

ECOFRIENDLY MANAGEMENT OF VASCULAR WILT OF LENTIL (*Lens culinaris* Medik.)

Thesis

Submitted to the

**G.B. Pant University of Agriculture & Technology,
PANTNAGAR-263 145 (U.S. Nagar), Uttaranchal, INDIA**



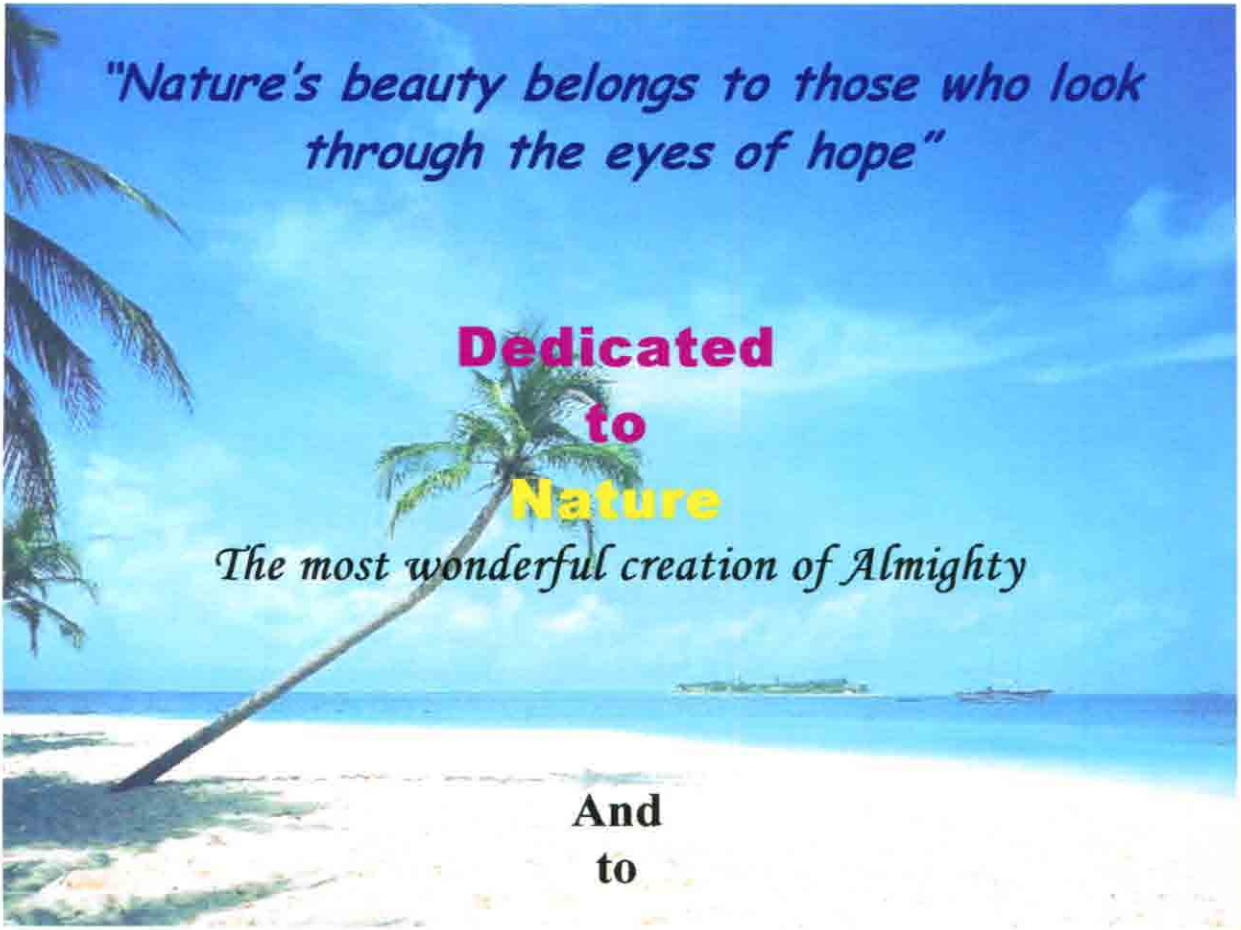
By

Shadma Ansari

***IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF***

**Doctor of Philosophy
(PLANT PATHOLOGY)**

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*"Nature's beauty belongs to those who look
through the eyes of hope"*

**Dedicated
to
Nature**

The most wonderful creation of Almighty

**And
to**

My Beloved Parents

*Who always insisted that there is no alternate
to sincerity and hard work*

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C E R T I F I C A T E

This is to certify that the thesis entitled **"ECOFRIENDLY MANAGEMENT OF VASCULAR WILT OF LENTIL (*Lens culinaris Medik.*)"** submitted in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** with major in **Plant Pathology** and minor in **ENTOMOLOGY** of the College of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona fide* research carried out by **Ms. Shadma Ansari, Id. No. 27831**, under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation have been duly acknowledged.

Pantnagar
August, 2003

(Y.P.S. Rathi)
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C E R T I F I C A T E

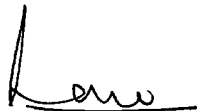
We, the undersigned, members of the Advisory Committee of **Ms. Shadma Ansari, Id. No. 27831**, a candidate for the degree of **DOCTOR OF PHILOSOPHY** with major in **Plant Pathology** and minor in **Entomology**, agree that the thesis entitled **“ECOFRIENDLY MANAGEMENT OF VASCULAR WILT OF LENTIL (*Lens culinaris* Medik.)”** may be submitted in partial fulfilment of the requirements for the degree.



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Introduction

Introduction

Pulses play an important role in Indian agriculture. Majority of Indian population is vegetarian and derive their dietary protein from pulses. India is one of the major pulse growing country in the world, accounting one third of total world area and one fourth of total world production. Besides increasing demand of pulses for food and feed in our country to ensure remunerative price of pulses and increase in yield, improved efficiencies of pulse farming is necessary for its sustainability in India. Pulse plant has deep penetrating root system, which enables it to utilize the limited available moisture more efficiently than other crops, and has ability to fix atmospheric nitrogen in symbiotic association with *Rhizobium* spp. These crops are not getting proper attention in production because of insufficient technology transfer for their production, susceptibility to number of abiotic/biotic factors and being less economical in comparison to wheat and rice to the farmers.

Lentil (*Lens culinaris* Medik.) is a valuable human feed. Dehulled lentil seeds contain 24-26 per cent protein, 1.3 per cent fat, 2.2 per cent ash, 3.2 per cent fibre and 57 per cent carbohydrate. It is a rich source of minerals containing 68 mg Ca, 300 mg phosphorus and 7 mg iron per 100 g seeds. It is also rich in vitamin C and riboflavin

(Masood Ali and Mishra, 2000). Being important source of protein its contribution to the diet is estimated to be 6.7 per cent in Asia and above 11.0 per cent in India (FAO, 1985). These crops are also rich in energy, minerals and certain vitamins. Needless to mention that in rice-wheat cropping system, the importance of pulses has been well recognized. The common pulses grown in India are: chickpea, lentil, fieldpea, *lathyrus* (kesari) and rajmash during *rabi* season and pigeonpea, mungbean, urdbean, mothbean, cowpea, ricebean during *kharif* season. Among these, lentil is one of the man's oldest food crops. It belongs to family Leguminosae and sub family Papilionidae. The most probable place of origin of lentil is from Turkey to South Iran. *Lens* is a latin word which refers to the shape of the seed of lentil. Lentil is known by different names in various countries such as *lentille* (French), *linse* (German), *lenteja* (Spanish) a *das* (Arabic), *mercimek* (Turkish), *hiramame* (Japanese) and *masur* /masoor (Hindi) (Masood Ali and Mishra, 2000).

Globally, lentil shares only 5 per cent of the total area under pulses (71 million hectares). It occupies 3.3 million hectares area with an annual production 2.82 million tonnes (Fig.1 A, B). It is predominantly grown in Asia, which accounts for 80 per cent and 75 per cent of the global area and production, respectively. It is also an important crop in North and Eastern Africa, North-Central America, Southern Europe, India, Turkey, Canada, Bangladesh, Iran, Nepal,

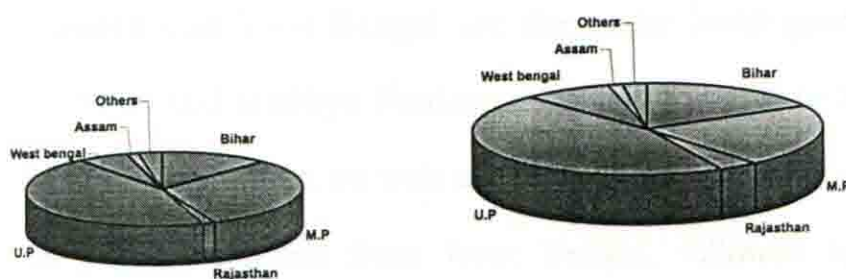
☐ Africa
 ☐ Asia
 ☐ India
 ☐ North America
 ☐ Europe



Global area and production of lentil

Fig.1

☐ Bihar
 ☐ M.P
 ☐ Rajasthan
 ☐ U.P
 ☐ West bengal
 ☐ Assam
 ☐ Others



Area and production of lentil in major states of India(2000-2001)

Fig. 2

Syria, Pakistan, China, Ethiopia, Morocco, Spain, Chile, Argentina, Colombia, New Zealand, Jordan and Australia are important lentil growing countries in descending order. Highest productivity (2111 kg ha⁻¹) has been reported from Australia, followed by Canada (1265 kg ha⁻¹), China, Syria, Turkey, Spain and Bangladesh (825 kg ha⁻¹) (Masood Ali and Mishra, 2000).

In India, lentil is the second most important winter pulse crop. The area of production and productivity during 2000-2001 was 1.41 million hectares, 0.86 million tonnes and 611 kg ha⁻¹, respectively (Appendix I and Fig. 2 A, B) (Anonymous, 2002). Being a cool-season crop, lentil production is mostly confined to northern and central India. Bihar, Haryana, Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh and West Bengal are the major lentil producing states. Uttar Pradesh and Madhya Pradesh together contribute 78 per cent towards total national area as well as production. Highest productivity (901 kg ha⁻¹) was reported from West Bengal, followed by Bihar and Uttar Pradesh during 2000-01 (Masood Ali and Mishra, 2000).

The main constraints responsible for low yield of lentil are the bioaggressors besides the non-availability of seed of improved high-yielding varieties to farmers, preference of most farmers to grow bold-seeded lentils which are comparatively poor yielders, tendency of Indian farmers to grow lentil on neglected marginal land with poor fertility and other inputs, cultivation of lentil mainly under rainfed conditions, etc.

The potentially damaging diseases (bioaggressors) of lentil are wilt, root rot complex, collar rot, rust and ascochyta blight. Lentil is infected by some viruses, which may become a threat for its cultivation under conditions favourable to the vector. Besides above, bacteria and nematodes are also important pathogens of lentil. The diseases of lentil not only affect yield but cause deterioration of seed quality.

Lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis* Vasudeva and Srinivasan, is a major disease of lentil that occurs in patches, covering large area and resulting tremendous loss to the crop yield. There are several soil-borne plant pathogens, associated with the disease, like *Rhizoctonia solani* Kuhn., *Rhizoctonia bataticola*, *Sclerotium rolfsii* Sacc. and *Phytophthora* spp. The proportion of plants, which die due to wilt of lentil, has been reported to vary from 25 per cent in seedling stage (Kannaiyan and Nene, 1972) to 50 per cent in flowering stage (Khare *et al.*, 1972). A loss of 37.5 per cent grain yield per hectare due to wilt was reported by Fleischmann (1937).

The concept of sustainable agriculture aims at maintaining an increasing production trend to ensure food security with future dimensions while preserving the underlying resource base. The changed agricultural scenario in India has to focus our attention to manage plant diseases, cut crop losses, avoid wide fluctuations in production and sustain the high levels of productivity.

Lentil crop is vulnerable to infection at all the growth stages. Therefore, it is practically impossible to control all the pathogens by the

use of chemicals alone. Nevertheless, biological control has been found as most effective and promising for the management of the disease. The resistance in cultivars also collapses with the development of new pathogen race(s). Judicious use of chemicals has also become very important to save the environment. As such, the feasible approach for the management of the soil-borne diseases is the development of an ecofriendly, integrated management strategy.

Considering the importance of wilt disease in *Tarai* area of Uttaranchal and Uttar Pradesh, the proposed study was undertaken on various aspects including the eco-friendly management of the wilt with the following objectives:

1. Isolation of pathogen(s) from diseased plants, their identification and pathogenicity test.
2. Histopathological studies.
3. To study the efficacy of selective bioagents against the pathogen.
4. To study the efficacy of fertilizers against the pathogen.
5. To study the efficacy of micronutrients and plant extracts against the pathogen in *in vitro* and *in vivo*.
6. To study the efficacy of different biocontrol agents against the pathogen through monoculture, dual culture and to find out the effect of volatile/non-volatile compounds on the pathogen in laboratory.

7. To study the effect of alum on wilt pathogen alone, or in combination with biocontrol agents and zinc.
8. Role of environmental factors on wilt incidence under field condition.
9. To study the effect of date of sowing on wilt incidence.
10. Screening of lentil germplasm lines in disease sick plots.
11. To explore the feasibility of integration of biocontrol agents with fungicides, and to work out an integrated disease management strategy.

Review
of
Literature

Review of Literature

2.1 The Disease

2.1.1 Historical background

Prissyajnyak first reported lentil wilt caused by *Fusarium* spp. from Russia (U.S.S.R.) in 1931. After three years, the occurrence of such a disease was reported from undivided Bengal, India (Anonymous, 1934). Fleischmann described wilting of lentil plants from Hungary in 1937 but no evidence of the pathogen was reported. Affected plants showed yellow discoloration, shriveling and finally collapse in windy weather, especially in acid soils. Carrera (1940) reported infection of lentil plants by *F. orthoceras* in Uruguay. Padwick (1941) observed wilted lentil plants in experimental plots in Delhi and Karnal and found these plots infected with *Fusarium* spp. The disease was later found damaging the lentil crop in Kanpur under natural conditions (Dey, 1948). However, the first authentic and published record of vascular wilt of lentil seems to be that by Vasudeva and Srinivasan (1952), who studied the disease in detail and noted about 67 per cent incidence of the disease in a field trial at Delhi.

2.1.2 Geographical distribution

The potentially damaging diseases of lentil are wilt, root-rot complex, collar rot, rust, ascochyta blight, sclerotinia blight, botrytis

grey mold and downy mildew. Lentil is also infected by some viruses, which may become a threat for its cultivation under conditions favourable to the vector. Bacteria and nematodes are also important pathogens of this crop.

Out of several diseases, wilt is widespread in most of the lentil growing countries of the world, particular in America (Wilsen and Brandsberg, 1965), Bangladesh (Bakr, 1991), Argentina (Ravenna and Negri, 1979), Brazil (Veiga *et al.*, 1974), India (Vasudeva and Srinivasan, 1952), Italy (Tosi and Cappelli, 2001), France (Moreau, 1978), Czechoslovakia (Ujevic *et al.*, 1965), Myanmar and Pakistan (Bahl *et al.*, 1991), Nepal (Karki, 1991), Syria (Bayaa *et al.*, 1986; El Ahmed and Mouselli, 1986), Uruguay (Carrera, 1940), USSR (Kotova *et al.*, 1965), etc.

In India, the first incidence of lentil wilt was reported from Bengal in 1934 (Anonymous, 1934). The association of *Fusarium* spp. with lentil wilt was reported from the experimental plots at Delhi and Karnal (Padwick, 1941). Later on, the disease was observed at Kanpur (Dey, 1948). Now, it is widely distributed in all the states, wherever lentil is cultivated.

2.1.3 Economic importance

The plants collapse before seed formation, if they are infected early in the season. Seeds produced on the infected plants are few in numbers, germinate poorly and produce weak seedlings. Erskine *et al.* (1989) reported strong correlation between wilt incidence and

grain yield. The regression study showed that every 10 per cent increase in wilt incidence caused a yield loss of 112 kg/h which comes to 8.77 per cent.

Many workers have reported the mortality of plants due to wilt of lentil. Extent of mortality varies from 25 per cent in seedling stage (Kannaiyan and Nene, 1972) to 50 per cent in flowering stage (Khare *et al.*, 1972). A loss of 37.5% of lentil grain/ha was reported by Fleishmann (1937) primarily due to wilt complex.

Under natural conditions, the disease incidence has been reported as high as 50-78 per cent in Madhya Pradesh (Khare *et al.*, 1972; Agrawal *et al.*, 1991), 70 per cent in areas of Czechoslovakia (Bajdeva and Sinsky, 1990) and 13.2 per cent in South Syria (El Ahmed and Mouselli, 1986, 1987). In Northwest Syria, an overall crop loss of 12 per cent has been reported due to lentil wilt (Bayaa *et al.* 1986).

2.2 Symptomatology

The lentil wilt pathogen affects the plant at three stages (Vasudeva and Srinivasan, 1952; Claudius and Mehrotra, 1973a; Khare *et al.*, 1979) i.e. pre-emergence stage, post-emergence stage and adult stage.

Under early infection, pre and early post-emergence mortality is noticed in patches showing seedling wilt. The vascular wilt is observed in case of plants infected at an advanced stage i.e. at blooming. At this

stage the damage to crop is heavy. The growth of infected plant is checked, leaves shrink or get curled starting from the lower part of the plants and extending upwards. Epinasty may be visible in some cases. Leaves become yellow and the whole plant gets dried. Root system is poorly developed and becomes brown. Secondary roots sometimes proliferate above the affected level. Walls of some of the xylem vessels in the root are discoloured brown and contain fungal hyphae particularly if the plant is infected at an advanced stage of its growth.

Vasudeva and Srinivasan (1952) reported that in a broadcast crop, wilt occurs in isolated patches, more or less circular in outline, which enlarge as the disease advances. When the crop is sown in rows the disease appears to progress along the lines.

Shulindin (1950) reported that in the former USSR the symptoms of the disease were inconspicuous in young seedlings but after 12-18 days the growth of infected seedlings was inhibited, leaves remained smaller, abnormally dark green and closer to the stem compared to those of healthy plants. Plant growth is reduced, remained mostly sterile or produced few pods with small and undeveloped seeds and subsequently the plants died. Infected roots of some young plants produced brownish red lesions and numerous conidia in the affected cortex and in tissues which showed no external symptoms.

El Ahmed and Mouselli (1987) differentiated the symptoms of vascular wilt (*F. oxysporum*) and root-rot (*F. solani*) of lentil in Syria.

They reported that both the diseases were characterized by yellowing and defoliation of leaves and drying of plants, however vascular wilt involves vascular tissues while in case of root-rot, roots rotted completely. Khare *et al.* (1976) differentiated fusarium wilt with collar rot of lentil caused by *Sclerotium rolfsii* in India. Stunting of plants, yellowing and drooping of leaves, followed by drying and death of the whole plant, characterized the former. In a few cases partial drying of the plant was also observed. The stem of diseased plants mostly exhibited reddish discolouration. In case of collar rot, sudden drooping of leaves at an early stage, followed by drying of the plants was observed. Affected old plants turned yellow, drooped and withered.

2.3 The Pathogen

Fusarium species are ubiquitous fungi found not only in the temperate and tropical areas of the world but also occur in such diverse environment as the arctic and deserts. These are saprophytes that attack plants and animals. On plants, *Fusarium* causes cortical rots, head blights, leaf spots, root rots, fruit rots, cankers, dieback and vascular wilt diseases. Of all the diseases caused by *Fusarium*, vascular wilt is the most important disease caused by formae speciales of *F. oxysporum* (Nelson, 1964). This f. sp. attacks over 100 species of gymnosperm and angiosperm crops like tomato, cabbage, flax, banana, pea, cotton, etc. (Bosland, 1988).

Several species of *Fusarium* infect the lentil plants (Khare *et al.* 1979) but vascular wilt is caused by *F. oxysporum* f. sp. *lentis* (Vasudeva and Srinivasan, 1952).

2.3.1 Taxonomy and nomenclature

Fusarium oxysporum, as we know it today, was included in the section *Elegans* by Wollenweber and Reinking (1935). Within this section they described three subsections *orthocera*, *constrictum* and *oxysporum*. Later on, Synder and Hansen (1940) formulated a species concept in which all members of the section *Elegans* constitute one natural species, *F. oxysporum*, Schlect. Due to this change in nomenclature, Chattopadhyay and Sengupta (1967) suggested the name *F. oxysporum* f. sp. *lentis*, which is now widely accepted. The morphological and cultural characters of the fungus resembles those isolates of *Fusarium*, which cause wilt of pea and chickpea, but the fungus was found to be host specific and was pathogenic only to lentil, so called as f. sp. *lentis* (Setti and Bouznad, 1998).

2.3.2 Morphology and cultural characters

Vasudeva and Srinivasan (1952) described the fungus as: stroma thin, plectenchymatous, not sclerotial, erumpent, pale to purple in colour. Aerial mycelium abundant, woolly, white, sometimes turning pink, collapsing after 3-4 weeks, becoming gelatinous and tough. Pinnotes as well as sporodochia is absent. The pathogen produces three

types of asexual spores. Micro conidia have been reported to be single celled, hyaline, ovoid, cylindrical, oblong or slightly curved and measuring $6.7 \times 3.7 \mu\text{m}$ (Khare *et al.*, 1971) and $8.5 \times 2.7 \mu\text{m}$ (Vasudeva and Srinivasan, 1952). Macro conidia ^{are} nearly straight to fusiform, falcate, slender, thin-walled with indistinct septa, mostly single celled, rarely 1-5 septate and $10-50 \times 5-6.7 \mu\text{m}$ in size. These conidia ^{are} formed freely on mycelium at the ends of free conidiophores. Base of conidia papillate or tending to foot-cell formation (Vasudeva and Srinivasan, 1952). Chlamydospores are terminal or intercalary, borne on mycelium, rarely on conidia, spherical to pyriform, smooth, hyaline, rich in protoplasmic contents, one celled, occasionally 2-celled, abundantly formed on certain media but rare to none on others (Vasudeva and Srinivasan, 1952).

The fungus grew well on Potato Dextrose Agar (PDA) at 27-30°C but not at 9°C and 35°C as reported by Vasudeva and Srinivasan (1952). In Syria, the best growth and sporulation was recorded at 22.4°C on PDA (Erskine *et al.* 1990). It can withstand a wide range of pH optimum being at 4.6-5.8. It grew rapidly in sand with 1 per cent ground maize compared to sand + maize + soil, indicating the favourable effect of aeration on fungal growth.

Sharma and Agnihotri (1971) and Kumar *et al.* (2000) reported that the fungus grew well on natural as well as synthetic media but Richard's medium supported best growth. Mannitol as the carbon

source, sodium nitrate as the nitrogen source, C : N ratio 26 : 1 and pH level of 4.8 (Sharma and Agnihotri. 1971) to 5.3 (Kumar *et al.* 2000) were found best for growth.

2.3.3 Host range

Padwick (1941) reported that the lentil wilt pathogen could infect lentil as well as chickpea but the chickpea isolate did not infect lentil. Others have reported the lentil wilt pathogen to be highly host specific in nature (Vasudeva and Srinivasan, 1952; Khare, 1980; Kannaiyan and Nene, 1978a) infecting only lentil and not others like chickpea, pea, lathyrus, pigeonpea, broadbean, blue lupine, cowpea, soybean, black gram, green gram, french bean, paddy, wheat, sorghum, maize, sugarbeet, cotton, linseed, sunflower, mustard and brinjal. However, Haware and Nene (1982) reported that some crops might carry the pathogen without showing visible symptoms and act as symptom less carrier of the pathogen.

2.3.4 Variability

Though the lentil wilt pathogen is highly host specific, great variability exists in it. Sharma and Agnihotri (1972) identified three isolates 'A', 'B' and 'C' on the basis of their virulence on lentil. In Madhya Pradesh, Claudius and Mehrotra (1973a) isolated 14 cultures of *F. oxysporum* f.sp. *lentis* at Sagar and found them differ from each other with respect to their virulence on two varieties of lentil.

The isolates were differentiated from each other on the basis of colour/pigmentation of mycelium and substrate, growth habit, morphology of micro- and macro-conidia and chlamydospores (Khare, 1980); preference for different nutrients, especially carbon, nitrogen, sulfur and phosphorus (Kushwaha *et al.*, 1974); optimum temperature required for their growth (Dhingra *et al.*, 1974); growth on different solid media (Khare *et al.*, 1975); optimum pH level for their growth (Khare, 1980); sensitivity to different fungicides in regard to their growth and macro-conidia formation (Agarwal and Khare, 1975b, 1977a) as well as their virulence in lentil. Kannaiyan and Nene (1978b) reported strain variability in *F. oxysporum* f.sp. *lentis*. Bhalla *et al.* (1984) identified a more virulent strain 82A of *F. oxysporum* causing complete necrosis of lentil seedlings within 7 days.

El Morsy *et al.* (1997) isolated fungi from wilted fababean, lentil and chickpea plants collected from different regions of Egypt. Isolates of *F. oxysporum* collected from each crop were grouped depending on their characteristics. They obtained 6 groups of isolates from lentil, which therefore, was subdivided into 4 types.

2.3.5 Host- parasite interaction

The pathogen *F. oxysporum* f.sp. *lentis* enters the host through natural openings, such as emergence of secondary roots, or through wounds, which expose xylem (Khare, 1980) and ultimately it colonizes the metaxylem and stops the supply of water and mineral ions, resulting in loss in turgidity and finally wilting (Saxena *et al.* 1992).

The vascular tissues of lentil roots infected with *F. oxysporum* f.sp. *lentis* showed the presence of inter- as well as intra-cellular mycelium. The xylem vessels are plugged with the mycelial growth and formation of tyloses. In some cases only a few hyphae were detected in the vessels but their walls and the adjoining tissues develop browning. Chlamydospores- like bodies has also been detected in the vessels (Agarwal and Prasad, 1997).

Bhalla *et al.* (1992) reported that *F. oxysporum* infects roots of lentil by entering through juncture of epidermal cells within 8 h after inoculation with or without forming appressoria. Prior to entry of hyphae, the cells became irregular in shape with granular cytoplasm. Hyphae in cortical cells were observed 10-12 h after inoculation, the walls of which were ruptured. Hyphae proliferated extensively in cortex within 24 h but the endodermis and vascular cells were free of hyphae for at least 72 hours.

2.4 Epidemiology

In Czechoslovakia, Ujevic *et al.* (1965) isolated *F. oxysporum* from lentil seeds. In India, Khare *et al.* (1979) also reported the fungus to be associated with the seeds of lentil. However, Erskine *et al.* (1990) did not find lentil wilt pathogen associated with seeds obtained from wilt-affected plants. They considered the seed-borne nature of the disease related to either an external contamination or via trash.

Khare (1980) reported that seed transmission of the wilt pathogen is extremely important in the transfer of inoculum from infested to uninfested areas, while Mehrotra and Claudius (1973c) reported that the competitive saprophytic ability of *F. oxysporum* f.sp. *lentis* is very high because of which it can survive in soil.

Sharma *et al.* (1971) and Saxena and Khare (1988b) reported that soil pH of 7.5 to 8.0 has been found favourable for lentil wilt development while Vasudeva and Srinivasan (1952) and Kaushal *et al.* (1998) observed that it could tolerate a wide range of pH and produced good growth at 4.6-5.8 pH.

Lentil wilt is also affected by soil moisture. It is favoured by soil moisture of 25 per cent water holding capacity (Vasudeva and Srinivasan, 1952; Sharma *et al.*, 1971; Saxena and Khare, 1988b). They also reported that with an increase in soil moisture the disease incidence decreases, showing no wilt at 75 per cent or more moisture level of soil. Khare (1980) also noted reduction in the disease due to higher soil moisture.

Vasudeva and Srinivasan (1952) observed the wilt incidence of 7.5, 96.4 and 41.7 per cent at soil temperature range of 13-23°C, 17-31°C and 13-38°C, respectively. They also reported that the disease appeared in November but with a decline in temperature it had almost ceased in December-January and again appeared in February and reached up to its maximum in March with an increase in temperature. Saxena and Khare (1988b) also reported the maximum disease at

20-30°C. Kaushal and Sharma (1998) reported highest wilt incidence (75.1%) at temperature range of 24-27°C and the lowest (13.0%) in the temperature range of 13-16°C.

2.5 Disease Management

2.5.1 Date of sowing

Various workers reported that lentil wilt is greatly influenced by various environmental factors. Sowing time of the crop also affects the disease incidence. Myalova (1973) reported infection of lentil by *F. oxysporum* to be seven times more severe in early sowing than in late ones. At Pantnagar, least disease was observed when crop was sown on 15 December while adult stage wilt was less in crops sown on 15 November (Kannaiyan and Nene, 1975a). Bhatt and Sharma (1992) also reported more seedling wilt in a crop sown upto the third week of October and lesser, in late sown crops. Izquierdo and Morse (1975) recorded more wilt incidence on early sown crop. In the former USSR, severe wilt incidence was reported by Shulindin (1950) in the spring-sown crop compared to that sown in summer. However, in Syria late sowing of lentil (February) increased wilt incidence compared to normal sowing in December (Anonymous, 1987) due to different temperature regime (Erskine *et al.* 1990).

2.5.2 Host nutrition

Nutrients affect the severity of disease not only by influencing root physiology and host resistance but also by affecting the interaction

between host, pathogen and the antagonists, each of which may also be affected independently by the availability of nutrients (Sivasithamparam, 1996). Effect of host nutrition in relation to plant diseases has been studied by Huber and Watson (1974); Perrenoud (1977, 1990); Huber (1980); Graham (1983); Graham and Webb (1991); Fageria *et al.* (1991); Gupta (1999); Sarkar *et al.* (2000) and Chandrasehar *et al.* (2001).

Nitrogen, phosphorus and potassium are known as primary plant nutrients; calcium, magnesium and sulphur, as secondary nutrients and iron, manganese, copper, zinc, boron, molybdenum and chlorine as trace elements or micronutrients. The primary and secondary nutrient elements are known as major elements. The micronutrients are required in small quantities, but they are as important as the major elements in plant nutrition (Handbook of Agriculture, 1997).

The effect of nutrients on disease resistance may be attributed to (i) effect on plant growth that can influence the microclimate in a crop and thereby affect infection and sporulation of the pathogen, (ii) effects on cell walls and tissues, as well as on the biochemical composition of the host, (iii) the rate of growth of the host which may enable plants to escape infection in their susceptible stages, and (iv) effects on the pathogen through alteration in the soil environments (Colhoun, 1973).

The information available in literature pertaining to the effect of nutrient is given below :

(i) Zinc (Zn)

Zinc in the ionic form (Zn^{+2}) or in the form of a complex with a chelating agent, salts or complexes can easily be absorbed directly through leaves. It is a metal component and serves as a functional, structural or regulatory co-factor of a large number of enzymes (Vallee and Wacker, 1970; Vallee and Galles, 1984 and Marschner, 1986). The most prominent physiological role of Zn is the inter-relationship with auxin. It is thereby essential for cell elongation and growth, as well as being functional in respiration and enzyme regulation (Huber, 1980).

Zinc application has suppressive effects on soil-borne diseases (Graham, 1983). With Zn deficiency, a leakage of sugars and inhibition of protein synthesis occurs with large increase in free amino acids which in turn favors disease development. Sarkar *et al.* (2000) conducted an experiment to check the influence of boron, zinc and iron on sun hemp wilt and reported that zinc @ 10 kg/ha + iron @ 2 kg/ha resulted in the reduction of wilt incidence. Singh (1948) and Pradhan *et al.* (1999) reported that boron, zinc and manganese restrict the saprophytic ability of *Fusarium udum* (pigeonpea wilt).

Mehrotra and Claudius (1973a) found that pre-soaking of lentil seeds in 80-ppm concentration of Zn and Mn salt reduced fusarium wilt. The laboratory studies conducted by Gupta (1999) on root pathogens (*Sclerotium rolfsii* [*Corticium rolfsii*], *Fusarium oxysporum* f.sp. *ciceri* and *Rhizoctonia bataticola* of chickpea showed that out of 6 micronutrients tested, zinc inhibited the growth of all the 3 pathogens.

(ii) Calcium

Calcium combines with pectin in the plant to form calcium pectate, which is an essential constituent of the cell wall. It also promotes the activity of soil bacteria concerned with the fixation of free nitrogen or the formation of nitrates from organic forms of nitrogen. Furthermore, it is necessary for the development of a good root-system. By the base exchange action it renders more soluble, some of the insoluble salts of other nutrients, and thus promotes plant growth. With low supplies, plant roots die at the tips or remain short or stubby, thus adversely affecting plant growth, particularly of legumes.

Chandrasehar *et al.* (2001) reported the role of calcium on fusarium wilt disease (*F. oxysporum* f.sp. *lycopersici*) of tomato. They observed that Ca at 1000 ppm was highly effective in inhibiting the fungal growth. They further reported that pectinase enzyme production decreased with increased Ca levels and the toxin production of pathogen was inhibited at all Ca levels.

Yamazaki *et al.* (1999) reported the effect of calcium concentration in nutrient solution before and after inoculation with *Ralstonia solanacearum* against bacterial wilt of tomato. They reported that the disease was not affected by the concentration of Ca before inoculation but a higher concentration of Ca after inoculation reduced the disease severity.

(iii) Magnesium

Magnesium is an essential constituent of chlorophyll and is a part of many enzymes. It is also necessary for the formation of oil. Magnesium Sulphate has been reported to be good source of sulphur and hence finds place in standard nutrient media like Czapek's, Richard's, Coon's, Brown's, Elliott's and Houston's media, supporting the fungal growth, however Ganacharya and Wanker (1976) reported that when $MgSO_4$ was added to the media it didn't increase the growth of *Fusarium oxysporum* causing potato wilt.

(iv) Ammonium

Ammonium is a source of nitrogen for plant nutrition, and only leguminous crops can directly use the free nitrogen with the help of symbiotic bacteria *Rhizobium* spp. Other plants derive nitrogen from the soil in the form of nitrogen and ammonium. Nitrogen and ammonium are produced in the soil by the action of micro-organisms on the soil organic matter. Non-symbiotic micro-organisms can fix free nitrogen of the air and make it available to plants in ammonium and nitrate forms. It encourages the vegetative growth of the plants by imparting a healthy green colour to the leaves. It also controls the efficient utilization of phosphorous and potassium to some extent.

(v) Fertilizers

Nitrogen (N), as the fourth most abundant element in plants, has been extensively studied in relation to host nutrition and disease

severity because of its essential requirement for plant growth, its limited availability in soil, and its effect on cell size and cell wall thickness. It promotes vigorous growth, delays crop maturity, and is essential for the production of amino acids, protein, growth hormones, new protoplasm, phytoalexin and phenols (Huber, 1980).

Being the legume, the major portion of N requirement of lentil crop is met through biological nitrogen fixation. Malik *et al.* (1991) carried out field trials to determine the effect of NPK on seed yield and quality of *Lens culinaris* cv. Masoor 85 and reported that the fertilizer application 25 kg N + 50 kg P + 50 kg K per ha produced higher yield and protein contents in seed.

Hoffland *et al.* (2000) reported no effect of N concentration on susceptibility to *F. oxysporum* causing tomato wilt. Taya *et al.* (1988) reported that increased level of nitrogen fertilizers enhanced severity of dry root rot (*Rhizoctonia bataticola*) in chickpea while phosphate levels decreased it and the combined effect of both N and P subsided the root rot incidence.

Goudar *et al.* (1998) found neem cake and compost effective, in reducing the wilt incidence of pigeonpea (*F. udum*) and improving seed germination. They recorded highest per cent survival of plants when neem cake was amended to the soil, followed by compost, FYM and groundnut oil cake. Dickerson (1999) also reported that compost could suppress damping off and wilt of chilli.

Compost extracts made from livestock manures showed varied degree of protection against cucumber wilt (*F. oxysporum* f.sp. *cucumerinum*) as reported by LiPing *et al.* (1999). The relative efficacies was found as pig, horse and cow manure and the possible mechanism involves the inhibition of conidial germination by the compost extracts and the antagonistic effect of microorganisms in the compost. Orlikowski *et al.* (1998) reported that the application of Antifung (vermicompost) as a substrate drench immediately after the planting of carnations into peat decreased the spread of fusarium wilt (*F. oxysporum* f.sp. *dianthi*) by 50 per cent during 12 and 24 weeks of cultivation.

Raviv *et al.* (1998) determined the effect of compost on pathogen *F. oxysporum* f.sp. *basilici* causing wilt of sweet basil (*Ocimum basilicum*) and reported that compost reduced the severity of the visual symptoms of wilt. Dhital *et al.* (1997) reported that the soil amended with 428kg/ha urea and 5t/ha lime can effectively control the bacterial wilt of potato caused by *Ralstonia solanacearum*.

Phosphorous fertilizer plays key role in atmospheric nitrogen fixation in grain legumes. It imparts hardness to shoot, improves root development and nodulation (Singh and Prasad, 1997). Sharma *et al.* (2001) determined the efficacy of four chemical fertilizers viz., super phosphate, urea, DAP potash and two organic manures, against wilt disease in cumin, caused by *F. oxysporum* var. *cumini*. They reported

that the frequency of mycoflora was less in treatments of superphosphate and organic manure (goat dung) whereas it was more in other treatments. Increased rate of phosphate significantly increased the growth of the plants and grain yield in chickpea. Application of 60 kg P_2O_5 /ha brought about 22.1 per cent increase in grain yield over 20 kg P_2O_5 /ha (Raju and Verma, 1984). In Punjab, Azad *et al.* (1995) reported that maximum yield of lentil (1225 kg/ha) was harvested when the soils were fertilized with 60 kg P_2O_5 + 40 kg K_2O .

Singh and Singh (1997) reported that potassium application significantly increased dry matter production, grain yield, test weight, carbohydrate and ash content over control. Sharma *et al.* (1993) recorded the highest mean seed yield (744 kg/ha) of lentil when urea was applied as 18 kg nitrogen, SSP as 46 kg P_2O_5 , muriate of potash as 20 kg K_2O and $ZnSO_4$ 25 kg/ha .

Singh (1983) reported differential response of chickpea to potassium application. Murumkar and Chavan (1985) observed that K-deficiency reduced most growth parameters and also altered the nutrient and carbohydrate content in chickpea.

2.5.3 Biological control

Biological control is the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man (Cook and Baker, 1983; Mukhopadhyay, 1996). In simple words, biological control is the use, by

man, of any organism for pathogen suppression. Biological control as a strategy and physiology for reducing crop loss from plant disease is not new to agriculture (Cook, 1982). Even the earliest farmers practiced biological control by rotating their crops, burrowing disease infested crop residues and fertilizing with organic manures. These and many other traditional practices give effective disease control by allowing the time and opportunity for biological destruction of disease organisms. The challenge today is how to achieve effective biological control of pests in modern agriculture where farmers must specialize with one or two crops, use minimal or no-till to save fuel and stop soil erosion; and where organic fertilizer are unavailable, in convenient or too expensive. Pesticides are necessary at present but are not a long-term solution to crop health. Besides its non-target effects, petroleum based pesticides will become even more expensive and some are now losing their effectiveness (Cook, 1982).

So, it can be achieved either by promoting the native antagonists to reach a density sufficient to suppress pathogen(s) or by introducing alien antagonists. Although some of the earlier work was related to promoting indigenous antagonists by using organic amendments or other cultural practices. Recently the antagonists are being isolated, multiplied and introduced to soil or in seed in specific counts to achieve a successful biological suppression of a disease. Today biological control research has reached a state where we have commercial formulations of

biocontrol agents sold in the market of several countries; (Papavizas, 1985; Baker, 1986, 1987; Gabriel and Cook, 1990) and there remains no doubt that biological control has become an integral part of integrated disease management.

Several reports have appeared in the literature concerning potentiality of *Trichoderma* spp. as biological control agents for the control of plant diseases. Dennis and Webster (1971 a, b, c) reported that among many potentially antagonistic soil inhabitants, the members of the genus *Trichoderma* have gained considerable success.

Baker and Cook (1974) reported that majority of antagonists are saprophytes. Saprophytes are more broadly adopted to the environment and are able to use a greater range of nutrients than the plant parasites, a situation favourable for the biological control.

(i) *Trichoderma harzianum* (Rifai) (Teleomorph-*Hypocrea albobulva* Berk and Br, 1873)

Trichoderma harzianum was isolated from the soil of the Sheffield University, Botany Department Garden, Taptonville Road, Sheffield, England, November 1963, M.A. Rifai-T008 colonies grew rapidly and form at first a smooth surfaced, watery white and sparse mycelial mat but soon aerial hyphae may develop on their surface. The conidial areas are whitish green, turning to bright green but ultimately they appear rather dull green. The reverse of the colony, however, remains colourless. The hyphae are septate, branched, smooth walled,

colourless and 1.5-12.0 μm in diameter. Intercalary or sometimes terminal chlamydospores are produced in the mycelium. They are mostly globose, smooth walled, colourless and vary from 6 to 12 μm in diameter. The main branches of conidiophores produce numerous side branches. Phialide are short, sickle shaped, narrower at the base than at the middle attenuated abruptly towards the conidial end sharp pointed neck and measure $5.7 \times 3.5 \mu$. The phialospores are produced singly or successively and accumulate at the tip of each phialide (Rifai, 1969; Cook and Baker, 1983).

Sub division	:	Deuteromycotina
Form class	:	Deuteromycetes
Form sub class	:	Hyphomycetidae
Form order	:	Moniliales
Form family	:	Moniliaceae
Form subfamily	:	Gliosporae
Genus	:	<i>Trichoderma</i>
Species	:	<i>harzianum</i>

(Alexopoulos *et al.*, 1996)

Dercole *et al.* (1984) observed four types of antagonism in between the isolate of *T. harzianum* and *Fusarium* on PDA. They were:

- i. Relief formation in the content zone between the fungi.
- ii. Lytic phenomenon
- iii. Complete overlapping of the pathogen by the antagonist.
- iv. Variable behaviour

Vajnn (1985) reported that in dual cultures of *Fusarium* and *Trichoderma* species, there was a necrotrophic mycoparasitic relationship between *F. oxysporum* and *Trichoderma pseudokoningii* and *T. longobranhianin*. Mukhopadhyay and Kaur (1990) found *T. harzianum* inhibitory to the growth of *Fusarium oxysporum*. De and Chaudhary (1999) reported that *Gliocladium virens*, *T. harzianum* or *T. viride* reduced the wilt incidence of lentil caused by *F. oxysporum* f.sp. *lentis* by 79 per cent and increased yield by 140, 224 and 241 per cent, respectively.

Niknejad *et al.* (2000) conducted an experiment to check the efficacy of *Trichoderma* spp. on the control of fusarium wilt of tomato caused by *F. oxysporum* f.sp. *lycopersici*. They reported that the most effective antagonist was T₂ (*T. harzianum*) and the least effective was T₄ (*T. viride*). The antagonists had a positive effect on plant height and weight. According to Dhedhi *et al.* (1990), *T. harzianum* is antagonistic *in vitro* to *F. oxysporum* f.sp. *ciceri* causing vascular wilt of chickpea.

Trichoderma preparation has been reported to increase vigor and emergence of oat seedlings (Wu, 1977). Wheat bran saw dust preparation of *T. harzianum* has been reported to promote the growth of various crops, which included brinjal (Chaturvedi, 1986), lentil (Saxena, 1987; Shreshtha, 1992), tomato (Nagpal, 1987), chickpea (Kaur, 1988; Khilari, 1994), pigeonpea (Mehrotra, 1988) and cauliflower (Mukherjee *et al.*, 1989).

Dennis and Webster (1971 a, b) studied antagonistic properties of species of *Trichoderma* by an agar layer technique. They reported that gliotoxin and viridin were not produced, but other chloroform soluble non-volatile antibiotics including *Trichodermin* and other peptide antibiotics like suzukacillin, alamethicine and dermadine, which were active against certain fungi and bacteria, were detected. Some isolates of *Trichoderma* produced acetaldehyde as a volatile compound. Tronsmo and Dennis (1978) reported inhibition of growth of *Botrytis cinerea* Pers. exfr. and *Mucor mucedo* Fr. by non-volatile and volatile metabolites, produced by *Trichoderma* spp. Chet and Baker (1981) reported that *T. harzianum* released active B (1-3) glucanase and chitinase when grown on *R. solani* cell walls (Appendix 2).

ii) ***Trichoderma virens* (= *Gliocladium virens* Miller, Giddens and Foster, 1957)**

Petch (1939) studied the genus *Gliocladium corda*. Isolation and characterization of *T. virens* was done from forest and cultivated soil of Georgia by Miller *et al.* (1957). It belongs to:

Sub division	:	Deuteromycotina
Form class	:	Hyphomycetes
Form sub class	:	Hyphomycetidae
Form order	:	Moniliales
Form family	:	Moniliaceae
Form subfamily	:	Gliosporae

Genus : *Trichoderma*

Species : *virens*

(Morquer *et al.* 1963; Alexopoulos *et al.* 1996)

Singh and Mukhopadhyay (2000) reported that *Gliocladium virens* and its gamma radiation induced mutants (M-3 and M-7) effectively control the wilt complex of lentil caused by *R. solani*, *S. rolfsii* and *F. oxysporum* f.sp. *lentis*. *Gliocladium virens* exhibited antagonistic effect against the wilt complex pathogens. Mukherjee and Tripathi (2000), while working on wilt complex of frenchbean reported that the antagonist produced both volatile and non-volatile metabolites that inhibited the growth of pathogen.

iii) **Pseudomonads**

Straight or curved rods, which are motile, characterize pseudomonads by polar flagella. They are chemoorganotrophs and gram negative in stain reaction. Cell size measuring $0.5-1.0 \times 1.5-4.0 \mu$, non-spore formers with no resting stage.

The genus *Pseudomonas* consists of a very diverse assemblage of bacteria, in which some are pathogenic and some are non pathogenic. Most of the species of non-pathogenic pseudomonads have the ability of plant growth promotion and biological control of phytopathogenic fungi, in which *Pseudomonas fluorescens* and *P. putida* are most important (Weller, 1988; Dube, 1995; Lam and Gaffney, 1993).

Pseudomonas fluorescens belongs to:

Kingdom	:	Prokaryotae
Division	:	Gracillicutes
Class	:	Proteobacteria
Order	:	Eubacteriales
Family	:	Pseudomonadaceae
Genus	:	<i>Pseudomonas</i>
Species	:	<i>fluorescens</i>

(Plougherty and Lamberti, 1954)

These are typically gram negative, chemoheterotrophic, and motile rods with one or more polar flagella. They are distinguished from other *Pseudomonads* by their ability to produce a water-soluble yellow green pigment. Several strains of fluorescent pseudomonads are found in soil and on young growing plant roots. They are known to secrete a large number of secondary metabolites that may affect the growth and health of plants (Leisinger and Margaraff. 1979).

The mechanism of action of bacterial biocontrol agents are by production of antibiotics, siderophores, ammonium cyanide and hydrolytic enzymes (Lam and Gaffney, 1993). In addition to this, they may also provide protection through induction of host plant defense mechanism; elimination of plant signals that trigger pathogen development or competition for nutrients (Lam and Gaffney, 1993).

Fluorescent pseudomonads have many promising properties as biocontrol agents (Appendix 3). They effectively and efficiently colonize the growing underground plant organs (e.g. roots, hypocotyls and tubers) and their nutritional requirement is simple as they can utilize a large number of organic substrates commonly found in the root exudates. They are known to produce a variety of secondary metabolites toxic to pathogenic fungi and bacteria, and then, reduce the rhizosphere population of deleterious microorganisms. They also produce some plant growth regulators thus; these strains enhance the plant growth (Elad and Chet, 1987; Polonenko *et al.*, 1987; and Chang Senk *et al.*, 1988). Some strains of fluorescent pseudomonads also induce systemic resistance and reduce plant diseases (Van Peer *et al.*, 1991).

Inam-ul-Haq (2001) evaluated different strains of *Pseudomonas fluorescens* against chickpea wilt alone or in combination and showed that among five different isolates tested, isolate no. 1, 2, 4, 5 and 9 were capable of suppressing *F. oxysporum* f.sp. *ciceri* and isolates I2 + I5, I1 + I2 + I5, I5 + I1, I5 + I9 and I5 + I4 were the best combination for the biocontrol pathogen as they showed 98.0, 97.4, 96.7, 91.7 and 92.4 per cent decrease in disease incidence over control, respectively. Gehlot *et al.* (2002) reported that *Pseudomonas fluorescens* reduced the disease intensity of wilt of chilli caused by *Fusarium solani* by 37 per cent and it increased the plant biomass and yield of chilli.

Field evaluation of *P. putida* (PDBCAB 19) and *P. fluorescens* (PDBCAB 2) against wilt and root-rot of chickpea was carried out by

Rangeshwaran *et al.* (2001). They reported that root-rot incidence was prevalent upto 60 days of plant growth whereas fusarial wilt was observed from 60 days. *P. putida* gave lowest wilt incidence (3.3%) as compared to check and the combination of both biocontrol agents produced 5.8 and 7.5 per cent suppression of root-rot and wilt incidence, respectively.

Ramamoorthy *et al.* (2002) found that *P. fluorescens* isolate PF₁ protect tomato plants from wilt disease caused by *F. oxysporum* f.sp. *lycopersici*. They reported that phenolic compounds accumulated in bacterized tomato root tissues and the activities of phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase also increased in the tissues challenged with *F. oxysporum* f.sp. *lycopersici* one day after pathogen challenge. The results suggest that induction of defense enzymes involved in phenylpropanoid pathway and accumulation of phenolics and PR-proteins might have contributed to restriction of invasion of pathogen in tomato roots.

2.5.4 Chemical control

Attempts were made by various scientists to control wilt pathogens by chemicals. Kovacikova (1970) first obtained effective control of lentil wilt by treating the seeds with Captan (0.2%) or Thiram (0.2%). Khare *et al.* (1971) reported considerable reduction in wilt incidence in field when seeds were treated with Benlate or Chloroneb + Thiram @ 1: 400 w/w. Sharma *et al.* (1971) and Agarwal *et al.* (1974)

found that Benlate, Ceresan wet, Mercuric chloride, Thiride and mixture of Captan, Thiram and PCNB were highly inhibitory to *F. oxyspoum* f.sp. *lentis*.

Kannaiyan and Nene (1972) found effective control of lentil wilt by seed treatment with Triarimol (0.05% a.i.) or Benomyl (0.1% a.i.) under field conditions. Seed treatment with 0.15 per cent boric acid and KMNO₄ reduced infection by 20-70 per cent and yield increased by 22-23 per cent (Myalova, 1973). *F. oxysporum* was effectively controlled by seed treatment with Orthiocide 75 @ 6 g/kg seeds (V'Rbanov, 1973) and thiophanate methyl (DeCallonne *et al.*, 1975). In glass house study, *R. solani*, *F. solani* and *Pythium ultimum* were controlled with Topsin or Tetrachlor (Shatla *et al.*, 1974).

Out of the eleven fungicides tested only Captan eliminated all the fungi associated with lentil seeds. Thiram and Bavistin were also effective against wilt pathogens of lentil (Vishunawat and Shukla, 1982). Saxena (1987) studied the effect of various fungicides on lentil wilt pathogens *in vitro* and observed that Bavistin and Vitavax @ 10, 25, 50 and 100-ppm were effective against *R. solani* and *F. oxysporum* f.sp. *lentis*.

Ahmed and Ahmed (2000) and Chaudhary *et al.* (2000) evaluated several pesticides (insecticides, fungicides and weedicides) on population dynamics of *F. oxysporum* f.sp. *lentis* and recorded the lowest population 75-105 days after treatment with Carbendazim.

Helmy *et al.* (2001) reported that application of Topsin M and Vitavax/Thiram as seed dressing significantly controlled damping-off of medicinal plant (*Ruta graveolens* L.) caused by *Fusarium moniliformae*, *F. oxysporum* and *Sclerotinia sclerotiorum* in Egypt.

2.5.5 Host resistance

2.5.5.1 Varietal screening

Resistance is fundamental attribute of all living systems. Plant essentially offers active resistance that largely determines the outcome of host parasite interaction. Chemical constituents of plant help in strengthening or weakening the barrier of host resistance altering its metabolism. Fleischmann (1937) was the first to suggest development of a wilt resistant population of lentil by the mass selection method and drew attention to the superiority of small -seeded lentils over the large types. Shulindin (1950) also reported the same in the former USSR.

In India, Khare and Sharma (1969) tested 82 varieties and collections of lentil for wilt incidence in the field and noted great variability for this trait. Thus, 2000 lines were screened at Jabalpur under field and pot culture conditions during 1970-74. Of these, 33 entries were resistant (Khare *et al.*, 1976). Later, some more LWS lines were found resistant to wilt with less than 5 per cent incidence (Khare *et al.*, 1979). Tiwari and Singh (1980) scored lentil lines against wilt under field conditions and identified JL 500, which was used as parent in the lentil-breeding programme. Some of the Jabalpur lines i.e.

LWS (JL 500) and LWS 21 (JL 674) were tested at Pantnagar, which exhibited immune reaction against five out of seven pathogenic strains of *F. oxysporum* f.sp. *lentis* (Kannaiyan and Nene, 1978b). Kannaiyan and Nene (1976) reported that of the 32 lines found immune to wilt, JL 80, Pusa 3 and Pant 234 showed a high degree of tolerance to wilt, collar rot and root rot pathogens. Pandey *et al.* (1988) reported nine lines resistant to wilt complex of lentil. They also developed a variety 'Pant L 406' in 1980, by selection from indigenous genetic stock line 'P 495'. It was found immune to race 5 and resistant to race 3 and 6 of *F. oxysporum* f.sp. *lentis*.

Singh *et al.* (1994) recommended small -seeded lentil variety Pant L 4 for cultivation in the northwestern plains of India. This variety is resistant to wilt, rust and ascochyta blight and has a production potential of 3.2 tonnes/ha. Singh *et al.* (1997) developed bold -seeded lentil cultivar Narendra Massor-1 from the cross Precoz × L, 9-12, that showed consistently higher seed yield than the controls as well as resistance to rust [*Uromyces fabae* (*U. viciae-fabae*)] and wilt (*Fusarium*).

Lentil varieties and germplasm have been screened against wilt at different research centers under the All India Co-Ordinated Pulses Improvement Programme (AICPIP). The resistant sources in lentil against wilt have been identified in other countries also, especially in Czechoslovakia (Kovacikova and Suchanek, 1974), Uruguay (Izquierdo and Morse, 1975), Russia (Voluzneva and Arkhpov, 1985), Bulgaria, Egypt and Syria (Appendix 4).

Sarkar *et al.* (2001) registered six lentil germplasm lines by ICARDA with the identification code L as L-422 (ILL-422), L-813 (ILL-813), L-1220 (ILL-1220), L-1462 (ILL-1462), L-2313 (ILL-2313) and L-2684 (ILL-2684). These lines were found resistant to lentil wilt (*F. oxysporum* f.sp. *lentis*).

2.5.5.2 Inheritance of resistance

Kamboj *et al.* (1990) reported that resistance to wilt in Pant L 234 is controlled by 2 dominant duplicate genes that are non-allelic to 2 independent dominant genes with a complementary gene detected in resistant lines JL 446 and LP 286. A third dominant gene complementary to dominant genes in JL 446 and LP 286 is present in susceptible lines JL 641 and L, 9-12. The third gene complementary to 2 genes in resistant lines is non-allelic to the third complementary gene in susceptible lines.

Eujayl *et al.* (1998) investigated the inheritance of resistance to lentil against wilt in a cross between resistant (ILL 5588) and susceptible (L692-16-1(S)) lines. They reported that resistance to wilt was conditioned by a single dominant gene (*fw*) in the population. The map location of the *fw* locus was also identified for the first time through linkage to a Random Amplified Polymorphic DNA (RAPD) marker (OPK-15900) at 10.8 cM. Two other RAPD markers (OP-B17800 and OP-D15500) identified by bulked segregant analysis were associated in the coupling phase with the resistant trait.

2.5.5.3 Mechanism of resistance

Kumar and Jalali (1985) noted differences in seed leachates of wilt susceptible (JG-62) and resistant (CPS-1) genotypes. Susceptible cultivars exude greater amounts of sugars and amino acids than the resistant ones. Root exudates may also determine resistance/susceptibility of the plant to wilt. The conidia of the fungus showed 9 per cent germination in root exudates of resistant cultivar (CPS-1) as compared to 95.4 per cent in presence of exudates from susceptible cultivar (JG-62). The mycelial growth was also adversely affected in exudates from resistant than susceptible cultivars (Haware and Nene, 1984).

Gupta *et al.* (1985) reported that root exudates from susceptible cultivars stimulated the mycelial growth and conidial germination of the wilt fungus whereas, those from the resistant cultivars inhibited them. Aspartic acid, asparagines and histidine were detected only in exudates of the susceptible cultivars, whereas cystine was consistently observed in the exudates of resistant variety. The concentrations of N, P, K, Na, S and Zn were more in resistant plants as compared to susceptible ones, when analyzed chemically.

2.5.5.4 Disease grading

The percentage of dead plants has been used as a criterion for scoring test lines of lentil against wilt. The lines were then grouped into various categories (Khare *et al.* 1976) as:

Resistant	:	0-5 per cent wilting
Moderately resistant	:	5-10 per cent wilting
Susceptible	:	10-30 per cent wilting
Highly susceptible	:	above 30 per cent wilting

2.5.5.5 Induced resistance

It's a phenomenon where a plant has been infected by a microbial agent/chemical. It acquires enhanced resistance to subsequent infection. Constitutive barriers (physical and chemical) present in plant prior to infection are collectively responsible for natural resistance in plants. When a physical, chemical or biological agent induces the production/accumulation of defense components in the host or activates defense mechanism, it is regarded as induced or acquired resistance (Purkayastha, 1998). Induced resistance in plants can be activated by preinfection with necrotizing pathogen or pretreatment of plants with either natural or synthetic chemicals (Kessmann *et al.*, 1994; Agrios, 1997; Sticher *et al.*, 1997). Induced resistance is distinguished from conventional chemicals in plant protection by the lack of toxicity of the inducing agents towards the pathogen. The protection of the plants is not based on the elimination of the pathogen but rather on activation of plant defense mechanism or on the enhancement of their activity (Kessmann, 1994; Sticher *et al.*, 1997).

Yamazaki (1999) reported that application of higher concentration of calcium after inoculating pathogen *Ralstonia solanacearum* into host

tomato seedlings showed reduction in disease severity. It was concluded that the concentration of Ca in the host, especially in the cell walls, before infection may not directly^{be} involved in the Ca -dependent resistance of tomato seedlings to bacterial wilt. Chaudhary (2000) tested the effectiveness of four different non-conventional chemicals of diverse nature at concentration of 10^{-3} to 10^{-6} M in controlling fusarium wilt of chickpea in pot experiment. Among these chemicals, indole acetic acid, cycloheximide and cycocel showed very strong protective effect at lower than at higher concentration in susceptible chickpea cultivar ICC-4951, reducing wilt symptoms by 45 to 57 per cent, preventing mortality appreciably and also limiting vascular colonization by the pathogen.

Plants have been used to control plant pathogen and diseases in various ways. Plant parts and their products have been tested by different methods. Their use is safe and more rewarding. The practice of using plants in controlling plant diseases is very old as references are made in 'Atharaveda' and 'Rigveda'. The science of medicine for plants is described in 'Vrakshayurveda' written by Surapala. Seed treatment was done with powder and ash of amla, sesame, mustard, barhati, vidanga etc. before planting (Ray Chaudhary, 1964; Sadhale *et al.*, 1996).

Garlic (*Allium sativum* L.) has a long history of reputed value and actual use for its medicinal, antimicrobial and pesticidal properties (Fliermans, 1973 and Amonkar and Banerji, 1971). Extracts from garlic bulbs have been used against several human diseases, viz., snakebite,

hemorrhoids, rheumatism, abdominal pains and skin infections (Moore and Atkins, 1977). It has been found to be insecticidal, antiprotozoal, antibacterial and antifungal by several scientists (Barone and Tansey, 1977; Cavallito *et al.* 1945; Buck and Suter, 1944; Rippon, 1974 and Stoll and Seebeck, 1951). The antimicrobial activity is believed to be due to allicin (Cavallite *et al.* 1944).

Upadhyay and Gupta (1990) reported that at 20 per cent extract and 5 per cent ethanol extract, *Allium sativum* inhibited linear growth of *Curvularia lunata* by 44.4 and 55.6 per cent, respectively, while at 10 per cent aqueous solution it gave only 46.6 per cent inhibition as compared to *Datura alba* which gave 87.8 per cent inhibition at 10 per cent aqueous solution and 11.1 per cent and 42.2 per cent inhibition at 20 per cent extract and 5 per cent ethanol extract respectively.

Solanum nigrum, a common leafy vegetable, is routinely cooked as food in South India and considered as medicine (heals ulcer) as reported by Kalai-Chelvan and Sumathi (1992). It produces alkaloids that are antimicrobial agents (Kalai-Chelvan and Nagarajan, 1992) and protects the plants against the invasions of pathogenic colonization. Nearly 100 *Solanum* alkaloids have been isolated so far from nearly 250 species of the family Solanaceae. *S. nigrum* has solarmargine, a steroid that is inhibitory to *Dreschlera oryzae* and *Curvularia lunata*. The reason behind this was supposed to be that low molecular weight solarmargine could have inhibited the fungi by infecting the membrane energy metabolism and with biosynthesis of essential enzymes.

There are fairly a large number of reports on the effectiveness of neem (*Azadirachta indica* A. Juss) plant against insect pests of agricultural importance (Jotwani and Srivastava, 1981). Dharamvir and Sharma (1985) reported 100 per cent inhibition in growth of *Alternaria alternata* at 10 per cent concentration of neem oil.

Antifungal property is neither a family character nor genus one. It varies from family to family, within a family from genus to genus and within a genus from species to species. The plant species, which partially inhibit one fungus, may found to be completely inhibitory to other one (Shekhawat and Prasada, 1971). In case of family Cruciferae, the isothiocyanates present in the plant tissues are responsible for the antifungal activity (Walker *et al.*, 1937). Presence of protocatechuic acid and catechol in onion is responsible for the bursting of young hyphae of the fungus (Walker, 1923). Shekhawat and Prasada (1971) reported the antifungal properties of the extracts of *Allium cepa* L., *Allium sativum* L., *Mentha piperita* L. and *Datura stramonium*, against *Alternaria tenuis*, *Helminthosporium* sp. and *Curvularia penniseti*.

Agarwal and Gupta (1969) also observed antifungal activity of bulb extracts of *Allium sativum*. Anonymous (1972) reported the presence of essential oils in *A. sativum* and *Ocimum sanctums* and fixed oils and cannabinal in *Datura alba* and *Cannabis sativa*, respectively.

Chaurasia and Dayaram (1990) reported that the bulb extract of onion and garlic was found inhibitory to mycelial growth and selerotial

production of *Rhizoctonia solani*. Similar results were also obtained by Sundraj *et al.* (1998). Standard extract (SE) and 1: 10 dilution of SE of datura, garlic, onion, neem and ginger inhibited the teliospore germination of *Neovosia indica* (Sharma and Nanda 2000). Singh *et al.* (1979) reported that aqueous garlic leaf extract inhibit the growth of *Fusarium oxysporum* f.sp. *ciceri* and *Sclerotinia sclerotiorum* in liquid medium. Tansey and Appleton (1975) also reported the inhibition of *Fusarium solani* by applying garlic extract *in vitro*.

Gupta and Singh (1981) reported 97.6 per cent inhibition of teliospore germination at 500-ppm concentration of *Datura stramonium*. Singh *et al.* (1979) also reported complete control of hill bunt of wheat by seed treatment with extracts from *Datura stramonium*. Reddy (1987) reported 42 per cent inhibition of aflatoxin production by *Aspergillus flavus* using extracts of onion bulbs, 40 per cent by extracts of garlic bulbs and 20 per cent inhibition by using neem leaf extract.

Gupta and Bansal (2003) tested leaf extracts of 7 plant species against fenugreek wilt caused by *F. oxysporum* under pot conditions and reported that all leaf extracts except that of *Ailanturs excelsa* showed significant effect over control and the maximum germination of fenugreek seeds was observed with *Atropa belladonna* (90.66%) followed by *Azadirachta indica* (87.99%) and *Lantana camara* (87.99%).

Orlikowski *et al.* (2001) tested the biological activity of grape fruit extract in the control of *F. oxysporum* f.sp. *cyclaminis* and

F. oxysporum f.sp. *dianthi*, causing fusarium wilt of carnation. They reported that Biosept 33SL (containing 33% of grapefruit extract) @ 40 Fg GE /cm³ when amended with PDA, inhibited mycelial growth by about 50 per cent and drenching of carnation with 165 Fg GE/cm³ immediately after planting resulted, drastical decrease in number of colony forming units of the pathogen and increased healthiness of plant stand by about 50 per cent.

Rodriguez and Montilla (2002) tested different concentrations of Citrex (*Citrus paradisi* seed extract) against *F. oxysporum* f.sp. *lycopersici* and reported that it reduced wilt incidence by 85 per cent when the roots were dipped in its solution and was applied in the soil weekly, followed by the combined application to the soil and foliage which resulted in 64 per cent reduction.

3.6 Integrated Disease Management

An integrated control that denotes the rational use of all available control measures should be considered especially with a crop, which is attacked, simultaneously by numerous types and kinds of pathogens (Elad *et al.*, 1980).

Integrated disease management (IDM) with its pragmatic approach is slowly but surely transforming modern disease management from the rigid confines of conventional chemical control to flexible, eco-friendly, multicomponent nouveau protocols. It is an alternative to the problems, which have been caused due to:

- Widespread introduction of monogenic 'r' cultivars.
- The misuse of chemical (pesticides).
- Destruction of natural population of biological control agents.
- Abandonment of cultural control practices.
- Pesticide resistance in pathogens.
- Environmental pollution, etc.

It has been reported that the integration of biological seed treatment with lower dose of fungicide improved the degree of disease control. De and Chaudhary (1999) assessed the effect of seed treatment using fungicide alone or in combination with different biocontrol agents as well as kerosene oil against *F. oxysporum* f.sp. *lentis* and reported that *Bacillus subtilis* in combination with carboxin (Vitavax) showed 66 per cent wilt control and 45 per cent increase in yield, whereas in separate combinations with *Gliocladium virens*, *T. harzianum* or *T. viride*, reduced wilt incidence by 79 per cent and increased yield by 140, 224 and 241 per cent, respectively. *Pseudomonas fluorescens* in combination with *T. viride* and carboxin (Vitavax) decreased wilt incidence by 65 per cent and increased yield by 229 per cent. Kerosene oil reduced wilt by 31-46 per cent and increased yield by 48-52 per cent, it also reduced seed germination by 16-17 per cent.

Roy *et al.* (1998) reported that wilt complex disease of banana can be controlled by integrating some control measures like flooding, application of inorganic fertilizers (NPK @ 225, 75 and 60 kg/ha,

respectively) in soil amended with organic compound (compost/cake), and pesticides.

Naik and Sen (2002) reported the management of fusarial wilt of watermelon by rhizosphere manipulation of non-cucurbitaceous crops. They concluded that among the seed exudates, garlic, radish and onion were detrimental to germination of micro conidia of *F. oxysporum* reducing 30-70 per cent germination.

Ahmed *et al.* (2002) integrated host resistance with planting date and fungicide seed treatment to manage lentil wilt and reported that lentil genotype had a greater effect on the onset and duration of fusarium wilt than planting date or fungicide seed treatment. The percent terminal wilt and areas under the disease progress curve were lowest during November plantings for all genotypes.

The individual and combined effect of plant extracts, biological control agents and chemicals on foliar anthracnose and pod blight of soybean (caused by *Colletotrichum truncatum*) was studied by Chandrasekaran and Rajappan (2002). They reported that alum at 1 and 0.1 per cent with leaf extract of *Lawsonia inermis* showed 100 per cent reduction in pod blight *in vitro* while in the pot experiment it gives 10 per cent and 7 per cent reduction of anthracnose and pod blight, respectively and it was *at par* with the disease incidence by seed treatment + foliar spray of *L. inermis* (1%) + *T. viride* 4 (0.4%) + alum (0.1%).

Materials

and

Methods

Materials and Methods

The field experiments were conducted consecutively for two *rabi* crop seasons 2001-2002 and 2002-2003 at Crop Research Centre (CRC) of G.B. Pant University of Agriculture & Technology., Pantnagar (Uttaranchal). A wilt susceptible variety, L, 9-12 of lentil (*Lens culinaris* Medik.) was used through out the investigation.

Pantnagar falls in the humid and subtropical (*Tara*) climate of North West Plain Zone (NWPZ). The zone lies at the foothills of Shivalik range in lower Himalayas. It is situated at 29°N latitude and 79.73°E longitude, at an altitude of 243.8 meters above the mean sea level (MSL).

3.1 Observation of Symptoms

The sequential development of symptoms of the disease were studied on naturally infected plants in experimental plots at CRC as well as on artificially inoculated plants in glasshouse during present investigations.

3.2 The Pathogen

3.2.1 Isolation

Lentil seedlings showing symptoms of wilt were collected from the field and were washed thoroughly with water and thereafter placed in between blotting papers to remove excess moisture, if any. The infected

parts were cut aseptically into small pieces and surface sterilized with mercuric chloride (0.1%) for one minute followed by two to three washing with sterilized distilled water and then placed aseptically on solidified PDA in petriplates. The inoculated petriplates were incubated at $25 \pm 1^{\circ}\text{C}$ for 8 days. Mycelial growth appeared on PDA. A single spore was transferred on PDA and was sub cultured to maintain pure culture. The pure culture was maintained in the refrigerator at 5°C for further studies.

3.2.2 Pathogenicity test

The test pathogen was multiplied separately on sand maize meal medium (Vasudeva and Srinivasan, 1952). Maize meal 10 g, sand 90 g and sufficient water to moisten the medium was thoroughly mixed in a tray and placed in 250 ml capacity Erlenmeyer flasks and autoclaved. Each flask containing 100 g sand maize meal mixture was inoculated with a 8 mm mycelial disc of pure culture of the fungus and incubated at $25 \pm 2^{\circ}\text{C}$ for 10 days. Flasks were shaken intermittently so as to get a fairly uniform growth of the inoculum on the medium.

3.2.3 Soil inoculation

Fungus colony grown on sand maize meal medium was used for artificial inoculation studies. Sand maize meal medium with uniform fungal growth was mixed with autoclaved soil @ 6 g/kg soil and filled in 5 kg capacity plastic pots. Thereafter, inoculated pots were kept for 3 days for the establishment of the pathogen in the soil. Ten surface

disinfected seeds of a susceptible lentil cultivar L, 9-12 were sown in each pot at equi distance. Pots were watered regularly and maintained with appropriate moisture level. After germination, the data were recorded for diseased seedlings commencing from 7th day of sowing upto 30 days. Reisolation of the causal fungus was confirmed on PDA.

3.3 The Antagonists

The following antagonists were screened for their effectiveness against wilt causing pathogen: *Trichoderma harzianum* Rifai, *Pseudomonas fluorescens* and *Pseudomonas* strain 82.

T. harzianum was procured from Rice Pathology Laboratory while *Pseudomonas fluorescens* and *Pseudomonas* strain 82 were procured from culture collection of Biocontrol Laboratory, Department of Plant Pathology. All the cultures were multiplied on PDA and stored in a refrigerator at 10°C for further studies.

3.3.1 Screening of the antagonists

The antagonism of *T. harzianum* against the test pathogen was studied by employing dual culture technique (Mortan and Stroube, 1955). Twenty ml of sterilized and melted PDA was poured into 90 mm sterilized petriplates. After the solidification of medium, the petriplates were inoculated with 6 mm disc of test pathogen and the antagonist taken from the edge of a 3-day-old culture and placed towards the edge opposite to each other (approximately 4 cm away). Four replications

were maintained, while in case of *Pseudomonas fluorescens* antibiosis technique was used.

3.3.2 Mass multiplication of antagonists

The fungal antagonists were multiplied on sterilized pre boiled sorghum grains. In this medium sorghum grains, anhydrous dextrose and water was taken in ratio of 1: 0.05: 1.25 w/w/v. Erlenmeyer conical flasks of 250 ml capacity were filled with pre boiled sorghum grains and were inoculated with 3 mm disc cut from 3 days old culture of antagonists maintained on PDA petriplates. Conical flasks were incubated at $28 \pm 2^{\circ}\text{C}$ for 20 days and thereafter, colonized sorghum grains were air dried and powdered. The powder was sieved through 80-mesh sieve.

King's B liquid medium was used for mass multiplication of *Pseudomonas fluorescens*.

3.3.3 Composition of medium

For the multiplication of *Trichoderma*, modified *Trichoderma* selective medium (TSM) (Mukherjee *et al.* 1989) was used in the present study.

Composition of TSM :

MgSO ₄ .7H ₂ O	:	0.2 g
K ₂ HPO ₄	:	0.9 g
KCl	:	0.15 g

NH ₄ NO ₃	:	1.0 g
Glucose	:	3.0 g
Chloramphenicol	:	0.25 g
Apron 35 SD	:	0.015 g
PCNB	:	0.2 g
Rose Bengal	:	0.15 g
Agar	:	20.0 g
Water	:	1.0 lt

For *Pseudomonas fluorescens* King's B medium (KMB) was used.

Composition of KMB

Proteose peptone	:	20.0 g
Potassium sulphate	:	1.5 g
Magnesium sulphate	:	1.5 g
Glycerol	:	8.0 ml
Agar	:	20.0 g
Distilled water	:	1000 ml
Cycloheximide	:	75.0 mg
Novobiocin	:	45.0 mg
Penicillin	:	75.0 mg
pH	:	7.0

Antibiotics (cycloheximide, Novobiocin and penicillin) were dissolved in 3.0 ml of 90 per cent ethanol and added into the medium .

after autoclaving when medium was cooled to 40°C. The pure culture was maintained by frequent sub culturing on King's medium B and preserved in refrigerator at 5°C for further studies.

3.4 Histopathology

Healthy and wilted lentil plants of 45 days old crop were taken from field and the transverse sections were cut with a microtome to study the root/stem anatomy of healthy and wilted plants. The micro technique includes:

i) Killing and fixation

The stem was taken, washed thoroughly and surface sterilized with 0.1 per cent HgCl₂ solution for 1 minute. After that sections were cut having both healthy and wilted part and the callus tissue was fixed in FAA for 24 hours.

ii) Dehydration

The callus tissue was dehydrated in ethyl alcohol series for different time intervals and depicted in the table dehydration series.

S. No.	Ethyl alcohol (%)	Distilled water (ml)	Time interval (h)
1.	70	30	12
2.	90	10	1
3.	95	5	1
4.	100	0	1

iii) Cleaning and dehydration

After dehydration the tissues were processed in xylene and absolute alcohol series for different time intervals as:

S. No.	Xylene (ml)	Alcohol (%)	Time (min.)
1.	25	75	60
2.	50	50	45
3.	75	25	30
4.	100	0	10

iv) Infiltration and embedding

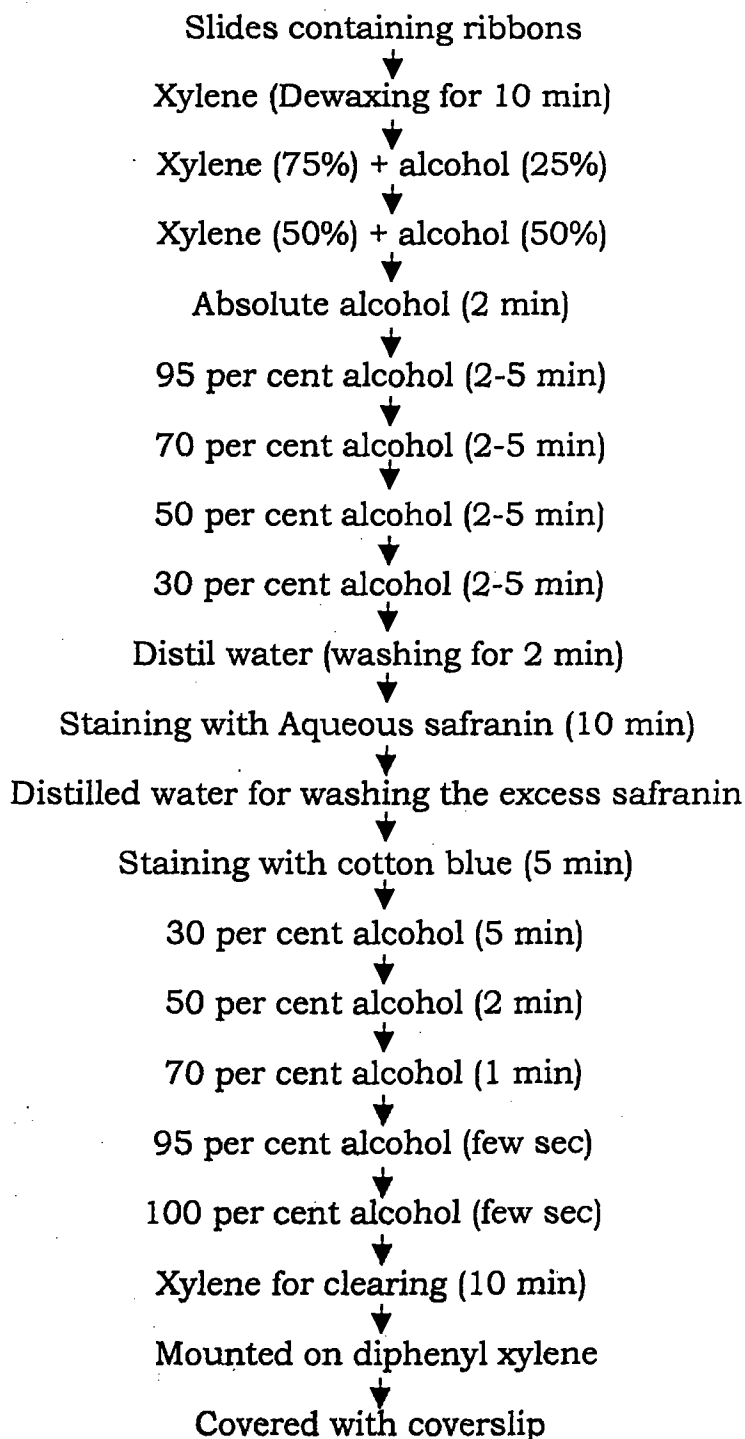
The callus tissue was infiltrated with paraffin wax (55-58°C). Small paraffin chips were gradually added to vials and the excess xylene was removed from the vials by decanting and the vials were kept in an oven at 58°C. Paraffin chips were added till the removal of xylene from the tissue. The infiltrated tissue with pellet was poured into the paper boats and kept for cooling and hardening at room temp. The process of enclosing the tissue in a block of solidified paraffin is called embedding.

v) Section cutting

The block was divided into small pieces each containing the callus tissue and trimmed into rectangular shape. Each piece was fixed on block holder with the help of a heated scalped. After coating, the block holder was affixed to the clamp on the rotary microtome and 10 μ m thick sections were cut. The ribbons were collected, divided into small pieces and fixed to slide.

vi) Staining and mounting

Sections were stained with safranin/aniline blue and mounted in diphenyl xylene.

vii) Flow diagram for staining procedure

3.5 Effect of Non-Volatile Compounds on Test Pathogen

Potato Dextrose Agar medium was used for production of antibiotics by different isolates of *Trichoderma* spp., employed in present investigation. Erlenmeyer flasks (250 ml) containing 100 ml medium were inoculated with 0.6 cm diameter mycelial disk cut from the margin of 3 days old culture of *T. harzianum* and were incubated at $28 \pm 2^\circ\text{C}$ for 12 days. Flasks were shaken horizontally on every alternate day. After 12 days the mycelial mat was first filtered through Whatman No. 1 filter paper and then using 3 G and 5G bacteria proof filters to eliminate the phialospores of *T. harzianum*. Ten ml of this extract was mixed in 90 ml of melted ($40 \pm 2^\circ\text{C}$) PDA and poured in sterilized petridishes. After solidification, 0.6 cm disk of *F. oxysporum* f.sp. *lentis* was placed in the center of each dish. Four replications were maintained. Dish inoculated with test pathogen but not amended with extract of *T. harzianum* served as check. Colony diameter was recorded after 48 hours of incubation at $28 \pm 2^\circ\text{C}$, and finally per cent inhibition of mycelial growth was calculated using following formula:

$$I = \frac{C - T}{C} \times 100$$

where,

I = per cent inhibition

C = radial growth in check (mm)

T = radial growth in treated plates (mm)

3.6 Effect of Volatile Compounds on Test Pathogen

T. harzianum was grown on PDA in 90 mm diameter petridishes for 9 days at $28 \pm 2^\circ\text{C}$. After a given period i.e. 2, 4, 6 and 8 days, the lid of dish was replaced by the bottom containing 20 ml and inoculated centrally with test pathogen. The two dishes were taped together with 1" wide cellophane adhesive tape. The control was maintained with test pathogen at the upper one and with out *T. harzianum* at the lower one. The colony diameter was measured and compared to that with the control plates after 48 h.

Per cent inhibition of the mycelial growth as a result of addition of culture filtrate was calculated by using the formula:

$$I = \frac{C - T}{C} \times 100$$

where,

I = per cent inhibition

C = radial growth in check (mm)

T = radial growth in treated plates (mm)

3.7 Effect of Micronutrients

Four micronutrients, calcium as CaCl_2 , magnesium as MgSO_4 , zinc as ZnSO_4 and ammonium as ammonium salt were tested against *F. oxysporum* f.sp. *lentis*. Five different concentrations (0, 100, 200, 500 and 1000 ppm) were mixed in 100 ml PDA medium and poured in 90 mm petridishes. A disc of 3 days old culture was placed in the centre.

After 48 hours radial growth of pathogen was measured. Media without micronutrients served as check.

3.8 Disease Management

3.8.1 Effect of fertilizers on disease incidence and grain yield

The field trials were conducted during *rabi* 2001-2002 and 2002-2003 crop season in RBD with four replications. The plot size was 3.0 × 1.5 m². Cultivar L, 9-12 was sown in each plot at 30 cm apart. The details of fertilizers along with their doses are given below for each experiment and were applied in the plots before sowing.

Treatments	Dose
Diammonium phosphate (DAP)	100 kg/ha
Single super phosphate (SSP)	290 kg/ha
Compost	2.160 kg/m ²
Single super phosphate (SSP) + urea	1:1
Urea	40 kg/ha
Check (no fertilizer)	0

Data on disease incidence was recorded at 10-day interval starting from disease appearance till maturity of the crop. Yield (kg/ha) and 1000-grain weight (g) was also recorded.

3.8.2 Effect of micronutrients on disease incidence and grain yield

Experiment was conducted during *rabi* 2001-2002 and 2002-2003, in RBD with four replications. The field preparation, plot size and sowing was done in similar way as discussed in previous experiments.

The micronutrients were obtained from Soil Science Department of the university. The details of micronutrients along with their dose used in the present study are given below. These were applied just before the sowing of lentil seeds in plots.

Treatments	Dose (kg/ha)
Calcium chloride (CaCl_2)	5.00
Magnesium sulphate (MgSO_4)	5.00
Ammonium salt	1.00
Zinc sulphate (ZnSO_4)	25.00
Check	0.00

Observations on disease incidence were recorded at 10-day intervals till the physiological maturity of the lentil crop. Grain yield (kg/ha) and 1000-grain weight (g) was also recorded.

The above micronutrients were also tested against wilt incidence under glass house condition. The soil was artificially inoculated with the wilt pathogen multiplied in sand-maize-meal medium in the laboratory. Ten seeds of susceptible cultivar L, 9-12 were sown in each pot. There were four treatments along with check each having 3 replications. Data on percent germination and mortality of seedlings was recorded at 10-day interval.

3.8.3 Efficacy of plant extract and detergent

The following plant extract were tested against wilt pathogen after selecting effective dilution:

Treatments	Dilution
<i>Allium sativum</i>	1: 10
<i>Azadirachta indica</i>	1: 10
<i>Datura stramonium</i>	1: 10
<i>Solanum nigrum</i>	1: 10
Detergent (surf)	2: 1000
Check	-

Preparation of crude plant extracts

Fresh plant material (1.0 g) was washed, frozen (30 min) and homogenized in distilled sterilized water (1: 1 w/w) and then filtered through double layer of sterilized muslin cloth. These extracts were designated to be 100 per cent concentration.

Surface sterilized lentil seeds were dipped in the freshly prepared solution of above extracts and chemical, separately for 24 hours. Five seeds were sown in each pot filled with *F. oxysporum* f.sp. *lentis* infested soil. The observations were recorded for seed germination, seedling mortality and thereafter per cent disease control was calculated.

3.8.4 *In vitro* bioassay of plant extracts

Five different plant species namely *Allium sativum*, *Allium cepa* var. *viviparium*, *Datura stramonium*, *Mentha spicata* and *Solanum nigrum* were assessed against lentil wilt pathogen, by bioassaying their extracts in cold water, alcohol and acetone with three concentrations at

5, 10 and 20 per cent. The percentage inhibition of spore germination was calculated after 48 hours at $25 \pm 2^\circ\text{C}$.

3.9 Integrated Disease Management (IDM)

An experiment was conducted in the glass house and subsequently in the field to study the role of bioagents and chemicals either alone or in combination, on wilt incidence of lentil.

In the glass house experiment, six treatments were taken along with check. These were: *Trichoderma harzianum*, *Pseudomonas fluorescens*, *Pseudomonas* strain 82, vitavax + *T. harzianum*, vitavax alone and vitavax+thiram. The efficacy of these treatments, on percent germination and mortality of lentil seedlings was observed. For this, the soil was artificially inoculated with wilt pathogen, multiplied in the sand-maize-meal medium in the laboratory. Three replications with 10 seeds per pot were taken. Data was recorded at 10-day intervals and finally disease control was calculated.

Similar experiment was conducted on IDM during *rabi* 2001-2002 and 2002-2003, crop seasons in RBD with four replications. The plot size was $3.0 \times 1.5 \text{ cm}^2$ with row spacing of 30 cm. Seeds of susceptible cultivar L, 9-12 was sown on 27 November (2001-2002 crop season) and 12 November (2002-2003 crop season). The seeds were treated with bioagents and chemicals 24 hrs prior to sowing. *T. harzianum*, *Pseudomonas fluorescens* and *Pseudomonas* strain 82 were

used alone @ 5 g/kg seed, vitavax alone was applied @ 2.5 g/kg seed and vitavax along with *T. harzianum* was treated in 1 : 4 ratio whereas vitavax with thiram was used in 1 : 2 ratio.

Observations on incidence of wilt disease were recorded at 10-day intervals starting from first appearance of the disease in the field till maturity of the crop. Grain yield (kg/ha) and 1000-grain weight were also recorded.

In another experiment, conducted under glass house, the above biocontrol agents were tested alone with zinc and alum and also in combination with zinc and alum both. Three replicates, each having 5 seeds per pot was treated with these agents. A check was also maintained with these treatments. The data was recorded at 10-day intervals on per cent germination and mortality of lentil seedlings.

3.10 Data Analysis

The data was analysed statistically at the computer centre of G.B. Pant University of Agriculture and Technology, Pantnagar using Completely Randomized Design (CRD) and Randomized Block Design (RBD). Data recorded in percentage were first angular transformed to arcsine values before analysis and the treatments were compared by the means of critical differences (CD) at 0.05 level of significance.

Experimental Results

Experimental Results

4.1 Symptomatology

The symptoms of wilt may occur at any stage during plant growth. The plants may show partial wilting or complete wilting. At pre-emergence stage, seedlings don't emerge above soil surface and immediately after seed germination they die. Sometimes seeds rot even before germination. On splitting such seeds, browning at the micropylar end is visible. The seedlings infected 4-5 days after emergence exhibit browning of radicles. They remain stunted, droop and finally collapse.

With the advancement of season when infection occurs at adult stage, the plants show symptoms of true vascular wilt. The leaves turn yellow and curl from the lower part of the plant upwards and the growth of the plant is checked. The upper top of the plant droops and ultimately infected plant dies. Roots of the affected plant turn yellowish-brown to dark brown partially or wholly (Plate 1 A). No fungal growth was observed on the root surface but upon splitting the root, the walls of the xylem vessels show discolouration (Plate 1 B). The root nodules are poorly developed on infected plants.

4.2 The Pathogen

4.2.1 Isolation and Identification

The isolated fungus was identified as *F. oxysporum* f. sp. *lentis* on the basis of the morphological and cultural characteristics. The fungus

Plate - 1



Healthy (L) and Wilted (R)



Split roots showing discolouration



Lentil plants showing typical wilt symptoms

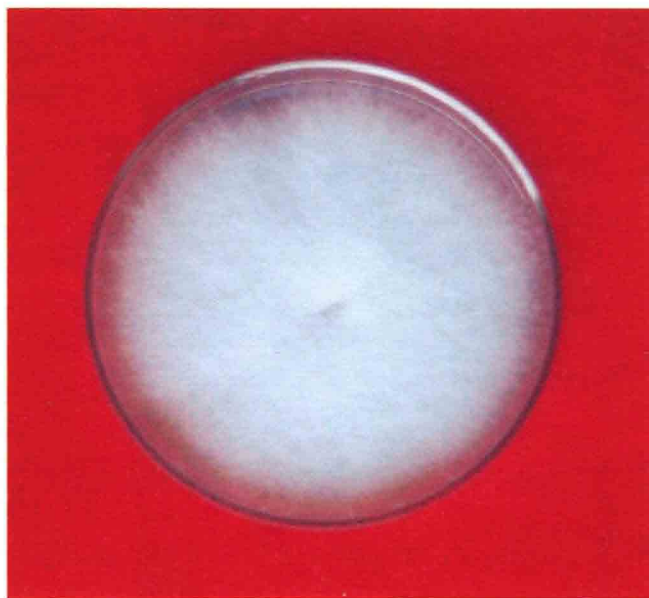
produces whitish mycelium on PDA (Plate 2 A). The mycelium of the fungus was cottony at the beginning but it turned pinkish within 3-4 days. The mycelium was septate and produced both macro-and micro-conidia (Plate 2 B). Macro-conidia were hyaline sickle shaped with both the ends pointed, 3 to 5 septate $3.5-4.5 \times 25-39 \mu\text{m}$. Micro-conidia were mostly single celled, few two celled, ellipsoid to ovate, measuring $2.5-3.5 \times 5.0-11.0 \mu\text{m}$. Chlamydospores were thick walled, spherical, terminal or inter calary and formed singly or in pairs. (Plate 2 C).

4.2.2 Pathogenicity test

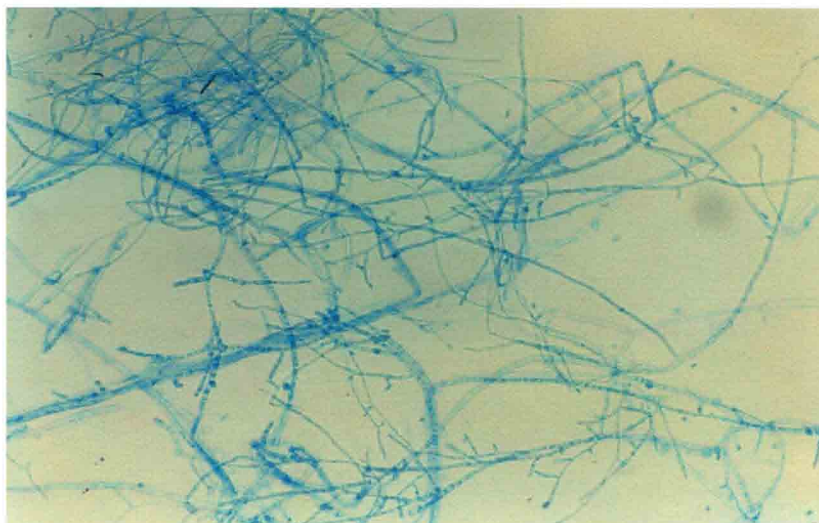
The pathogenicity test was determined by artificial inoculation on susceptible lentil plants raised in plastic pots in glasshouse. The typical symptoms of the disease appeared after 30 days of sowing. The symptoms were identical to those recorded and described in naturally infected lentil plants (Plate 1 C).

4.2.3 Mass multiplication of fungus for soil inoculation

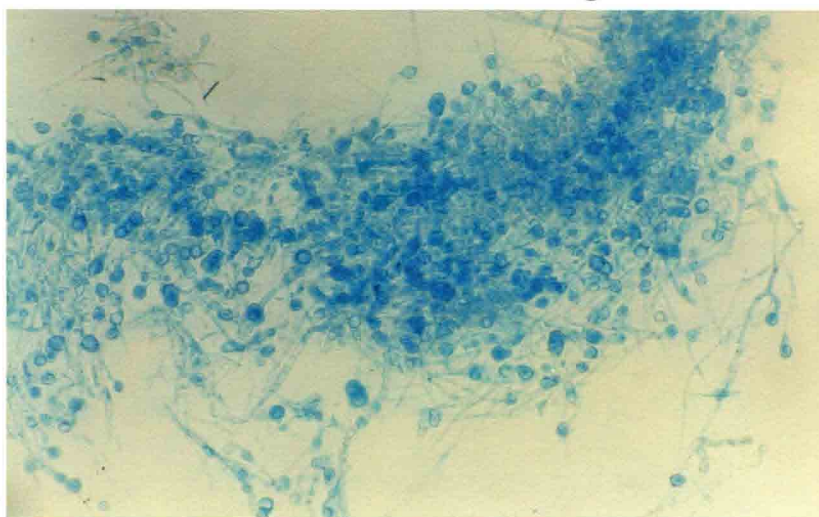
The fungus was multiplied on sand-maize-meal medium. The medium was autoclaved at 1.05 kg/cm^2 for 30 minutes. The sterilized medium was aseptically inoculated with pure culture of *F. oxysporum* f. sp. *lentis*. The inoculated flasks were incubated at $25 \pm 1^\circ\text{C}$ for 15 days. The fungus produced abundant conidia and mycelium on medium that was further used in different experiments conducted in the glasshouse.



A. Whitish cottony growth of *F. oxysporum* f.sp. *lentis* on PDA



B. Macro and micro conidia of fungus



C. Chlamydospores of fungus

Plate -2 : Morphological characteristic of *F. oxysporum* f. sp. *lentis*

4.3 Antagonists

4.3.1 Morphology and mass multiplication of fungal antagonists

4.3.1.1 Morphology

***Trichoderma harzianum* Rifai**

The fungal colony on PDA was fast growing, floccose, tufted and white to green in appearance (Plate 3 A). Conidiophores were erect, ramified and verticillate with flask shaped phialide. Phialospores were smooth, green, sub-globose to short obovoid, $2.4-3.2 \times 2.2-2.8 \mu\text{m}$. Chlamydospores were intercalary, globose, hyaline and smooth walled (Plate 3 B).

4.3.1.2 Mass multiplication

The antagonists grew profusely when inoculated on sorghum grain medium. The medium in Erlenmeyer flasks was completely covered with mycelium and profuse conidia and/or chlamydospores of the fungal bioagent.

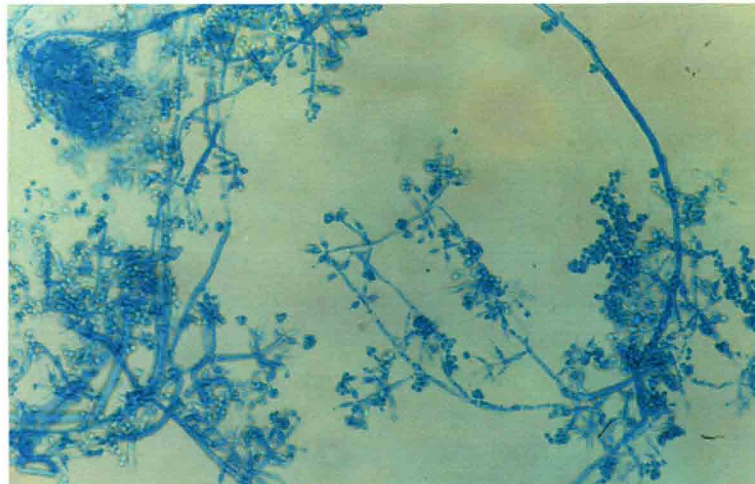
4.3.2 Bacterial antagonists

Pseudomonas fluorescens

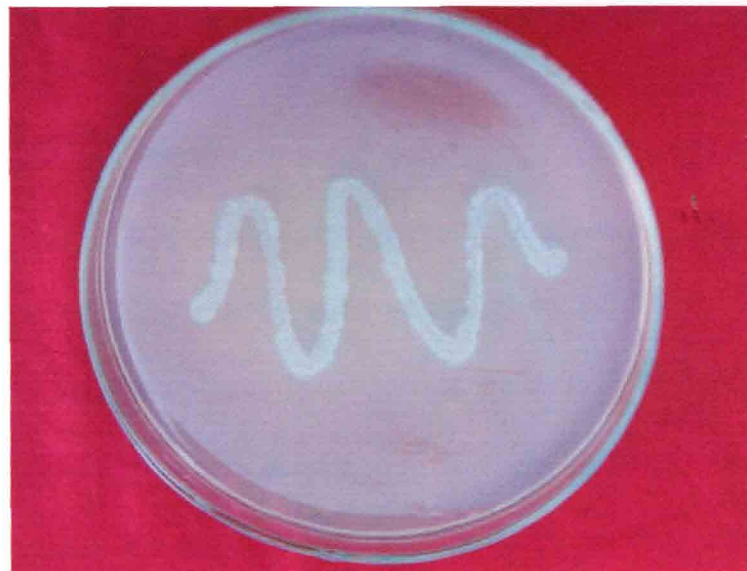
Pseudomonas fluorescens produced off-white or cream coloured colonies on King's B medium after 48 hours of incubation at $28 \pm 2^\circ\text{C}$. When the colonies grew older, the surroundings of the colonies showed a greenish hue. This was due to siderophores production.



A. Mycelial growth of *T. harzianum* on PDA



B. Conidia and conidiophore of *T. harzianum*



C. Colony of *Ps. fluorescens* on king's B medium

Plate -3 : Morphological characteristic of Antagonists

4.4 Histopathological Studies

The histopathology of roots of healthy and infected plants revealed that the *Fusarium oxysporum* gained entry through natural openings or secondary roots/rootlets. The infected plants had greater number of secondary roots as compared to healthy plant roots. Internal structure of healthy roots [Plate 4 D, E, F] revealed that cork cambium cells were more compact and multilayered as compared to infected one. The phellogen was fully developed while in the infected roots [Plate 4 A,B,C]fungus colonized the metaxylem and stopped the supply of water and minerals. The diameter and the thickness of metaxylem was more in infected root tissues as compared to the healthy root tissues.

4.5 Disease Management

4.5.1 Cultural practices

4.5.1.1 Effect of date of sowing and environmental factors on disease incidence and grain yield

The field experiments were conducted consecutively during *rabi* 2001-2002, and 2002-2003 crop seasons to observe the effect of dates of sowing on wilt incidence and grain yield of lentil, if any. During 2001-02 sowing was done late in the season (27 Nov.) while during 2002-03 crop season early sowing (12 Nov.) was done.

The results revealed (Table 1, 2; Fig. 3, 4) that disease incidence was decreased when sowing was done slightly late in the season. In early sown crop (12 Nov.) there was maximum wilt incidence, which

Table 1 : Effect of late sowing on disease incidence of lentil wilt at different weather factors during 2001-02 crop season

Month	Week	Disease incidence (%)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)	Wind Speed (km/h)
Dec.01	51	2.23	14.0	79.5	0.4	0.7
	52	1.85	13.7	73.5	0	0.9
Jan. 02	1	0.69	11.95	71.0	0	0.6
	2	1.65	13.50	69.5	0	1.2
	3	2.14	14.6	81.0	58.0	1.4
	4	1.49	12.9	69.0	2.6	1.7
	5	1.41	12.8	69.5	0	2.3
Feb.02	6	1.63	16.7	74.0	17.6	3.2
	7	0.76	15.9	74.5	42.0	1.7
	8	2.84	18.5	68.0	7.2	2.6
	9	1.74	17.1	70.0	2.2	3.2
Mar. 02	10	1.72	19.0	66.5	0	1.6
	11	2.32	19.5	66.5	0	2.1
	12	4.17	24.2	60.0	0	0.9
	13	3.39	23.7	55.5	0	1.7
Apr. 02	14	2.72	24.9	55.5	29.0	2.0
	15	2.93	25.8	51.5	0	1.9
	16	4.03	29.5	41.5	0	3.3
	17	3.45	27.5	49.5	54.0	6.3
	18	3.79	28.7	50.0	0	4.4

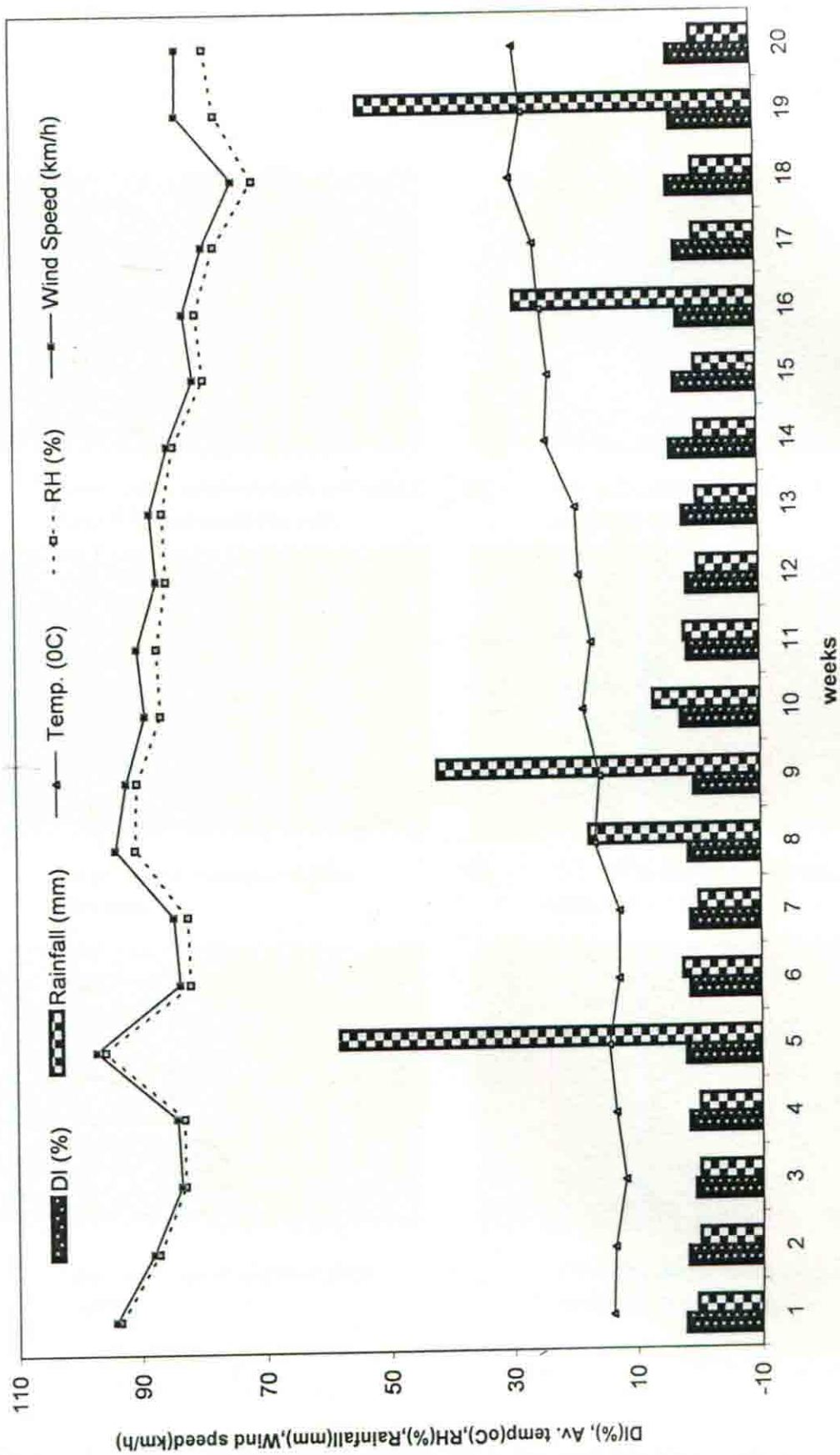
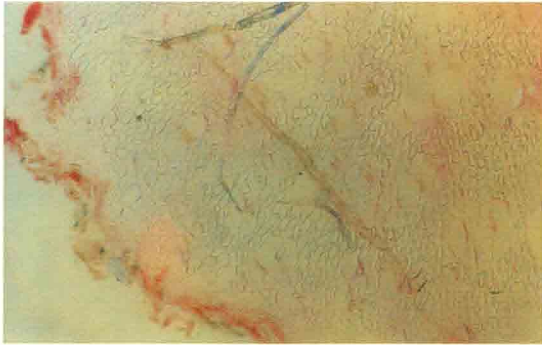
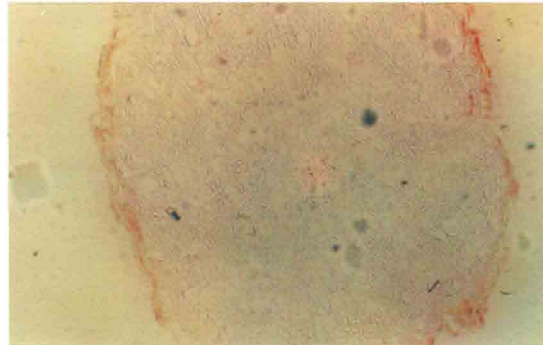


Fig. 3 : Effect of Late sowing on disease incidence of Lentil wilt at different weather factors during 2001-02 crop season



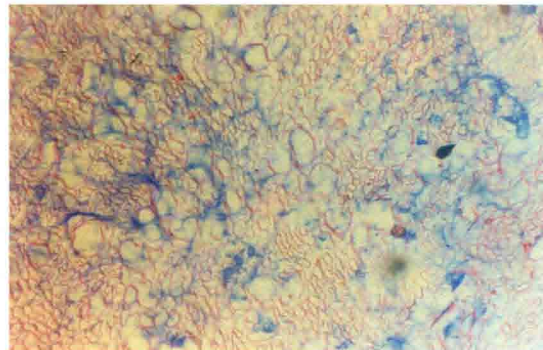
A. Loose cork cambium cells with mycelium of fungus inside the root



D. T.S. of healthy root with compact cork cambium cells



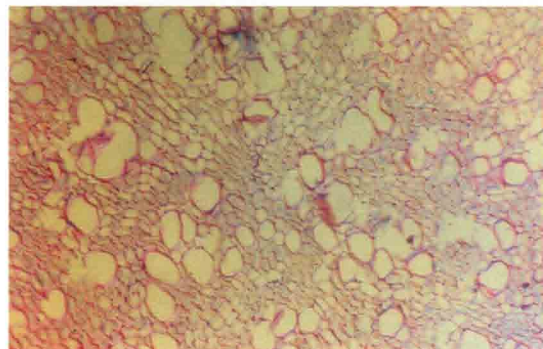
B. Large metaxylem vessels with mycelium



E. T.S. of healthy root showing narrow metaxylem vessels



C. Large and ruptured metaxylem vessels



F. T.S. of healthy root showing thin and narrow metaxylem vessels

Plate -4 : T.S. of healthy and wilt infected roots of lentil plants

Table 2 : Effect of early sowing on disease incidence of lentil wilt at different weather factors during 2002-03 crop season

Month	Week	Disease incidence (%)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)	Wind Speed (km/h)
Dec.02	49	15.48	16.3	66.5	0	1.4
	50	13.13	15.8	68.0	0	1.5
	51	12.42	15.6	72.0	0	2.7
	52	8.76	13.2	81.0	6.4	4.0
Jan. 03	1	14.93	11.3	85.5	56.6	3.7
	2	7.78	7.0	88.5	0	3.3
	3	8.03	9.3	87.5	0	3.2
	4	11.11	10.9	85.2	0.2	2.4
Feb.03	5	15.93	13.7	82.0	32.2	4.8
	6	13.26	15.0	68.5	0	1.9
	7	16.21	15.4	73.0	1.0	2.2
	8	5.69	14.8	77.5	57.4	5.4
Mar. 03	9	20.28	18.2	74.5	9.8	4.1
	10	0.86	15.2	70.5	0	5.6
	11	1.97	20.4	67.5	0	3.4
	12	2.92	21.3	65.0	0	3.8
Apr. 03	13	2.83	22.7	63.5	9.0	4.4
	14	3.70	23.5	54.5	0.4	4.6
	15	6.72	27.1	48.5	0	3.9
	16	4.37	27.6	44.0	1.2	4.7
	17	3.30	28.9	46.5	25.2	6.8

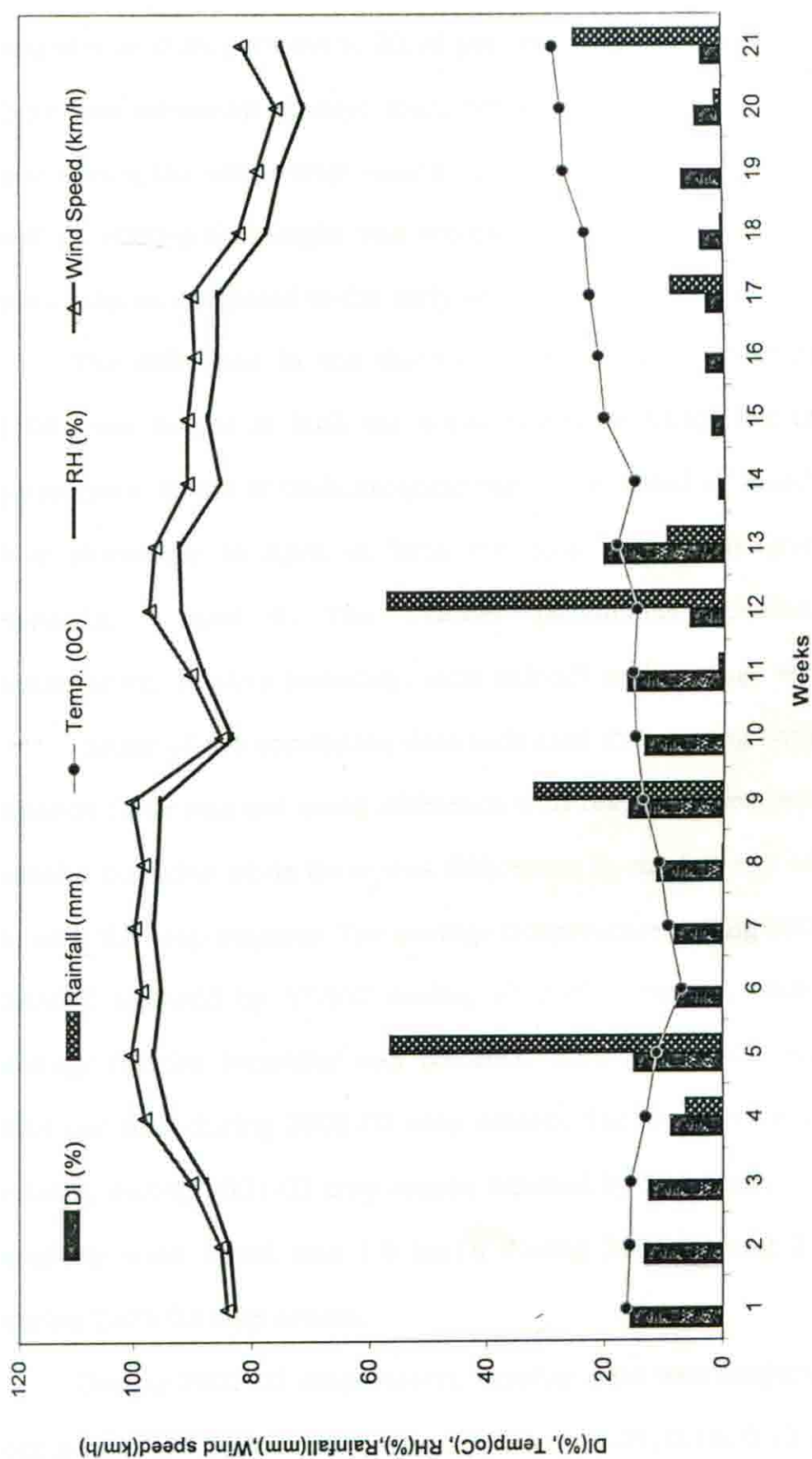


Fig. 4 : Effect of early sowing on disease incidence of Lentil wilt at different weather factors during 2002-03 crop season

ranged from 0.86 per cent to 20.28 per cent, while in the late sown crop there was minimum disease incidence ranging from 0.69 to 4.17 per cent during the whole crop season. Comparatively higher grain yield as well as 1000-grain weight was recorded in all the treatments in late sown crop as compared to the early sown crop.

The difference in the disease incidence, grain yield as well as 1000-grain weight in both the seasons was probably due to weather parameters. Effect of meteorological factors recorded at weekly interval from November to April in both the crop seasons is given in the Appendix 5 and 6. The weather parameters included average temperature, relative humidity, total rainfall and average wind speed.

Study of the correlation data indicated that during both the crop seasons there was not much difference with respect to temperature and relative humidity while there was differences in rainfall and wind speed in both the crop seasons. The average temperature during 2001-02 was 18.68°C followed by 17.9°C during 2002-03, whereas, 66.5 per cent average relative humidity was recorded during 2001-02, followed by 68.4 per cent during 2002-03 crop season. There was total 238.6 mm rainfall, during 2001-02 crop season followed by 201.4 mm in 2002-03, similarly wind speed was 1.9 km/h during 2001-02 and 3.47 km/h during 2002-03 crop season.

During 2001-02 crop season, weather data was subjected for the calculation of 4 partial regression coefficient, 0.34, 0.19, 0.13 and -3.05

values were obtained for the independent variables viz., temperature ($^{\circ}\text{C}$), relative humidity (%) rainfall (mm) and wind speed respectively on the dependent variable, the disease incidence. Based on the partial regression coefficient a linear equation was derived to predict the disease incidence, depending upto the weather conditions prevailing during the crop season.

$$Y = a + (0.34) X_1 + (0.19) X_2 + (0.13) X_3 + (-3.05) X_4$$

where,

Y = disease incidence

a = 3.93,

X_1 = average temperature ($^{\circ}\text{C}$)

X_2 = average relative humidity (%)

X_3 = total rainfall (mm)

X_4 = average wind speed (km/h)

Positive correlation was observed with temperature, relative humidity and rainfall whereas negative correlation was observed with wind speed.

During 2002-03 crop season, positive correlation was observed with temperature and relative humidity while rainfall and wind speed showed negative correlation. The weather data was subjected for the calculation and values of 4 partial regression coefficients 0.21, 0.32, -0.81 and -0.49 were obtained for the independent variables viz.,

temperature (°C), relative humidity (%), rainfall (mm) and wind speed (km/h) respectively on the dependent variable, the disease incidence.

Based on the partial regression coefficient a linear equation was derived to predict the disease incidence, depending upon the weather conditions prevailing in the season.

$$Y = a + (0.21) X_1 + (0.32) X_2 + (-0.81) X_3 + (-0.49) X_4$$

where,

$$a = 13.79$$

$$Y = \text{disease incidence}$$

$$X_1 = \text{average temperature (°C)}$$

$$X_2 = \text{average relative humidity (\%)}$$

$$X_3 = \text{total rainfall (mm)}$$

$$X_4 = \text{average wind speed (km/h)}$$

These equations gave close rating of disease incidence to the prevailing weather conditions in both the crop seasons. Such attempt would help in predicting disease incidence in a given locality.

4.5.1.2 Effect of fertilizers

The results obtained on the effect of various fertilizers on the disease incidence are presented in (Table 3; Fig. 5; Plate 5)

The disease incidence was significantly less in all the treatments compared with check during 2002-03 crop season, however, during 2001-02, significantly lower disease incidence (1.08 per cent) was recorded in

Table 3 : Effect of fertilizers on disease incidence, grain yield and 1000-grain weight

Treatment	Dose (kg/ha)	Disease Incidence (%)		Decline in DI (%)		Grain Yield (Kg/ ha)		Increase in grain yield (%)		1000-grain weight (g)	
		2001-02	2002-03	2001-02	2002-03	2001-02	2002-03	2001-02	2002-03	2001-02	2002-03
DAP	100	2.51 (8.92)*	6.40 (14.65)	45.2	34.4	392.05	352.50	70.6	19.5	14.56	13.81
SSP	290	1.61 (7.27)	3.99 (11.53)	64.8	59.1	439.88	398.75	91.3	35.2	13.93	14.15
Compost	2150	2.94 (9.83)	4.25 (11.88)	35.8	56.5	376.63	392.50	63.8	33.0	14.52	13.93
SSP+Urea	145 + 145	1.08 (5.81)	3.16 (10.23)	76.4	67.6	543.88	436.37	136.4	47.9	14.65	14.29
Urea	40	3.89 (11.32)	5.39 (13.42)	15.1	44.8	307.50	373.75	33.7	26.7	14.18	13.29
Check		4.58 (12.21)	9.76 (18.21)			230.0	295.00			12.88	12.94
CD (=0.05)		2.29	0.54			43.13	27.16			0.81	0.80
CV		16.47	2.67			7.49	4.81			3.82	0.39

* Figures in parenthesis are angular transformed values.

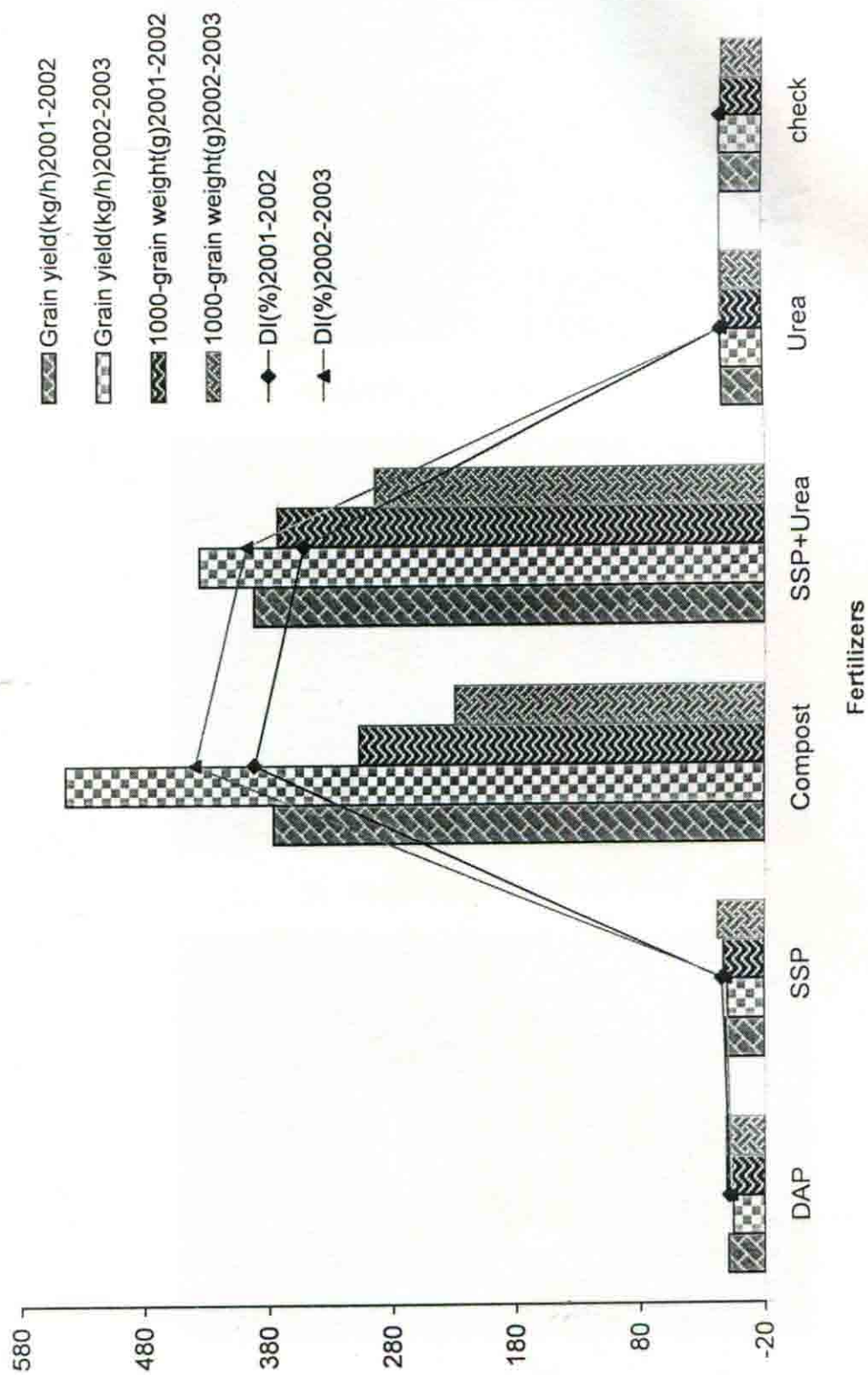


Fig. 5 : Effect of fertilizers on disease incidence, grain yield and 1000-grain weight



A. Single Super Phosphate (SSP) + Urea



B. Single Super Phosphate (SSP)



C. Check

Plate -5 : Influence of fertilizers on vascular wilt of lentil

SSP +urea followed by SSP alone (1.61 per cent). Maximum disease incidence of 3.89 per cent was recorded in urea followed by 2.94 per cent in compost treatment while in the check there was 4.58 per cent disease incidence. SSP in combination with urea showed highest decline in disease incidence (76.4%) followed by SSP alone (64.8%) over check. The above combinations resulted ⁱⁿ highest grain yield (543.8 kg/ha) followed by SSP alone (439.8 kg/ha). The lowest yield was recorded in urea (307.5 kg/ha). The maximum increase in grain yield was also observed from SSP + urea treated plots (136.4%) followed by SSP alone (91.3%) over check. Maximum 1000-grain weight (14.65 g) was recorded in SSP + urea followed by DAP (14.56 g), while the lowest 1000-grain weight (13.93 g) was in SSP alone.

During 2002-03 again SSP+urea resulted in lower disease incidence (3.16%) followed by SSP alone (3.99%), while the maximum disease incidence (6.40%) was recorded in DAP treated plots.

The per cent decline in disease incidence was maximum in SSP + urea (67.6%) followed by SSP alone (59.1%), while it was minimum (34.4%) in DAP. The yield was also highest in SSP + urea (436.37 kg/ha) followed by SSP alone (398.75 kg/ha). Minimum yield (352.5kg/ha) was recorded in DAP treatment.

4.5.1.3 Effect of micronutrients on per cent germination and mortality

The results are presented in (Table 4; Fig. 6; Plate 6).

Table 4 : Effect of micronutrients on percent germination and mortality of lentil

Micronutrients	Germination (%)	Increase in germination (%)	Mortality (%)	Disease control (%)
CaCl ₂	86.7	52.9	15.13(22.75)	63.2
MgSO ₄	96.7	70.5	17.04(24.20)	58.5
Ammonium Salt	80.0	41.1	20.50(26.77)	50.1
ZnSO ₄	100.0	76.4	6.67(12.29)	83.8
Check	56.7	–	41.10(39.83)	–
CD(=0.05)	17.35		10.71	
CV	10.97		22.62	

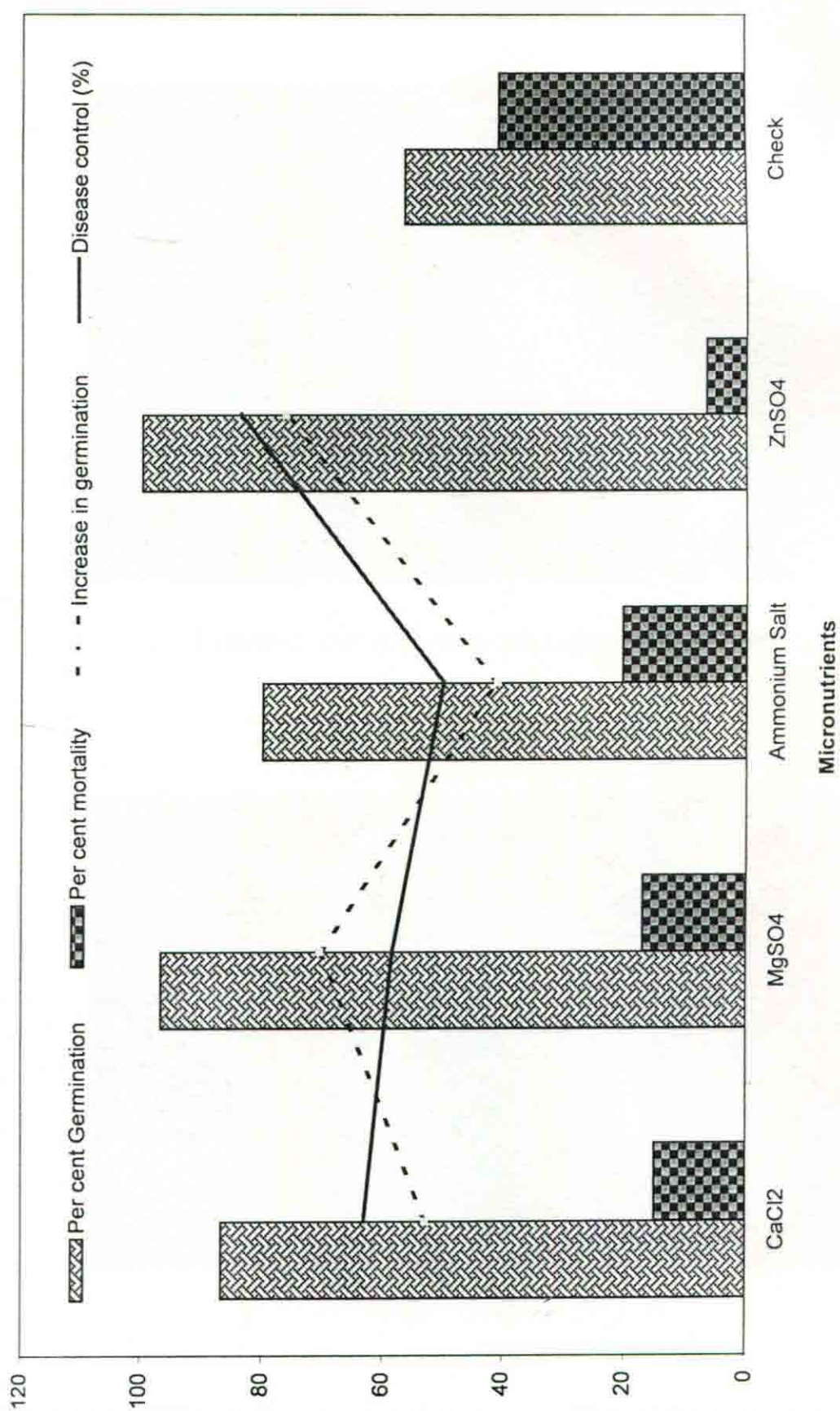
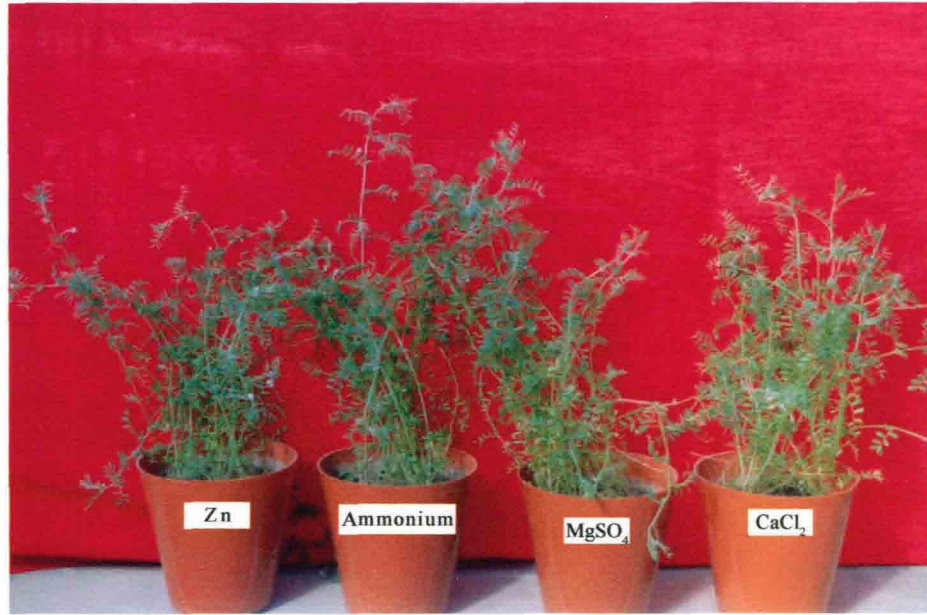


Fig. 6 : Effect of micronutrients on per cent seed germination and mortality of Lentil



A. Treatments : Zinc, Ammonium salt, magnesium and calcium



B. Check

Plate -6 : Effect of micronutrients on wilt incidence under glass house condition

Table 5 : Effect of micronutrients on radial growth of *Fusarium oxysporum* f.sp. *lentis* at different concentrations

Micronutrients	Radial Growth (mm) at different concentrations					Mean
	100 ppm	200 ppm	500 ppm	1000 ppm	0 ppm	
MgSO ₄	67.2(55.0)	65.7(54.2)	63.2(52.7)	60.9(51.3)	90.0(71.6)	69.4(56.9)
ZnSO ₄	61.6(51.7)	58.8(50.1)	56.6(48.8)	59.8(50.6)	90.0(71.6)	65.4(54.6)
Ammonium Salt	72.8(58.5)	73.8(59.2)	78.7(62.5)	80.9(64.1)	90.0(71.6)	79.2(63.1)
CaCl ₂	66.1(54.4)	67.1(55.0)	62.8(52.4)	60.5(51.0)	90.0(71.6)	69.3(56.9)

CD of micronutrients (0.05) = 0.24
 CD of concentration (0.05) = 0.27
 CD micronutrient × conc. (0.05) = 0.53
 CV = 0.56

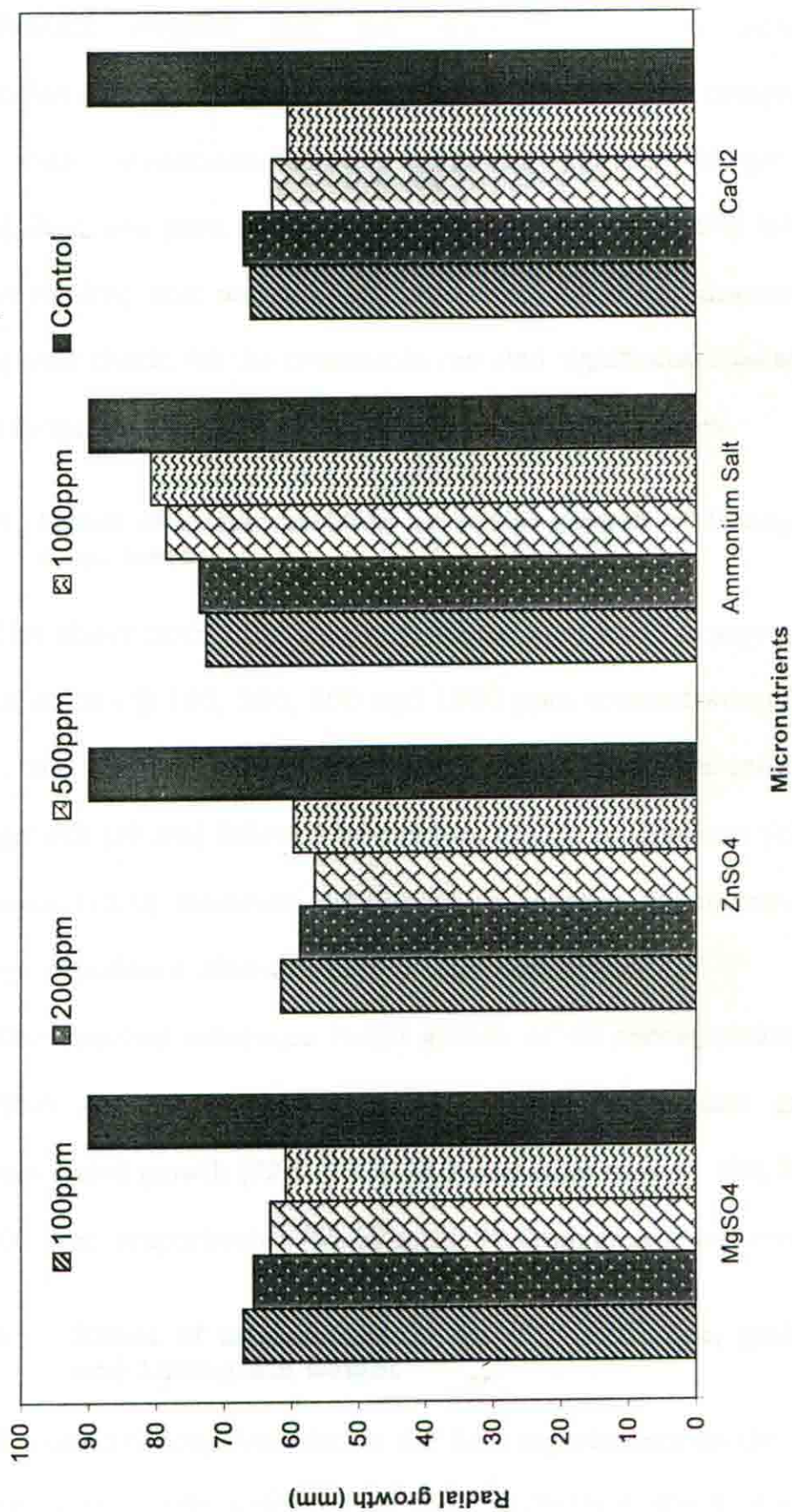


Fig. 7 : Effect of micronutrients on radial growth of *Fusarium oxysporum* f.sp. *lentis*

Results revealed that zinc showed maximum increase in germination (76.4%) followed by magnesium (70.5%) when compared with check while ammonium showed minimum increase in germination (41.1%). Zinc also gave the maximum disease control (83.8%) followed by calcium (63.2%) and ammonium resulted in minimum disease control (50.1%) over check. All the treatments resulted significant disease control as well as increase in germination when compared with check.

4.5.1.4 Effect of micronutrients on radial growth of *F. oxysporum* f. sp. *lentis*

The above micronutrients were tested against *F. oxysporum* f. sp. *lentis* in *in vitro* @ 100, 200, 500 and 1000 ppm concentration (Table 5; Fig. 7). The results revealed that zinc showed maximum inhibition of radial growth (27.3%) followed by calcium (23.0), magnesium (22.9) and ammonium (12.0). However, differences in between the micronutrients were non-significant when compared with each other.

Zinc resulted minimum radial growth at all concentrations (61.6, 58.8, 56.6 and 59.8 mm, respectively) while ammonium gave the maximum radial growth (72.8, 73.8, 78.7 and 80.9 mm) at 100, 200, 500 and 1000 ppm, respectively while in check 90.0mm growth was recorded.

4.5.1.5 Effect of micronutrients on wilt incidence, grain yield and 1000-grain weight

The observations recorded in the field experiments on the effect of micronutrients on wilt incidence are given in (Table 6; Fig. 8; Plate 7).

Table 6 : Effect of micronutrients on disease incidence, grain yield and 1000-grain weight

Treatments	Dose (kg/ha)	Disease incidence (%)		Decline in disease incidence (%)		Grain Yield (Kg/ha)		Increase in grain yield (%)		1000-Grain Weight (g)	
		2001-02	2002-03	2001-02	2002-03	2001-02	2002-03	2001-02	2002-03	2001-02	2002-03
CaCl ₂	5	1.55 (7.13)	9.75 (18.19)	62.8	39.6	322.50	296.25	72.8	115.0	14.14	13.09
MgSO ₄	5	1.32 (6.57)	9.78 (18.22)	68.3	39.4	353.95	287.50	90.7	109.0	13.95	12.93
Ammonium Salt	1	3.21 (10.31)	11.06 (19.42)	23.0	31.5	303.88	278.75	62.8	102.7	13.62	12.79
ZnSO ₄	25	1.24 (6.34)	8.58 (17.03)	70.0	46.8	516.13	310.63	176.5	125.9	14.42	13.21
Check		4.17 (11.78)	16.15 (23.69)			186.65	137.50			12.72	12.55
CD (=0.05)		0.95	0.37			60.28	45.80			0.74	0.12
CV		7.35	1.25			11.63	11.34			3.48	0.60

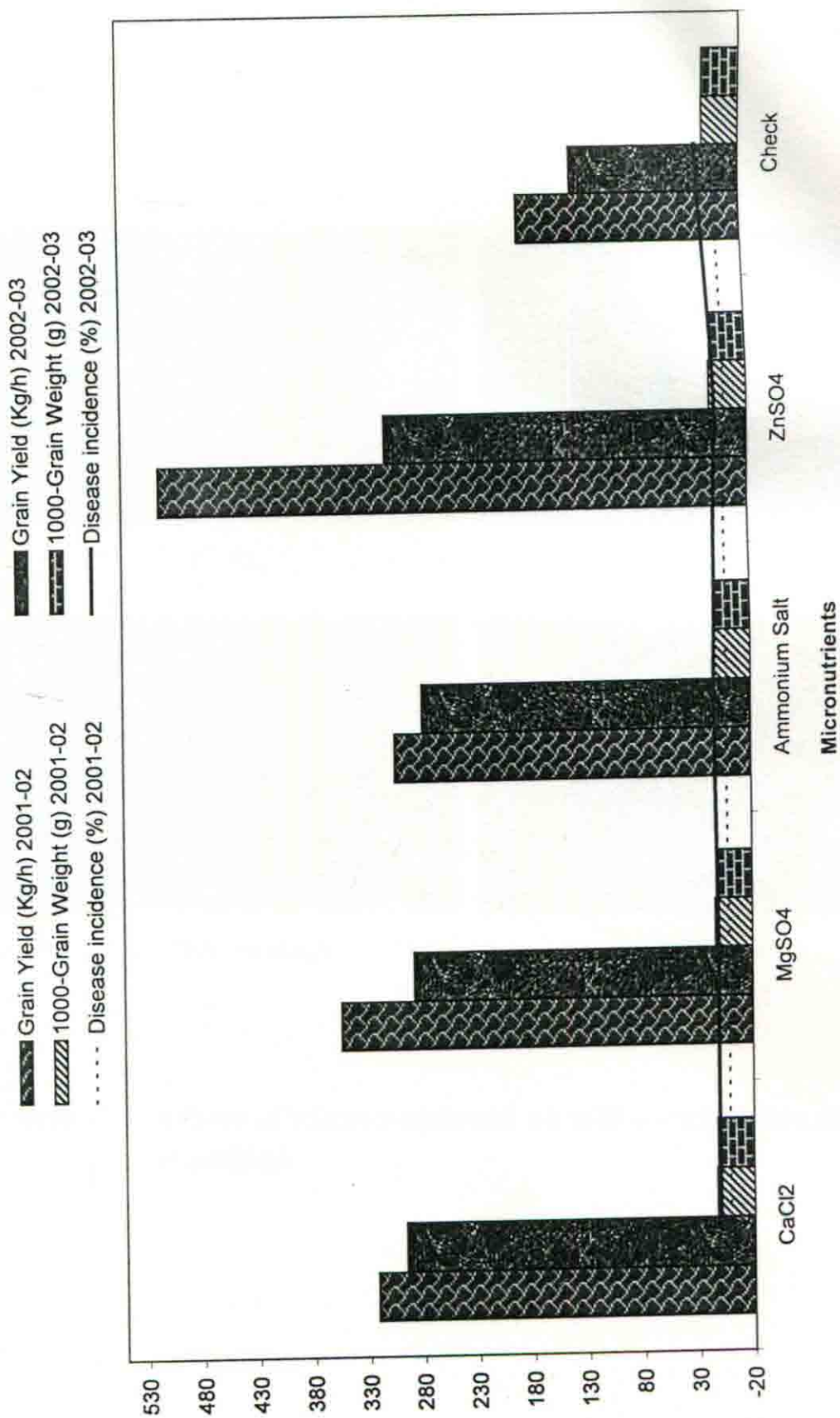


Fig. 8 : Effect of micronutrients on disease incidence, grain yield and 1000-grain weight



A. Zinc



B. Magnesium



C. Ammonium salt



D Check

Plate -7 : Effect of micronutrients on wilt incidence in the field condition

All the treatments were found highly significant in reducing the wilt incidence as compared to check in both the crop seasons. Zinc applied @ 25 kg/ha resulted the lowest disease incidence (1.24 and 8.58%) in both the crop seasons followed by magnesium sulphate (1.32%) in the first year and calcium chloride (9.75%) in the second year trial.

During 2001-02 crop season, zinc sulphate exhibited maximum disease control (70.3%) followed by magnesium sulphate (68.3%) over check. Other treatments were also effective but remained non-significant. Highest grain yield (516.3 kg/ha) and 1000-grain weight (14.42 gm) was also recorded in zinc treated plots. However, there were no significant differences between ZnSO_4 , CaCl_2 and MgSO_4 . The maximum increase in yield (176.5%) over check was recorded in ZnSO_4 treated plots.

During 2002-03 crop season, similar results were obtained and ZnSO_4 was found best treatment giving maximum yield (310.6 kg/ha), maximum disease control (46.8%) as well as maximum 1000-grain weight (13.21 g), followed by calcium which resulted 296.2 kg/ha grain yield, 39.6 per cent disease control and 13.09 g 1000-grain weight.

4.5.2 Biological control

4.5.2.1 Dual Culture

The fungal antagonist *T. harzianum* was screened for its antagonistic potential against the fungal pathogen by dual culture technique in two ways.

Table 7 : Radial growth of biocontrol agent (*Trichoderma harzianum*) and *Fusarium oxysporum* f. sp. *lentis* in dual culture (Simultaneous inoculation)

Incubation period (h)	Radial growth (mm)	
	<i>T. harzianum</i>	<i>F. oxysporum</i> f. sp. <i>lentis</i>
48	23.43(28.94)	14.93(22.70)
72	40.95(39.78)	21.85(27.86)
96	54.96(47.86)	28.55(32.28)
120	67.95(55.56)	37.05(37.48)
144	75.40(60.28)	38.30(38.21)
CD(=0.05)	2.59	2.59
CV	3.71	5.43

Table 8 : Radial growth of biocontrol agent (*Trichoderma harzianum*) and *Fusarium oxysporum* f. sp. *lentis* in dual culture (After 3 days of inoculation)

Incubation period (h)	Radial growth (mm)	
	<i>T. harzianum</i>	<i>F. oxysporum</i> f. sp. <i>lentis</i>
24	16.33(23.81)	35.93(36.77)
48	27.55(31.65)	40.07(39.24)
72	41.20(39.90)	45.23(42.24)
96	63.50(52.90)	53.03(46.75)
CD(=0.05)	4.85	6.02
CV	8.50	9.48

The antagonist inhibited the growth of fungus completely after 144 hr of incubation when both were inoculated at the same time (Table 7; Plate 8 A, C). The radial growth of 75.40 mm was recorded for *T. harzianum* while 38.30 mm of the test fungus. The average diameter of *T. harzianum* was 23.43, 40.95, 54.96, 67.95 and 75.40 mm, respectively at 48, 72, 96, 120 and 144 hr.

When the antagonist was inoculated three days after inoculation of pathogen (Table 8) the inhibition in growth of the pathogen was observed after 96 h of inoculation. The average radial growth of fungus was 35.93, 40.07, 45.23, 53.05 mm at 24, 48, 72 and 96 h, respectively, while the radial growth of antagonist was recorded 16.33, 27.55, 41.20 and 63.50 mm at these interval and the inhibition was recorded when the antagonist attained radial growth of 63.50 mm and the fungus attained 53.03 mm radial growth.

4.5.2.2 Effect of volatile compounds released by *T. harzianum* on the radial growth of the test fungus

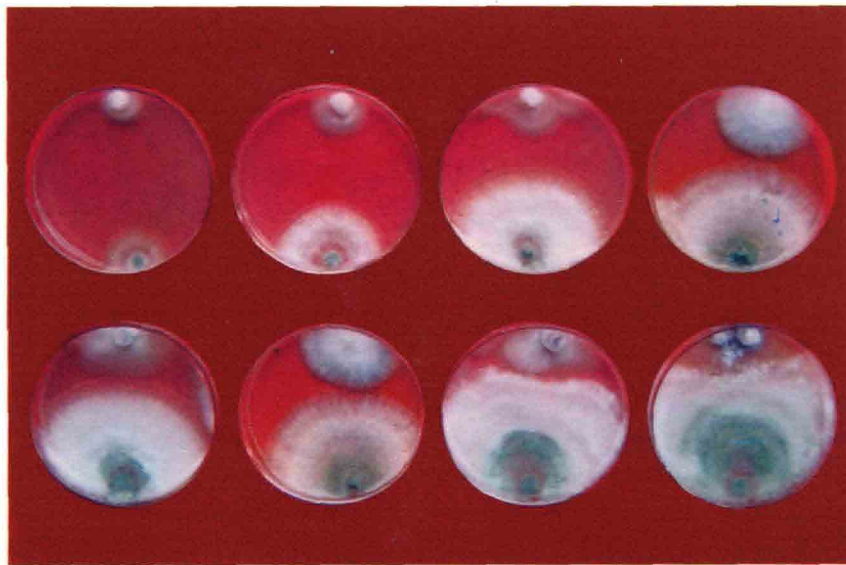
Volatile compounds of 2, 4, 6 and 8 days old culture of *T. harzianum* in PDA significantly inhibited the radial growth of test pathogen. Maximum inhibition in radial growth of pathogen was 89.3 per cent, when 2 days old culture was used. As the culture of *T. harzianum* got older its effect on growth of pathogen was slightly reduced (Table 9; Plate 8 B).

Table 9 : Effect of volatile compounds produced by *T. harzianum* on radial growth of *F. oxysporum* f.sp. *lentis* and per cent inhibition in growth

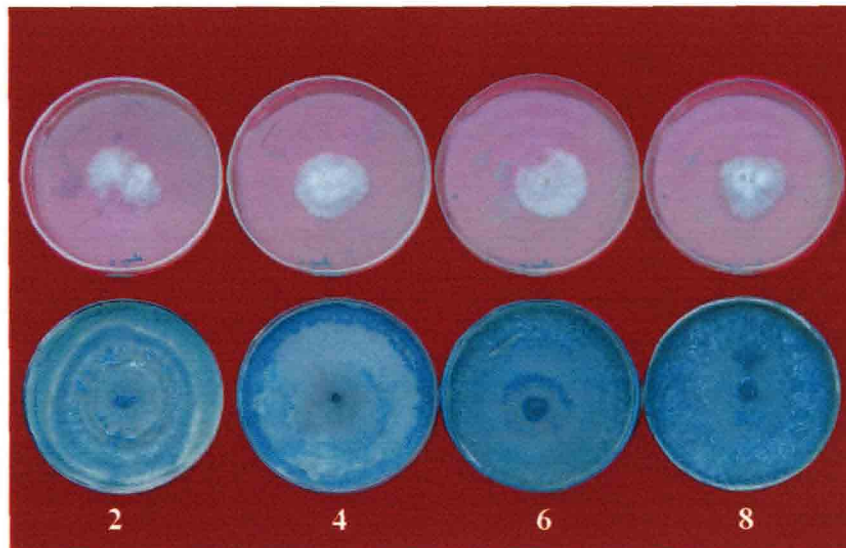
Age of Culture (in Days)	Average diameter of <i>F. oxysporum</i> f.sp. <i>lentis</i> (mm)	Inhibition in growth (%)
2	9.67(18.11)	89.3
4	27.70(31.76)	69.2
6	57.06(49.06)	36.6
8	70.93(57.38)	21.2
Check	90.00	-
CD (=0.05)	0.94	
CV	1.27	

Table 10 : Effect of Non-volatile compounds produced by *T. harzianum* on radial growth of *F. oxysporum* f.sp. *lentis* and per cent inhibition in growth

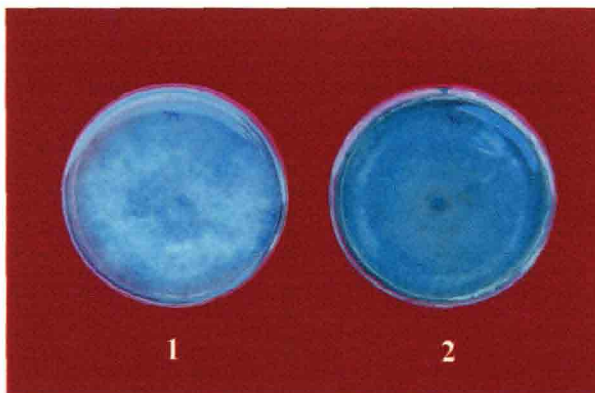
Incubation Period (in Hr.)	Average diameter of <i>F. oxysporum</i> f.sp. <i>lentis</i> (mm)	Inhibition in growth (%)
24	27.06	20.3
48	25.13	25.9
72	23.47	30.9
96	22.97	32.3
Check	33.95	-
CD (=0.05)	0.96	
CV	1.98	



A. Antagonism between *T. harzianum* & *F. oxysporum* f.sp. *lentis* (Dual culture)

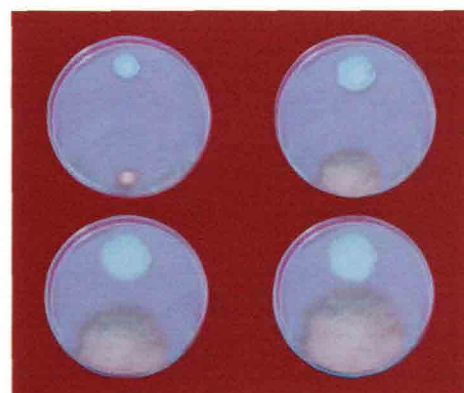


B. Effect of volatile compounds produced by 2, 4, 6 and 8 days old culture of *T. harzianum* on *F. oxysporum* f.sp. *lentis*



C. Check

1. *F. oxysporum* f. sp. *lentis*
2. *T. harzianum*



D. Antagonism between *Ps. fluorescens* & *F. oxysporum* f. sp. *lentis*

Plate 8 : *In vitro* antagonism of bioagents against *Fusarium oxysporum* f. sp *lentis*

4.5.2.3 Effect of non-volatile compounds

The effect of twelve-day-old culture filtrate of *T. harzianum* resulted in significant inhibition of growth of *F. oxysporum* f. sp. *lentis* when compared with control. Results indicated that *T. harzianum* produced diffusible non-volatile antibiotics that inhibited the growth of pathogen. After 96 h of incubation, the radial growth of fungus was 22.97 mm and the per cent inhibition was 32.3% (Table 10).

4.5.2.4 Effect of bacterial antagonist *Pseudomonas fluorescens* on the radial growth of test fungus

The bacterial antagonist *P. fluorescens* was tested against pathogen through dual culture technique (Table 11). The results indicated that *P. fluorescens* produced clear inhibition zone of 9.3 mm of the fungus. There was no growth of the fungus mycelium at the site of inhibition zone (Plate 8 D).

4.5.3 Host Resistance

4.5.3.1 Evaluation of lentil entries against wilt disease under field conditions

In order to find out resistant source(s) against vascular wilt of lentil, available lines of lentil were screened during *rabi* 2001-02 and 2002-03 under field conditions where disease incidence remained very high during the crop season.

The observations are presented in Table 12, 13. The data revealed that among the tested entries none was found resistant to wilt. During

Table 11 : Radial growth of biocontrol agent (*Pseudomonas fluorescens*) and pathogen (*Fusarium oxysporum* f.sp. *lentis*) in dual culture

Incubation Period (h)	Radial Growth (mm)		Inhibition Zone (mm)
	<i>P. fluorescens</i>	<i>F. oxysporum</i> f.sp. <i>lentis</i>	
24	8.90(17.35)	25.07(30.04)	-
48	16.50(23.96)	32.03(34.47)	-
72	21.17(27.38)	44.90(42.07)	-
96	23.17(28.77)	59.57(50.52)	-
120		74.33(59.56)	-
144		80.17(63.56)	9.3
CD(=0.05)	1.38	1.02	
CV	3.00	1.23	

Table 12 : Evaluation of entries against lentil wilt under field conditions during 2001-2002 crop season

Sl. No.	Entry	Incidence of wilt/root rot/collar rot (%)	Grading
1.	DPL-518	24.2	S
2.	IPL-128	23.7	S
3.	LL-818	17.5	S
4.	LL-4646	27.6	S
5.	LL-874	29.3	S
6.	IPL-139	27.1	S
7.	IPL-133	23.3	S
8.	IPL-131	38.8	HS
9.	IPL-401	27.5	S
10.	DPL-58	29.4	S
11.	ILL-43	32.0	HS
12.	ILL-2022	25.0	S
13.	ILL-2580	24.6	S
14.	ILL-5845	23.2	S
15.	ILL-7978	26.8	S
17.	LH97-32	23.9	S
18.	LH84-8	17.7	S
19.	IPL-134	35.8	HS
20.	NDL-94	30.3	HS
21.	LH97-32	34.3	HS
22.	LL-775	32.1	HS
23.	KLB97-3	24.0	S
24.	L-4076	24.0	S
25.	L-5256	29.0	S
26.	NDL-1	41.5	HS

27.	IPL-127	26.0	S
28.	KLS-217	20.7	S
29.	KLS-218	23.8	S
30.	IPL-132	30.7	HS
31.	IPL-203	14.9	S
32.	IPL-202	17.8	S
33.	VL-502	19.0	S
34.	HUL-57	11.9	S
35.	IPL-403	16.8	S
36.	LH97-44	15.9	S
37.	LH97-48	16.3	S
38.	KL-303	10.6	S
39.	LH84-8	10.0	MR
40.	NDL99-1	15.1	S
41.	L-4591	29.2	S
42.	IPL-201	16.5	S
43.	L-4660	18.7	S
44.	LL-876	29.9	S
45.	L-4645	17.5	S
46.	L-4659	12.8	S
47.	VL-120	15.3	S
48.	L-4247	17.9	S
49.	L-4592	25.7	S
50.	IPL-402	15.8	S
51.	IPL-302	18.9	S
52.	L,9-12 (check)	50.5	HS

Table 13 : Evaluation of entries against lentil wilt diseases under field conditions during 2002-2003

Sl. No.	Entry	Incidence of wilt/root rot/collar rot (%)	Grading
1.	IPL519	8.80	MR
2.	L-4694	6.66	MR
3.	L4663	6.00	MR
4.	Sehore 74-3	22.2	S
5.	KLEB-2004	12.50	S
6.	L-4619	11.11	S
7.	IPL305	33.20	HS
8.	IPL406	20.0	S
9.	IPL81	22.1	S
10.	IPL405	13.5	S
11.	L4650	11.11	S
12.	L4618	21.11	S
13.	IPL306	17.11	S
14.	L184-8	8.67	MR
15.	LH2K-27	15.50	S
16.	L4649	9.12	MR
17.	IPL206	29.47	S
18.	IPL207	26.67	S
19.	L4595	27.14	S
20.	L4596	24.58	S
21.	IPL404	11.85	S
22.	DPL15	18.07	S

23.	DPL62	10.70	S
24.	KLB97-5	9.65	MR
25.	IPL304	10.0	MR
26.	KLB320	10.54	S
27.	PL06	8.69	MR
28.	L4648	14.18	S
29.	L4617	8.77	MR
30.	HUL62	17.24	S
31.	L4076	13.33	S
32.	IPL518	17.85	S
33.	L4661	15.83	S
34.	KLS86-13	28.57	S
35.	L 9-12 (Check)	50.0	HS
36.	IPL204	6.97	MR
37.	KLS221	11.92	S
38.	L4147	19.56	S
39.	HUL57	20.00	S
40.	NDL96-24	16.67	S
41.	NDL99-1	21.12	S
42.	L4594	10.38	S
43.	HUL58	17.24	S
44.	KLS219	23.07	S
45.	L, 9-12 (Check)	50.0	HS

2001-02, only one line LH 84-8 was moderately resistant to wilt, showing 10 per cent disease incidence. Lines IPL-131, L-9-12, NDL-94, LH 97-32, LL-775, NDL-1 and IPL-132 were highly susceptible and the rest were susceptible to wilt, exhibiting 10.6 (KLB-303) to 29.9 per cent (LL-876) wilt incidence.

While during 2002-03 crop season, IP-519, L-4694, L-4663, L184-8, KLB 97-5, IPL-304, PL-06, L-4617 and IPL-204 were moderately resistant to lentil wilt. Only one line IPL-305 (33.20%) was found ^{highly} susceptible to wilt while rest lines were susceptible to wilt showing 10.38 (L-4594) to 29.47 per cent (IPL-206) disease incidence.

4.5.3.2 Induced Resistance

4.5.3.2.1 Efficacy of plant extracts and detergent

My observations on the efficacy of different plant extracts on lentil wilt under glasshouse revealed that all the treatments significantly improved per cent seed germination over check (Table 14; Fig. 9).

Among these, garlic (*Allium sativum*) leaf extract resulted 96.7 per cent germination followed by neem leaf extract (93.3%). Minimum germination (80.0%) was recorded in the extract of *Solanum nigrum*. However, non-significant difference was recorded between garlic and neem extract. Garlic extract resulted 45.2 per cent increase in germination over check while neem, datura, detergent and *Solanum* resulted 40.1, 35.1, 25.1 and 20.1 per cent, respectively.

Table 14 : Effect of plant extracts and detergent on per cent germination and mortality of lentil

Sl. No.	Treatments	Germination (%)	Increase in Germination (%)	Mortality (%)	Disease control (%)
1.	Allium sativum	96.7	45.2	7.03	76.7
2.	Neem	93.3	40.1	10.73	64.4
3.	Datura stramonium	90.0	35.1	10.83	64.1
4.	<i>Solanum nigrum</i>	80.0	20.1	12.63	58.1
5.	Detergent	83.3	25.1	15.73	47.8
6.	Check	66.6	-	30.17	-
CD (=0.05)		13.28		10.34	
CV		8.59		39.16	

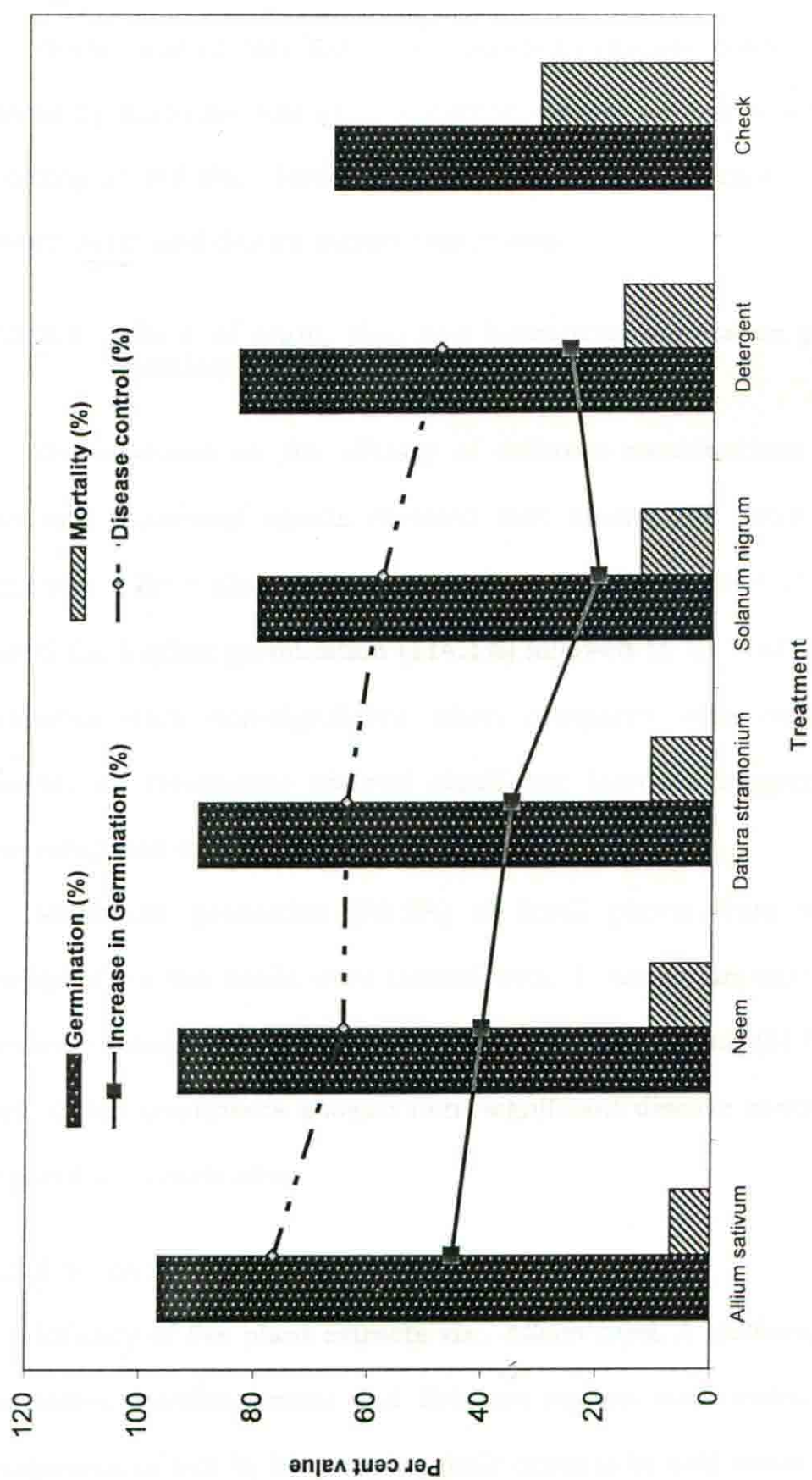


Fig. 9 : Effect of plant extracts and detergent on per cent seed germination and mortality of lentil

Garlic leaf extract exhibited maximum disease control (76.7%) followed by neem (64.4%) while minimum disease control was recorded in detergent (47.8%). However, there was non-significant difference between neem and datura extract treatments.

4.5.3.2.2 Effect of alum, zinc and biocontrol agents on per cent germination and mortality of lentil

Observations on the efficacy of different combinations of zinc, alum and biocontrol agents revealed that among the treatments *T. harzianum* + Zn + alum (99.8%) as well as *P. fluorescens* + Zn + alum showed the highest germination (114.1%) followed by Zn + alum. Other treatments were non-significant when compared with each other. However, all treatments showed significant increase in germination when compared with check (Table 15; Fig. 10).

Maximum protection (78.2%) of lentil plants from wilt was recorded when the seeds were treated with *T. harzianum* and Zn and alum were mixed in soil, followed by *T. harzianum* + alum (61.8%) over check. Other treatments showed non-significant disease control when compared with each other.

4.5.3.2.3 In vitro bioassay of plant extracts

Efficacy of five plant extracts viz., *Allium cepa*, *A. sativum*, *Datura stramonium*, *Mentha spicata* and *Solanum nigrum* was tested for the management of wilt by bioassaying their extracts in cold water, alcohol

Table 15 : Effect of alum, zinc and biocontrol agents on percent germination and per cent mortality of lentil

Treatment	Germination (%)	Germination (%)	Mortality (%)	Disease control (%)
<i>T. harzianum</i> +Zn	86.7	85.6	38.33(38.07)	37.3
<i>P. fluorescens</i> +Zn	80.0	71.3	41.67(39.99)	31.8
<i>Pseudomonas</i> Strain 82+Zn	73.3	56.9	44.43(41.75)	27.3
<i>T. harzianum</i> +alum	86.7	85.6	23.33(28.85)	61.8
<i>P. Fluorescens</i> +alum	80.0	71.3	32.77(34.83)	46.4
<i>Pseudomonas</i> Strain 82+alum	80.0	71.3	25.00(29.99)	59.1
Zn+ alum	93.3	99.8	28.33(31.93)	53.6
<i>T. harzianum</i> +Zn+alum	100.0	114.1	13.33(17.71)	78.2
<i>P. fluorescens</i> +Zn + alum	86.7	85.6	35.00(36.15)	42.7
<i>Pseudomonas</i> Strain 82+Zn+alum	100.0	114.1	33.33(35.00)	45.5
Alum	86.7	85.6	31.67(33.85)	48.2
Check	46.7	-	61.11(56.75)	-
CD (=0.05)	18.33		18.85	
CV	12.90		31.44	

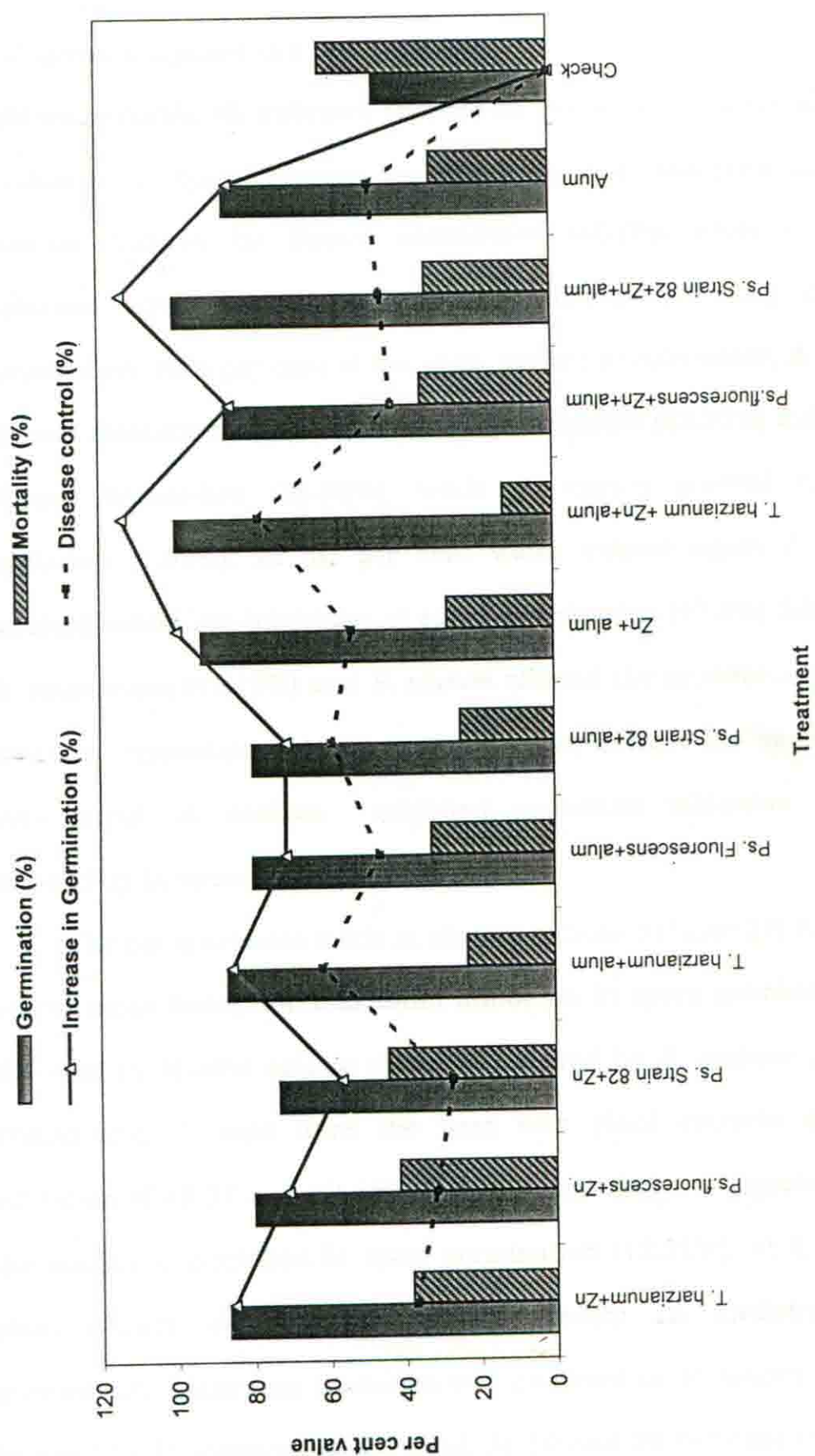


Fig. 10 : Efficacy of alum, zinc and biocontrol agents on per cent seed germination and mortality of lentil

and acetone against the test pathogen. The results of plant extracts in cold water (Table 16) indicated that, on an overall mean basis maximum inhibition in spore germination (53.76%) was observed in *Allium sativum* followed by *Datura stramonium* (46.0%) while extract of *Solanum nigrum* resulted in minimum inhibition (10.32%) in spore germination. At 5 per cent of the plant extract in cold water, *A. sativum* showed maximum inhibition in spore germination (33.23%) followed by *Datura stramonium* (25.98%), while *S. nigrum* showed minimum inhibition (4.99%). At 10 per cent water extract again *A. sativum* resulted maximum inhibition of spore germination (47.0%) followed by *D. stramonium* (41.17%) and *S. nigrum* showed the minimum inhibition (11.09%), respectively. At 20 per cent water extract the same results were found. *A. sativum* exhibited maximum inhibition (81.05%) followed by *D. stramonium* (70.85).

The plant extracts made in alcohol indicated (Table 17) that on an overall mean basis, the maximum inhibition in spore germination was observed in *Mentha spicata* (61.43%) followed by *A. sativum* (51.94%). *Datura* and *A. cepa* were the next best plant extracts exhibiting inhibition of 46.37 and 40.43 per cent, respectively. *S. nigrum* resulted in the minimum inhibition in spore germination (12.21%). At 5 per cent, plant extract was assayed for its efficacy in inhibiting spore germination. Maximum inhibition was observed in *M. spicata* (35.05%) followed by *D. stramonium* (25.46%). At 10 and 20 per cent extracts in

Table 16 : Effect of water extract of plants on spore germination of *Fusarium oxysporum* f. sp. *lentis*

Plant Species	Inhibition (%) in spore germination at different conc.			Mean
	5%	10%	20%	
<i>Allium cepa</i>	11.75(20.04)	21.83(27.85)	30.42(33.47)	21.33
<i>Allium sativum</i>	33.23(35.19)	47.00(43.28)	81.05(64.19)	53.76
<i>Datura stramonium</i>	25.98(30.64)	41.17(39.91)	70.85(57.32)	46.00
<i>Mentha spicata</i>	17.24(24.53)	28.10(32.01)	50.29(45.17)	31.88
<i>Solanum nigrum</i>	4.99(12.90)	11.09(19.45)	14.87(22.68)	10.32
Check	0.00(0.00)	0.00(0.00)	0.00(0.00)	-
CD(=0.05)	0.37	0.32	0.27	
CV	1.03	0.66	0.42	

Table 17 : Effect of alcohol extract of plants on spore germination of *Fusarium oxysporum* f. sp. *lentis*

Plant Species	Inhibition (%) in spore germination at different conc.			Mean
	5%	10%	20%	
<i>Allium cepa</i>	20.36(26.82)	42.59(40.74)	58.35(49.81)	40.43
<i>Allium sativum</i>	25.04(30.03)	52.31(46.33)	78.47(62.35)	51.94
<i>Datura stramonium</i>	25.46(30.30)	49.03(44.45)	64.64(53.52)	46.37
<i>Mentha spicata</i>	35.05(36.29)	65.29(53.91)	83.94(66.38)	61.43
<i>Solanum nigrum</i>	6.67(14.96)	12.88(21.03)	17.09(24.42)	12.21
Check	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00
CD(=0.05)	0.15	0.42	0.45	-
CV	0.37	0.68	0.59	-

alcohol similar trend was observed. *M. spicata* showed the maximum inhibition (65.29%, 83.94%) followed by *A. sativum* (52.31% and 78.47%), respectively, while *S. nigrum* showed the minimum inhibition (12.88% and 17.09%) in spore germination at 10 and 20 per cent concentration.

The extracts of plants made in acetone (Table 18) again indicated the supremacy of *A. sativum* over other plant extracts tested. On mean basis, it showed maximum inhibition (63.25%) in respect of spore germination followed by *D. stramonium* (50.60%). At 5 per cent concentration, *A. sativum* resulted in maximum inhibition (37.14%) followed by *D. stramonium* (32.92%). At 10 per cent as well as at 20 per cent similar trend was observed and again *A. sativum* showed maximum inhibition (64.21% and 88.39%) followed by *D. stramonium* (40.79% and 78.10%), respectively. At all concentrations *S. nigrum* showed minimum inhibition of spore germination (7.24, 9.01 and 14.47%) at 5, 10 and 20 per cent concentrations, respectively.

4.6 Integrated Disease Management

Effect of fungicides and biocontrol agents were assessed under glasshouse conditions on per cent germination and disease control (Table 19; Fig. 11; Plate 9). The results revealed that *T. harzianum*, *Pseudomonas* strain 82 and Vitavax in combination with Thiram resulted 100 per cent germination followed by Vitavax alone and

Table 18 : Efficacy of acetone extract of plants on spore germination of *Fusarium oxysporum* f. sp. *lentis*

Plant Species	Inhibition (%) in spore germination at different conc.			Mean
	5%	10%	20%	
<i>Allium cepa</i>	18.68(25.61)	26.01(30.66)	58.19(49.72)	34.29
<i>Allium sativum</i>	37.14(37.55)	64.21(53.26)	88.39(70.08)	63.25
<i>Datura stramonium</i>	32.92(35.01)	40.79(39.69)	78.10(62.10)	50.60
<i>Mentha spicata</i>	23.39(28.93)	36.57(37.18)	60.58(51.12)	40.18
<i>Solanum nigrum</i>	7.24(15.61)	9.01(17.46)	14.47(22.36)	10.24
Check	0.00(0.00)	0.00(0.00)	0.00(0.00)	–
CD(=0.05)	0.32	2.31	0.46	
CV	0.77	4.37	0.60	

Table 19 : Effect of Integrated disease management on per cent germination and mortality of lentil

Treatments	Germination (%)	Increase in germination (%)	Mortality (%)	Disease control (%)
<i>T. harzianum</i>	100.0	130.78	16.66(23.85)	65.38
<i>Pseudomonas</i> strain 82	100.0	130.78	13.33(21.14)	72.30
<i>P. fluorescens</i>	86.67	100.0	10.37(15.35)	78.5
Vitavax	96.7	123.17	17.40(24.37)	63.8
Vitavax+ <i>T. harzianum</i>	96.7	123.17	3.33(6.14)	93.1
Vitavax+Thiram	100.0	130.78	10.00(14.99)	79.2
check	43.33	-	48.13(43.93)	-
CD (=0.05)	19.98		16.79	
CV	12.62		44.11	

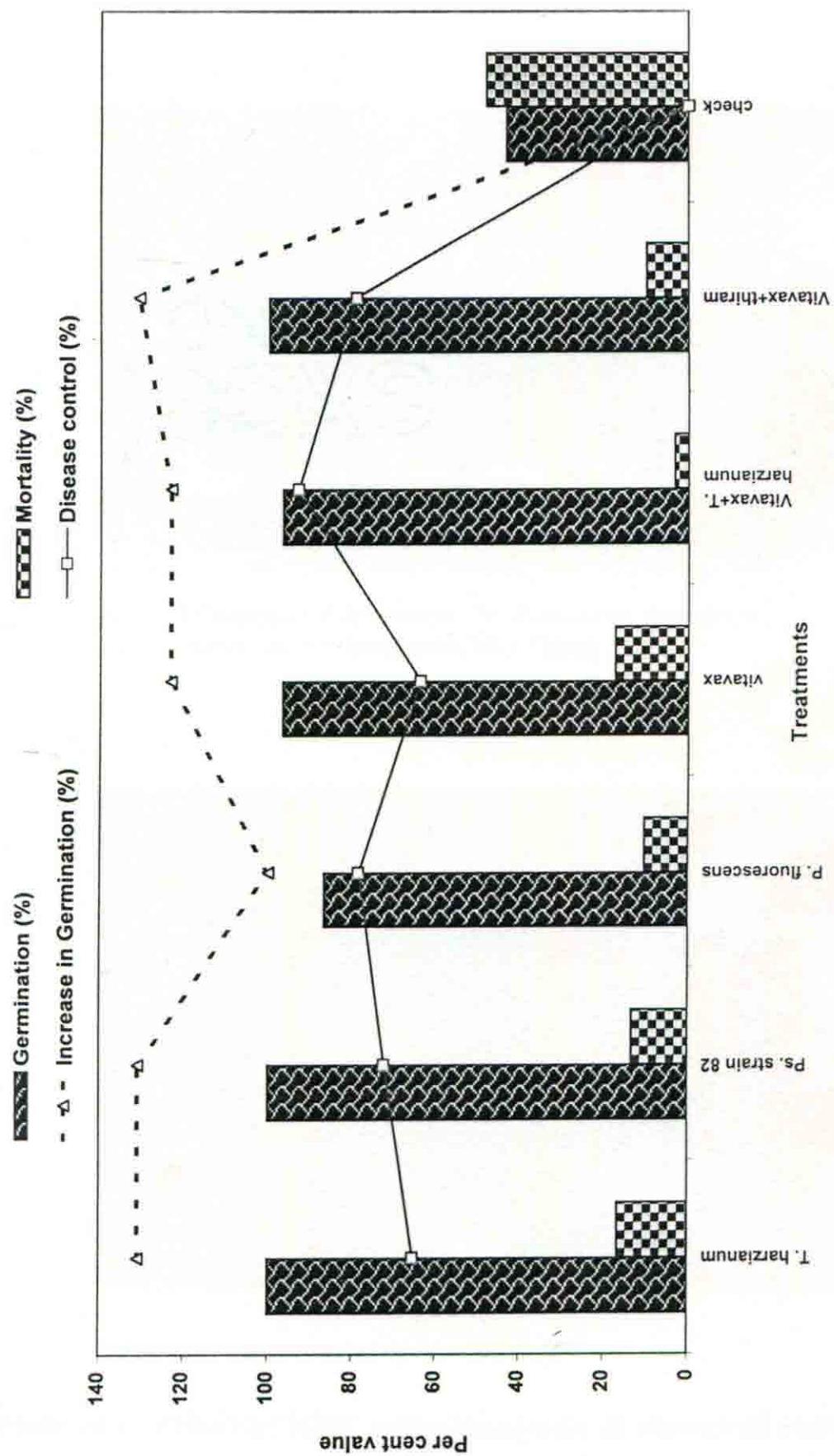
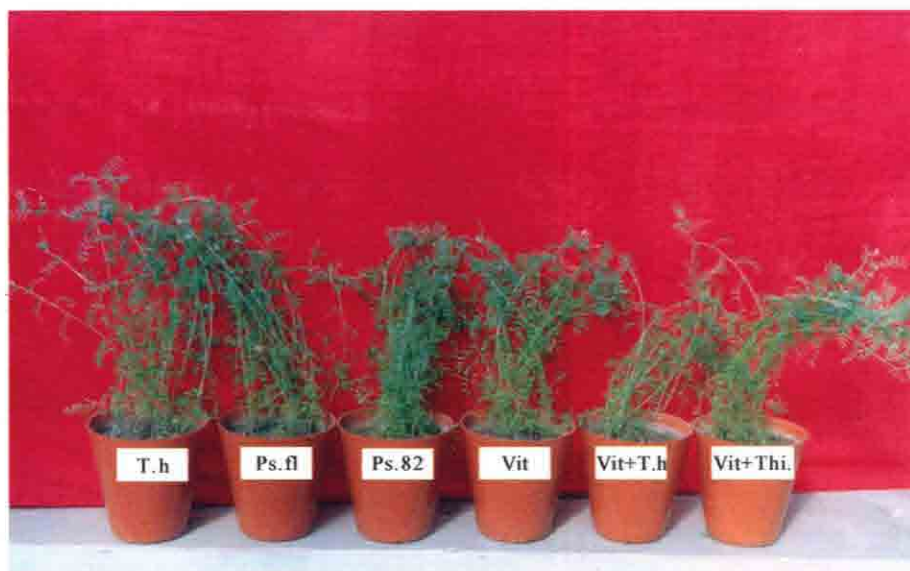
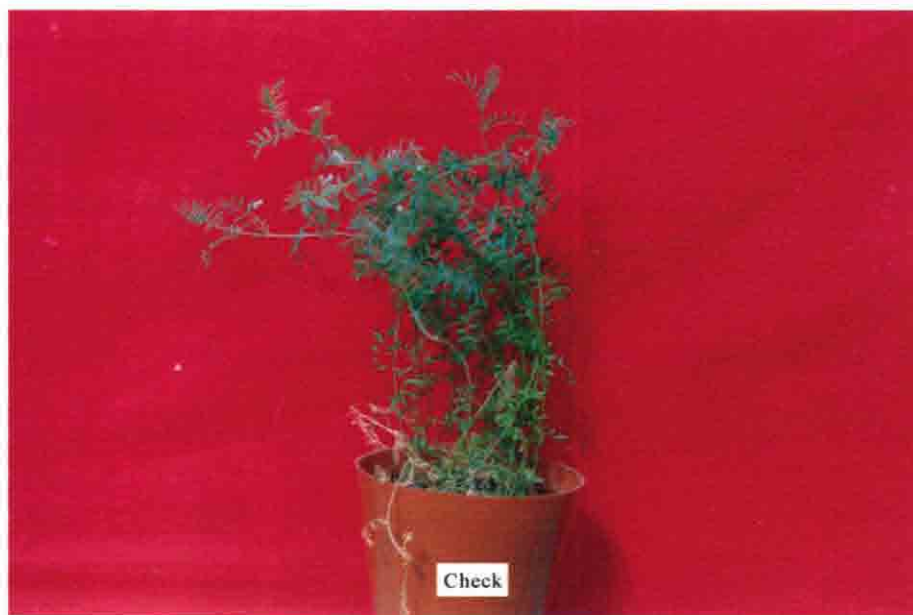


Fig. 11 : Effect of Integrated disease management on per cent seed germination and mortality of lentil plants under glasshouse conditions



A. Treatments : *T. harzianum*, *Ps. fluorescens*, Ps. strain 82, vitavax, vit + *T. harzianum*, Vit + Thiram



B. Check

Plate -9 : Effect of IDM with bioagents & chemical under glass house

Vitavax in combination with *T. harzianum*. The increase in germination was 130.78 and 123.17 per cent, respectively, in the above treatments over control, while Vitavax + *T. harzianum* showed lowest mortality (3.33%) and maximum disease control (93.1%) followed by Vitavax + Thiram which gave 10 per cent mortality and 79.2 per cent disease control. *P. fluorescens* showed 78.5 per cent disease control followed by *P. strain* 82 (72.30%) and *T. harzianum* (65.38%). Vitavax alone showed lowest disease control (63.8%), however, all treatments were found to be significant when compared with check.

Further studies were carried out in the field in two successive crop seasons to study their effect on disease incidence; grain yield (kg/ha) and 1000-grain weight (g) of lentil. The results are presented in Table 20; Fig. 12 and Plate 10.

During 2001-02 crop season the data recorded, revealed that Vitavax in combination with *T. harzianum* showed the lowest disease incidence (0.88%) followed by *T. harzianum* alone (1.27%) and Vitavax + Thiram (1.44%). *P. fluorescens* showed 1.84 per cent disease incidence while high disease incidence (2.22%) was recorded in Vitavax alone when compared with other treatments. The highest grain yield (558.7kg/ha) was recorded in Vitavax + *T. harzianum* followed by *T. harzianum* alone (516.0 kg/ha). It was minimum (348.5kg/ha) when the seeds were treated with Vitavax alone @2.5g/kg seed.

Table 20 : Effect of biocontrol agents and fungicides on wilt incidence, grain yield and 1000-grain weight

Treatment	Dose	Disease incidence (%)		Decline in disease incidence (%)		Grain yield (kg/ha)		Increase in grain yield (%)		1000-grain weight (g)	
		2001-02	2002-03	2001-02	2002-03	2001-02	2002-03	2001-02	2002-03	2001-02	2002-03
<i>T. harzianum</i>	5g/kg seed	1.27(6.43)	9.19(17.65)	66.8	52.6	516.00	358.25	91.9	192.4	13.41	12.64
<i>Pseudomonas</i> strain 82	5g/kg seed	1.60(7.23)	12.58(20.77)	58.2	35.1	451.25	311.25	67.8	154.1	13.99	12.19
<i>P. fluorescens</i>	5g/kg seed	1.84(7.77)	9.64(18.08)	51.9	50.3	446.38	350.75	65.9	186.3	13.38	12.42
Vitavax	2.5g/kg seed	2.22(8.55)	11.39(19.72)	42.0	41.3	348.50	323.25	29.6	163.8	13.89	12.31
Vitavax+ <i>T. harzianum</i>	1:4	0.88(5.33)	7.90(16.32)	77.0	59.3	558.75	453.50	107.7	270.0	14.11	13.79
Vitavax+thiram	1:2	1.44(6.79)	8.64(17.09)	62.4	55.4	496.00	403.25	84.4	229.1	14.04	13.04
Check		3.83(11.27)	19.39(26.12)	-	-	268.98	122.50	-	-	11.85	11.58
CD(=0.05)		0.99	0.53			46.00	13.04			0.55	0.27
CV		8.82	1.84			7.02	2.65			2.77	1.45

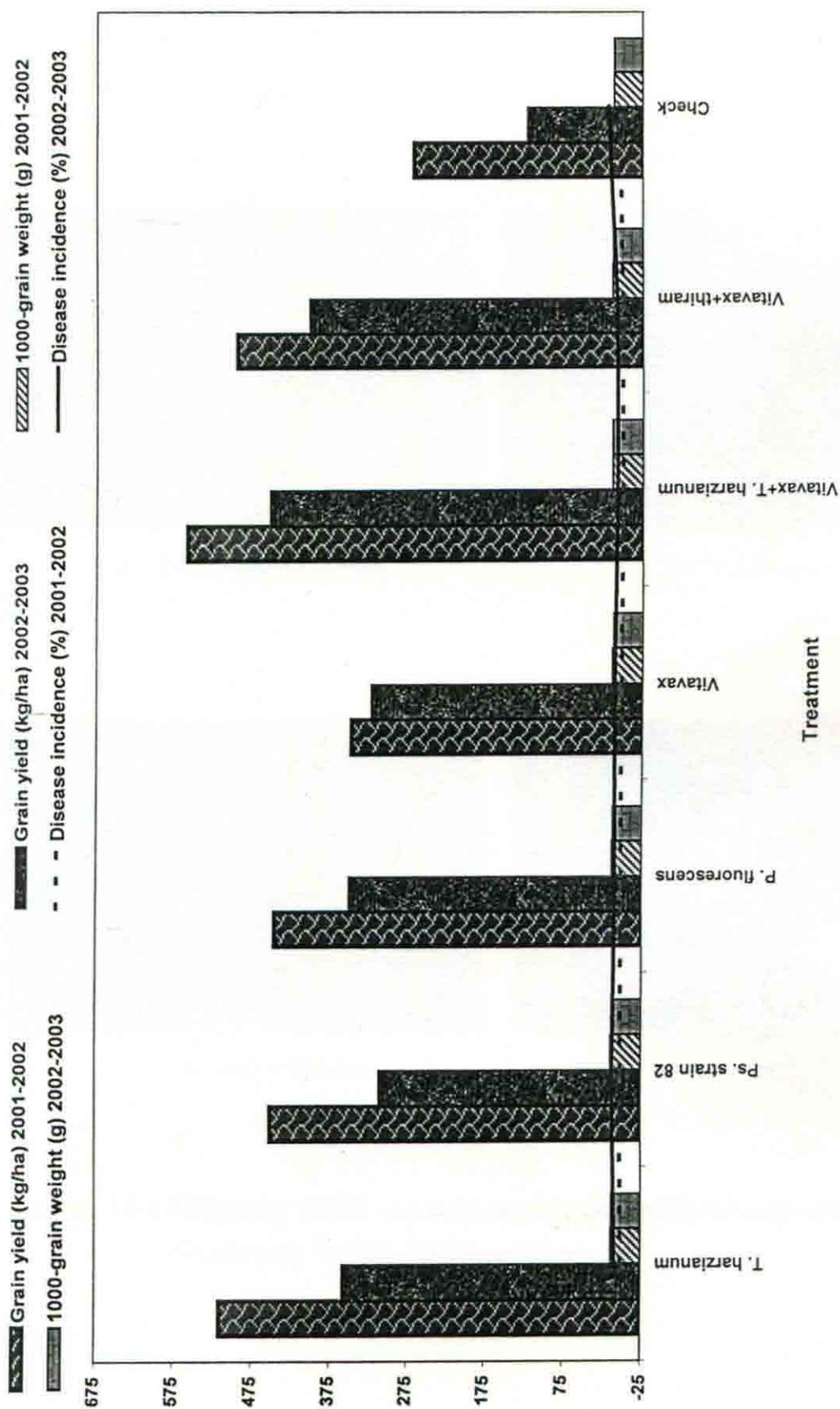


Fig. 12 : Effect of biocontrol agents and fungicides on wilt incidence, grain yield and grain weight during 2001-02 and 2002-03 crop season



A. *T. harzianum* + vitavax



B. *T. harzianum*



C. Vitavax + Thiram



D. Check

Plate -10 : Effect of IDM on wilt incidence with bioagents & chemicals in the field condition.

The maximum reduction in disease incidence (77.0%) and per cent increase in grain yield (107.7%) was recorded in Vitavax + *T. harzianum* while it was minimum in Vitavax alone, where 42.0 per cent reduction in disease incidence and 348.50% increase in grain yield was recorded. Maximum grain weight (14.11g) was recorded in Vitavax + *T. harzianum* treated seeds followed by Vitavax + Thiram (14.04 g). However, other treatments showed non-significant differences when compared with each other.

During 2002-03 crop season, the results indicated that Vitavax + *T. harzianum* again resulted the minimum disease incidence (7.90%) like previous year, followed by Vitavax + Thiram (8.64%), while it was maximum (12.58%) in *P. fluorescens* strain 82. Highest grain yield (453.50kg/ha) and 1000-grain weight (13.79g) was recorded in Vitavax + *T. harzianum*. The per cent decline in disease incidence (59.3%) and increase in grain yield (270%) was recorded in Vitavax + *T. harzianum* treated plots over check. In general there was difference in disease incidence between the two crop seasons and it was probably due to the difference in dates of sowing of the crop.

Effect of fungicides and biocontrol agents were also analyzed under glasshouse condition on per cent germination and disease control of lentil plants. The results revealed that *T. harzianum*, *P.* strain 82 and Vitavax in combination with Thiram gave 100 per cent germination followed by Vitavax alone and Vitavax in combination with

T. harzianum. The increase in germination was 130.78 and 123.17 per cent respectively in the above treatments over control, while Vitavax in combination with *T. harzianum* showed lowest mortality (3.33%) and maximum disease control (93.1%) followed by Vitavax + Thiram which gave 10 per cent mortality and 79.2 per cent disease control. *P. fluorescens* showed 78.5 per cent disease control followed by *P. strain* 82 (72.30%) and *T. harzianum* (65.38%) Vitavax alone showed lowest disease control (63.8%), however, all treatments were found significant when compared with check.

Discussion

5.1 Symptomatology

The plants infected with *Fusarium oxysporum* f. sp. *lentis* produced characteristic symptoms on different plant parts at different stages of plant growth. There was no seed germination because of pre-emergence mortality and on splitting the non-germinated seeds, browning at the micropylar region was observed. The seedlings infected 4-5 days after emergence showed browning of radicles. At the adult stage, typical wilt symptoms were observed. Same type of symptoms were observed by Vasudeva and Srinivasan (1952); Claudius and Mehrotra (1973a); Khare *et al.* (1979). Later on, the leaves turn^{ed} yellow and curl^{ed} from the lower part of the plant upwards and the growth of the plant was checked. The crown droops and ultimately it dies. Roots of the affected plant turn^{ed} yellowish-brown to dark brown partially or wholly. On splitting the main roots, xylem vessels showed brown discoloration and the root nodules were poorly developed. Shulindin (1950) also observed the similar symptoms and reported that the pathogen was usually confined to the cortex but vascular elements were invaded in advanced stages of the disease.

5.2 The Pathogen

5.2.1 Isolation and identification

The fungus was isolated from the infected root of lentil plant showing characteristic symptoms of wilt and thereafter identified as

Fusarium oxysporum Schlect f.sp. *lentis* (Vasudeva and Srinivasan) Gorden on the basis of morphological and cultural characteristics.

Fungal colonies on PDA were white, cottony with moderate aerial mycelial growth, which turn^{ed} the medium pinkish within 3-4 days. The mycelium of the fungus was septate and produced three types of spores. Macro conidia were sickle shaped 3 to 5 septate with both the ends pointed. Micro conidia were mostly single celled; few conidia were two celled, ellipsoid to ovate. Chlamydospores were thick walled, spherical and terminal or intercalary and thick-walled. My findings are in accordance with Vasudeva and Srinivasan, (1952) and Khare *et al.* (1971) who reported that *Fusarium oxysporum* f.sp. *lentis* produced three types of spores of different morphological characters.

5.2.2 Pathogenicity test

The pathogenicity test was conducted in glasshouse on susceptible lentil cultivar L, 9-12 and Koch's postulates were confirmed. The re-isolation of the pathogen revealed similar morphological and cultural characteristics as described by several workers.

5.2.3 Mass multiplication of fungus for soil inoculation

In the present investigation the fungus *Fusarium oxysporum* f. sp. *lentis* was multiplied on sterilized sand-maize meal medium, on which the fungus produced abundant mycelium and conidia. Singh *et al.* (1979) have also used the same medium for mass multiplication of the fungus.

5.3 Antagonists

5.3.1 Morphology and mass multiplication of fungal antagonists

5.3.1.1 Morphology

Trichoderma harzianum Rifai grown on PDA, showed similar morphological characteristics as reported by Rifai (1969) and Cook and Baker (1983). The colonies on PDA grew rapidly, smooth surfaced in the beginning, watery white and sparse mycelial mat but soon aerial hyphae developed on their surface. The conidia were whitish green, turning to bright green but ultimately appeared dull green. The reverse of the colony on petriplates however, remained colourless.

Pseudomonas fluorescens produced off-white or cream coloured colonies on King's B medium. When the colonies grew older, the surroundings of the colonies gave a greenish hue. It is due to the siderophores production. Fravel (1998) also observed the production of siderophores which gave it green colour, hence called as fluorescent pseudomonads.

5.3.1.2 Mass multiplication

Fungal antagonist *T. harzianum* was multiplied on sorghum grain medium on which it produced abundant mycelium and conidia and/or chlamydospores. Kumar (1996) and Mukherjee (1998) have also used the same medium for the mass multiplication of *T. virens* and *T. harzianum*. Multiplication of *P. fluorescens* was done on King's B medium.

5.4 Histopathological Studies

Histopathological studies of the infected and healthy lentil root exhibited the presence of inter-as well as intracellular mycelium. In some sections few hyphae were detected in the vessels. Internal structure of healthy roots showed compact and multilayered cork cambium cells and fully developed phellogen while in the infected root sections loose cork cambium and thick metaxylem with more diameters was observed. My findings are in conformity with Bhalla *et al.* (1992) and Agarwal and Prasad (1997).

5.5 Disease Management

5.5.1 Cultural practices

5.5.1.1 Effect of date of sowing and environmental factors

Early sowing resulted in more disease incidence than late sowing. Myalova (1973) also reported severe (7 times) wilt incidence in early sowing than in late ones. Similar findings were also reported by Shulindin (1950); Kannaiyan and Nene (1975a); Izquierdo and Morse (1975) and Bhatt and Sharma (1992) who recorded that wilt incidence decreased when the sowing was delayed. Therefore, late sowing i.e., second fortnight of November seems to be the best timing for sowing of lentil in *Tarai* regions of Uttaranchal and Uttar Pradesh.

The low incidence during 2001-02 crop season might be due to the unfavourable environmental condition especially soil temperature,

moisture in soil, soil pH and rainfall which are essential for disease development in comparison to crop sown during 2002-03. The findings are in conformity with Vasudeva and Srinivasan (1952); Saxena and Khare (1988b); Erskine *et al.* (1990) and Kaushal and Sharma (1998).

5.5.1.2 Effect of fertilizers and nutrients

The observations recorded on the effect of fertilizers and micronutrients on incidence of vascular wilt and grain yield of lentil revealed that among the fertilizers, SSP in combination with urea was most effective in reducing the per cent wilt incidence as well as increasing grain yield in both the crop seasons. Phosphorous with nitrogen in judicious amount have been reported effective in reducing tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Raj and Kapoor, 1995). Taya *et al.* (1988) reported that increased level of nitrogen fertilizers enhanced severity of dry root rot (*R. solani*) in chickpea while phosphate levels decreased it and combined effect of both N and P subsided the root rot incidence, however, Hoffland *et al.* (2000) reported no effect of N concentration on susceptibility to *F. oxysporum* causing tomato wilt. Azad *et al.* (1995) also reported that maximum yield of lentil (1225 kg/ha) when 60 kg P₂O₅ + 40 kg K₂O/ha was applied.

Among the micronutrients tested ZnSO₄, MgSO₄, CaCl₂ and Ammonium salt, zinc was found most effective in reducing the wilt incidence and increasing the grain yield in both the crop seasons in

comparison to check. All the treatments were found effective in reducing the wilt incidence in natural as well as in the artificially inoculated (glasshouse) conditions. Grahm (1983) observed suppressive effects of zinc on soil-borne diseases, with zinc deficiency a leakage of sugars and inhibition of protein synthesis occurred with large increase in free amino acids which in turn favoured disease development. Sarkar *et al.* (2000) also reported that zinc @ 10 kg/h + iron @ 2 kg/h gave the best result in reducing the sun hemp wilt. Singh (1984) and Pradhan *et al.* (1999) also reported that boron, zinc and manganese restricted the saprophytic ability of *Fusarium udum* causing wilt of pigeonpea. The laboratory studies also confirmed the results of Gupta (1999) that zinc was the best among all the micronutrients tested, in reducing the root-rot pathogens [*Sclerotium rolfsii* (*Croticum rolfsii*), *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia bataticola*].

Zinc is a metallic component and serves as a functional, structural and regulatory cofactor of large number of enzymes (Vallee and Wacker, 1970; Vallee and Galles, 1984 and Marshner, 1986). The most prominent physiological role of Zn is in its inter-relationship with auxin (Huber, 1980), which made the plants nutritionally and physiologically stronger and resistant and probably reduced incidence of lentil wilt.

Magnesium is an essential constituent of chlorophyll. Chakrabarty and Taneja (1990) in their investigation also reported that

increase in quantity of boron and magnesium increased curd weight and reduced disease incidence in cauliflower. Calcium combines with pectin^{to} form an essential constituent of the cell wall. It also promotes the activity of soil bacteria concerned with the fixation of free nitrogen or the formation of nitrates from organic forms of nitrogen. The calcium was found more effective to inhibit the growth of *Fusarium oxysporum* f. sp. *lentis* at higher (1000 ppm) concentration. Chandrasehar *et al.* (2001) also reported the similar results.

5.5.2 Biological control

5.5.2.1 Dual culture

Before proceeding for the work on biological control of plant pathogen, the first step is to choose a potential antagonist, effective against target pathogen to get success for controlling the disease in the field. Generally the selection is done by dual culture technique (Elad *et al.*, 1982; Mukhopadhyay, 1995 and Mukherjee and Mukhopadhyay, 1995).

Species of *Trichoderma* exhibited good results in glasshouse as well as in field trials for the control of chickpea wilt (Kaur and Mukhopadhyay, 1992). Vajnn (1985) reported that in dual cultures of *Fusarium* and *Trichoderma* species, there was a necrotrophic mycoparasitic relationship between *F. oxysporum* and *T. pseudokoningii* and *T. longobranhianum*. Similar observations were recorded in the dual culture conducted in the laboratory under present studies.

Mukhopadhyay and Kaur (1990) also found *T. harzianum* inhibitory to the growth of *Fusarium oxysporum* f.sp. *ciceri*.

Trichoderma harzianum was found most effective against *F. oxysporum* f. sp. *lentis* through the production of volatile and non-volatile compounds. Dennis and Webster (1971a, b) reported that *Trichoderma* produced some chloroform soluble non-volatile antibiotics including trichodermin and other peptide antibiotics like suzukacillin, alamethicine and dermadine which were effective against certain fungi and bacteria while some isolates produced acetaldehyde as a volatile compound. Tronsmo and Dennis (1978) also reported inhibition of growth of *Botrytis cinerea* Pers. Exfr. and *Mucor mucedo* Fr. by non-volatile and volatile metabolites produced by *Trichoderma* spp.

Pseudomonas species produced a variety of secondary metabolites toxic to pathogenic fungi and bacteria (Elad and Chet, 1987; Polonenko *et al.*, 1987 and Chang Senk *et al.*, 1988). In the dual culture clear inhibition zone was formed and produced siderophores (Fravel, 1988). Effectiveness of *T. harzianum* against some soil-borne pathogens infecting pulses have been reported by Rodger (1993), Inam-ul-Haq (2001) and Rangeshwaran *et al.* (2001).

5.5.3 Host resistance

5.5.3.1 Evaluation of lentil entries against lentil wilt

Among the tested entries, none was found resistant against lentil wilt; however, LH 84-8 appears moderately resistant exhibiting lowest

disease incidence (10%) during 2001-2002 crop season. While during 2002-2003, out of 50 entries tested, eleven entries were moderately resistant against lentil wilt.

There is a need for further evaluation of more number of entries against vascular wilt under glasshouse condition so as to get the lines with high degree of resistance that can be used as donor(s) for breeding of disease resistance programme in future.

5.5.3.2 Induced resistance

5.5.3.2.1 *In vivo* evaluation of plant extracts and detergent

When a physical, chemical or biological agent induces the production/accumulation of defense components in the host or activates defense mechanism it is regarded as induced or acquired resistance (Purkayastha, 1998). Today, use of plant extracts for activating the resistance system of the host plant or by prevention or suppressing the multiplication of pathogen inside the host plant system is gradually gaining importance for the management of the diseases. Therefore, the studies were undertaken to observe the effect of plant extracts as inhibitor of lentil wilt pathogen. The data recorded revealed that leaf extracts of garlic and neem were highly effective in reducing the wilt incidence of lentil when applied as seed treatment. Garlic leaf extract resulted 45.2 per cent increase in germination and 76.7 per cent disease control over check while neem leaf extract resulted 40.1 per cent increase in germination and 64.4 per cent protection against wilt

pathogen. Singh *et al.* (1979) have also reported fungicidal effect of neem and garlic on wilt pathogens of gram *Fusarium oxysporum* f. sp. *lentis*. Gupta and Bansal (2003) have also reported 87.99 per cent increase in germination of fenugreek seeds against *Fusarium oxysporum*.

Detergent also plays an important role in the management of plant diseases as it increases the surface area as well as solubility of minerals. The protection of the plants is not based on the elimination of the pathogen but rather on activation of plant defense mechanism or on the enhancement of their activity (Kessmann, 1994 and Sticher *et al.*, 1997).

5.5.3.2.2 *In vitro* bioassay of plant extracts

The leaves of higher plants are most susceptible to infection by pathogens. However, a large number of these plants are able to escape infection, thereby indicating the presence of some fungistatic substance(s) in them. Therefore, the studies were undertaken in this direction. *In vitro* evaluation of some plant leaf/bulb extracts for their activities against wilt pathogen showed the supremacy of garlic leaf water extract, which resulted maximum inhibition of spore germination of *Fusarium oxysporum* f. sp. *lentis* at all concentrations (5, 10 and 20%) followed by *Datura* and *Mentha spicata*. Similar results were also reported by Agarwal and Gupta (1969); Anonymous (1972); Singh *et al.* (1979); Upadhyay and Gupta (1990) and Sharma and Nanda (2000).

The extracts made in alcohol indicated the maximum inhibition by *Mentha spicata* followed by *A. sativum*. Shekhawat and Prasada (1971) also reported the antifungal nature of *Mentha piperita* against *Alternaria tenuis*, *Helminthosporium* sp. and *Curvularia pennegeti*, however, Upadhyay and Gupta (1990) reported more inhibition (55.6%) of linear growth of *Curvularia lunata* at 5 per cent ethanol extract.

The extracts made in acetone again showed the supremacy of *A. sativum* over other extracts exhibiting 63.25 per cent reduction in spore germination followed by *Datura stramonium* and *Mentha spicata*. Similar findings were reported by Shekhawat and Prasada (1971); Upadhyay and Sharma (1990) and Sharma and Nanda (2000) who worked on inhibition of teliospore germination of *Neovossia indica*.

The results indicated that antifungal or fungistatic property is neither a family character nor a generic one. It varies from family to family, genus to genus and species to species. The fungistatic activity of the extract may be due to the presence of natural chemical inhibitors in the plant tissues (Buck and Suter, 1944; Cavallite *et al.*, 1944; Cavalitto *et al.*, 1945; Agarwal and Gupta, 1969; Anonymous, 1972; Rippon, 1974 and Barone and Tansey, 1977;).

5.6 Integrated Disease Management

Modern agriculture has tended to apply ever-large amounts of pesticides to attain the yield potential set by the climate, the agronomic

inputs and the genetics of cultivars. Meeting the future challenge for a protective but sustainable agriculture will require the use of all available strategies that are effective, economical, safe and compatible (Cook and Baker, 1983).

In the present investigation, effect of different combinations of alum, zinc and biocontrol agents were analyzed under glasshouse condition and the result indicated that *T. harzianum* in combination with zinc and alum as well as *P. fluorescens* in combination with zinc and alum showed the highest germination (114.1%) followed by zinc + alum (99.8%). *T. harzianum* along with zinc and alum also exhibited the highest disease control (78.2%) followed by *T. harzianum* + alum over check. There were no reports found in the literature in this context however, Chandrasekaran and Rajappan (2002) reported the maximum reduction of pod blight of soybean caused by *Colletotrichum truncatum* through the application of alum in combination with leaf extract of *Lawsonia inermis*, under laboratory condition. They further reported that using leaf extract of *Lawsonia inermis* in combination with *T. viride* and alum could also control pod blight.

Integration of biological and chemical methods seems to be a very promising way of controlling plant pathogens with a minimal interference with biological equilibrium. For the integration, the two systems must be compatible. In the present investigation, it was observed that use of systemic fungicide along with biocontrol agent

(Vitavax + *T. harzianum*) was highly effective in managing the lentil wilt exhibiting only 0.88 per cent wilt incidence in 2001-2002 and 7.90 per cent in the successive crop season, followed by *T. harzianum* alone in the first year experiment while during second year experiment Vitavax along with Thiram was the second best treatment giving 8.64 per cent disease incidence. Highest grain yield was also recorded in the above treatments.

Considering all the factors i.e. canopy, cost-benefit ratio, effectiveness in controlling disease etc. it was observed that biocontrol agents in combination with zinc, alum and chemical as well as seed treatment with Vitavax and Thiram is quite effective against vascular wilt of lentil. Mukhopadhyay (1987, 1990); Mukhopadhyay and Mukherjee (1991); Roy *et al.* (1998); De and Chaudhary (1999) and Chandrasekaran and Rajappan (2002) have also observed the similar results.

Summary
and
Conclusion

Summary and Conclusion

Vascular wilt of lentil caused by *Fusarium oxysporum* f.sp. *lentis* is an important disease of lentil, being destructive in many parts of India, resulting heavy yield losses. Gradual, sometimes sudden yellowing, withering and drying of leaves followed by drying of the entire plant or a large portion of it characterize the most striking feature of wilt disease.

Lentil wilt tolerant cultivars and chemicals are being used effectively by lentil growers to reduce the losses caused by lentil wilt pathogen. But due to non-availability of seeds of highly resistant and stable cultivars in interior areas and more over its cost limits the use only to the progressive farmers of our country. The hazardous and non-judicious use of pesticides in polluting the environment and entering in the food chain of animals and human beings has become one of the most important concerns of the today's environmentalists and public health authorities. A very attractive and promising approach to combat plant disease is by integration of biological methods and a low non-hazardous dose of fungicide. In recent years, considerable success has been achieved against many pathogens by the use of non-conventional chemicals and nutrients i.e. by induction of systemic resistance.

The present investigations were undertaken to find out an ecofriendly, effective and economically feasible method to protect the crop from vascular wilt in *Tarai* area of Uttaranchal state. The salient findings are summarized below:

1. Histopathological studies showed that cork cambium cells were compact and multilayered in healthy roots while the infected roots have loose cork cambium, thick and wide metaxylem and mycelial growth of the fungus.
2. Early sown crop i.e. during first fortnight of November was more prone to wilt infection as compared to late sown crop (end of November).
3. Soil application of micronutrients helped in managing the lentil wilt. Zinc was highly effective in reducing the per cent wilt incidence and increasing grain yield. CaCl_2 and MgSO_4 were also found effective in reducing the wilt incidence while ammonium salt was found ineffective in reducing the wilt incidence.
4. The fungal antagonist *Trichoderma harzianum* tested against *F. oxysporum* f.sp. *lentis* in dual culture clearly form^{ed} a band at the site of contact between the pathogen and the antagonist. Further the growth of antagonist overlapped the mycelium of the test fungus after 144h, when both were inoculated simultaneously and 96h, when antagonist was inoculated 3 days after inoculation of test fungus. The volatile as well as non-volatile compounds

were also found effective in reducing the growth of the fungus. However, the production of volatile compounds decreased with the increase in the age of cultures. Similarly, the bacterial antagonist *P. fluorescens* was also found effective in dual culture producing a clear inhibition zone between the bioagent and the fungus.

5. Among all the tested entries, not a single one showed resistant reaction to wilt pathogen. During 2001-02 crop season entry (LH 84-8) was found to be moderately resistant and during 2002-03 crop season out of 50 entries screened, eleven entries were found moderately resistant to the disease.
6. Garlic leaf extract was found to be highly effective in reducing wilt incidence (76.7%) followed by neem leaf extract (64.4%). It also enhanced the per cent seed germination.
7. Of the five plant extracts evaluated *in vitro*, garlic leaf extract prepared in water and acetone showed maximum inhibition of spore germination of *F. oxysporum* f.sp. *lentis* at all concentrations (5,10 and 20%) followed by *Datura stramonium* while the extracts of *Mentha spicata* and garlic leaves prepared in alcohol exhibited maximum inhibition of spore germination at all concentrations(5,10 and 20%), respectively.
8. Integrated disease management strategies using alum, zinc and biocontrol agents in the glasshouse studies revealed that the

maximum increase in seed germination was recorded in *T. harzianum* + zinc + alum as well as *P. fluorescens* + alum + zinc. The highest disease control was observed when the seeds were treated with *T. harzianum* + zinc + alum followed by *T. harzianum* + alum.

9. Under field conditions during 2001-02 crop season, maximum disease control was achieved when the seeds were treated with the Vitavax + *T. harzianum* followed by *T. harzianum* alone and during 2002-03 crop season Vitavax + *T. harzianum* was again found effective in controlling the disease followed by Vitavax + Thiram.

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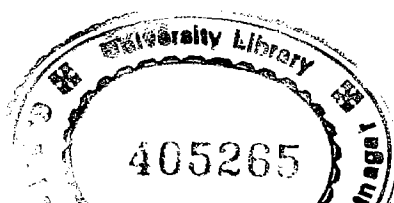
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Appendices

Appendix 1

All India area, production and yield of lentil (Masur) from 1970-71 to 2000-01

<i>Year</i>	Area (million ha)	Production (million ton)	Yield (kg/ha)
1970-71	0.75	0.37	497
1971-72	0.63	0.30	481
1972-73	0.83	0.37	447
1973-74	0.93	0.41	440
1974-75	0.95	0.46	480
1975-76	0.94	0.46	492
1976-77	0.85	0.39	461
1977-78	0.88	0.38	436
1978-79	1.01	0.45	443
1979-80	0.85	0.32	377
1980-81	0.93	0.47	498
1981-82	0.95	0.50	525
1982-83	1.00	0.49	491
1983-84	0.94	0.53	566
1984-85	0.98	0.55	557
1985-86	1.09	0.66	607
1986-87	1.09	0.66	607
1987-88	1.04	0.66	635
1988-89	1.08	0.73	679
1989-90	1.11	0.71	635
1990-91	1.19	0.85	717
1991-92	1.19	0.80	674
1992-93	1.20	0.79	657
1993-94	1.18	0.75	632
1994-95	1.16	0.78	678
1995-96	1.26	0.71	569
1996-97	1.37	0.96	702
1997-98	1.29	0.80	623
1998-99	1.39	0.94	675
1999-2000	1.46	1.08	738
2000-2001	1.41	0.86	611

Appendix 2

Commercial formulation of some fungal biocontrol agents

Formulations	Special features	Reference
BIMAB-T	<i>Trichoderma viride</i> based mycofungicide for protection of pruning wounds	Rodger, 1993
F-STOP	<i>Trichoderma</i> based product for control of damping off of peas and beans	Rodger, 1993
Soil gard/ Gliogard	<i>Gliocladium virens</i> based product for controlling many soil-borne diseases	Cook, 1993
Trichodex	<i>Trichoderma harzianum</i> Rifai (T-36) based biopesticide used for controlling gray mold of cucumber and grapewine (<i>Botrytis cineria</i>)	Rodger, 1993
WRC-API (WRC-GL 21)	<i>Gliocladium virens</i> based product for control of <i>Pythium</i> and <i>Rhizoctonia</i> root rots on ornamental plants	Ristaino and Thomas, 1997
Trichostar	<i>Trichoderma harzianum</i> based biopesticide for controlling <i>R. solani</i> , <i>Fusarium</i> , <i>Sclerotinia</i> diseases	Mukhopadhyay, 1996
Gliostar	<i>G. virens</i> based product for controlling root rot and damping off diseases	Mukhopadhyay, 1996
Antogon TV, Ecofit	<i>T. viride</i> based bio-pesticides for controlling root rots pathogens of oil-seeds and pulses	Jeyarajan, 1997
Root shield, Biotrek	<i>T. harzianum</i> T-22 (RRL-AG 2) based for controlling <i>R. solani</i> , <i>Pythium</i> , <i>Fusarium</i> , <i>Sclerotinia</i> , on beans, cotton, potato, sorghum, tomatoes, cabbage, corn and peanut	Ristaino and Thomas, 1997
Biodac	<i>Trichoderma</i> and <i>Gliocladium</i> based biopesticide for controlling damping-off caused by <i>R. solani</i>	Lewis et al., 1998

Appendix 3

Commercial formulations of bacterial biocontrol agents

Commercial products	Biocontrol agents	Pathogen	References
Galltrol-A	<i>Agrobacterium radiobacter</i>	<i>Agrobacterium tumefaciens</i>	Becker and Schwinn, 1993
Quantum-4000	<i>Bacillus subtilis</i>	<i>Sclerotium rolfsii</i>	Becker and Schwinn, 1993
Conguer	<i>Pseudomonas fluorescens</i>	Bacterial pathogens of mushroom	Rodger, 1993
Diegall	<i>Agrobacterium radiobacter</i>	<i>Agrobacterium tumefaciens</i>	Becker and Schwinn, 1993
GUS-4000	<i>Bacillus subtilis</i>	<i>Rhizoctonia</i> , <i>Fusarium</i> , <i>Gaumannomyces</i> (cereals)	Becker and Schwinn, 1993
Mycostop	<i>Streptomyces griseoviridus</i> K-61	<i>Rhizoctonia solani</i>	Rodger, 1993
Epic-Gus 376	<i>Bacillus subtilis</i> MBI-600	<i>R. solani</i> , <i>Fusarium</i> , <i>Alternaria</i> (wheat, cotton, peanut)	Ristaino and Thomas, 1997
Pf ALR 2	<i>Pseudomonas fluorescens</i>	<i>Rhizoctonia solani</i>	Ravindran and Udhyasekaran] 1996
Dagger-G	<i>Pseudomonas fluorescens</i> G-105)	<i>Pythium ultimum</i> , <i>R. solani</i>	Ristaino and Thomas, 1997
Victus	<i>Pseudomonas fluorescens</i> N (1B-12089)	<i>Pseudomonas tabaci</i> (mushroom)	Ristaino and Thomas, 1997
Deny (formerly Burkholderia blue circle) SMP Pcp Wi	<i>Pseudomonas cepacia</i>	<i>Rhizoctonia</i> , <i>Pythium</i> , <i>Fusarium</i> spp. (cotton, sunflower, sorghum, soybean)	Thojmas, 1997
Bio save-10 Bio save-11	<i>Pseudomonas syringae</i> ECC-10, ECC-11	<i>Penicillium expansum</i> , <i>P. italicum</i> , <i>Botrytis cineria</i> , <i>Geotrichum</i> (apple, lemon, grape, pears and oranges)	Ristaino and Thomas, 1997

Appendix 4

Wilt resistant lines/accessions of lentil reported from different countries

Country	No. of entries tested	Resistant entries	Reference
Bulgaria	16	Naslada, Zhana, Anisiya and Standard Tadzhikskaya 95	Mikhov <i>et al.</i> (1987)
Egypt	12	H 5-6-81, H 4-4-81, F 29, R 270 and F 300	Omar <i>et al.</i> (1988)
India	2000	L 642, P 338, Pant 638, Pusa 1, Pusa 7, LG 7, LP 6, 18, 119, 208; LWS 1, 3, 5 to 8, 10, 13, 14, 22, 36, 38 to 42, 44, 48, 52, 62, 77, 79	Khare <i>et al.</i> (1976)
	–	LWS 4, 15, 20, 21, 24, 25, 26, 50	Khare <i>et al.</i> (1979)
	158	32 were immune including JL 80, Pusa 3, Pant 234 (tolerant to collar rot and root rot also)	Kannaiyan and Nene (1976)
	–	LWS 15, 21 were immune to 5 strains of the pathogen	Kannaiyan and Nene (1978b)
	49	PL 81-18, 81-74, 81-85, 81-230, 781-268, 81-270, 81-340, 81-371	Pandey <i>et al.</i> (1988)
	–	PL 77-2; LG 170, 171, 175, 178, 224, 4125, 4162, 4163; LL 299; LH 82-6; HUL 8; PDL 1; Pant L 406, 639	AICPIP
	–	L 4076, JPL 555	S.C. Agrawal (unpublished data)
	58	PL 406, LG 170, LG 189 and Sehore 74-7 were resistant under artificial inoculation	Kaur and Sharma (1993)
	300	SL 420, 8996, 914, LC 202, 270, 271	Singh and Singh (1994)

Contd...

Appendix 4 : Contd...

Country	No. of entries tested	Resistant entries	Reference
Syria	162	ILL accessions 5582, 6688, 5722, 5751, 5845, 5996, 6050, 6207, 6215 to 6217, 6220, 6223, 6226, to 6229, 6233, 6234, 6239, 6243, 6249, 6250, 6409, 6430, 6435, 6474	Bayya and Erskine (1990)
	220	Accessions belonging to <i>Lens culinaris</i> sub sp. <i>orientalis</i> , <i>L. nigricans</i> sub. sp. <i>nigricans</i> , <i>L. nigricans</i> sub. sp. <i>ervoides</i>	Erskine and Saxena (1991)
	221	Three accession each of <i>L. culinaris</i> sub. sp. <i>orientalis</i> , <i>L. nigricans</i> sub. sp. <i>nigricans</i> and 2 of <i>L. nigricans</i> sub. sp. <i>ervoides</i> were resistant in plastic house but in sick in plot ILWL 79 and ILWL 113 of <i>L. culinaris</i> sub. sp. <i>orientalis</i> and ILWL 138 of <i>L. nigricans</i> sub. sp. <i>ervoides</i> were resistant	Bayaa et al. (1995)

Appendix 5

The weekly average temperature, relative humidity, rainfall and wind speed recorded at Pantnagar during 2001-2002

Month	Week	Temperature (°C)	Relative humidity (%)	Rainfall (mm)	Wind Speed (Km/hr)	Evaporation (mm)
November 2001	45	20.4	66.0	25.4	1.3	3.8
	46	19.7	73.0	-	0.4	2.4
	47	19.2	66.5	-	0.5	1.9
	48	16.6	72.0	-	0.8	1.8
December 2001	49	10.7	75.5	-	1.0	1.5
	50	14.7	80.0	-	1.7	1.3
	51	14.0	79.5	0.4	0.7	1.2
	52	13.7	73.5	-	0.9	2.3
January 2002	1	11.9	71.0	-	0.6	1.4
	2	13.5	69.5	-	1.2	1.8
	3	14.6	81.0	58.0	1.4	2.5
	4	12.9	69.0	2.6	1.7	2.1
February 2002	5	12.8	69.5	-	2.3	2.7
	6	16.7	74.0	17.6	3.2	3.6
	7	15.9	74.5	42.0	1.7	3.4
	8	18.5	68.0	7.2	2.6	3.4
March 2002	9	17.1	70.0	2.2	3.2	3.9
	10	19.0	66.5	-	1.6	4.0
	11	19.5	66.5	-	2.1	4.0
	12	24.2	60.0	-	0.9	4.3
April 2002	13	23.7	55.5	-	1.7	5.3
	14	24.9	55.5	29.2	2.0	5.8
	15	25.8	51.5	-	1.9	5.1
	16	29.5	41.5	-	3.3	7.7
Average	17	27.5	49.5	54.0	6.3	8.8
	18	28.7	50.0	-	4.4	9.1
Average		18.7	66.5	238.6	1.9	3.7

Appendix 6

The weekly average temperature, relative humidity, rainfall and wind speed recorded at Pantnagar during 2002-2003

Month	Week	Temperature (°C)	RH (%)	Rainfall (mm)	Wind Speed (Km/hr)	Evaporation (mm)
November 2002	45	20.1	66.5	-	1.4	3.1
	46	19.3	66.5	-	3.0	3.3
	47	18.7	62.5	-	1.3	2.7
	48	17.5	64.5	-	1.0	2.5
December 2002	49	16.3	66.5	-	1.4	2.8
	50	15.8	68.0	-	1.5	2.3
	51	15.6	72.0	-	2.7	2.2
	52	13.2	81.0	6.4	4.0	2.2
January 2003	1	11.4	85.5	56.6	3.7	1.3
	2	7.0	88.5	-	3.3	1.1
	3	9.3	87.5	-	3.2	0.9
	4	10.9	85.0	0.2	2.4	1.1
February 2003	5	13.7	82.0	32.2	4.8	1.7
	6	15.0	68.5	-	1.9	2.1
	7	15.4	73.0	1.0	2.2	2.2
	8	14.8	77.5	57.4	5.4	3.3
March 2003	9	18.2	74.5	9.8	4.1	3.0
	10	15.2	70.5	-	5.6	3.3
	11	20.4	67.5	-	3.4	3.0
	12	21.3	65.0	-	3.8	3.8
April 2003	13	22.7	63.5	-9.0	4.4	4.3
	14	23.5	54.5	0.4	4.6	5.2
	15	27.1	48.5	-	3.9	6.7
	16	27.6	44.0	1.2	4.7	6.9
	17	28.9	46.5	25.2	6.8	8.4
	18	27.1	50.0	2.0	6.9	6.9
Average		17.9	68.4	201.4	3.5	3.3

Vita

The authoress was born on August 18, 1977 at Almora (Uttaranchal). She got her early education up to 12th from Adam's Girls Inter College, Almora. Thereafter she was admitted in Aligarh Muslim University, Aligarh in 1993, for her Bachelor of Science degree (Honours in Zoology) and completed in 1996 and Masters of Science in Agriculture, Plant Protection in 2000. In the same year she joined Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (Uttaranchal) for Ph. D. In Agriculture (Plant Pathology), and completed the program in 2003. The authoress has also qualified for ICAR -NET, 2000 in Entomology and recipient of Merit scholarship during the Master's program and university fellowship during Ph. D. programme.

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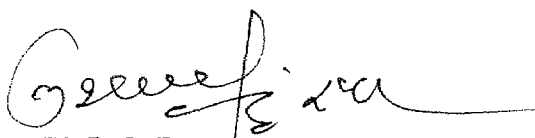
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ABSTRACT

Pulses play an important role in Indian agriculture. Among all pulses, lentil (*Lens culinaris* Medik) is a valuable human feed. Globally, it shares only 5 per cent of the total area under pulses. The main constraints responsible for low yield of lentil are wilt, root-rot complex and collar rot etc. Out of these diseases, lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis* is a major one, which gives 37.5 percent grain yield loss per hectare. Thus keeping this in mind, experiments were conducted to study the effect of different management strategies against wilt incidence.

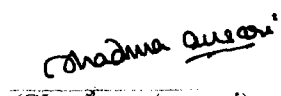
The wilt infected plants show typical symptoms of wilting as the leaves turn yellow and curl from the lower part of the plant upwards and the growth of the plant is checked. The histopathological studies of healthy roots showed compact and multilayered cork cambium cells while the infected roots showed loose cork cambium, thick and wide metaxylem and mycelial growth of the fungus. Early sown crop shows more wilt infection as compared to late sown. Zinc was found to be highly effective in reducing the wilt incidence followed by calcium and magnesium. Among botanicals garlic extracts in water and acetone showed maximum inhibition of spore germination of the pathogen. In case of germplasm screened, only one entry (LH84-8) was found to be moderately resistant against wilt during 2001-2002, while during 2002-2003 out of 50 entries tested, 11 entries showed moderate resistance.

As an integrated management strategies *Trichoderma harzianum* along with zinc and alum showed highest disease control under glass house condition. Under field conditions, maximum disease control was achieved when seeds were treated with Vitavax+ *T. harzianum* followed by *T. harzianum* alone during 2001-2002 crop season while during 2002-2003 crop season the second best treatment was Vitavax+ Thiram. If we use the best compatible integration of the fungicides, micronutrients, fertilizers, bioagents and moderately resistant cultivars selected from this study, we can achieve the eco-friendly management of lentil wilt.



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