Integrated Effect of Oil Cakes, Bioagent and Nematicide on Root Knot Namatode and Root Nodulation in Lentil

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Effect of different oil cakes along with bioagents/nematicide was evaluated on root knot nematode (*Meloidogyne* spp.) and root nodulation in lentil verity K-75. The results revealed that combination of oil cakes (neem cake, mahua cake, groundnut cake, sunflower cake) along with carbosulfan (nematicide) was found very effective on both reducing nematode population and increasing root nodulation in lentil. It have also found in lowering down the disease of fusarium wilt and root knot complex on lentil verity K-75 with different parameters *viz.*, root lengths (8.13, 8.50, 7.51, 6.67 cm), root weight (4.36, 4.52, 4.15, 3.21), shoot length (36.68, 37.45, 34.75, 29.37 cm), shoot weight (17.12, 14.72, 12.43, 10.52 gm), root knot index(0.95, 1.00, 0.85, 1.00), minimum no. of root gall per plant (15.10, 20.00, 46.20, 20.20), maximum no. of rhizobium nodules per plant (50.00, 52.05, 65.10, 42.05) and minimum nematode population(30.26, 31.15, 30.15, 25.56). Out of four cakes combination with different bioagents the best results have been found in neem cake followed by mahua cake.

Keywords: Lentil, oil cakes, bioagents and nematicide, root-knot nematode, root nodule, efficacy.

Lentil or Masoor (*Lens culinaris* Medik) is one of the multipurpose *rabi* pulse crop of India that originated in East and Mediterranean region. It is called "poor man's meat" because lentil seeds contain 22 -34.6 per cent protein (Adsule *et al.*, 1989). In India, lentil crop occupies 1.35 million hectares area with annual production of 0.96 million tones and having 693 kg/ha productivity (Anonymous, 2011). Lentil is mostly grown in India as a rainfed crop by marginal farmers on their marginal lands and it is mainly cultivated in Uttar Pradesh, Madhya Pradesh, Chhattisgarh, Jharkhand, Bihar, Rajasthan, Haryana and West Bengal. These states together accounts for 85 per cent and 90 per cent of the total area and production, respectively (Dixit et al., 2011). Lentil crop is mainly damaged by fungal, bacterial, viral and nematode diseases. Wilt disease is caused by Fusarium oxysporum Schlecht. is the most devastating disease of lentil, causing yield loss up to 50 per cent in India (Khare, 1980). In addition, if the soil is infested with root-knot nematode (Meloidogyne spp.), then the losses accredited are much higher. Plant- parasitic nematodes causing severe damage to plant growth of lentil and mungbean (Ali, 1989). The disease complex of wilt fungus and root knot nematode reduces the crop yield significantly. Hence, the study was conducted to evaluate the effect of different oil cakes along with bioagents/nematicide on root knot nematode (Meloidogyne spp.) and root nodulation

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in lentil. It is much economical and effective than individual chemical or bioagent or oil cakes etc.

MATERIALS AND METHODS

Estimation of nematode population in soil

Cobb's sieving and decanting technique was followed for extraction of nematodes. For which, 200 gram of the soil was taken in a container and mixed thoroughly with water. Hard particles and stones, or any other raw materials were removed by stirring the suspension and was then passed through a set of sieves of 250, 45 and 37 μ m pore size.

The aliquot or filtrate was poured in a Petri dish containing tissue paper and enough water as to keep the tissue paper moist. The Petri dishes were kept for three days to extract nematodes. Care was taken to prevent drying of the tissue paper. The nematode suspension collected in the Petri dish was examined by means of research stereoscopic binocular microscope. The different plant parasitic nematodes present in the suspension were identified. Their number present in the suspension were determined by taking the average number of nematodes present in five different one ml aliquots of nematode suspension. **Evaluation of efficacy of oil cakes along with bioagents/nematicide**

A pot culture experiment was carried out to evaluation of efficacy of oil cakes along with bioagents / nematicide on root knot nematode of lentil grown in the earthen pots containing sick soil. The commercially available plants products/ oil cakes likes neem cake (NC), mahua cake (MC), groundnut cake (GC), sunflower cake (SC), bioagents like *Trichoderma viride*, *T. harzianum*, *T. virence*, *Pseudomonas fluorescens*, *Paecilomyces lilacinus* and a nematicide like carbosulfan (0.1%) were used in the experiment.

Sterilized pot mixture was taken in six kg capacity pots and rooted terminal cutting of lentil were planted separately (two cuttings per pot). After proper establishment, the treatments were applied to the pots. Simultaneously *Meloidogyne incognita* was inoculated @ 1000 j₂ per kg of soil around the roots of lentil plants. The treatments were replicated thrice in completely randomized design (CRD) as follows: Observations were recorded on plant growth parameters *viz.*, shoot

length (cm), root length (cm), shoot weight (g), root weight (g), number of galls per plant, final nematode population and root knot index using the following 1 to 5 scale (Taylor and Sasser, 1978). Where, 0: No galls per plant root system, 1: 1-2 galls per plant root system, 2: 3-10 galls per plant root system, 3: 11-30 galls per plant root system, 4: 31-100 galls per plant root system, and 5: More than 100 galls per plant root system. The experiment was conducted by using lentil variety K-75 planted in 15 cm diameter pots. Twenty five days after the date of planting (after rooting), suspension containing desirable number of infective juveniles of the nematode were inoculated to the root region. The nematode suspension was determined by taking the average number of juveniles present in five different one milliliter aliquots of the suspension. Uniform quantity of the suspension containing desired number of juveniles was used for inoculation.

Three replications were maintained for each treatment and the pots were placed in glasshouse and suitably randomized design (CRD). The temperature during the experimentation was $27 \pm 2^{\circ}$ C. The pots were watered daily and observations were recorded in respect of shoot length, root length, shoot weight, root and shoot weight and average number of juveniles present in each of five aliquot of suspensions. These were recorded at the 55th days of termination of experiment. Root length, root weight, and shoot weight were determined at the end of the experiments after depotting. The root system was gently retrieved after loosening the soil. Nematode population in the soil was recorded immediately after termination of the experiments.

Collection of cultures of root-knot nematode maintenance and build up of inoculums-Root-knot infested lentil plants were collected from the experimental plots of the Department of Plant Pathology C.S. Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh. Root portion was carefully removed from the soil and washed gently under running tap water. Egg masses were picked and kept for hatching in Petri dish. After 24-36 hours, juveniles hatched were used to inoculate lentil grown in sterilized soil sand mixture in green house. These plants were served as culture plants. After giving sufficient time to complete 3-4 generations of the nematode, plants were uprooted carefully. The root system was washed to remove adhered soil particles. The galls containing egg masses were used to get inoculums of the pathogen for further studies.

Identification of prevailing root knot nematode species

The galled root system from the above culture plants was immersed in a beaker containing 0.1 per cent cotton blue in lactophenol and left over night for clearing (Southey, 1986). The roots infected by root knot nematode were washed. The females were dissected out from the well developed galls of the roots under the Stereo binocular microscope and were transferred to a drop of lactophenol taken on a clean glass slide. The portion of the females was carefully cut with a sharp razor blade and body content was cleaned. The perineal region was trimmed and mounted for observations under oil immersion objective. At least ten slides were prepared containing the perineal pattern of the nematode. The identification of species was made on the basis of characters of perineal pattern as described by Eisenback et al. (1981).

Hatching of juveniles and inoculation

The egg masses from stock culture were transferred carefully to a wire gauge sieve containing two layers of facial tissue paper trimmed down to edge of wire gauge and kept in a Petri dish holding sufficient water to remain in contact with the bottom of Petri dish. After 24 hours, the content of Petridish was emptied into a beaker, diluted to a suitable volume and population counts were made with the help of fenwick's multi-chamber counting slide. Based on the requirement, the suspension was diluted with sterile water.

Second stage juveniles were obtained from egg masses collected from heavily infested lentil plants by incubating large number of egg masses at room temperature in water. After 48 hours of incubation, the second stage juveniles in water were collected in a 100 ml beaker and volume of water was made up to 50 ml. The nematode suspension was bubbled with the help of 10 ml pipette and an aliquot of one ml was transferred to counting dish for counting the juveniles under Stereo binocular microscope. Five aliquots were examined from each sample and average population was calculated. For inoculation, larval levels were adjusted with water so as to add equal volume of nematode suspension in each treatment to give desired inoculum level. The required number of juveniles in the water was added to potted seedlings to the two cm deep holes made on the rhizosphere. A similar treatment given to the inoculated check plants except that only water was used without nematode.

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Estimation of nematode population from root samples

Root samples of known quantity (5g) were directly observed under Stereo binocular microscope for counting adult females of sedentary nematodes and same was processed using blending and Baermann's funnel method for the extraction of active forms of sedentary as well as migratory nematodes. After incubation of 48 hours, the volume of suspension was made to 250 ml out of which 10 ml pipetted out and used for counting nematode. The roots were stained in a boiling solution of 0.05 per cent lactophenol cotton blue for one minute and allowed to cool for few minutes before washing gently under running tap water. The total nematode counts were expressed per 5 g root. The nematodes were identified based on key provided by Taylor and Sasser (1978).

Counting of egg masses

The number of egg masses of root-knot nematode per root system was counted by exposing the infected roots to 0.25 per cent trypan blue for three minutes as per the procedure given by Sharma and Kumar (1991).

RESULTS AND DISCUSSION

Effect of different oil cakes in combination with bio agent/nematicide was tested for management of root knot nematode and root nodule formation. Data presented in table 1, 2, 3 and 4 revealed that all the combination treatment were found effective for management of the root knot nematode.

Effect of neem cake (NC) with different bio agents on root knot nematode and root nodulaton in lentil

The data on effect of neem cake in combination with bio agent/nematicide on growth parameters of lentil was presented on Table 1. It was observed that combination neem cake + carbosulfan was found significantly superior to other treatments in terms of maximum shoot length (36.68 cm) and root length (8.13 cm), highest fresh shoot weight (17.12 g) and root weight (4.36 g), lowest root-knot index (0.95) and number of rhizobial nodules/plant (50.00) followed by neem cake + *T. harzianum* treatment with shoot length (35.77 cm) and root length (8.11 cm), fresh shoot weight (14.87 g) and root weight (4.12 g), root-knot index (1.00) and increase the number of rhizobial nodules/plant (48.10). While Minimum shoot length (28.00 cm) and root length (4.0 cm), fresh shoot weight (8.50 g) and root weight (3.00 g), rootknot index (5.00) and number of rhizobial nodules/ plant (12.00) was found in control. Other treatments were found moderately effective against root knot nematode. Minimum number of root gall/plant (15.10) and nematode population/200 g of soil (30.26) were recorded with treatment neem cake + carbosulfan followed by neem cake + *T. harzianum* treatment with number of root gall/plant (36.25) and nematode population/200 g of soil (46.16). Whereas maximum root gall (95.00/ plant) and nematode population (125.20/200g of soil) was recorded in control. Results were reported by Devi and Das (1998) who observed that neem cake was found significantly superior in reducing *M. incognita* population and increased yield of carrot.

Table 1. Effect of combination of neem cake and bio-agents/nematicide against root knot nematode in lentil

Treatments	Plant length (cm)		Fresh plant weight (g)		Root- knot	No. of root galls	No. of rhizobium	Nematode population
	Shoot	Root	Shoot	Root	index	/plant	nodules /plants	
N.C. + Trichoderma viride	35.48	8.03	13.06	3.68	1.41	41.00	45.00	60.00
N.C. + Trichoderma harzianum	35.77	8.11	14.87	4.12	1.00	36.25	48.10	46.16
N.C. + Trichoderma virens	34.75	6.46	12.98	2.95	1.59	46.55	41.00	70.10
N.C.+ Paecilomyces spp.	32.60	6.10	13.56	2.86	1.50	40.00	37.13	66.20
N.C.+Bacillus thuringiensis	31.93	4.42	13.17	2.68	2.00	57.00	25.00	76.24
N.C. + Pseudomonas fluorescens	30.12	4.11	12.62	2.27	3.00	74.25	20.10	102.00
N.C. + carbosulfan	36.68	8.13	17.12	4.36	0.95	15.10	50.00	30.26
Check	28.00	4.00	11.12	2.00	5.00	95.00	12.00	125.20
C.D. (0.05)	2.081	0.802	0.815	0.463	0.294	4.381	2.386	7.784
S.E. (d)	1.005	0.388	0.390	0.225	0.147	2.105	1.149	3.745

N.C. = Neem cake

Table 2. Effect of combination of mahua cake and bio-agents/nematicide against root knot nematode in lentil

Treatments	Plant length (cm)		Fresh plant weight (g)		Root- knot	No. of root galls	No. of rhizobium	Nematode population
	Shoot	Root	Shoot	Root	index	/plant	nodules /plants	
M.C. + Trichoderma viride	34.41	6.50	14.00	4.10	1.75	44.14	35.07	60.00
M.C. + Trichoderma harzianum	34.70	7.00	14.52	4.30	1.50	35.13	41.19	52.12
M.C. + Trichoderma virens	31.91	6.28	13.50	4.00	2.15	78.16	25.03	91.00
M.C.+ Paecilomyces spp.	28.51	5.93	11.27	3.29	2.10	68.00	28.27	85.10
M.C.+ Bacillus thuringiensis	28.47	4.87	10.93	3.27	2.00	67.10	30.07	76.00
M.C. + Pseudomonas fluorescen	s27.36	4.35	9.48	3.15	2.17	81.12	20.15	98.17
M.C. + Carbosulfan	37.45	8.50	14.72	4.52	1.00	20.00	52.05	31.15
Check	23.15	4.00	8.50	3.00	5.00	107.13	14.00	135.00
C.D. (0.05)	1.577	0.581	1.003	0.340	0.345	4.296	3.908	7.723
S.E. (d)	0.760	0.278	0.481	0.164	0.172	2.066	1.878	3.716

M.C. = Mahua cake

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Effect of Mahua cake (MC) with different bio agents on root knot nematode and root nodulaton in lentil

The data on effect of mahua cake in combination with bio agent/nematicide on growth parameters of lentil was presented on Table 2. It was observed that mahua cake + carbosulfan treatment was found significantly superior to other treatments in terms of maximum shoot length (37.45 cm) and root length (8.50 cm), highest fresh shoot weight (14.72 g) and root weight (4.52 g), lowest root-knot index (1.00) and number of rhizobial nodules/plant (52.05) followed by mahua cake + *T. harzianum* treatment with shoot length (34.70

cm) and root length (7.00 cm), fresh shoot weight (14.52 g) and root weight (4.30 g), root-knot index (1.50) and number of rhizobial nodules/plant (41.19). While minimum shoot length (23.15 cm) and root length (4.0 cm), fresh shoot weight (8.50 g) and root weight (3.00 g), increase root-knot index (5.00) and number of rhizobial nodules/plant (14.00) was found in control. Other treatments were found moderately effective against root knot nematode. Minimum number of root gall/plant (20.00) and nematode population /200 g of soil (31.15) were recorded with treatment mahua cake + carbosulfan followed by mahua cake + *T. harzianum* followed

Table 3. Effect of combination of ground nut cake and bio-agents/nematicide against root knot nematode in lentil

Treatments	Plant length (cm)		Fresh plant weight (g)		Root- knot	No. of root galls	No. of rhizobium	Nematode population
	Shoot	Root	Shoot	Root	index	/plant	nodules /plants	
G.C. + Trichoderma viride	30.33	6.18	11.05	3.22	2.00	98.10	35.05	66.00
G.C. + Trichoderma harzianum	32.15	7.01	11.82	4.07	1.40	75.10	45.00	55.10
G.C. + Trichoderma virens	29.83	5.63	10.12	2.83	2.12	102.00	32.13	69.12
G.C.+ Paecilomyces	24.80	4.46	8.05	2.33	1.95	94.10	37.00	60.00
G.C.+ Bacillus thuringiensis	24.30	3.76	7.73	1.85	2.10	100.02	40.00	80.15
G.C. + Pseudomonas fluorescens	20.82	2.42	7.08	1.18	2.80	113.00	35.00	85.10
G.C. + Carbosulfan	34.75	7.51	12.43	4.15	0.85	46.20	65.10	30.15
Check	20.00	2.00	7.00	1.00	5.00	132.00	10.25	138.00
C.D. (0.05)	1.661	0.326	0.804	0.324	0.418	7.161	3.668	6.267
S.E. (d)	0.802	0.147	0.392	0.152	0.208	3.444	1.764	3.013

G.C. = Groundnut cake

Table 4. Effect of combination of sunflower cake and bio-agents/nematicide against root knot nematode in lentil

Treatments	Plant length (cm)		Fresh plant weight (g)		Root- knot	No. of root galls	No. of rhizobium	Nematode population
	Shoot	Root	Shoot	Root	index	/plant	nodules /plants	
S.C. + Trichoderma viride	29.21	5.12	9.17	2.83	2.00	73.12	20.10	72.15
S.C. + Trichoderma harzianum	29.37	6.06	10.22	3.18	1.60	43.00	32.62	64.13
S.C. + Trichoderma virens	27.02	4.35	8.35	2.71	2.25	95.00	17.45	87.00
S.C.+ Paecilomyces	24.87	3.52	6.85	2.12	1.90	69.10	25.75	68.00
S.C.+ Bacillus thuringiensis	23.92	4.05	6.95	2.01	1.75	50.10	30.10	62.10
S.C. + Pseudomonas fluorescens	23.16	3.15	5.61	1.22	3.00	108.00	12.32	90.30
S.C. + Carbosulfan	29.37	6.67	10.52	3.21	1.00	20.20	42.05	25.56
Check	21.00	3.00	5.00	1.00	5.00	130.00	10.00	135.00
C.D. (0.05)	2.924	0.434	1.241	0.237	0.399	8.512	1.649	8.226
S.E. (d)	1.404	0.205	0.597	0.100	0.187	4.091	0.793	3.955

S.C. = Sunflower cake

by with number of root gall/plant (35.13) and nematode population /200 g of soil (52.12). Whereas maximum root gall (107.13/plant) and nematode population (135.00/200g of soil) was recorded in control. Soil amendment with combination of mahua cake + bioagent provided improved physical health of the soil.

Effect of ground nut cake (GC) with different bio agents/nematicide on root knot nematode and root nodulaton in lentil

The data on effect of ground nut cake in combination with bio agent/nematicide on growth parameters of lentil were presented on Table 3. It was observed that ground nut cake + carbosulfan treatment was found significantly superior to other treatments in terms of maximum shoot length (34.75 cm) and root length (7.51 cm), highest fresh shoot weight (12.43 g) and root weight (4.15 g), lowest root-knot index (0.85) and number of rhizobial nodules/plant (65.10) followed by ground nut cake + T. harzianum treatment with shoot length (32.15 cm) and root length (7.01 cm), fresh shoot weight (11.82 g) and root weight (4.07 g), root-knot index (1.40) and number of rhizobial nodules/plant (45.00). While minimum shoot length (20.00 cm) and root length (2.0 cm), fresh shoot weight (7.00 g) and root weight (1.00 g), root-knot index (5.00) and number of rhizobial nodules/plant (10.25) was found in control. Other treatments were found moderately effective against root knot nematode. Minimum number of root gall/plant (46.20) and nematode population /200 g of soil (30.15) were recorded with treatment ground nut cake + carbosulfan followed by ground nut cake + T. harzianum followed by with number of root gall/ plant (75.10) and nematode population /200 g of soil (55.10). Whereas maximum root gall (132.00/ plant) and nematode population (138.00/200g of soil) was recorded in control.

Effect of sunflower cake (SC) with different bio agents/nematicide on root knot nematode and root nodulaton in lentil

The data on effect of sunflower cake in combination with bio agent/nematicide on growth parameters of lentil was presented on Table 4. It was observed that sunflower cake + carbosulfan treatment was found significantly superior to other treatments in terms of maximum shoot length (29.37 cm) and root length (6.67 cm), highest fresh shoot weight (10.52 g) and root weight (3.21 g), lowest root-knot index (1.00) and number of rhizobial nodules/plant (42.05) followed by sunflower cake + T. harzianum treatment with shoot length (29.37 cm) and root length (6.06 cm), fresh shoot weight (10.22 g) and root weight (3.18 g), root-knot index (1.60) and number of rhizobial nodules/plant (32.62). While minimum shoot length (21.00 cm) and root length (3.00 cm), fresh shoot weight (5.00 g) and root weight (1.00 g), increase root-knot index (5.00) and number of rhizobial nodules/plant (10.00) was found in control. Other treatments were found moderately effective against root knot nematode. Minimum number of root gall/plant (20.20) and nematode population /200 g of soil (25.56) were recorded with treatment sunflower cake + carbosulfan followed by sunflower cake + T. harzianum followed by with number of root gall/ plant (43.00) and nematode population /200 g of soil (64.13). Whereas maximum root gall (130.00 plant) and nematode population (135.00/200g of soil) was recorded in control.

Comparison of efficacy of different oil cakes+ bio agents/nematicide

Nematode root gall index

Ground cake + carbosulfan treatment was found significant over other treatments in reducing nematode infection with lowest root gall index of 0.85 followed by neem cake + carbosulfan treatment with root gall index of 0.95. Among oil cake + bioagent treatments, neem cake + *Trichoderma harzianum* treatment having minimum root gall index (1.00) followed by ground nut cake + *Trichoderma harzianum* treatment (1.40). While maximum root gall index (5.00) was observed in control.

Root nodulation

Maximum root nodulation was observed in groundnut cake + carbosulfan treatment (65.10 *Rhizobiunm* root nodules per plant) followed by neem cake + carbosulfan treatment with 50.00 *Rhizobium* root nodules per plant. Among the oil cake + bioagent treatments neem cake + *Trichoderma harzianum* treatment shown maximum root nodulation (48.10 root nodules per plant) followed by ground cake + *Trichoderma harzianum* treatment with 45.00 root nodules per plant. While minimum number of root nodules were observed in control (10.00 root nodules per plant). The similar results were reported by Reddy, 1975 and Khan *et al.*,1973. They reported that neem cake + carbosulfan was significantly effective against root knot nematode. The results are directly proportionate to the better growth of crop and also in reducing nematode population. Seedling bare root dip for 6 h in carbosulfan (25 ST) and triazophos (40 EC) @O.1% increased the yield by 43 and 42% respectively with low gall index 3.5 and 3.8 as against 5 in control root knot nematode in tomato (Vadhera *et al.*, 2000). Bhat *et al.* (1998) studied the combined application of *P. lilacinus* and oil cakes for protection of chickpea against *M. incognita.*

Therefore, on the behalf of soil health and environment safety and economic feasibility a treatment of oil cake + bioagent combination or oil cake + reduced dosage of nematicide application serves better alternative to manage root knot nematode.

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