

## Interaction between Nematode and Fungi and its Management causing Vascular Wilt of Lentil

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### ABSTRACT

Interaction between wilt, root rot and root knot complex in lentil was evaluated and formulated its management strategies. Population of plant parasitic nematodes in rhizosphere was observed as pre - and post- sowing of of lentil and found that when variety K-75 was grown, the population of *Meloidogyne* sp. was increased to be 950, 1000, 1550, after 30, 60 and 90 days, respectively as compared to population 450, in case of essential population before growing of crop. Whereas the population in the rhizosphere of VL-516 was 600, 1750 and 2550, respectively, in 30, 60 and 90 days after sowing in compare to 450 of initial population before growing different varieties of lentil. Pathogenic ability of different inoculums levels of *Meloidogyne incognita* on plant growth was studies and showed that severe reduction of length at 5000 level of juvenile was 9 cm in comparison of check (14.57 cm), there was gradual reduction was observed. The reduction in root length was also higher at 5000 larval level in comparison of check. Reduction in shoot weight was also present with increasing order of larval population with reduction of 0.80 g, 0.65 g, 0.44 g and 0.23 g on level of 100, 500, 1000 and 5000 inoculums level, respectively. The results in respect of reactions of different lentil varieties against root-knot nematode and observed that variety NDL-9302, showed maximum shoot length (33.00 cm) and root length (8.55 cm) followed by VL-519 (8.15 cm) recorded, whereas minimum shoot length in (10.00 cm) and root length (3.15 cm) was observed with variety RLG- 101. The cultivar RLG-101 also exhibited maximum number of root galls/root system (115), and root-knot index (5.00). There was nematode population was recorded highest under cultivar of RLG- 101 (515) and minimum was observed in VL-1 (80.20). Interaction between *Meloidogyne incognita* with *Fusarium oxysporum* f. sp. *lentis* and *Rhizoctonia solani* showed that decreased shoot (10.18 cm) and root length (2.17 cm), decreased fresh weight of shoot (4.46 gm) and root length (0.86 gm), increased the no. of root galls/plant (70.00), reduced the number of rhizobium nodules/plant (9.30), increased root-knot index (4.00) and Nematode population (45.00) and highest wilting percentage (46.43 %) per 200 g soil. The effect of combination of thiram + bavistin + vitavax + carbosulfan + nemanin results highest shoot length (39.87 cm), fresh shoot weight (10.70 g), lowest for root-knot index (0.95), decreased the number of root galls/plant (21.12), increased the number of rhizobium nodules/plant (50.00) and lowest nematode larvae (15.13).

#### Keywords

Lentil, Nematode,  
fungi, Interaction,  
Management

#### Article Info

##### Accepted:

10 February 2021

##### Available Online:

10 March 2021

## Introduction

Lentil or Masoor (*Lens culinaris* Medik) is one of the oldest valuable *rabi* pulse crop of India. 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 mongbean (Ali, 1989). The disease complex of wilt fungus and root knot nematode reduces the crop yield significantly. Hence, the study of interaction between wilt, root rot and root knot complex in lentil and its management. It is much economical and effective than individual chemical or bioagent or oil cakes etc.

## Materials and Methods

Samples of soil and roots of lentil crop were collected from infected fields from the rhizosphere to the root depth, and about 10-15 spots were selected randomly for taking soil and root samples representing the whole field. Later, from the different locations of fields,

the number of plants wilted was counted and the mean wilt incidence was expressed in percentage. Whenever required, the complete wilted plants were also collected. The per cent disease incidence was calculated by using the following formula:

$$\text{Per cent disease (PCD)} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Each sample was filled in polythene bag and tied with a rubber band labeled immediately. Information pertaining to the locality, crop history, etc. was also obtained along with the samples. Samples of soil and roots were analyzed on the day of collection or after keeping for a few days in refrigerator.

The nematode population from soil and root samples was estimated. Fungal propagules were isolated from soil samples, while root samples were used for detection of the associated fungus with wilted plants. Galled root system was scored by using disease rating scale (0 to 5 scale) given by Taylor and Sasser (1978). The severity of root-knot was calculated for each district by using the following formula:

$$\text{Disease severity (DS)} = \frac{\text{Number of infested root samples from a district}}{\text{Total number of root samples collected from a district}} \times 100$$

## Estimation of nematode population in soil

Cobb’s sieving and decanting technique was followed for which 200 ml 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  $\mu\text{m}$  pore size. The aliquot or filtrate was poured in a Petri

dishes 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 stereo-scopic 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.

### **Isolation of fungi from plant material**

Tissue isolation technique was employed for isolation of *Fusarium oxysporum* f. sp. *lentis* and *Rhizoctonia solani* from plants showing typical symptoms of wilt. The infected plant parts (from root and collar region) were washed thoroughly in running tap water repeatedly till they were free from adhering soil. The infected parts were cut into bits of 5 to 6 mm size and surface sterilized in 0.1 per cent mercuric chloride for a minute.

This was followed by repeated washing in sterile water. The bits were transferred aseptically on to a sterilized Potato dextrose agar (PDA) slants. The slants were incubated at room temperature ( $27 \pm 1^{\circ}$  C) for 72 hours. Pure culture of *Fusarium oxysporum* f.sp.*lentis* and *Rhizoctonia solani* obtained were maintained on PDA medium. Subsequent sub culturing was done once in 30 days on PDA slants which were preserved in refrigerator at  $4^{\circ}$  C for further experimental use.

### **Isolation of fungi from soil samples**

The propagules of fungi were estimated by plating the rhizosphere soil using dilution plate technique. *Fusarium* specific medium (Nash and Synder, 1962) was used for plating

fungi and *Fusarium* sp. from soil at a dilution of  $10^3$ .

All the plates were incubated at room temperatures. Counts were made in all the dilutions in three replicate plates and fungal colonies developed on the agar plates were counted 5-7 days after incubation and the number of colony forming units (cfu) were calculated and expressed per gram of oven dry soil.

The fungal inoculum was multiplied on sterilized sand maize meal agar medium in 1000ml flasks containing 400 g in each flask. A loopfull of fungus from actively growing culture was inoculated into each flask and incubated at room temperature for 15 days. The fungal culture multiplied on sand maize meal agar medium was used for inoculation to the soil.

### **Fungal inoculation**

At the time of inoculation, the fungus culture maintained on sand maize meal medium was added around the root system by removing a small quantity of soil and replacing it again after inoculation.

### **Pathogenicity of *M. incognita* on lentil by using different inoculum levels**

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.

### **The inoculum levels maintained for the pathogenicity test**

A check, 0, 100, 500, 1000, 5000, infective juveniles in 3 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 number present in each of five aliquot of suspensions and computed for total quality of suspension obtained by processing 200 ml of soil. These were recorded at the 55<sup>th</sup> 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 systems were washed free of soil. 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 were carefully cut with a sharp razor blade and body content were 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 gauze 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 fen wicks 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 upto 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 numbers of juveniles in the water were 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.

#### **Estimation of nematode population from root samples-**

Root samples of known quantity (5g) were directly observed under Stereobinocular 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. Nematode count from this was finally converted to 5 g root by multiplying with a common factor of 25 to observe presence of second stage larvae and other developing stages.

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. Stained roots were then kept in plain lactophenol for few to 48 hours for

differentiation and examined under Stereobinocular microscope.

The stained nematodes were counted and converted to 5 g root. 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).

#### **Effect of interaction between *Meloidogyne incognita* with *Fusarium oxysporum* f. sp. *lentis* and *Rhizoctonia solani* in lentil**

To study the effect of simultaneous inoculation of *M. incognita* with *F. oxysporum* f. sp. *lentis* and *R. solani* with singly or in sequential on plant growth, host infestation, nematode multiplication and disease development, a pot culture experiment was designed under green house condition by adding 15 fungal culture of thousand freshly hatched second stage juveniles of root-knot nematode was applied individually to 25 days old rooted cuttings of lentil grown in 1:1 sterile sand soil moisture in 45 cm diameter earthen pots.

Inoculation of all the said pathogens was made singly or in combination in sequence as per the following treatments:

Inoculation with *M. incognita* alone.

Inoculation with *F. oxysporum* f.sp. *lentis* alone

Inoculation with *Rhizoctonia solani* alone

Inoculation with *F. oxysporum* f.sp. *lentis* and *R. solani*

Inoculation with *M. incognita* seven days prior to inoculation of *F. oxysporum* f.sp.*lentis*

Inoculation with *F.oxysporum* f.sp. *lentis* seven days prior to inoculation of *M. incognita*

Inoculation with *F. oxysporum* f. sp. *lentis* and *R. solani* seven days prior to inoculation of *M. incognita*

Inoculation with *M. incognita* + *F. oxysporum* f.sp. *lentis* simultaneously.

Inoculation with *M. incognita* + *F. oxysporum* f. sp. *lentis* and *R. solani* both

Check (Control).

Observations were recorded on plant growth parameters like shoot length, root length, fresh shoot weight, root weight, root-knot index, number of rhizobium nodules and number of galls per plant, final nematode population in soil as well as in roots and per cent decrease incidence. The rating of root-rotindex was done following the scale suggested by Chidananda Prabhu (1987) as given below:

0=,Healthy, no symptoms at all

1= A slight discolouration at the collar regions – roots healthy

2= Discolouration up to 1-1 <sup>1</sup>/<sub>2</sub>inch from ground level, tap roots discoloured, secondary root still healthy

3= Complete discolouration of tap root 2-2 <sup>1</sup>/<sub>2</sub> inch discoloration of stem, roots started getting discoloured.

4= Majority of secondary roots discoloured, root tips disinfected

5= All roots rotting- complete discoloration

### **Screening different of lentil varieties for their resistance to *Meloidogyne incognita* with *Fusarium oxysporum* f. sp. *lentis* and *Rhizoctonia solani***

Pot culture studies were conducted in the glass house to test the reaction of eleven different lentil varieties of root-knot nematode with *F. oxysporum* f. sp. *lentis* and *R. solani*. These varieties were selected based on their popularity in Uttar Pradesh. The lentil grown in the 45 cm diameter pot containing 2.5 kg of soil and sand (1:1) proportions and 500 freshly hatched second stage juveniles and 15 g of fungal culture were inoculated to each pot 30 days after planting and covered with the soil.

The observations were recorded at 120 days after inoculation with respect to shoot length, root length, number of galls/root, nematode population. Root rot index and root knot index were calculated using 1-5 rating scale (Anonymous, 1993).

### **Infection scale**

Based on number of galls developed the varieties were rated as per the number of galls per plant scale reaction), which are 0= Highly resistant, 1-10= Resistant, 11-30= Moderately resistant, 31-100= Susceptible, 101 and above=Highly susceptible

The following eleven varieties of lentil were used for screening against root-knot nematode (*M. incognita*) with *Fusarium oxysporum* f. sp. *lentis* and *R. solani* complex:- VL-1, VL-138, VL-516, VL-519, K-75, SL-224, RLG-101, RLG-105, PL-408, NDL-9302 and HUL-60,

Observations will be 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, root rot index and root knot index

using the following scale (Taylor and Sasser, 1978) 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

### **Management of disease complex using cakes, bioagents and chemicals**

A pot culture experiment was carried out for the management of root-knot nematode with wilt and root-rot of lentil grown in the earthen pots containing sick soil.

The commercially available plants products like Neem cake, bio agents like *Trichoderma viride*, *Pseudomonas fluorescens*, *Paecilomyces lilacinus* and chemicals like carbo sulfan and vitavax power (Carboxin + Thiram) were used in the experiments.

Sterilized pot mixture was taken in six kg capacity pots and rooted terminal cutting of lentil were planted separately @ 2 cuttings per pot.

After proper establishment, commercially available plant products viz. different cakes, bioagents like *Trichoderma viride*, *Pseudomonas fluorescens*, *Paecilomyces lilacinus* were applied to the soil and chemicals like carbosulfan and vitavax power at their respective dosages, dipped in the solution at the time of transplanting and drenched individually as well as in combinations into the respective sets of pots.

Simultaneously *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *lentis* were inoculated @ 1000 j<sub>2</sub> per kg and 15 g of joint culture per kg of soil, respectively around the roots of lentil plants. The treatments were replicated thrice in completely randomized design as follows:

## **Results and Discussion**

### **Population of pre-and post- sowing of plant parasitic nematodes in 200 g soil.**

The data on the population of different plant parasitic nematode genera recorded in field soil before and after growing of lentil varieties K-75 and VL-516 are presented in Table 1. The data observed that prior to sowing of lentil crop, the soil samples harvested 450, 400, 250, 90, 70, 50, 100, 10, 30 and 20 in number of *Meloidogyne* sp., *Rotylenchus* sp., *Pratylenchus* sp., *Helicotylenchus* sp., *Hoplolaimous* sp., *Tylenchorhynchus* sp., *Xiphinema* sp., *Longidorus* sp. and some others free living species.

When variety K-75 was grown the population of *Meloidogyne* sp. was increased to be 950, 1000, 1550, after 30, 60 and 90 days, respectively as compared to population 450, in case of essential population before growing of crop. Whereas the population in the rhizosphere of VL-516 was 600, 1750 and 2550, respectively, in 30, 60 and 90 days after sowing in compare to 450 of initial population before growing different varieties of lentil. In case of other nematodes same general trend was observed that the initial population was increased by increasing the growing age of the both lentil variety.

The data on frequency and relative abundance of fungi in rhizosphere of lentil showed that *F. oxysporum* f. sp. *lentis*, *F. solani*, *R. solani*, *R. bataticola*, *S. sclerotiorum* were more abundant. Some more common important fungi viz., *Sclerotinia rabeae*, *S. rolfsii* were isolated, other than *T. harzianum*, *T. viride* and *T. virens* from rhizosphere of lentil. Further, the frequency of these fungi was also observed more in VL-516, in comparison of K-75 at 30, 60 and 90 days of observations. The frequency of *R. bataticola* was found more in both the lentil varieties and it was

responsible for rotting and physiological wilting.

The same trend stands true regarding the rhizospheric microflora of lentil, as the view expressed by Fawcett (1931) that nature does not work with pure culture. The antagonistic and synergistic effects have been also observed by the combined inoculum of fungi and root-knot nematode. There were some variations in the frequency of the occurrence of pathogens that may be due to their saprophytic nature. Therefore it is advisable for extra inoculation of these fungi may cause sporulation of pathogenic fungi (Fazal *et al.*, 1994; Srivastava and Singh, 1991; Mahapatra and Swain, 2001).

#### **Pathogenic ability of different inoculum levels of *Meloidogyne incognita* on plant growth characters in lentil.**

Pathogenicity of *Meloidogyne incognita* was tested as per treatments given in Table 2. The seed of lentil variety K-75 was grown in 15 cm pots during the year 2008 to 2012 by applying different inoculums level of second stage juveniles of *Meloidogyne incognita* i.e. 100, 500, 1000 and 5000 per kg soil. The data on plant growth character, root-knot index, root galls/plant, rhizobium nodules/plant and nematode population/ 200 g soil was recorded. The reduction and increment in plant growth character, root-knot index, root galls/plant, rhizobium nodules/plant and nematode population/ 200 g soil was also recorded. The first symptoms on inoculated plant was showed yellowing, stunting and drooping of leaves, the scorching of leaves was started on the margin of leaves to inward after 35 days of inoculation infected plant showed reduced shoot and root length, the health of plant was gradually reduced as per inoculums level initial to higher. It is further clear that effect of higher inoculums level was more effective for the incidence of root-knot affected plants were

sowing nutritional deficiency. Affected plants showed spreading nature during the hot pot of the day and recovered during morning and evening period. The seed of affected plant were shriveled and deformation of flowers was present. Mostly main and lateral root system was bearded swollen, spherical to elongated root-knot. The size and shape of root galls varied from swelling with smaller than a pin head to a swelling many times. Thickness of the root on which they were developed the colour of gall was light creamy in the beginning and later on change to dark brown along with necrotic lesion in the advantage stage. The whitish egg mass was present on the surface of root-knot at the time of breaking of gall with the help of needles large number of females and eggs were shown in the view of binocular. The root system was reduced, its growth with the proliferation of roots and could be easily uprooted on pulling.

Result showed that severe reduction of length at 5000 level of juvenile was 9 cm in comparison of check (14.57 cm), there was gradual reduction was observed. The reduction in root length was also higher at 5000 larval level in comparison of check. Reduction in shoot weight was also present with increasing order of larval population with reduction of 0.80 g, 0.65 g, 0.44 g and 0.23 g on level of 100, 500, 1000 and 5000 inoculums level, respectively.

Similar trend in reduction of weight was observed in case of root weight, the highest reduction in root weight was recorded at 5000 larval level only 0.17 g in comparison of 0.40 g at 100 larval levels. A considerable reduction in root length was recorded on all inoculation level and the increment was present according to the increment in population level in case of higher inoculums level. The plants were so shallow, rooted and proliferated root presented with severally with that could very easily be pulled out. It is



noteworthy that a considerable increment in root weight was recorded 100 to 5000 inoculums level. The highest increment was 80 and lowest 17 per cent. The increment was whiteness by formation of galls on increasing the inoculums level of nematode juveniles due to hypertrophic and hyperplastic reaction. The data on rhizobial nodules also revealed that the reduction in rhizobial nodules was in according increment to inoculums levels of nematode larvae. This was an account of hypertrophic and hyperplastic reaction of root-knot nematode during the parasitism on 5000 inoculums level of nematode a higher reduction of 80 per cent. The bacterial nodulation was recorded which was present significant, even on lowest inoculums level of 100 larvae the reduction was 13 per cent recorded. The data on root-knot index was revealed that on 100, 500, 1000 and 5000 inoculums level of nematode second stage juvenile. There was 37.15 galls formation as initiation of gall formation was observed the highest, root-knot index was 4.43 under 5000 J<sup>2</sup> level was recorded. However, lowest 2.42 root-knot index was recorded with inoculums level of (100). The data on pod weight showed that, there was gradual reduction in pod weight with increased inoculums levels.

The data on larval population assessed after termination of experiment in 200 gm soil after 75 days of germination showed that 779.5, 2776, 4992 and 14115.25 nematode larvae were recorded with the increment of larval level The data observed significant increment of population level of nematode larvae with the increment of 100, 500, 1000 and 5000 J<sup>2</sup>, respectively.

### **Reaction of different lentil varieties against root-knot nematode (*Meloidogyne incognita*).**

Eleven lentil varieties were evaluated to test their reaction against root-knot nematode

(*Meloidogyne incognita*) and data are presented in Table 3. The results indicated that variety NDL-9302, showed maximum shoot length (33.00 cm) and root length (8.55 cm) followed by VL-519 (8.15 cm) recorded, whereas minimum shoot length in RLG- 101 (10.00 cm) and root length (3.15 cm) was observed. Cultivar RLG-101 exhibited maximum number of root galls/root system (115), and also the maximum root-knot index in this variety of lentil (5.00), and lowest root-knot index was recorded under cultivar VL-1 (1), whereas root-rot index were present maximum in cultivar of RLG- 101 (5.00) and minimum VL-1 (1.55). There was nematode population was recorded highest under cultivar of RLG- 101 (515) and minimum was observed in VL-1 (80.20).

The minimum Root-knot index and root-rot index in varieties, VL-1, VL-138, RLG-105 and HUL-60 showed resistant reaction. Whereas VL-516, VL-519 and NDL-9302, proved moderately resistant and varieties viz. K-75, SL-224 and PL-408 were rated susceptible. There was only one variety, RLG-101 which was recorded as highly susceptible.

The decreasing of root-knot index in resistant varieties was due to genetically resistant or may be due to presence of alkaloid compounds and root exudates in the root system as reported by Singh *et al.*, (2010). Reason of increasing shoot and root length may be due to suppression of nematode population and inhabitant nature of nematode organism against resistance varieties the main reason behind it nematode larvae not more attacked and feeder with resistant varieties it was due to the presence of genetical character or individual varietal character, further it is advisable to the breeders and advanced farmers those who working under breeding programme, these four resistant varieties viz., VL-1, VL-138, RLG-101 and HUL-60 may be incorporated under breeding programmed.

**Interaction between *Meloidogyne incognita* with *Fusarium oxysporum* f. sp. *lentis* and *Rhizoctonia solani*.**

The data recorded on the effect of various treatments on growth parameters viz. plant length, root-knot index, number of rhizobium nodules/plant, number of root-galls /plant and nematode population and wilt were presented in Table 4. All the treatments significantly increased the plants length, fresh plant weight, number of rhizobium nodules/plant and decreased in root-knot index, number of root-galls/plant and nematode population per 200 g soil.

Shoot length of lentil was recorded, when nematode, *Fusarium oxysporum* f. sp. *lentis* and *Rhizoctonia solani* inoculated individually results 25.37, 24.02, 20.05 cm shoot length, respectively in comparison of check (32.12 cm). These results showed that *Fusarium oxysporum* f. sp. *lentis* was more virulent in comparison of nematode and *Rhizoctonia solani*. When all the three inoculated together the highest reduction was recorded (10.18 cm) in comparison to check (32.12 cm). It is evident that the shoot length was significantly reduced with the simultaneous inoculation of nematode and *Fusarium oxysporum* f. sp. *lentis*, was recorded by higher 63.41 per cent.

In case of nematode prior and *Fusarium oxysporum* f. sp. *lentis* after, this reduction was enhanced by 59.37 per cent, in comparison of check. When *Fusarium oxysporum* f. sp. *lentis* inoculated prior to nematode, shoot length was decreased by 54.82 per cent. When nematode and *Rhizoctonia solani* were simultaneously inoculated with nematode prior seven days to *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lentis* prior to seven days of reduction was significantly present for shoot length 68.08, 63.04 and 60.30 per cent respectively, there was also significant reduction was recorded in

comparison to check in case of shoot length. In case of shoot and root weight, significant reduction was found in comparison to check. In case of simultaneous inoculation of nematode with *Fusarium oxysporum* f. sp. *lentis* and *Rhizoctonia solani* was more virulent in comparison of prior fungus to nematodes, whereas virulence was more prevalent when the entire three organisms was inoculated simultaneously.

Perusal data of Table 4 showed infection of all the pathogens reduced rhizobium nodulation in separately. Inoculation of nematode, *Fusarium oxysporum* f. sp. *lentis* and *Rhizoctonia solani* tend to observed 47.20, 26.50 and 21.50 number of rhizobium nodules, respectively. In *Fusarium oxysporum* f. sp. *lentis* and *Rhizoctonia solani*, nematode was more virulent for the reduction of rhizobium nodulation and all the three treatments were significantly different from check. In case of simultaneous inoculation of nematode + *Fusarium* and nematode + *Rhizoctonia* recorded 36.70 and 39.50, rhizobium nodules, respectively. Combination of nematode + *Rhizoctonia* found more effective for the reduction of rhizobial nodules, whereas inoculation of nematode prior in of *Fusarium* and after nematode, after and nematode prior and *Rhizoctonia* after, rhizobium prior and nematode after, recorded 33.13, 27.15, 22.13 and 21.75 number of rhizobium nodules respectively.

It is evident from the Table 4, when all the combination of nematode + *Fusarium* + *Rhizoctonia* inoculated simultaneously recorded only 9.30 rhizobium nodules. There was all the combination were significantly different over the check. The reason of reduction in rhizobium nodules due disturbance of physiology of the plant in all the pathogens, and *Rhizoctonia solani* was more virulent in comparison of nematode and *Fusarium* (Fazal *et al.*, 1994).

**Table.1** Population of pre- and post- sowing of plant parasitic nematodes in rhizosphere of lentil/200 g soil in lentil varieties of K-75 and VL-516

Sl. No.	Nematode isolated	Pre-sowing population	Days after sowing					
			30 days		60 days		90 days	
			K-75	VL-516	K-75	VL-516	K-75	VL-516
1.	<i>Meloidogyne</i> sp.	450	950.00	600.00	1000.00	1750.00	1550.00	2550.00
2.	<i>Rotylenchus</i> sp.	400	200.00	460.00	850.00	1400.00	1000.00	2000.00
3.	<i>Pratylenchus</i> sp.	250	90.00	150.00	400.00	750.00	800.00	1500.00
4.	<i>Helicotylenchus</i> sp.	90	75.00	130.00	120.00	290.00	200.00	1000.00
5.	<i>Hoplolaimous</i> sp.	70	45.00	75.00	80.00	200.00	150.00	500.00
6.	<i>Tylenchus</i> sp.	50	30.00	70.00	65.00	150.00	70.00	300.00
7.	<i>Tylenchorhynchus</i> sp.	100	80.00	195.00	150.00	320.00	200.00	500.00
8.	<i>Xiphinema</i> sp.	10	15.00	40.00	35.00	80.00	65.00	232.50
9.	<i>Longidorus</i> sp.	30	25.00	65.00	40.00	115.00	70.00	260.00
10.	Others spp.	20	15.00	35.00	35.00	90.00	65.00	230.00
	C.D.	16.895	19.708	29.840	29.840	38.510	38.263	69.514
	S. E. (d)	8.233	9.603	14.543	14.542	18.767	18.649	33.879

**Table.2** Pathogenic ability of different inoculums levels of *Meloidogyne incognita* on plant growth characters in lentil

Sl. No.	Inoculum levels J <sup>2</sup> /plant	Plant length (cm)		Plant weight (g)		Pod weight (g)/plant	Average % reduction	Root-knot index	Root galls/plant	Rhizobium nodules/plant	Final nematode population/200 g soil
		Shoot	Root	Shoot	Root						
1.	Check	14.57	6.80	1.50	0.65(1.50)	0.47	00.00 (00.284)	00.00	00.00	50.00	00.00
2.	100	13.37	5.20	0.80	0.40 (0.80)	0.15	15.57 (23.191)	2.42	37.15	9.17	779.50
3.	500	12.55	4.70	0.65	0.35 (0.60)	0.10	30.12 (33.276)	2.98	39.25	7.13	2776.00
4.	1000	10.15	3.10	0.44	0.21 (0.44)	0.04	38.00 (38.057)	4.15	41.00	4.12	4992.00
5.	5000	9.00	2.90	0.23	0.17 (0.23)	0.02	50.17 (45.096)	4.43	45.32	2.15	14115.25
	C.D.(0.05)	0.936	0.737	0.133	0.137	0.041	2.500	0.885	1.820	2.903	2267.37
	S.E.(d)	0.425	0.338	0.063	0.055	0.059	1.150	0.408	0.837	1.335	1040.55

**Table.3** Reactions of different lentil varieties against root-knot nematode (*Meloidogyne incognita*)

Sl. No.	Varieties	Shoot length (cm)	Root length (cm)	No. of galls/root	Nematode population/ 200 ml soil	Root-knot index	Root-rot index	Reaction
1.	VL-1	25.45	6.15	5.55	80.20	1.00	1.55	R
2.	VL-138	26.15	6.50	6.75	87.00	1.25	1.50	R
3.	VL-516	27.10	7.20	17.75	175.00	3.00	2.90	MR
4.	VL-519	30.15	8.15	19.50	185.00	3.00	2.90	MR
5.	K-75	17.15	4.60	75.15	460.00	4.00	4.25	S
6.	SL-224	18.25	5.00	57.20	475.00	4.00	4.50	S
7.	RLG-101	10.00	3.15	115.00	515.00	5.00	5.00	HS
8.	RLG-105	28.10	7.55	3.55	101.00	2.00	2.40	R
9.	PL-408	15.50	4.10	25.10	455.00	4.00	4.15	S
10.	NDL-9302	33.00	8.55	20.10	180.00	3.00	3.25	MR
11.	HUL-60	30.50	7.25	2.50	90.00	2.00	2.15	R
	C.D.(0.05)	1.582	0.442	3.966	19.486	0.361	0.171	
	S.E.(d)	0.778	0.215	1.946	9.574	0.177	0.074	

**Table.4** Interaction between *Meloidogyne incognita* with *Fusarium oxysporum* f. sp. *lentis* and *Rhizoctonia solani* complex on lentil

Sl. No.	Treatments	Plant length (cm)		Fresh plant weight (g)		No. of root galls/plant	No. of rhizobium nodules/plant	Root-knot index	Nematode population	Wilting %
		Shoot	Root	Shoot	Root					
1.	Check	32.12	10.50	15.12	7.90	0.00	57.70	0.00	0.00	4.96
2.	N. alone	25.37	7.50	9.93	5.75	63.00	47.20	2.10	29.20	9.54
3.	F. alone	24.02	6.70	9.50	4.37	0.00	26.50	0.00	0.00	30.75
4.	R. alone	20.05	6.62	8.24	4.07	0.00	21.50	0.00	0.00	45.75
5.	N+F simultaneously	13.87	4.87	6.73	3.15	35.00	36.70	1.50	24.00	28.20
6.	N+R simultaneously	14.51	5.42	6.97	3.75	30.00	39.50	1.45	17.15	20.70
7.	N- F after	12.05	4.66	5.97	3.00	36.72	33.13	1.97	24.00	24.66
8.	F- N after	11.75	3.92	5.55	2.88	32.15	27.15	2.00	26.00	26.56
9.	N-R after	10.75	3.45	5.30	2.42	34.76	22.13	2.30	30.76	30.09
10.	R-N after	10.25	3.02	5.10	2.16	29.15	21.75	2.75	32.15	34.42
11.	N+F+R simultaneously	10.18	2.17	4.46	0.86	70.00	9.30	4.00	45.00	46.43
	C.D	0.986	0.303	0.527	0.242	12.904	2.824	0.828	2.224	2.279
	SE (d)	0.484	0.145	0.255	0.100	6.321	1.380	0.408	1.092	1.116

N = Nematode, F = *Fusarium oxysporum* f. sp. *Lentis*, R = *Rhizoctonia solani*,

**Table.5** Effect of different fungicides and nematicides with and without combination against root knot-fungus interaction on lentil variety K-75

Sl. No.	Treatments	Plant length (cm)		Fresh plant weight (g)		Root-knot index	No. of root galls/plant	No. of rhizobium nodules/plants	Nematode population
		Shoot	Root	Shoot	Root				
1.	Thiram	33.25	5.60	8.90	4.40	3.00	130.10	11.00	160.00
2.	Bavistin	34.25	5.80	9.10	4.70	2.90	125.25	15.00	150.00
3.	Vitavax	35.35	6.00	9.40	5.30	2.70	122.00	20.00	130.10
4.	Carbosulfan	35.87	6.20	9.50	5.00	2.20	115.00	25.05	119.00
5.	Nemarin	36.50	6.40	9.60	5.40	1.90	98.74	27.10	100.13
6.	Thiram + carbosulfan	37.12	6.80	9.80	5.70	1.50	91.00	27.50	95.00
7.	Thiram + nemarin	37.50	6.90	9.90	6.00	1.30	71.23	28.70	75.15
8.	Bavistin + carbosulfan	38.00	7.10	10.10	6.20	1.40	81.25	30.00	60.10
9.	Bavistin + nemarin	38.10	7.30	10.20	6.60	1.20	55.00	30.25	40.10
10.	Vitavax + carbosulfan	38.25	7.45	10.25	6.70	1.25	65.55	35.50	32.19
11.	Vitavax + nemarin	38.37	7.60	10.30	5.60	1.00	32.12	45.00	26.76
12.	Thiram + bavistin + vitavax + carbosulfan+ nemarin	39.87	7.75	10.70	7.00	0.95	21.12	50.00	15.13
13.	Check	23.00	4.00	6.00	3.60	5.00	170.00	9.00	190.00
	C.D. (0.05)	1.221	0.548	0.554	0.336	0.485	11.376	2.883	9.180
	S.E. (d)	0.604	0.266	0.276	0.169	0.241	5.611	1.424	4.529

Perusal of data regarding study of nematode population, when fungi were inoculated with nematode showed gradual reduction of population of nematodes were present in case of nematode prior to *Fusarium* (24.00) and nematode prior of *Rhizoctonia* (30.76), population in case of simultaneous inoculation of *Rhizoctonia* with nematode recorded nematode population (17.15). When combination of all the three pathogen nematode, *Fusarium* and *Rhizoctonia* inoculated, that population of nematode was highest (45) in 200 g soils that were highest in comparison of other treatments. This treatment was superior for the supplement of nematode population when *Rhizoctonia* inoculated prior 9 days to the nematode recorded much more (32.15) was near to the all the combination, whereas inoculated together, main reason behind it, both the fungi may provide supplement to the nematodes (Anwar and Verma, 1993 and Duwedi *et al.*, 1992).

Perusal of data regarding root gall per plant under separate inoculation of nematode (63) root galls were recorded, these galls were higher in number with comparison of all two combination of nematode and fungi it may be due to toxicity of organisms nematode and both fungi *R. solani* and *Fusarium* whereas, combination of nematode + *Fusarium* recorded number of root galls (70) these root galls happens more in comparison of all the treatment it showed that both the fungi were antagonist to each other and may gives supplement to the organisms of *M. incognita* (De *et al.*, 2001 and Mahapatra and Swan, 2001).

Perusal of data regarding nematode population showed when fungi were inoculated with nematode gradual reduction of population of nematode was reduced. In case of nematode prior to *Fusarium* (24.00) and nematode prior to *Rhizoctonia* (30.76). Whereas, prior inoculation of *Fusarium* to nematode (26.00)

and prior inoculation of *Rhizoctonia* to nematode recorded (32.15), respectively. In case of simultaneous inoculation of all the three pathogens recorded population of nematodes was 45.00, respectively. In case of study of wilt per cent at the time of inoculation of all three pathogens of *Fusarium* was found more virulent and wilted lentil plants 46.43 per cent, in comparison of *Rhizoctonia* recorded wilt per cent was 45.75, respectively, in comparison of other treatments separately in case of combined inoculation with nematode + *Fusarium* and nematode + *Rhizoctonia* recorded 28.20 and 20.70 per cent, respectively. Here the combination of nematode + *Fusarium* was more destructive in comparison of nematode + *Rhizoctonia*, whereas in nematode inoculated before *Fusarium* received 24.66 per cent wilting comparing with nematode, prior to *Rhizoctonia* and after nematode 34.42 per cent wilting. When combination of all the three pathogen inoculated nematode + *Fusarium* + *Rhizoctonia* were found more effective in comparison of all the treatments and recorded 46.43 per cent wilting, respectively.

Regarding the wilt percent there was negligible (9.54 %) wilt was present when nematode present alone. It was recorded by (30.75 %) with *Fusarium* above that was more (21.21 %) in comparison of nematode with *Fusarium* alone. It was further recorded by (45.75 %) with *Rhizoctonia* above that was again increased by (36.21 %) comprising with nematode in above treatment. *Rhizoctonia* showed separately more virulent effect due to its toxic effect. In the combination of all the pathogen recorded heavier wilting (46.43 %).

There was main reason behind it was due to supplement given to nematodes by fungus and individual toxicity of all the pathogens against to the lentil host (Singh *et al.*, 1981, Fazal *et al.*, 1994, Kamalwanshi, 1993 and Chanchal and Chabra, 1984).

### **Effect of different fungicides and nematicides with and without combinations against root knot-fungus interaction.**

Data on effect of different fungicides and nematicides with and without combinations against root knot-fungus interaction on lentil variety K-75 recorded on shoot length and root length in cm, fresh shoot and root weight in g, root-knot index (0-5), number of root galls/plant, number of rhizobium nodules/plant and nematode population/200 g soil as bellow in Table 5.

The effect of combination of thiram + bavistin + vitavax + carbosulfan + nematicide was highest shoot length (39.87 cm) followed by vitavax + nematicide (38.37 cm), vitavax + carbosulfan (38.25 cm), comprising with bavistin + nematicide (38.10 cm), bavistin + carbosulfan (38.00 cm), thiram + nematicide (37.50 cm), thiram + carbosulfan (37.12 cm), respectively.

Thereafter in case of individual treatments of nematicide was highest recorded (36.50 cm) followed by carbosulfan (35.87 cm), vitavax (35.35 cm), bavistin (34.25 cm) and thiram (33.25 cm), respectively. Similar trend was present in individual treatments and its combinations with on shoot length. These all results were significantly different over the check (23.00 cm); similar trend was also present in record of root length.

Perusal of data recorded on fresh shoot weight in gm was highest with thiram + bavistin + vitavax + carbosulfan + nematicide (10.70 g) followed by vitavax + nematicide (10.30 g), vitavax + carbosulfan (10.25 g), bavistin + nematicide (10.20 g), bavistin + carbosulfan (10.10 g), thiram + nematicide (9.90 g), thiram + carbosulfan (9.80 g), respectively. Whereas individual treatments was highest shoot weight recorded nematicide (9.60 g) and lowest was recorded in thiram (8.90 g), Similar trend was also present in record of root weight.

The effect of treatments in combination thiram + bavistin + vitavax + carbosulfan + nematicide (0.95) was recorded lowest for root-knot index, that was gradually increased with vitavax + nematicide (1.00) in compare to thiram + carbosulfan (1.50), whereas individual treatments in of nematicide recorded (1.90) and gradually increased up to individual treatment was thiram (3.00). All the treatments were significantly different over the check.

Data on number of root galls/plant recorded with combination thiram + bavistin + vitavax + carbosulfan + nematicide resulted was lowest (21.12) followed by vitavax + nematicide (32.12), bavistin + nematicide (55.00), vitavax + carbosulfan (65.55), thiram + nematicide (71.23), bavistin + carbosulfan (81.25) and thiram + carbosulfan (91.00), respectively. In case of individual treatments were recorded lowest by nematicide (98.75) followed by increasing tune of carbosulfan (115.00), vitavax (122.00), bavistin (125.25) and thiram (130.10), respectively. All the treatments were significantly different over the check.

The perusal of data on number of rhizobium nodules/plant were present in combinations of thiram + bavistin + vitavax + carbosulfan + nematicide (50.00), followed by in decreasing tune of vitavax + nematicide (45.00), vitavax + carbosulfan (35.50), bavistin + nematicide (30.25), bavistin + carbosulfan (30.00), thiram + nematicide (28.70) and thiram + carbosulfan (27.50), respectively. Whereas individual treatment of nematicide recorded highest number of rhizobium nodules/plant was (27.10) that were gradually decreased in others treatments and lowest in thiram recorded (11.00). All the result were significant different over the check.

Data on population of nematode larvae were recorded per 200 g soil in combination of thiram + bavistin + vitavax + carbosulfan + nematicide was noted (15.13) and lowest, there



was nematode population gradually increased in other treatment.

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#### How to cite this article:

Prem Shankar, S. K. Biswas, Santosh Kumar and Prem Naresh. 2021. Interaction between Nematode and Fungi and its Management causing Vascular Wilt of Lentil. *Int.J.Curr.Microbiol.App.Sci*. 10(03): 1125-1142.  
doi: <https://doi.org/10.20546/ijcmas.2021.1003.140>