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CERTAIN NON-EDIBLE OIL-SEEDS POSSESS SUBSTANTIAL NEMATICIDAL POTENTIAL

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ABSTRACT

Methanolic extracts of *Ricinus communis* and *Cassia fistula* seeds were tested against root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood. Results depicted absolute larval mortality, reduced and delayed egg hatch. Organic amendment of soil with *R. communis* and *C. fistula* seed meals also increased the growth parameters of guar crop. Organic amendment with *R. communis* @0.5 % concentration and *C. fistula* @0.3% concentration showed promising results on growth parameters of Guar plants. Moreover, all treatments significantly reduced the number of knots in roots of Guar plants. Phytochemical screening of methanolic extracts of *Ricinus communis* and *Cassia fistula* seeds revealed the presence of terpenoids, steroid, tannin, protein, coumarin, saponins, phenol, carbohydrate and flavonoids.

Key-words: Nematicidal activity, non-edible oil seed, phytochemicals, Root-knot nematode, guar and amaltas.

INTRODUCTION

Guar (*Cyamopsis tetragonoloba* L. (Taub.) is a drought tolerant plant which is largely cultivated in arid areas of US, India and Pakistan. Whereas guar leaves and young twigs are used as fodder its pods are used for human consumption. Also, guar gum (galactomannan) that is a derivative of guar plants has vast application in pharmaceuticals, cosmetics and textile industry (Undersander *et al.*, 2006). However, like any other crops, guar is also not free from diseases caused by plant pathogens, such as root knot nematodes *Meloidogyne* species which can cause severe damage to this crop and reducing its yields (Tripathi and Srivastava, 1975). *Meloidogyne* species infest roots of plants and obtain nutrients from adjoining cells. Host tissues swell up and roots appear to have knots on them. Growth of plants is affected because of the denatured root tissues. Plants cannot receive enough food and water (Abad *et al*, 2003). *Meloidogyne* species can affect more than five thousand species of crop plants (Trudgill and Blok, 2001). Synthetic chemical nematicides have been used for the control of plant pathogenic nematodes including root-knot nematodes. However, these chemicals pose serious health problems, environmental pollution and underground water pollution. For example ethylene di bromide (EDB) and Di-bromo chloropropane (DBCP) are banned in many countries because of their side effects on both humans and their environment (Oka *et al*., 2000). Because of these concerns including the increasing cost of chemicals there is a need to look for alternative means of nematode control. Plants and their products have been found to be effective against nematode infection (Uhlenbroek and Bijloo, 1958). Many plant pathologists do agree that organic amendments improve plants yields, and suppress its phytonematodes population (Muller and Gooch, 1982). Plant parts and the chemicals that are obtained from plants have been used to prevent plants from insects and pest infection. For instance, seed extracts of non-edible oil seeds have shown strong activities against hatching and mortality of root knot nematodes (Khurma and Singh, 1997; Radwan *et al.*, 2011). *Ricinus communis* L. belongs to family Euphorbiaceae is a plant that is indigenous to many parts of the world. Castor oil contains a wide range of chemical compounds steroids, saponins, alkaloids, glycosides and flavonoid and has traditionally been used as laxative, purgative, fertilizers and as fungicides (Jena and Gupta, 2012). In addition, seeds of many plant species such as *Acacia* sp., *Albizzia lebbak*, *Sesbania* sp., *Medicago* sp., *Phaseolus* sp., *Pisum* sp., *Pongamia* sp., *Sesbania* sp., and *Trigonella* sp. have shown strong nematicidal potential (Khurma and Chaudhry, 1999). In the present study we have tested the nematicidal potential of seed powder of *R. communis* and *C. fistula* against *M. incognita* egg hatch and its larval survival *in vitro* and growth of guar plants. We have also performed phytochemical analysis of *Ricinus communis* and *Cassia fistula* seeds.

MATERIALS AND METHODS

COLLECTION OF MATERIALS

Amaltas *(Cassia fistula* L.*)* and Castor (*Ricinus communis* L.) seeds were collected from University of Karachi Campus. Seed meals were prepared by grinding seeds in an electric grinder and powder was stored in an air tight bottle to avoid moisture.

PREPARATION OF METHANOL EXTRACT

Ten g of seeds powder were kept in 100mL of Methanol. After 15-20 days suspension was filtered through Whatman filter paper and left for evaporation. Residue left after evaporation was collected. This residual extract was then dissolved in DMSO to make 1000, 500 and 250 ppm solutions.

EGG HATCHING AND JUVENILE MORTALITY

Meloidogyne incognita (Kofoid and White) Chitwood, infected roots of egg plants (*Solanum melongena* L.) growing in a nearby agriculture plot were collected. Eggs of root knot nematodes were collected in distilled water as described by Hussey and Barker (1973).

One mL of egg suspension was poured into cavity blocks containing methanolic extracts. Treatments were replicated thrice. Egg suspension in sterilized water without methanolic extract served as control. After 24, 48, 72 and 96 hours emerging larvae were counted.

For larval mortality test *M. incognita* eggs were allowed to hatch for 24 h at room temperature. One mL of juvenile suspension was poured into each cavity block containing methanol extracts and replicated three times. Cavity block with juvenile suspension in distilled water served as control. After 24, 48, 72 and 96 h, the dead or static larvae in the cavity block under a low power stereoscope microscope were counted (Cayrol *et al*., 1989).

PHYTOCHEMICAL ANALYSIS:

Residue of methanolic extract left after evaporation was used for phytochemical analysis by using the standard methods (Harborne, 1998; Evans *et al.,* 2009; Pochapski *et al.,* 2011).

Terpenoids (Salkowski test): Residue of methanolic extract was mixed with 2 mL of $CHCl_3$ and H_2SO_4 (concentrated) was added. A reddish brown coloration at the interface indicates presence of terpenoids.

Steroids (Salkowski test): Residue of methanolic extract was dissolve in 2 mL H_2O and 2 mL $CHCl_3$ and 2 mL H_2SO_4 (concentrated) were added. Reddish brown ring at the junction of two liquids indicates presence of steroids.

Saponins (foam test): Residue of methanolic extract was dissolved in 5 mL H_2O and heated. Froth appearance in the test indicated the presence of saponins.

Proteins: Residue of methanolic extract was mixed with 1 mL H_2O and 1 mL H_2SO_4 (concentrated) was added. White precipitates indicated the presence of proteins.

Coumarins: Residue of methanolic extract was mixed with 3 mL NaOH (10%). Yellow coloration indicated the presence of coumarin.

Phenol: Residue of methanolic extract was mixed with few drops of 10% solution of Lead acetate. White precipitates in the solution indicate presence of phenol.

Phlobatannins: Residue of methanolic extract was mixed with 2 mL HCl (1%) and heated. Red precipitates indicated the presence of Phlobatannins.

Carbohydrates: Residue of methanolic extract was mixed with few drops of α naphthol solution in alcohol and H_2SO_4 (concentrated) was added from the side of the test tube. Violet ring formed at the junction of two liquid in test tube indicates presence of carbohydrates.

Flavonoids: Residue of methanolic extract was mixed with 5 mL of dilute ammonia followed by addition of H_2SO_4 (concentrated). The solution in the test tube turned yellow in color indicated the presence of flavonoids.

Tannins: Residue of methanolic extract was mixed with 2 mL of $FeCl_3$. Dark green coloration indicated the presence of tannins.

GREEN HOUSE EXPERIMENT

Soil used for the experiment was obtained from experimental plots of Department of Botany, University of Karachi. The soil was sandy loam and basic in reaction (pH ranged from 7.5 – 8.1) with maximum water holding capacity (MWHC) of 41% as determined by Keen and Raczkowski (1921). The total nitrogen as determined by the method of Mackenzie and Wallace (1954) was 0.07-0.10%. The pots were filled with soil @300g/pot, amended with seeds powder of *R. communis* and *C. fistula* @ 0.3 and 0.5% w/w/. The pots were placed in a greenhouse of the Department of Botany, University of Karachi, under natural sunlight, in a randomized design and there were three replicates of each treatment. Seeds of Guar were sown in pots filled with soil. Ten days after seedling emergence, the soil in each pot was inoculated with 2000 juveniles of *Meloidogyne incognita* by pouring the nematode suspension near the roots of each plant. After 8 weeks of nematode inoculation growth parameters such as shoot length, root length, shoot weight, root weight, number of nodules, shoot dry weight, root dry weight, number of galls per root system, amount of chlorophyll and carotenoids were recorded.

Estimation of Photosynthetic Pigments

Fresh guar leaves sample(0.1g) were crushed in 3 mL 80% acetone. Extract was centrifuged for 5 minutes at 5000 rpm and supernatant was collected. Absorbance was recorded at 663nm and 645nm for chlorophyll a & b estimation and 480 and 510 nm for carotenoids estimation, respectively (Maclachlan and Zalik, 1963). UV mini 1240 Spectrophotometer was used for absorbance estimation. Chlorophyll and carotenoids contents were estimated according to Arnon (1949) and Duxbury and Yentsch (1956). Amount of chlorophyll and carotenoids were expressed as $\mu g.g^{-1}$ fresh weight.

Data Analysis

Descriptive statistics and analysis of variance (ANOVA) with SPSS ver. 16 was performed for data analysis. Least Significant Difference (LSD) was calculated and Duncan's Multiple Range Test (DMRT) was employed to compare the means of treatments (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Methanolic extracts of *R. communis* and *C. fistula* seed meals were found to be highly effective against *M. incognita* eggs as it reduced the egg hatching. *R. communis* extracts when used at 500 and 1000 ppm concentration completely inhibited egg hatch, whereas *C. fistula* extract was only effective when used at 1000 ppm concentration (Fig.1). Whereas, both *R. communis* and *C. fistula* seeds methanolic extracts showed 100 percent larval mortality (Fig.1, 2). Khurma and Mangotra, (2004) also reported 100% larval mortality in aqueous extract of *C. fistula*. We found a significant increase in all growth parameters of guar plants at both concentrations (0.3 and 0.5%) of *R. communis* and *C. fistula* seed powder (Fig.3-14). The change in treatment concentrations gave a major impact on root weight, shoot weight and shoot length of plants. Number of knots found to be reduced more at 0.5 % organic amendment. Olaniyi *et al*. (2005) have proposed that increase in root weights of nematode infected plants is due to the formation of knots in roots caused by root knot nematode infection.

There was a significant increase in the number of nodules in non-nematode inoculated set of Guar plants than nematode inoculated plants. Castor seed meals have been reported to suppress root-knot nematode infection on tomato plants (Masood and Hussain, 1975). Initially it was thought, that a hemagglutinin protein present in the castor seeds was responsible for the nematicidal effects (Lis and Sharon, 1973). However, further research on castor seeds identified two agglutinating compounds, ricin and ricin us that may have caused the nematicidal effects (Lin and Li, 1980). Seeds of some plants of family Apiaceae were also tested against root knot nematode in vitro and in vivo and showed nematicidal activity (Siddiqui and Zaki, 2017). Olaniyi *et al*. (2005) suggested that release of some chemicals from the plant materials could have deleterious influence on the growth and development of root knot nematodes and this ability should be exploited for root knot susceptible crops. In the present study, *R. communis* and *C. fistula* seeds powder increased root dry weight when used at @0.5% concentration. However, dry weight of shoot, Chlorophyll "a", "b", carotenoids and total chlorophyll contents increased in plants treated with both concentrations of *R. communis* and *C. fistula* as compared to control.

Table 1. Phytochemicals of castor and *Cassia*.

Bioactive compounds	***Ricinus communis***	***Cassia fistula***
Terpenoids	++	+++
Steroids	+++	+++
Tannins	–	++
Coumarins	++	+++
Saponins	–	–
Phenols	+++	+++
Carbohydrates	++	–
Flavonoids	+	+++
Proteins	–	–
Phlobatannins	–	–

+ = indicates presence of phytochemicals; - = indicates absence of phytochemicals; ++ = shows moderate concentration; +++ = shows high concentration.

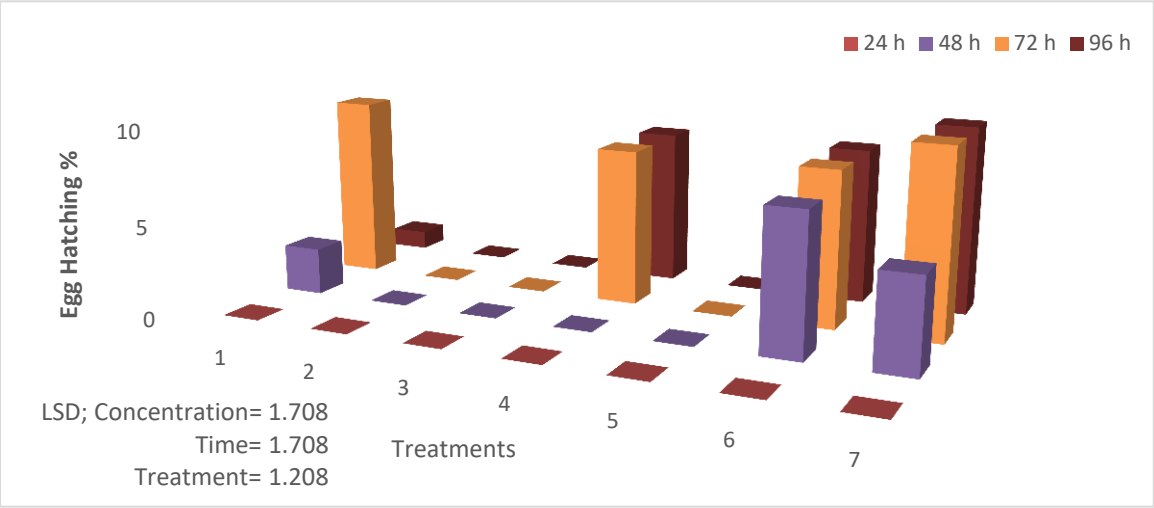

Fig.1. Effect of Methanolic extract of some non-edible oil seeds on Egg Hatching of *Meloidogyne incognita.*
1= control, 2= *Ricinus communis* 1000 ppm , 3= *R. communis* 500 ppm , 4= *R. communis* 250 ppm , 5= *Cassia fistula* 1000 ppm, 6= *C. fistula* 500 ppm, 7= *C. fistula* 250 ppm

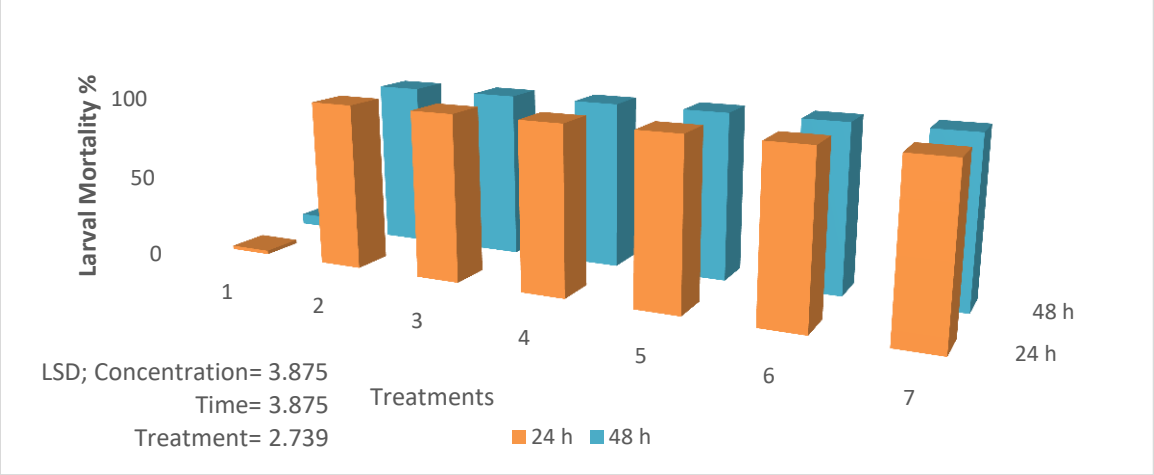

Fig.2. Effect of Methanolic extract of some non-edible oil seeds on larval mortality of *Meloidogyne incognita.*
1= control, 2= *Ricinus communis* 1000 ppm , 3= *R. communis* 500 ppm , 4= *R. communis* 250 ppm , 5= *Cassia fistula* 1000 ppm, 6= *C. fistula* 500 ppm, 7= *C. fistula* 250 ppm

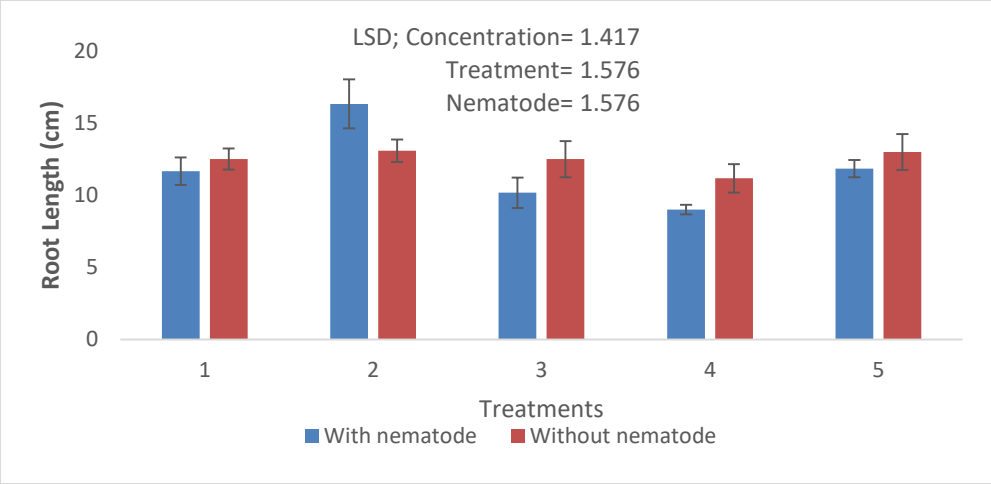

Fig.3. Effect of organic amendment of some non-edible oil seeds on Root length of Guar plants.
1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

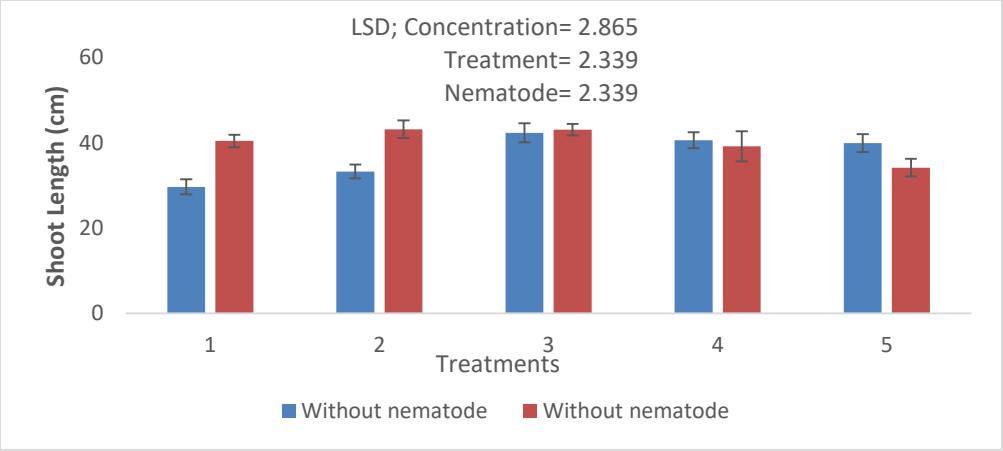

Fig.4. Effect of organic amendment of some non-edible oil seeds on Shoot length of Guar plants.
1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

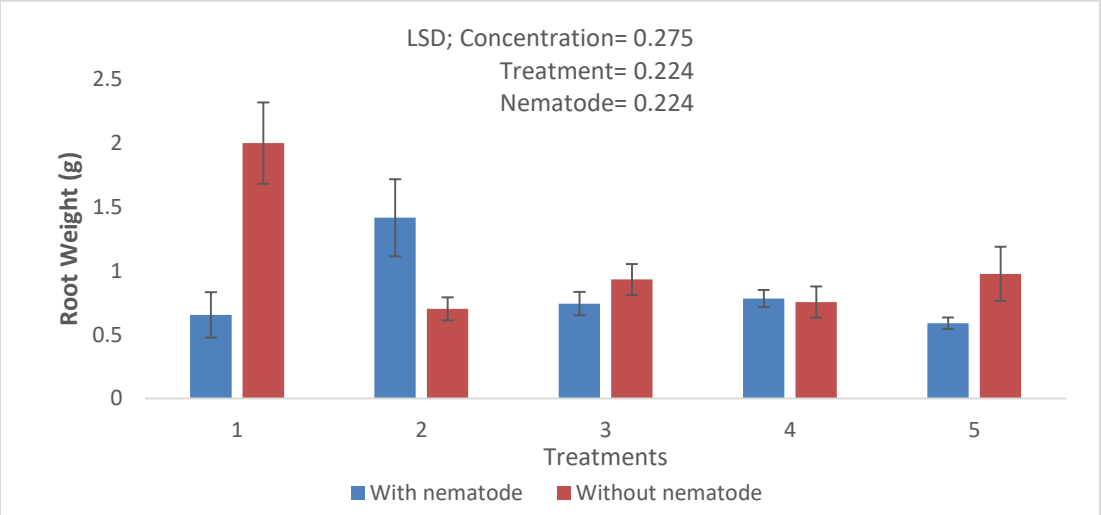

Fig.5. Effect of organic amendment of some non-edible oil seeds on Root Weight of Guar plants.
1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

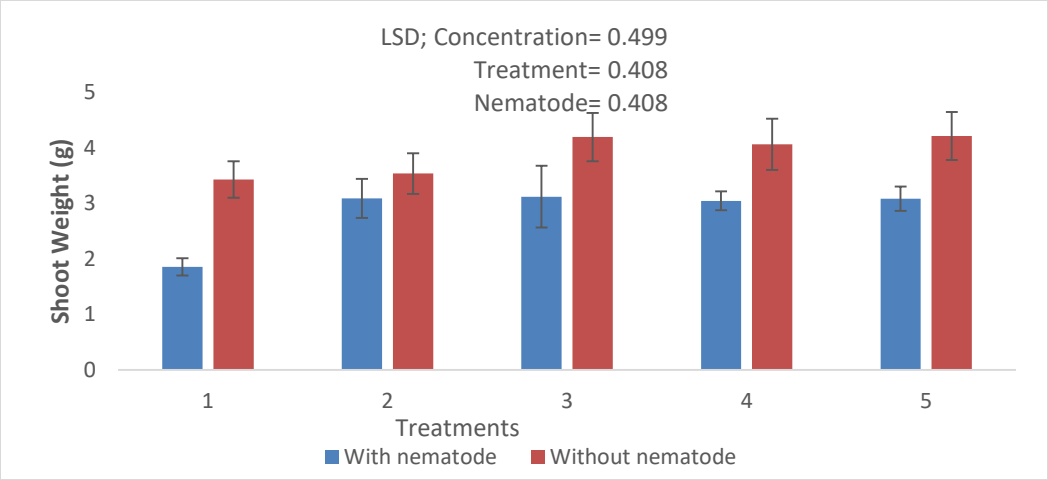

Fig.6. Effect of organic amendment of some non-edible oil seeds on Shoot Weight of Guar plants.
1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

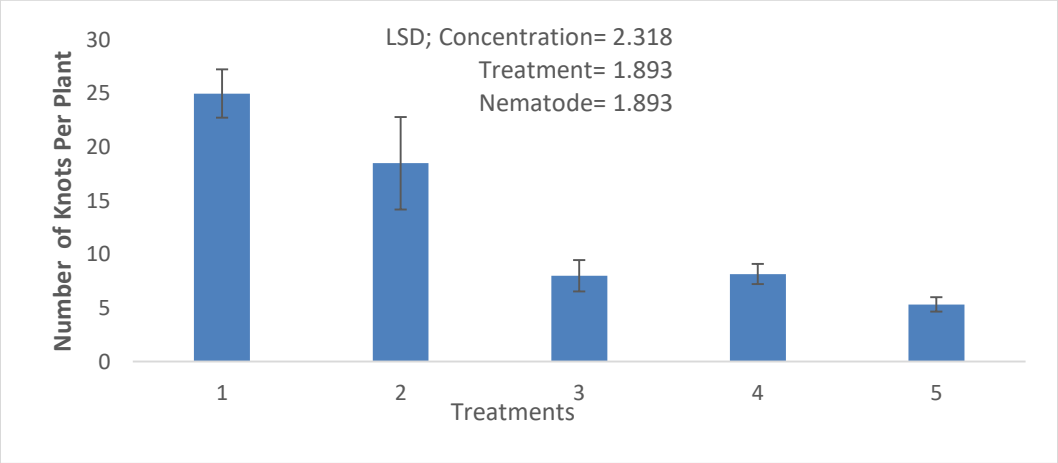

Fig.7. Effect of organic amendment of some non-edible oil seeds on Number of knots per plant in Guar.
1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

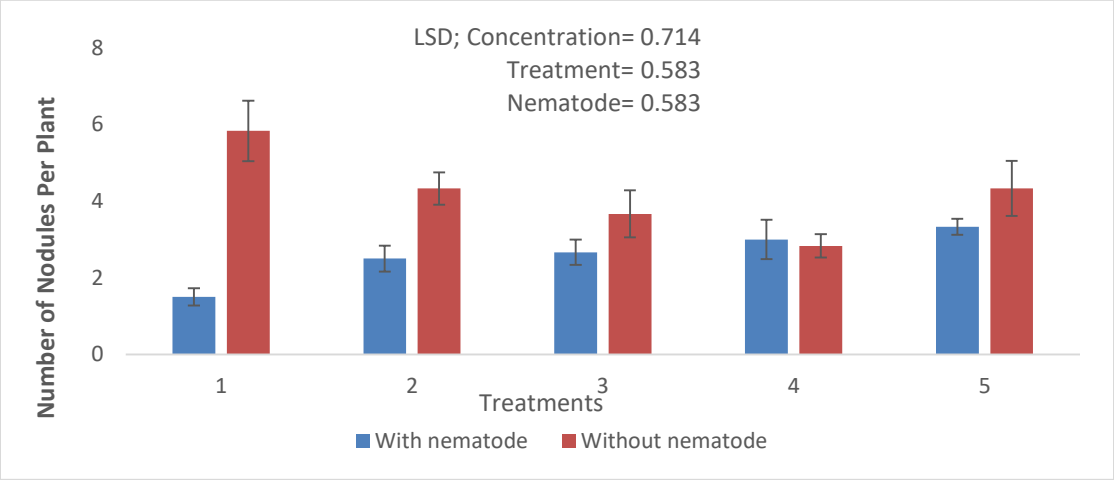

Fig.8. Effect of organic amendment of some non-edible oil seeds on Number of Nodules per plant in Guar.
1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

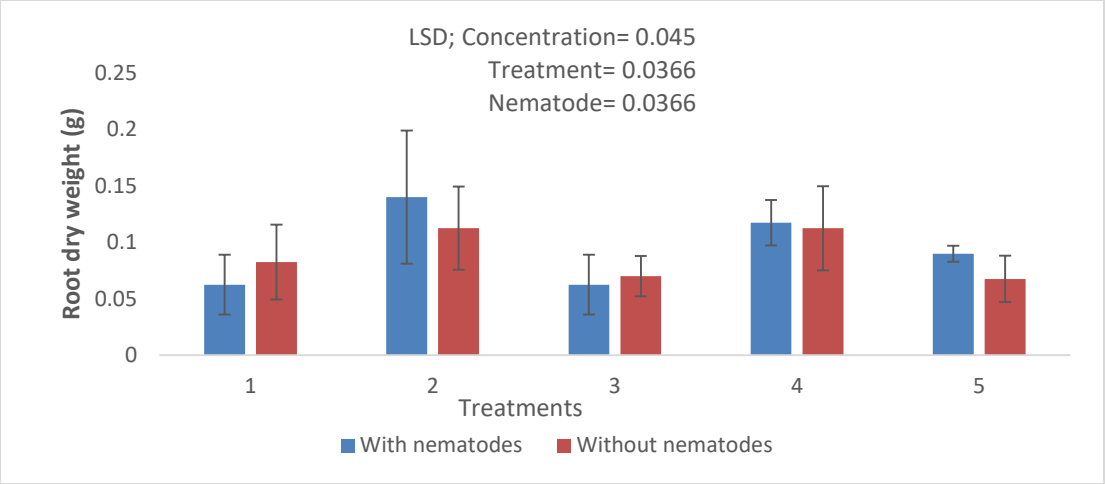

Fig.9. Effect of organic amendment of some non-edible oil seeds on Root dry weight of Guar plants.
1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

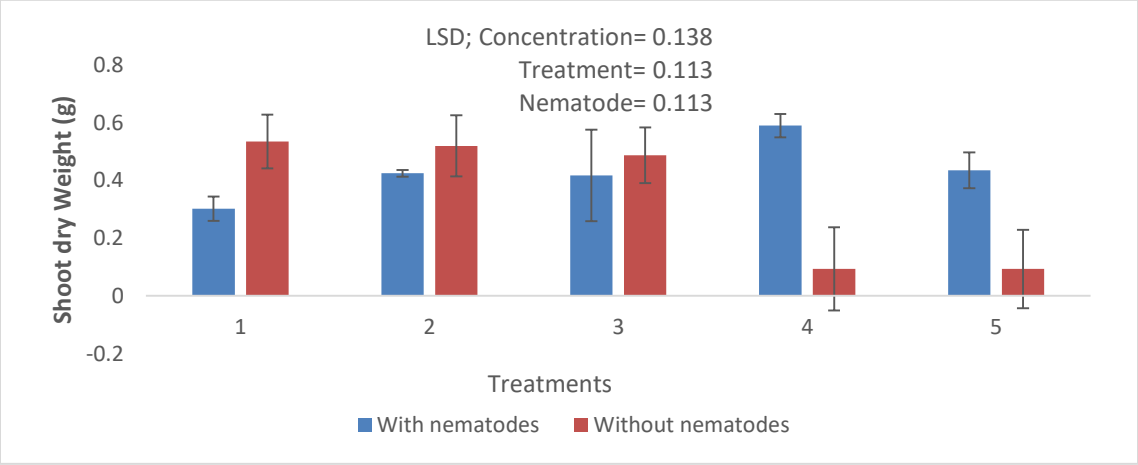

Fig.10. Effect of organic amendment of some non-edible oil seeds on Shoot dry weight of Guar plants.
1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

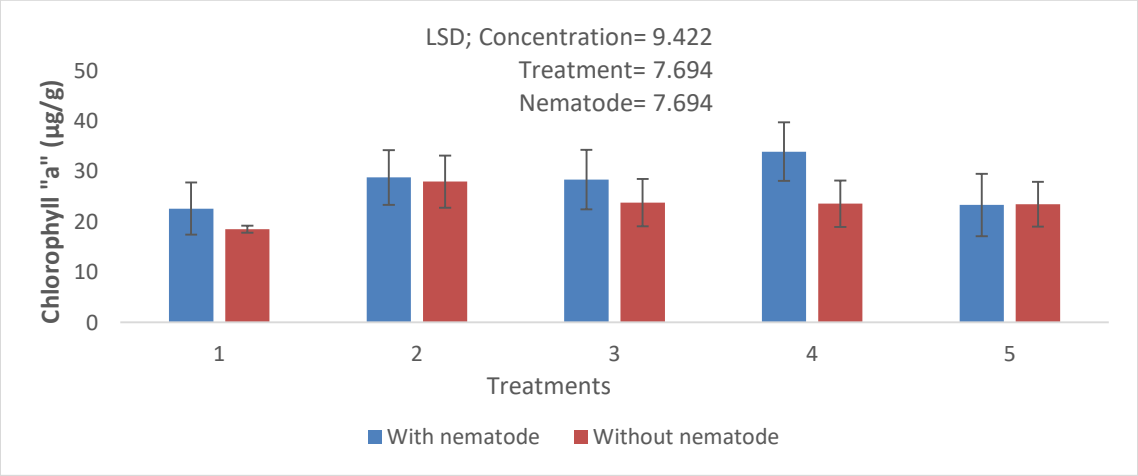

Fig.11. Effect of organic amendment Chlorophyll "a" (µg/g) content of Guar plants.
1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

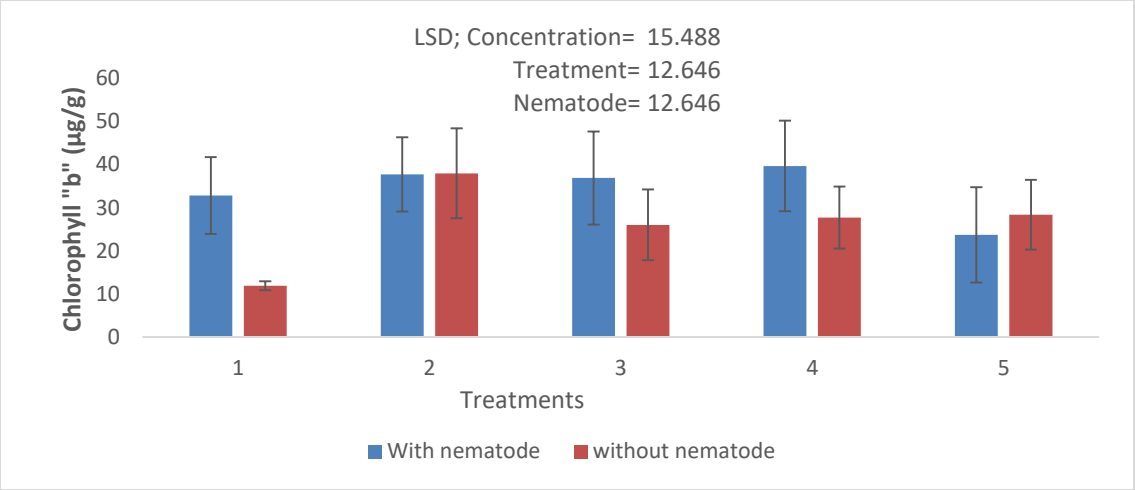

Fig.12. Effect of organic amendment of some non-edible oil seeds on Chlorophyll "b" (µg/g) content of Guar plants. 1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

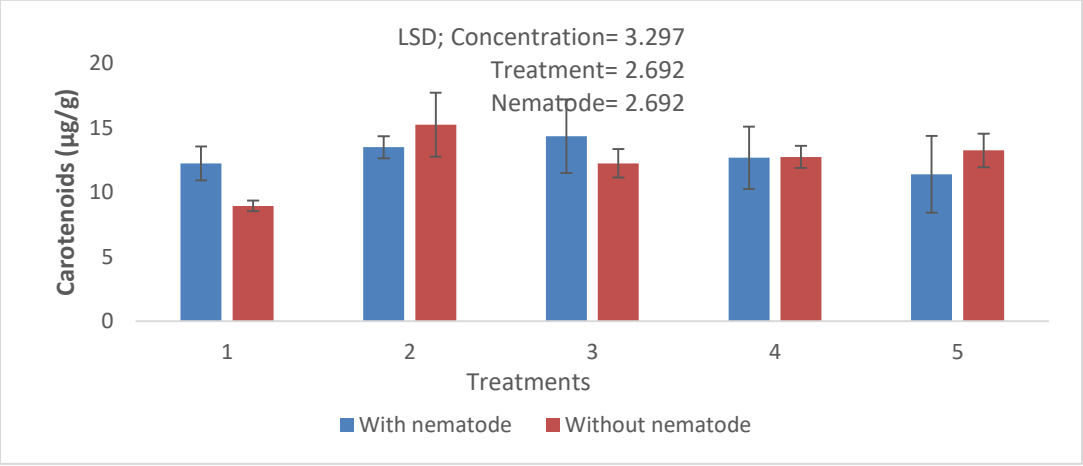

Fig.13. Effect of organic amendment of some non-edible oil seeds on Carotenoids (µg/g) content of Guar plants. 1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

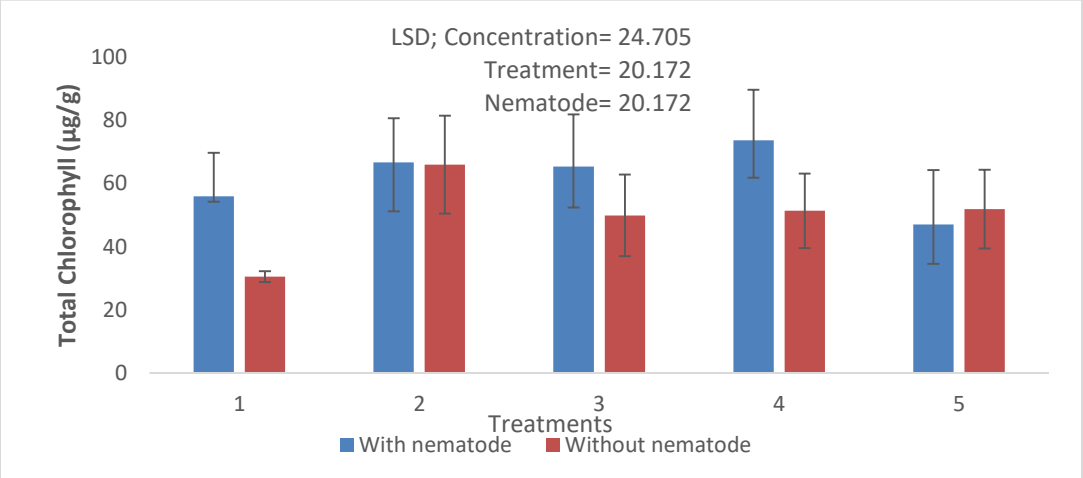

Fig.14. Effect of organic amendment of some non-edible oil seeds on Total Chlorophyll (µg/g) content of Guar plants. 1= Control, 2= *Ricinus communis* 0.3%, 2= *R. communis* 0.5%, 3= *Cassia fistula* 0.3%, 4= *C. fistula* 0.5%

Phytochemical can help identify chemical constituents of plant and their active ingredients (Bhandary *et al*., 2012). In this study, phytochemical screening of *R. communis* and *C. fistula* methanolic seed extracts showed the presence of terpenoids, steroid, tannin, protein, coumarin, saponins, phenol, carbohydrate and flavonoids (Table 1). Although we found the presence of terpenoids in all seed extracts and its concentration was highest in *C. fistula* extracts than R. *communis* extracts. Further, tannin was detected in *C. fistula* extracts but it was absent in *R. communis* extracts. In our study, we found organic solvent extracts were more potent than the aqueous extracts, the reason may be that the active substance that are present in plants can dissolve more and could be extracted more efficiently in organic but a less polar solvent than water; the compound that are present in *R. communis* and *C. fistula* extracts prefer non or less polar solvent.

The dried seeds of *R. communis* were effective in reducing the number of root knot nematodes in the soil as well as increasing shoot weight and root length. Seed meals of effective plant species those showed nematicidal activity could be considered as an alternative control of root knot nematodes.

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