Introduction
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Plants are the only higher organisms that harvest solar energy and convert it into chemical energy in the form of carbohydrates, proteins and fats. Plants make up the majority of earth’s living environment, providing food to all living organisms including humans. Plants when get infected by a pathogen grow poorly and exhibit various types of symptoms. Pathogens usually alter metabolism of plant cells through their enzymes, toxins, growth regulators and other substances. Some pathogens may multiply in xylem or phloem, thereby hampering translocation of water, minerals and metabolites. However, some pathogens affect only one variety of a plant and others affect hundreds of plant species.

Plant Pathology is a challenging, interesting and an important field of Life Sciences that has a practical and noble goal of protecting the food available for humans and all living organisms. The challenges ahead are reduction of food losses, improvement in food quality, in addition to safe guarding our environment.

It is estimated that pest and pathogens together destroy 31-42% yield annually of all important crops, worldwide. The losses are usually lower in developed countries and higher in developing countries. About 14.1% of the crops are lost to plant diseases which amounts approximately 220 billion dollars. As the world population is increasing, arable land and other natural resources are decreasing continuously, there is a need to control plant diseases effectively and safely.

Pulses, belonging to the family Fabaceae are also known as grain legumes. These are wonderful gift of nature, largely valued as food and fodder, and have been the mainstay of Indian agriculture. The unique features which make them indispensable are the ability of biological nitrogen fixation, deep root system, mobilization of insoluble soil nutrients and bringing qualitative changes in soil physical properties. Because of these characteristics legumes are considered as soil fertility restorers. In the predominantly vegetarian
population of India, pulses are an important source of dietary proteins (Jeswani and Vanchaik, 1968; Chand and Shrivastava, 1982). Protein content of pulses is several times of cereals and root tubers that can help to improve the protein intake of meals in which cereals and root tubers in combination with pulses are eaten (Kushwah et al. 2002).

Pulses play an important role in the economy of Indian agriculture by virtue of their ability to fix atmospheric nitrogen in symbiotic association with *Rhizobium* by improving soil health. They enrich nitrogen content of soil by adding approximately 14.50 metric tons of nitrogen annually. Pulses are the excellent source of nitrogen-rich green manure and are used as fodder and feed to the livestock. The deep penetrating root system enables them to utilize the limited available moisture more efficiently than many other crops and contributes substantially in loosening up of soil. They constitute an important component of crop rotation widely adopted by the farmers in different agroecological regions of the country, since legumes are known to benefit the succeeding crop in the rotation (Rego and Seeling, 1996). The seeds of pulses are of great importance as their low water content and impervious seed coats enhance their value for storage purposes, and increase their longevity. However, pulse crops have the ability to reduce the pH of the soil in the rhizosphere and make the micro-environment favorable for nutrient availability (Ali et al., 2002).

India has been distinctive in being one of the world’s largest producers of pulses, although the production is not adequate to ensure 80 g per capita availability, the minimum recommendation of World Health Organization (WHO), and of Food and Agriculture Organisation (FAO). In fact it has been dropped from 64 g during mid-fifties to less than 40 g at present. This has been attributed to stagnation in arable area (21 – 24 million hectares) with annual production of 10 – 13 million tons; and productivity 473 – 590 kg/hectares, over the past few decades, where the population has increased manifold.
Over a dozen pulse crops are grown in India, chickpea (*Cicer arietinum* L.) together with pigeon pea (*Cajanus cajan* L. Millsp.) accounts for 45 percent of the total pulse production in the country. The other important pulse crops are pea (*Pisum sativum* L.), green gram (*Vigna radiata* L. Wilczek), black gram (*V. mungo* L. Hepper), cowpea (*V. unguiculata* L. Walp.), mothbean (*V. aconitifolia* Jacq. Marechal), Lathyrus (*Lathyrus sativus* L.) horse gram (*Macrotyloma uniformus* Lam. Verdc.), and lentil (*Lens culinaris* Medik.). The major pulse growing states are Madhya Pradesh, Rajasthan, Uttar Pradesh, Maharashtra, Orissa, Bihar, Andhra Pradesh, Haryana, Tamil Nadu, West Bengal, Punjab and Gujarat. These are predominantly grown under rainfed and marginal rainfed lands with low inputs and without plant protection cover.

Lentil (*Lens culinaris* Medik.) is one of the very important nutritious food legumes, and grown as a winter season Rabi crop in India. It is cultivated for its seeds which have a relatively higher content of proteins, carbohydrates, and calories as compared to other legumes and is the most desired crop because of its high average protein content and fast cooking characteristics (Muehlbauer *et al*., 1985).

Seeds can be fried and seasoned for consumption; flour is used to make soups, stews, and mixed with cereals to make bread and cakes; and food for infants. Husks, dried leaves, stems, fruit wall and bran (residues), can be fed to livestock. Lentil seeds are the source of commercial starch for textiles and printing industries. Lentils are supposed to remedy constipation and other intestinal afflictions (Williams and Singh, 1988).

Protein concentration of lentil reportedly ranges from 22 - 34.6 %; 100 g dried seeds contain 340–346 calories, 12 % moisture, 22.2 g protein, 0.6 g fat, 65.0 g total carbohydrate and about 4 g fiber; 2.1 g ash, 68 mg calcium, 325 mg P, 7.0 mg Fe, 29 mg Na, 780 mg K, 0.46 mg thiamine, 0.33 mg riboflavin, and 1.3 mg niacin (Muehlbauer *et al*., 1985). Among the winter season leguminous crops, lentil is richest in the amino acids lysine, arginine, leucine and sulphur containing amino acids (Williams *et al*., 1994).
Lentils probably originated in the Near East and rapidly spread to Egypt, central and southern Europe, the Mediterranean basin, Ethiopia, Afghanistan, India and Pakistan, China and later to the new world including Latin America (Cubero, 1981; Ladizinsky, 1979). It is probably the oldest of grain legumes to be domesticated (Bahl et al., 1993). It is now cultivated in most subtropical and also in northern hemisphere such as Canada and Pacific Northwest regions.

The Botanical features of *Lens culinaris* Medik., can be described as annual bushy herb, slender, almost erect or suberect, much branched, softly hairy; stems slender, angular, 15 – 75 cm height. Flowers are small pale blue, purple, white or pink, in axillary 1 – 4 flowered racemes (Muehlbauer et al., 1985).

Seeds germinate best at temperature range of 18 – 21 °C; temperature above 27 °C is harmful; optimum temperature for growth and yield is around 24 °C. It tolerates annual precipitation of 2.8 – 24.3 dm, annual mean temperature of 6.3 – 27.3 °C and pH of 4.5 – 8.2. Lentil is propagated by seeds. Fields should be ploughed and harrowed to a fine texture. Seeds may be either broadcasted, or sown in drills at the rate of 25 – 30 cm apart. In India, planting starts late in November when sown in monoculture. Lentils may be intercropped with barley, castor, and mustard. Lentil flowers in 6 – 7 weeks after planting with early cultivars ready to harvest in 80 – 110 days; late cultivars reach maturity in 125 – 135 days (Muehlbauer et al., 1985).

In India, pulse yield ranges from 558 to 1,750 Kg/ha. The major producer of lentils in the world is India, with about 1,160,000 hectares producing 850,000 MT in 1994; while world production was 2.875 million MT on about 3.36 million hectares during the same year (FAO). Despite its immense importance, lentil remained an under-exploited and under researched crop until recently.

The natural soil environment constitutes a complex ecosystem that harbors a wide variety of microorganisms, as many as $10^6$–$10^8$ bacterial cells, $10^6$–$10^7$ actinomycetes cells, $5 \times 10^4$–$10^6$ fungal colony forming units (CFU),
10⁵–10⁶ protozoa, were to be present in one gram of soil taken from surface (Gottlieb, 1976), while 1×10⁷ nematodes in an area of 1m² of fertile soil. (Back et al., 2002). This makes the soil an open biological system.

Out of these organisms which inhabit the soil ecosystem, the plant parasitic nematodes are the most important, constituting about 12 – 15 % of the soil flora and fauna. Nematodes are found in the rhizosphere of plants and said to play a vital role in their growth and production. The first report of nematodes associated with plant diseases was made in England by Needham in 1743 in abnormally rounded wheat galls. The moist soil environment is favorable for the activities of plant parasitic nematodes (PPN) and for the growth and multiplication of pathogenic fungi.

Among various pests and diseases, phytoparasitic nematodes have emerged as major constraints in pulse production affecting both quality and quantity and proved to be the major biotic stresses to pulse production. The pulse production losses have drawn up the attention of scientist’s world over (Srivastava et al., 1974; Ngundo and Taylor, 1975; Melakeberhan et al., 1985, 1986; Chahal and Chahal, 1988; Kalita and Phukan, 1989). High population densities of these nematodes in the root or rhizosphere are often associated with patchy, stunted, chlorotic, and sickly growth of plants.

Among various phytoparasitic nematodes, the key target nematode pests of pulse crops are: root-knot nematodes (*Meloidogyne incognita, M. javanica*), pigeonpea cyst nematode (*Heterodera cajani*), root lesion nematode (*Pratylenchus thornei*) and reniform nematode (*Rotylenchulus reniformis*) which have been found as potent parasites causing potential damage to pulses resulting in economic losses in India. (Yadav, 1986). Annual yield loss in India is about 2.4 million tons annually, worth approximately 7.88 million dollars, considering total production of about 12 million tones and average price of Rs. 15 per Kg (Gaur et al., 2003).

Root-knot nematodes, *Meloidogyne* spp. are obligate sedentary endoparasites of many plant species. Their potential host range encompasses
more than 3,000 plant species, including pulse crops. *Meloidogyne* spp. are responsible for huge monetary (100 billion dollars per annum) losses, of which most economically important species are *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. *Meloidogyne incognita* occurs in temperature range from tropical to temperate countries, and is possibly the single most damaging crop pathogen in the world (Trudgill and Blok, 2001).

The above ground symptoms of the root-knot disease are chlorosis and stunting while underground are the formation of typical galls on the roots of susceptible host plants. Nutrient and water uptake are substantially reduced because of the damaged root system, resulting in weak and poor yielding plants. During parasitism root-knot nematode establishes and maintains an intimate relationship with its host. A mature female lays egg masses consisting of 200 – 250 eggs which hatch out into second-stage juveniles (J2) and invade the root in meristamatic region. They migrate intercellularly, first to the root apex and then to the vascular cylinder, where permanent feeding sites are established. The second-stage juveniles (J2) undergo three moults to develop into adults. The saccate females remain sedentary, producing large egg masses.

Nematode growth and reproduction depend on the establishment of specialized feeding sites within the root. Consequently, these nematodes do not kill the host cells from which they feed. Instead, they induce a redifferentiation process that leads to the formation of multinucleate feeding cells, named giant cells. Formation of giant cells is the outcome of repeated nuclear divisions of the initial feeding cells without cytokinesis. Each nematode triggers the development of 5 – 7 giant cells. These cells appear to be metabolically active, containing very dense cytoplasm which encloses mitochondria, plastids, ribosomes, well developed Golgi apparatus and smooth endoplasmic reticulum, generally organized in swirls. The central vacuole disappears and gives rise to many small vacuoles. In addition, cell wall ingrowths, typical of transfer cells are developed. Concomitant with giant cell formation, hyperplasia and
hypertrophy of the surrounding cortical cells lead to the formation of the typical root symptoms (Williamson and Hussey, 1996).

Root-knot disease of pulses is characterized by the presence of multiple knots on root system and can be easily seen with naked eyes. Size of knots is variable depending upon genotype reaction, nematode species and extent of infection (Sharma et al., 1994). Generally, M. javanica exhibits smaller knots than M. incognita, which produces profuse knotting all along entire root. Uneven growth in patches, poor stand, wilting, poor pod formation, less yield and less Rhizobium nodules are commonly associated with root-knot disease of pulses. In case of heavy infestation the plants may die, the symptoms advance with crop age and are more severe in low fertile soil (Catibog and Castillo, 1975).

Soil inhabiting plant parasitic nematodes live in an environment that is teeming with microorganisms such as bacteria, viruses and fungi etc., many of which are common components of soil biosphere. Since plant parasitic nematodes are in constant association with saprobic, parasitic and symbiotic microorganisms present in the rhizosphere, therefore, it is logical to consider interactions between these groups of organisms in terms of their combined effects on plant growth. As they occupy the same environmental niche, such organisms besides influencing the plants, are likely to influence each other as well. Most of the soil-borne plant diseases are often the result of interaction of two or more pathogens of the same or different groups resulting in disease complexes. Such pathogenic interrelationships often show additive, synergistic or neutralistic effects on plant disease development.

Our knowledge of nematode-fungal disease complexes has increased greatly since they were first reported in 1892, by Atkinson, at the Agriculture Experimental Station Auburn, Alabama. He wrote about the diseases of cotton and said: Multiple-parasitic associations are the usual norms under field conditions. Although some researches have been conducted in recent years, yet
there is great scope of making studies on the complex interactions among plant parasitic nematodes, soil microorganisms and host roots.

The fungus-nematode interactions are numerous and varied as they provide a wide open field for significant research. Weak parasitic fungal parasites can cause considerable damage, once they gain entry into plant roots, in the presence of feeding nematodes. The nematodes, therefore help the saprophytic fungi as invading organisms and increase their pathogenicity. Nematodes provide ready means of entry into the host for the fungus. Undoubtedly, this occurs when root-browsing nematodes cause superficial root injury and so enhance fungal access and enhance secondary pathogenicity of the roots.

The frequency of involvement of nematodes and fungi in disease complexes is reflected in the number of crops in which such complexes are recorded and as a single most destructive nematode species in the world; it is not surprising that *Meloidogyne incognita* has been so frequently reported in the disease complexes.

Nematodes interact with different soil borne fungi and cause disease complexes in pulse crops and have been found to increase the severity of wilt and other soil borne diseases. Some species of *Rhizoctonia* (*R. solani, R. bataticola*), and *Fusarium* (*F. solani*) and *Macrophomina* (*M. phaseolina*) are predominantly amongst the root-rot fungi known to interact with root-knot nematodes, *Meloidogyne* spp. (Ali and Singh, 2007).

*Macrophomina phaseolina* (Tassi) Goid. is a soilborne plant pathogenic fungus. It belongs to the anamorphic Ascomycetes and is characterized by the production of both pycnidia and sclerotia in host tissues and culture media. The pycnidial state was initially named *Macrophoma phaseolina* by Tassi, while Goidanich (1947) proposed the name *Macrophomina phaseolina*. The sclerotial state was described for the first time by Halsted as *Rhizoctonia bataticola*. The microsclerotia are black and are variable in size (50–150 μm) depending on the
The most striking symptoms of *Macrophomina phaseolina* are the sudden wilting and drying of the whole plant, most of the leaves remaining green. The stem and branches are then covered with black bodies and give the charcoal or ashy appearance of dead plants. Withering can be observed from seedling to maturing stage and is the result of necrosis of roots, stems and mechanical plugging of xylem vessels by microsclerotia, but also by toxin production, and enzymatic action (Kuti *et al.*, 1997; Jones and Wang, 1997).

A healthy plant is greatly influenced by the activities of non-pathogenic and rhizosphere inhabiting microorganisms, for example *Rhizobium* establishes a symbiotic association with the pulse crops. Root-knot nematode, soil-borne fungi and nitrogen fixing bacteria commonly occur together in the roots and rhizosphere of the same plant, each having a characteristic but often opposite effect on plant vigor. *Rhizobium* characteristically, stimulates plant growth, whereas nematode and fungi usually suppress it. Nematode and fungus infection may drastically affect the *Rhizobium*-pulse crop relationship.

A soil-borne fungus, *Paecilomyces lilacinus* (Thom.) Samson was reported at the International Potato Centre, Lima, Peru, that consistently and efficiently controlled the population of *Meloidogyne incognita*. The fungus *P. lilacinus* parasitizes the eggs as well as mature females of *M. incognita* thus destroying the embryos and killing females. The egg-parasitic fungus has been found to reduce *M. incognita* damage to pigeonpea and chickpea (Anver and Alam 1999). A wide host range of this fungus also has been reported for root infecting fungi, including *Macrophomina phaseolina*. (Shahzad and Ghaffar, 1989).

During the course of study a survey of Aligarh and adjoining pulse growing areas of Uttar Pradesh, India was made to evaluate plant parasitic nematodes and fungi (both from rhizosphere and rhizoplane) associated with the crop plants. In the present study a constant and concomitant occurrence of
Meloidogyne incognita (Kofoid and White) Chitwood, and Macrophomina phaseolina (Tassi) Goid. in the root and soil samples collected from lentil and chickpea fields has been observed. In areas where these pathogens were present singly, the number of damaged plants was comparatively less. These observations evoked an interest to study the problem and to determine whether the enhanced damage was the result of the interactions of these two pathogens.

In nature, infection of roots by soil borne root infecting fungi is often associated with infection by root-knot nematode Meloidogyne sp. A combined use of nematicide and fungicide are generally recommended. Keeping in view the cost of chemical pesticides and environmental hazards, the use of microbial antagonists to control soil borne pathogens has been suggested. One of the methods to overcome the nematodes and the disease complexes formed as a result of root-knot nematodes, Meloidogyne spp. and fungi is to combine the disease-suppressive activity of two (or more) beneficial microbes in a biocontrol preparation. Such combinations have potential for more extensive colonization of the rhizosphere and antagonism to a larger number of plant pathogens than individual applications. Conversely, microbes applied in combination also may have antagonistic interactions with each other as well. However, this approach has potential for overcoming the efficacy problems for Meloidogyne spp. and fungi in concomitance that occurs with the application of individual biocontrol agents.

This study was aimed to study the individual and interactive effects of both the pathogens and their biomanagement agents on Lentil cv. K 75, as the literature is very scanty on this problem of lentil.
The experiments conducted during the course of study are summarized as follows:

Section I
   A. Effect of different inoculum levels of *M. incognita* on growth, yield and nodulation of *L. culinaris*.
   B. Effect of different inoculum levels of *M. incognita* on chlorophyll and NPK contents of *L. culinaris*.
   C. Effect of different inoculum levels of *M. incognita* on galling and nematode reproduction on *L. culinaris*.
   D. Effect of different inoculum levels of *M. incognita* on the internal structure of infected roots of *L. culinaris*.

Section II
3. Pathogenic potential of *Macrophomina phaseolina* on *L. culinaris* (Medik.).
   A. Effect of different inoculum levels of *M. phaseolina* on growth, yield and nodulation of *L. culinaris*.
   B. Effect of different inoculum levels of *M. phaseolina* on chlorophyll NPK contents and root rot (%) of *L. culinaris*.

Section III
4. Study of disease complex involving *M. incognita* and *M. phaseolina* on *L. culinaris*.
   A. Effect of individual, sequential and simultaneous inoculations of *M. incognita* and *M. phaseolina* on growth, yield and nodulation of *L. culinaris*. 
B. Effect of individual, sequential and simultaneous inoculations of *M. incognita* and *M. phaseolina* on chlorophyll and NPK contents of *L. culinaris*.

C. Effect of individual, sequential and simultaneous inoculations of *M. incognita* and *M. phaseolina* on galling and nematode reproduction and rotting on *L. culinaris*.

**Section IV**

5. Studies on the effect of *Rhizobium* and *Paecilomyces lilacinus* on nematode, fungus and the root-knot and root-rot disease complex of lentil.

A. Effect of the biocontrol agents on plant growth, yield and nodulation.

B. Effect of the biomanagement agents on chlorophyll and NPK contents of plant.

C. Effect of *Rhizobium* and *P. lilacinus* on nematode galling, multiplication and disease severity.
Literature Review