted into two parts: i.e. the effect of bio efficacy of aqueous nodal part extract of stem of Aghada against egg hatching and juveniles (J2s) longevity. The concentration of Aghada aqueous nodal part extract of stem (10%, 20%, 30%, 40% and 50%) were prepared with distilled water separately and experiments was designed in such way that each petri dish has 9 ml of leaf extract from each concentration and also have 20 root-knot nematode J2s per Petri dish and kept for 24, 48, 72, 96 and 120 hours for incubation at room temperature. Each set of experiments have three-replication and the mortality of J2s were recorded at each interval. In another experiment, egg masses were collected from freshly infested roots of pomegranate and exposed them to the different concentrations of leaf extracts and observed the effect of nodal part extract of stem on juvenile hatching and on egg mass after each interval mentioned above. Each set of experiment have three-replication and data was subjected for statistical analysis.

RESULT

Identification of Root-knot Nematode Species:

Root-knot nematodes (RKN; Meloidogyne spp.) were previously diagnosed based on adult female perineal patterns [15,16], i.e., posterior region comprising the vulva-anus area (perineum), tail terminus, phasmids, lateral lines and surrounding cuticular striae; a set of characters that was originally proposed to distinguish among Meloidogyne incognita, M. javanica, M. arenaria and M. hapla [17].



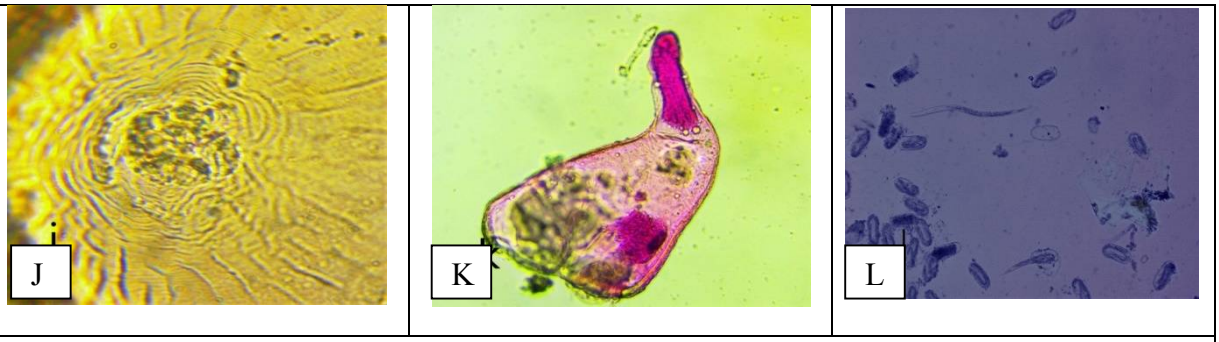


Fig 1.(A) Location of sampling field, (B) Soil Sample, (C) Root sample form, (D) Living juvenile, (E) Dead juvenile of RKNs, (F) Aghada plant. (G)Isolation of RKNs from soil, (H)Sampling plot, (I) Egg mass of Female, (J) Perineal pattern of female of RKN (K) Melon shaped female of RKN, (L) Infective juveniles of RKN hatched from eggs.

Bio efficacy studies of aqueous leaf extract of Aghada against Juvenile (J2) RKN's:

Study bioefficiency of plant extract of Aghada against RKN's (*Meloidogyne javanica*) of various concentrations as 10%, 20%, 30%, 40%, and 50% at different time 24H. , 48H. , 72H. , 96H. , and 120H.

Conc. / Time	Control	10%	20%	30%	40%	50%
24H.	0	45	51.65	71.5	80	91.65
48H.	0	58.3	66.65	75	86.65	100
72H.	5	81.3	91.65	100	100	
96H.	10	100	100			
120H.	20					

Table 1: Average (%) of Juvenile (J2) Mortality

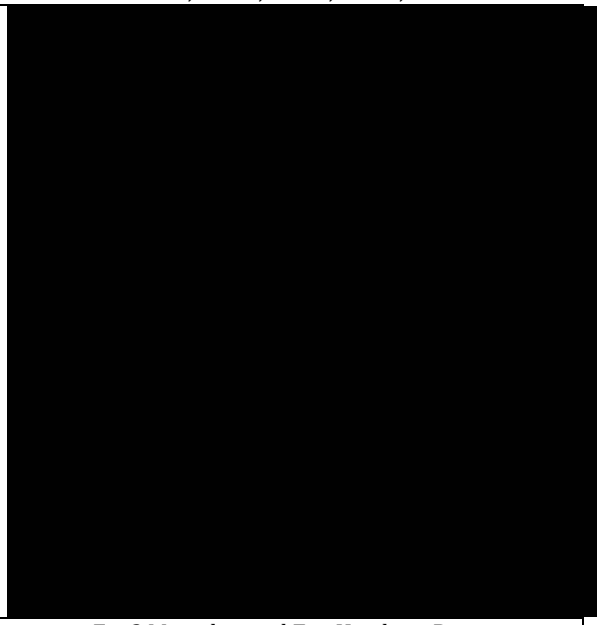


Fig.2 Mortality and Egg Hatching Rate

The result shows the *Achyaranthes aspera* recorded highest mortality of j2 *M. javanica* 50 % concentration indicating 91.65% mortality of juvenile observe *Achyaranthes aspera* in different concentration in 10% concentration 45% mortality, 20% concentration 51% mortality, 30% concentration 65% mortality and 40% concentration 80% mortality of j2 this is percent mortality were increase than control 0% mortality after 24hrs. of exposure time (Table 1). In 48 hrs. result shows the 50% concentration indicated 100% mortality of juvenile this is showing highest mortality as compared with control 0% than 40%contration 86.65 mortality 30% concentration 75% mortality of juvenile lowest mortality in 20% concentration 66.65% mortality and 10% concentration 58.3% mortality of juvenile this concentration into percent mortality were increase after 48hrs. of exposure time respectively. In 72hrs.result shows the 40%-30% concentration 100% mortality of juvenile as compared to control 5% lowest mortality in 20% concentration 91.65% and 10% concentration 81.3% mortality of juvenile this concentration percent mortality was increase after 72hrs. exposure time respectively. In 96 hrs. result shows the 10%-20% concentration 100% mortality of juvenile as compared to control 10% mortality the result shows the standard concentration of the extract represent maximum toxicity compare to the all dilution of extract or percent mortality time period aqueous extract of standard 50% concentration effect on J2 activity however concentration of 50% into 100% mortality (J2) in 72 hrs. exposure time were effective at reducing (J2) it has been concluded from this experiment extract of *Achyaranthes aspera* stem are a source of cheap and effective nematicides for the management of root knot nematode (Fig.2).

DISCUSSION

Finding of the research clearly suggested that weeds extract of the plant *Achyranthys aspera* were potential source of bionematicides against root knot nematode these result revealed that alkaloids, flavonoids, terpenoids and polyphenolic compounds of these weed might possess the Nematistatic as well as nematocidal activity the weed extract of stem into naturally present of these chemical. This study indicated that stem extract of *Achyranthys aspera* have various chemical constituent viz, alkaloids, flavonoids, phenols, tannins, saponins, phyto steroids and mucilage gum. Stem extract of this weed also revealed that plant is rich in alkaloids phenols terpenoids and flavonoids etc have high rate of nematocidal activity it is shown result possible that maximum mortality of second stage juveniles of *Meloidogyne javanica* in pomegranate root may be due to the presence of this chemicals in stem extract of *Achyranthys aspera*. The bioactivity of *Achyranthys aspera* against nematodes may be attributed to the presence of the alkaloids in its stem nodal part. Nematocidal property of some secondary metabolites (alkaloids, saponins, flavonoids, & glycosides) content extracted by these plant stem. During the last decade research on nematode control were focused on proposing strategies for inhibition or enhanced juvenile mortality. It has been observed that nematocidal activities were directly related to concentration of extract the result of this study is in agreement with other previous work. The result of the study showed that like *Achyranthys aspera* showed highest toxic effect against root knot nematode juveniles (J2) it is recommended that field trials be carried out to determine it's efficacy before recommending to farmers therefore it was concluded that the severe infection caused by *Meloidogyne javanica* could be lowered by the plant products in view of ecofriendly environment this has an advantage against expensive and hazardous chemical nematicides which have toxic effect on flora and fauna of environment plant products proved the way for the healthy and pollution free sustainable environment. The obtained results were correlates with finding of [12, 13, 14, and 15].

CONCLUSION

The present study shows nematocidal effect of *Achyranthys aspera* against root knot nematode *Meloidogyne javanica* infesting pomegranate. The bioefficacy study showed that 45%, to 100% juvenile mortality of Meloidogyne *javanica* in different concentration at different time *Achyranthys aspera* extract highly effective in reducing root knot development caused by *Meloidogyne javanica.* Finding of the research clearly suggested that extract of the plant *Achyranthys aspera* were potential source of bionematicides against root knot knot nematode, these results revealed that alkaloids, flavonoides, terpenoides and polyphenolic compounds of these weed might possess the nematistatic as well as nematicidal activity the weed extract of stem into naturally present of these chemical. During studies the aqueous extract of plant species were found in the toxic to root knot nematode egg and juveniles the *Achyranthys aspera* having nematostatic as well as nematicidal activity against root knot nematode. Utilization *Achyranthys aspera* plant would be the best ecofriendly and cheap strategy for nematode management in pomegranate.

REFERANCES

1. S.M. Vinodhini, T. Monisha, P. Praveen Arunachalam, S. Rajshree, P. Vignesh, E.G. Ebenezar and N. Seenivasan (2019).Effect of Plant Extracts on Root-Knot Nematode *Meloidogyne incognita* Infecting Tomato, Int.J.Curr. Microbiol. App.Sci, Vol. 8(6), pp. 373-378.
2. National Horticulture Board of India, (2018).
3. Ram Chandra , Vilas Tejrao Jadhav, Jyotsana Sharma (2010), Global scenario of pomegranate (*Punica granatum* L.) culture with special reference to India, Fruit, vegetables and cereal Science and biotechnology. Global science book, pp.7-18.
4. ICAR- (2018). National Research Centre on Pomegranate, Solapur.
5. Abi Beaulah, Mohamed Sadiq and Jaya Santhi , (2011). Antioxidant and antibacterial activity of Achyranthes aspera : An in vitro study, Scholars Research Library Annals of Biological Research, 2 (5).01-04
6. ***Priya***, G Kumar, L Karthik, KVB Rao. (2010)., Antioxidant activity of *Achyranthes aspera* Linn stem extracts. CL Pharmacologyonline 2 (2), 228-237.
7. Abhijeet Kasarkar, Archana Gavali, Nikita Kolekar, Shubhangi Kumbhar and Snehal Wadkar, (2018). Phytochemical screening of *Achyranthes aspera* L., *Amaranthus spinosus* L., *Parthenium hysterophorus* L., *Argemone mexicana* L., *Portulaca oleracea* L. and Euphorbia geniculata Ortega. from Kolhapur district of Maharashtra. International Journal of Herbal Medicine ; 6(3): 05-08.
8. Textbook of pharmacognosy 40th edition (2017)..
9. Cobb, N.A., (1918). Estimating the neem population of the soil. Agric. Tech. Cir. Bur. Pl. Industry. United States Department of Agriculture. No.1. p. 48.
10. Eisenback, J. D., H. Hirschmann, and A. C. Triantaphyllou. (1980). Morphological comparison of Meluidogyne female head structures, perineal patterns, and stylets. J. Nematol. Vol. 12, pp.300-313.

11. A. Asadi Sardari, A.A. Hojat Jalali, S. Bahraminejad & D. Safaee (2015) Effect of plant extracts on the mortality of root-knot nematodes' J2, *Meloidogyne javanica*, Archives of Phytopathology and Plant Protection, 48:4, 365-375.
12. Nour El-Deen AH1, Al-Barty Amal F , Darwesh Hadeer Y, and Al-Ghamdi AS .(2016). Eco-Friendly Management of Root-Knot Nematode, *Meloidogyne incognita* Infecting Pomegranate at Taif Governorate, KSA. RJPBCS 7(1) Page No. 1070.
13. Gulwaiz Akhter, Tabreiz Ahmad Khan. (2018). Evaluation of some plant extracts for nemato-toxic potential against juveniles of Meloidogyne incognita in vitro. The Journal of Phytopharmacology 2018; 7(2): 141-145.
14. María del Mar Montiel-Rozas, Miguel Ángel Díez-Rojo, Margarita Ros and José Antonio Pascual .(2020). Effect of Plant Extracts and Metam Sodium on the Soilborne Fungal Pathogens, Meloidogyne spp., and Soil Microbial Community Agronomy ,10, 513.
15. Karssen, G.; Van Aelst, A.C. (2001). Root-knot nematode perineal pattern development: A reconsideration. Nematology , 3, 95-111.
16. Eisenback, J.D.; Hunt, D.J. (2009). General Morphology. In Root Knot Nematodes; Perry, R.N., Moens, M., Starr, J.L., Eds.; CABI: Wallingford, CT, USA, pp. 18-54.
17. Chitwood, B.G. (1949). Root-knot nematodes"-Part I. A revision of the genus Meloidogyne Goeldi, 1887. Proc. Helminthol. Soc. Wash. 15, 90-104.

CITATION OF THIS ARTICLE

M Bharat_, D Bhushan, T Yogesh and S.P. Giri· Potential Role of Aqueous Stem Extracts of *Achyranthes aspera* L. on Root-Knot Nematode (*Meloidogyne javanica*) Parasitism in Pomegranate. Bull. Env. Pharmacol. Life Sci., Vol 11[1] December 2021 : 112-117.