EFFECT OF CHROMIUM AND NICKEL ON PATHOGENIC POTENTIAL AND MANAGEMENT OF *ROTYLENCHULUS RENIFORMIS* AND *FUSARIUM SOLANI* ON CHICKPEA (*CICER ARIETINUM* L.)

ABSTRACT

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

BOTANY

BY

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DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

2008

ABSTRACT

Increased industrialization and human activities have impacted on the environment through the disposal of waste containing heavy metals such as Cd, Pb, Cr, Ni, etc. Mine drainage, metal industries, refining, electroplating, dye and leather industries, domestic effluents, landfill leachate, agricultural runoff, and acid rain contribute such a kind of waste. All these metals are known to be highly toxic to plants and animals. Growth and activity of microorganisms including plant pathogens may be greatly influenced by the nature and concentration of heavy metals in soil, which in turn may influence the disease development in plants.

The focal theme of the present study is to assess the effect of two important heavy metal pollutants viz. chromium and nickel on pathogenic potential and management of reniform nematode, *Rotylenchulus reniformis* and the root-rot fungus, *Fusarium solani* infecting chickpea, *Cicer arietinum* var. Kranti. The results of the different experiments embodying the thesis are briefly presented as under:

1: IDENTIFICATION OF RACE OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* ASSOCIATED WITH CHICKPEA:

The results revealed that all the isolates of *R. reniformis* collected from chickpea fields were able to attack and multiply on castor, cowpea, cotton and mustard, but these populations were unable to infect bajra, therefore the populations of *R. reniformis* collected from different locations belonged to Race-3.

2: EFFECT OF CHROMIUM AND NICKEL ON THE HATCHING AND MORTALITY OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS IN VITRO*:

The results clearly indicated that both the heavy metals adversely affected the hatching of *R. reniformis*, Cr being more toxic than Ni. Not only the hatching of the nematode was inhibited but the heavy metals also caused significant mortality of the nematode. Both, hatching and mortality were found

to be directly proportional to the concentration of heavy metals. Inhibition in the hatching was minimum at the lowest concentration (25 ppm) and maximum at the highest concentration (400 ppm) for Cr and Ni. Hundred percent inhibition in the hatching was observed at 400 ppm of Cr. Similarly, the nematode mortality was also increased with an increase in the concentration of the heavy metals as well as the exposure period. The lowest mortality was recorded at 25 ppm of Cr at 12 h exposure period and 100% mortality was obtained in 400 ppm concentration of Cr at 96 h. The nematode mortality was lowest in 25 ppm Ni at 12 h and it increased to 71.0% in 400 ppm Ni at 96 h exposure period.

3: EFFECT OF CHROMIUM AND NICKEL ON THE GROWTH, SPORULATION AND HEAVY METAL UPTAKE OF *FUSARIUM SOLANI IN VITRO*:

The results revealed that the growth and sporulation of *F. solani* significantly decreased with an increase in the concentration of Cr or Ni except at 25ppm Cr and, 25 and 50 ppm Ni. Moreover, the growth and sporulation was significantly enhanced when the fungus was grown in the medium added with 25 ppm Ni. The chlamydospore formation initiated at and above 50 ppm Cr and 100ppm Ni, which increased further with an increase in the concentration of the heavy metals. Similarly, the uptake of heavy metals by *F. solani* was increased with an increase in their concentration. Overall, it was observed that Cr was more toxic to the fungus than Ni.

4: STUDIES ON POTENTIAL PATHOGENIC LEVEL OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* AND ROOT-ROT FUNGUS *FUSARIUM SOLANI* ON CHICKPEA:

The potential pathogenic level of reniform nematode and root-rot fungus, was determined by inoculating the seedlings of chickpea separately with different inoculum levels of *R. reniformis* (250, 500, 1000, 4000 and 8000 immature females per plant) and *F. solani* (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0g

mycelium + spores per plant). There was a gradual increase in the reduction of plant growth, nodulation, yield, chlorophyll content, protein content and water absorption capacity of roots of chickpea with increase in inoculum level of *R. reniformis* except at the inoculum level of 250 immature females per plant which slightly increased plant growth as compared to uninoculated plants. However, the significant reduction in the above mentioned parameters of chickpea plants was recorded at and above 1000 immature females of reniform nematode. The rate of nematode multiplication of *R. reniformis* decreased with an increase in the inoculum levels. The percentage of disease index of *F. solani* increased with increase in the inoculum level. Similarly, a direct correlation between increasing initial inoculum level with decreasing plant growth, yield, nodulation, chlorophyll content, protein content and water absorption capacity of roots was observed for the root-rot fungus, *F. solani.* However, the significant reduction in respective parameters of chickpea was observed at and above 3.0 g of *F. solani* / plant. Hence, the potential pathogenic level of *R. reniformis* and *F. solani* on chickpea was recorded as 1000 immature females of *R. reniformis* / plant and 3.0 g of *F. solani* / plant, respectively.

5: EFFECT OF CHROMIUM AND NICKEL ON PATHOGENIC POTENTIAL OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* AND ROOT-ROT FUNGUS, *FUSARIUM SOLANI* INFECTING CHICKPEA:

The results clearly showed that the plant growth, yield, nodulation, chlorophyll content of leaves, protein content of seeds and water absorption capacity of roots of chickpea decreased in the plants grown in soil treated with heavy metals either alone or in combination with the test pathogens. Chromium was found to be less toxic to chickpea plants and more toxic to nematode and fungus, whereas, nickel was less toxic to nematode and fungus, and more toxic to chickpea plant. With an increase in the concentration of heavy metals (Cr or Ni) from 25 to 200 ppm, there was a corresponding decrease in plant growth,

yield, nodulation, chlorophyll content, protein content and water absorption capacity, which was further ameliorated in the presence of either nematode or fungus. This reduction in plant growth and other parameters of chickpea was synergistically ameliorated when plants were inoculated with either nematode or fungus and grown in soil treated with different concentrations of either Cr or Ni, except the treatment of 25 ppm Cr with nematode and all concentrations of Cr with fungus, which were unable to show synergistic effect.

6: ACCUMULATION OF CHROMIUM AND NICKEL IN CHICKPEA PLANTS INFECTED WITH *ROTYLENCHULUS RENIFORMIS* AND *FUSARIUM SOLANI*:

It was interesting to note that Cr was accumulated by plants in lesser amount than Ni and the amount of heavy metals was more in roots than in shoots. The concentration of the heavy metal accumulation was more in inoculated plants than the uninoculated plants. Moreover, the heavy metals were accumulated in greater amounts by plants inoculated with *F. solani* than the plants inoculated with *R. reniformis*. It was further noticed that the accumulation of heavy metals in plants increased with an increase in the concentration of Cr or Ni.

7: EFFECT OF CHROMIUM AND NICKEL ON THE LIFE CYCLE OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* ON CHICKPEA:

Results revealed that the penetration, development and multiplication of reniform nematode, *R. reniformis* were inhibited and delayed by the presence of heavy metals viz. chromium and nickel as compared to control. The penetration of nematodes in control started within 1 day of inoculation which was however delayed by one day in plants grown in pots treated with either Cr or Ni. Females with slight swelling were first observed on 5^{th} day in control whereas such females were recorded on 8^{th} and 7^{th} day in plants treated with Cr and Ni, respectively. The fully swollen females, females with matrix and females with eggmasses were first recorded on 10^{th}, 14^{th} and 16^{th} day of

inoculation in the control plants, respectively. The corresponding stages of development of reniform nematode were first recorded on 12th, 18thand 20th day, and on 11th, 16th and 18th day of inoculation in plants treated with Cr and Ni, respectively. The average number of eggs per eggmass was significantly reduced in both Cr (48) and Ni (52) treated plants as compared to control (69). The eggs took 4 days to hatch into second stage juveniles in control, while in Cr and Ni treated soil, eggs hatched in 5 and 4 days respectively. The second stage juveniles were recorded on 20th day in control as against 25th day in Ni and 22nd day in Cr treated soil, respectively. The third stage female and male juveniles were recorded on 23rd day in control, but, these stages of development were recorded on 29th and 25th day of inoculation in Cr and Ni treated soil, respectively. Similarly, fourth stage female and male juveniles were recorded on 25th day in control, while in Cr and Ni treated soil these stages were recorded on 33rd and 28th day after inoculation, respectively. The immature females and adult males were recorded on 28th, 39th and 33rd day, respectively in the corresponding treatments. In this way the life cycle of *R. reniformis* on chickpea was delayed by 11 days and 5 days in the presence of Cr and Ni respectively as compared to control. The number of immature females and adult males were also significantly reduced in plants treated with either Cr or Ni as compared to control. The total population of the nematode was also reduced in Cr and Ni treated pots as compared to control on the day of recovery of immature females. The female and male ratio (female: male) was 1.17:1.00 in control as against 1.00:1.30 and 1.00:1.21 in Cr and Ni treated soil, respectively.

8: EFFECT OF CHROMIUM AND NICKEL ON THE EFFICACY OF OIL-CAKES, BIOCONTROL AGENTS AND BAVISTIN IN THE MANAGEMENT OF *ROTYLENCHULUS RENIFORMIS* AND *FUSARIUM SOLANI* INFECTING CHICKPEA:

The results clearly revealed that plant growth and yield of chickpea was significantly reduced in the pots individually treated with *R. reniformis*, *F. solani*, Cr and Ni. Moreover, these parameters of chickpea plants was synergistically reduced in the pots treated with the test pathogen (*R. reniformis* / *F. solani*) in combination with the heavy metal (Cr / Ni) except the plants grown in Cr-treated soil and inoculated with *F. solani* , in which the reduction in plant growth was not synergistic. It was found that the growth and yield of chickpea was significantly improved in presence of neem cake, mustard cake, *T. harzianum*, mahua cake, castor cake and sesame cake as compared to untreated-uninoculated plants. However, the application of linseed cake, *P. lilacinus* and Bavistin did not show any significant improvement in the plant growth and yield as against untreated-uninoculated plants. The best protection of chickpea plants against the *R. reniformis* was recorded by the application of *P. lilacinus* followed by Bavistin, neem cake, mustard cake, castor cake, *T. harzianum* and mahua cake. These treatments significantly reduced the reproduction factor of reniform nematode which consequently increased plant growth and yield of chickpea as compared to untreated-inoculated plants. Similarly, the best protection of chickpea plants against *F. solani* was recorded by the application of Bavistin, followed by *T. harzianum*, neem cake, linseed cake, mustard cake, castor cake and mahua cake. These treatments also significantly reduced the disease index of *F. solani* and increased the plant growth and yield of chickpea . The application of neem cake, mustard cake, castor cake, *T. harzianum* and mahua cake also reduced the damage caused by heavy metals (Cr / Ni). The application of neem cake, mustard cake, castor

cake, mahua cake, *T. harzianum*, *P. lilacinus* and Bavistin were found to be effective in managing the damage caused by combined effect of heavy metal (Cr / Ni) and *R. reniformis*. Similarly, the application of *T. harzianum,* neem cake, mustard cake, castor cake, mahua cake, linseed cake and Bavistin were also found to be effective in managing the damage caused by combined effect of heavy metal (Cr / Ni) and *F. solani*.

9: SCREENING OF CHICKPEA VARIETIES FOR RESISTANCE AGAINST *ROTYLENCHULUS RENIFORMIS, F. SOLANI,* CHROMIUM AND NICKEL:

There was an adverse effect of the test pathogens and heavy metals on the growth of chickpea varieties, irrespective the level of its resistance against pathogen or heavy metal. Out of 25 chickpea varieties, 8 were highly susceptible (Annegiri-1, KUSCR-2, Pant-186, Pragati, Pusa-1103, Pusa-120, Radhey and Vardan), 8 susceptible (Avarodhi, Gaut, Gulab, K-850, Phule G 96020, Pusa-1060, Vijay and XVSCR-2), 5 tolerant (CSJD, JG-74, Phule G 92028, Sadabahar and WCG-2 (Surya)), 3 moderately resistant (Gauraw, KGD-1168 and KWR-108) and one resistant (Phule G 8602) against the reniform nematode, *R. reniformis*, and 7 were highly susceptible (Annegiri-1, KUSCR-2, Pusa-1103, Radhey, Vardan, Vijay and XVSCR-2), 12 susceptible (Avarodhi, CSJD, Gauraw, Gaut, Gulab, K-850, Pant-186 , Phule G 92028, Phule G 96020, Pragati, Pusa-120 and Pusa-1060), 3 tolerant (JG-74, KGD-1168 and Sadabahar), 1 moderately resistant (WCG-2 (Surya)) and 2 resistant (KWR-108 and Phule- G 8602) against the root- rot fungus, *F. solani*. Similarly, out of 25 chickpea varieties, 15 were susceptible (Annegiri-1, CSJD, JG-74, Gauraw , KGD-1168, KUSCR-2, KWR-108, Phule G 92028, Phule G 96020, Pragati, Pusa-120 , Sadabahar , Vardan, Vijay, and XVSCR-2), 7 tolerant (Avarodhi, Gaut, Gulab, K-850, Pusa-1103, Pusa-1060 and WCG-2 (Surya)), two moderately resistant (Pant-186 and Radhey) and 1 resistant (Phule G 8602) to heavy metal, chromium, and 2 were highly susceptible (Pragati and XVSCR-

2), 19 susceptible (Annegiri-1, Avarodhi, CSJD, JG-74, Gaut, Gulab, K-850, KGD-1168, KUSCR-2, Phule G 92028, Phule G 96020, Pusa-1103, Pusa-120, Pusa-1060, Radhey , Sadabahar, Vardan, Vijay and WCG-2 (Surya)), 3 tolerant (Gauraw, KWR-108 and Pant-186) and one resistant (Phule G 8602) against the nickel.

It was interesting to note that the chickpea variety, Phule-G 8602 showed resistance against both the pathogens and heavy metals. Therefore, this variety was once again tested for its resistance to check whether the resistance persisted if the plants grown in the soil treated with either Cr or Ni even in the presence of either *R. reniformis* or *F. solani*. The results indicated that the variety Phule-G 8602 showed the resistance towards *R. reniformis, F. solani* and both the heavy metals (Cr and Ni) even when the same chickpea variety was grown in soil infested with the test pathogen (*R. reniformis/F. solani*) and contaminated with these heavy metals. Therefore, this variety may be recommended to farmers to grow in the fields infested with reniform nematode and root-rot fungus and contaminated with Cr and Ni after making field trials.

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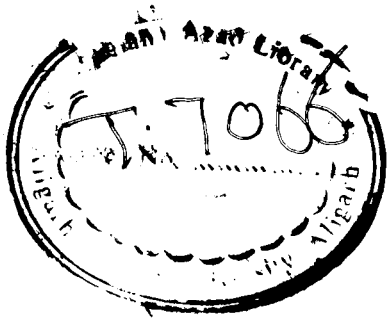

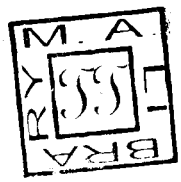

Dedicated to

SIR SYED AHMAD KHAN

(The Founder of Aligarh Muslim University, Aligarh)

Dated 08-05-2008

Certificate

*Certified that the thesis entitled "**Effect of chromium and nickel on pathogenic potential and management of Rotylenchulus reniformis and Fusarium solani on chickpea (Cicer arietinum L.)**" embodies the original research work carried out by **Mr. Mohd. Shaikhul Ashraf** under our guidance and supervision .The work has not been submitted elsewhere for the award of any other degree or diploma and can be submitted in the fulfilment of the requirements for the award of the degree of **Doctor of Philosophy** in **Botany**.*

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ACKNOWLEDGEMENT

Firstly, I am thankful and bow in gratitude to the ***Almighty Allah****, who provided and guided all the channels to work in cohesion and coordination and led me to this path of success.*

It is a great fortune, a matter of pride and privilege for me to express my profound sense of gratitude, indebtedness and sincere thanks to my supervisor, ***Dr. Tabreiz Ahmad Khan****, Reader, Department of Botany, Aligarh Muslim University, who shared with me his expertise and research insight. His noble guidance, constructed criticism, excellent supervision and benevolent behavior have been instrumental for me in getting this work completed.*

I am immensely thankful to my co-supervisor ***Dr. Rifaqat A.K. Rao,*** *Reader, Department of Applied Chemistry, Zakir Husain College of Engineering and Technology, Aligarh Muslim University for his excellent supervision, constant support and encouragement whenever required in completing this work.*

A grateful appreciation is extended to ***Prof. Ainul Haq Khan****, Chairman, Department of Botany, Aligarh Muslim University, for providing me all necessary and possible facilities available in the department to carrying out this work.*

For co-operation, support and a good friendly atmosphere I extend my deep sense of indebtedness to my lab colleagues, Mrs. Shweta Sharma, Miss. Iram Saba and Miss. Aasia Rashid Bhat and my seniors Dr. Rashida Parveen, Mr. Riyaz Ahmad Mir and Mr. Fiyaz Ahmad Ganai.

For giving me strength and support I would like to thank my friends Dr. Barket Ali, Dr. Bilal Habib, Mr. Aashiq Hussain Zargar, Mr. Naseem Ahmad, Dr. Mohd. Iqbal Bhat, Dr. Arshid Hussain Shah, Mr. Hilal Ahmad Bhat, Mr. Tanweer Ahmad Dar (KAS) , Dr. Kouser Parveen, Dr. Rafiq Ahmad Wani, Dr. Azam Musharaf , Mr. Mohd. Azhar Rather, Mr. Sajad Ali, Mr. Tanweer Azam and Mr. Merajul Islam.

No words could adequately express all that my parents have done for me throughout my life. They have always been a source of inspiration and whose constant admonitions help me in my academic pursuits. Their sacrifices, prayers, affection and love made me to reach this stage.

I am immensely thankful to my sister Mrs. Abidah Afzal for her love, encouragement and moral support.

Finally, I would like to thank my lovely Angel Mohammad Aasim Bhat for his endless love and encouragement throughout entire journey. Without whom I would have struggled to find the inspiration and motivation needed to complete this work.

(Mohd. Shaikhul Ashraf)

Introduction

Globally, chickpea (*Cicer arietinum* L.) is the third most important food legume, grown in over 40 countries representing all the continents. Over 95% of the area, production and consumption is in developing countries. Chickpea is the most important pulse crop of India and occupies 7.1 million ha with a production of 5.7 million tons, accounting for 30.9% and 39.9% of total pulse area and production, respectively. The main chickpea producing areas are the upper basin Ganga and Yamuna viz. Punjab, Haryana, Uttar Pradesh and Bihar and the adjoining tracts of central India viz. Rajasthan, Madhya Pradesh and Maharashtra (Anonymous, 2006).

Chickpea is an important food legume that provides many dietary nutrients and phytochemicals, especially in Asian and Middle Eastern countries. Although, predominantly consumed for its protein content, chickpea seeds also contain various carotenoids that can serve as vitamin 'A' precursors, and / or as an antioxidant molecules. On an average chickpea seeds contain 23% protein, 64% total carbohydrates, 47% starch, 5% fat, 6% crude fiber, 6% soluble sugar and 3% ash. The mineral component is high in phosphorus (340mg/100g), calcium (190mg/100g), magnesium (140mg/100g), iron (7mg/100g) and zinc (3mg/100g). Chickpea protein digestibility is the highest among the dry edible legumes. The lipid fraction is high in unsaturated fatty acids, primarily linolic and oleic acids. Chickpea is mainly used for human consumption and only a small proportion is used as feed. Chickpea is also known for its use in herbal medicine and cosmetics. Chickpea meets 80% of its nitrogen requirements from symbiotic nitrogen fixation and can fix up to140 kg N ha^{-1} from air. It also leaves substantial amount of residual nitrogen behind for subsequent crops and adds much needed organic matter to maintain and improve soil health, long term fertility and sustainability of the ecosystems (www.icrisat.org).

Chickpea suffers more than 50 diseases caused by fungi, bacteria, viruses and nematodes (Nene and Reddy, 1987), which lower the quality and quantity of the produce of this crop throughout the world.

Plant parasitic nematodes are one of the important limiting factors of plant growth and productivity, as they cause great destruction to plants singly or in collaboration with other pathogens and parasites. There is hardly any economic crop and for that matter any plant which is not being parasitized by one or more nematode species at a given time. The crop losses due to nematodes are not only in the form of reduced plant growth and yield but are also in the marketable quality of the produce. Plant parasitic nematodes cause US $ 125 billion of losses to world agriculture annually (Chitwood, 2003).

Plant parasitic nematodes are an important constraint to chickpea production and they are estimated to cause annual yield losses of nearly 14% world wide (Sasser and Freckman, 1987). According to Sharma and Sharma (1998), the major nematode pests of chickpea in the Indian sub-continent are the root-knot nematodes (*Meloidogyne incognita* (Kofoid & White) Chitwood , *M. javanica* (Treub) Chitwood and *M. arenaria* (Neal) Chitwood), lesion nematode (*Pratylenchus thornei* Sher & Allen) , reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira) and pigeonpea cyst nematode (*Heterodera cajani* Koshy).In addition to this association of other nematodes viz. *Helicotylenchus dihystera* (Cobb) Sher , *H. sharafati* Mulk & Jairajpuri , *Heterodera vigni* Edward & Misra, *Hirschmanniella mucronata* (Das) Khan *et al.*, *H. oryzae* (van Breda de Haan) Luc & Goodey , *Hoplolaimus dimorphicus* Mulk & Jairajpuri , *H. indicus* Sher ,*Tylenchorhynchus brevidens* Allen, *T. brevilineatus* Williams, *T. mashhoodi* Siddiqui & Basir , *T. vulgaris* Upadhyay *et al.* and *Xiphinema basiri* Siddiqi with chickpea have also been reported by several workers (Sitaramaiah , 1984 ; Sharma and Sharma ,1998).

The reniform nematode (*Rotylenchulus reniformis*), a semi-endoparasite, is largely known to be a serious problem of chickpea in tropics, semi tropics and warmer areas of temperate zone. As implied by common name, the mature females assume a reniform or kidney shape, then produce eggs in a gelatinous matrix, which usually occurs on the external surface of roots and are most frequently seen after a gentle wash of chickpea roots. Reniform nematode has been reported from chickpea plants grown in India, Syria, Tunisia, Ghana and Mediterranean countries (Lamberti, 1981; Oteifa, 1987; Ali, 1995).Reniform nematode is widely distributed in India and has wide host range. It has been reported on chickpea in Andhra Pradesh, Gujarat, Madhya Pradesh, Orissa, Uttar Pradesh and Bihar (Sharma and Sharma, 1998). In Uttar Pradesh the reniform nematode is wide spread in chickpea growing areas of Aligarh, Agra, Bulandshar, Ghaziabad, Kanpur and Mathura (Ali, 1995).

Moreover, chickpea is also a victim of large number of fungal diseases occurring from pre-emergence to the maturity of crop. The various fungal diseases of chickpea in India are wilt disease (*Fusarium oxysporum f.* sp. ciceri (Padwick) Snyd. & Hans.), dry root-rot *Rhizoctonia bataticola* (Taub.) Butler, wet root-rot (*R. solani* Kühn), collar-rot (*Sclerotium rolfsii* Sacc.), stem-rot (*Sclerotinia sclerotiorum* (Lib.) de Bary), foot-rot (*Operculella padwickii* Khesw.), black root-rot (*Fusarium solani* (Mart.) Sacc.) Ascochyta blight (*Ascochyta rabiei* (Pass.) Lab.), Alternaria blight (*Alternaria alternata* (Fr.) Keissler) and powdery mildews caused by *Leveillula taurica* (Lév.) Arnaud and *Erysiphe* Hedwg. Ex Fr. (Anonymous, 1992; Ali, 1995; Bhargava *et al.*, 1981).

Increased industrialization and human activities have impacted on the environment through the disposal of waste containing heavy metals. Mine drainage, metal industries, refining, electroplating, dye and leather

industries, domestic effluents, landfill leachate, agricultural runoff and acid rain contribute such a kind of waste (Aksu and Kutsal, 1990).

Heavy metals are defined as metals with a density higher than 5g cm^{-3}. Fifty three of the 90 naturally occurring elements are heavy metals, but not all of them are of biological importance. Based on their solubility under physiological conditions, 17 heavy metals may be available for living cells and of importance for organisms and ecosystems (Weast, 1984). Among these metals, Fe, Mo and Mn are important as micronutrients; Zn, Ni, Cu, V, Co, W and Cr are toxic elements with high or low importance as trace elements; As, Hg, Ag, Sd, Cd, Pb and U have no known function as nutrients and seem to be more or less toxic to plants and microorganisms (Nies, 1999). The heavy metals released as toxic effluents from smelters are deposited into near by ecosystem (Arnesen *et al.*, 1995) and migration of these contaminants into non-contaminated areas as dust or leachates through the soil and spreading of heavy metal containing sewage sludge are examples of events that contribute towards the contamination of our ecosystems (Atiemanav and Alok, 2004).

In recent years several studies have shown the harmful effects of metals on microbial diversity and activity in soil (McGrath, 1994). The heavy metal pollution has become a matter of concern in India over a last few decades. Industrialization in India gained a momentum with initiation of five-year development plan in the early 50's. The pollutants of concern include lead, chromium, mercury, uranium, selenium, gold, silver, copper and nickel. These toxic metals may be derived from mining operations, refining ores, sludge disposal, fly ash from incinerators, the processing of radioactive materials, metal plating or the manufacture of electrical equipments, paints, alloys, batteries, pesticides or preservatives. Heavy metals such as zinc, lead and chromium have a

number of applications in basic engineering works, paper and pulp industries, leather tanning, organochemicals, petrochemicals, fertilizers, etc. Major lead pollution is through automobiles and battery manufacturers. For zinc and chromium, the major application is in fertilizer and leather tanning, respectively (Trivedi, 1989). As man continues to add pollutants to the environment, they continue to be widely dispersed. As a result, plants, plant pathogens and plant pathogenesis are all being affected. At present, very little is known on the effects of pollutants on pathogenic diseases of plants. The pollutants may affect the pathogenesis in different way: it may be increased or decreased through a direct effect of the pollutant-induced changes in the host plant or through changes in other aspects of the environment. With all pollutants, the threshold concentration required to injure plants is affected by the resultant of duration of exposure × concentration of pollutant and a multitude of interacting biological and meteorological variables (Parveen, 1995).

Among the abiotic cause of plant diseases, industrial and other chemical pollutants are important in relation to crop cultivation (Heagle, 1982). The use of waste water containing heavy metals for irrigation cause phytotoxicity and predispose plants to pathogenic damage (Cole *et al.*, 1969). Moreover, several workers have investigated the effect of heavy metals on plant growth (Hagemeyer, 1999; Kukkola *et al.*, 2000), and microorganisms including plant pathogens such as bacteria (Rajapaksha *et al.*, 2004), fungi (Somashekar *et al.*, 1983; Parveen and Alam, 1998) and nematodes (Hafkenscheid, 1971; Haight *et al.*, 1982; Sturhan, 1986; Khan and Salam, 1990; Parveen and Alam, 1998; Khan *et al.*, 2006).

In India, Aligarh is a major lock-manufacturing city for more than 70 years. During this period, large amount of heavy metals emitting

from various lock manufacturing and electroplating industries has increased considerably during the last three decades. During a course of survey in various chickpea growing areas on both sides of Mathura Road, Aligarh district of Uttar Pradesh, more or less constant infections of reniform nematode, *Rotylenchulus reniformis* and root-rot fungus, *Fusarium solani* were observed on the roots of chickpea plants. Further investigation on the soil of these fields indicated the presence of high concentrations of heavy metals viz. chromium (94ppm) and nickel (118ppm). The association of either reniform nematode or root-rot fungus with heavy metals was invariably associated with the, stunting of plants, yellowing of leaves and ultimately death of certain plants. The source of irrigation in these fields is waste water coming from different industrial areas of Aligarh.

The information regarding the effect of heavy metals viz. Cr and Ni on reniform nematode, *R. reniformis* and root-rot fungus, *F. solani* infecting chickpea has not been furnished so far as indicated by the scanning of literature. So in order to furnish this information the present work was carried out to study the effect of chromium and nickel on pathogenic potential and management of *R. reniformis* and *F. solani* on chickpea var. Kranti with following objectives:

1. Identification of race of reniform nematode, *Rotylenchulus reniformis* associated with chickpea.
2. Studies on the effect of chromium and nickel on the hatching and mortality of *R. reniformis in vitro.*
3. Studies on the effect of chromium and nickel on the growth, sporulation and heavy metal uptake of *Fusarium solani in vitro.*

4. Studies on potential pathogenic level of reniform nematode, *Rotylenchulus reniformis* and root-rot fungus *Fusarium solani* on chickpea.

5. Studies on the effect of chromium and nickel on pathogenic potential of reniform nematode, *R. reniformis* and root-rot fungus *F. solani* infecting chickpea.

6. Studies on the accumulation of chromium and nickel in chickpea plants infected with *R. reniformis* and *F. solani.*

7. Studies on the effect of chromium and nickel on the life cycle of *R. reniformis* infecting chickpea.

8. Studies on the effect of chromium and nickel on the efficacy of oil-cakes, biocontrol agents and Bavistin in the management of *R. reniformis* and *F. solani* infecting chickpea.

9. Screening of chickpea varieties for resistance against *R. reniformis, F. solani*, chromium and nickel.

Review of Literature

India is basically an agriculture-based country, where agricultural technologies in practice are both traditional and modern. However, the former one is quite predominant. As far as the output is concerned, it is quite low as compared to the advanced countries. The main constraints for the low productivity of our crops are low rainfall, low fertilizer inputs, less availability of certified seeds, traditional agricultural systems in practice and biotic (pests, parasites and pathogens) and abiotic (environmental stresses including pollution problems) agencies. Moreover, the emergence of "Pollution Pathology" as a distinct discipline has drawn attention of various scientists worldwide. It reveals the interactive effects of pollutants and pathogenic agencies and their impact on crops (Rich, 1964; Heck, 1968; Heagle, 1973; Parveen and Alam, 1998).

The accumulation of metals in soils at high concentrations can be due to anthropogenic activities such as the application of sewage sludge. This practice has been widely used for nutrient recycling and is accepted for waste disposal in Agricultural soils (Sauerbeck, 1986). However, the addition of sewage sludge considerably increases the amount of heavy metals in the soil, which could be toxic for microorganisms as well as for plants (Chaudri *et al.*, 1993). The primary chemical change in soil is acidification, which increases the availability of heavy metals in the soil solution to toxic levels, which can persist for long periods of time. Therefore, there is an increasing concern about the possible toxic effects of heavy metals on the microbial population, plants and animals, especially after long term sludge application to soils. The metals in soil have deleterious effects on the metabolism of the soil microorganisms, which inturn affect the organic matter decomposition and microbial activities (Fritze *et al.*, 1989). Moreover, the excessive heavy metal concentration in soil has been reported to cause a decrease in microbial

population (McGrath *et al.*, 1995), changes in population structure (Bardgett *et al.*, 1994) and physiological activity (Cotrufo *et al.*, 1995). Growth and activity of microorganisms including plant pathogens may be greatly influenced by the nature and the concentration of heavy metal contamination in the soil, which inturn may influence the disease development in plants.

The present write-up is an attempt to gather hitherto scattered informations on the effect of heavy metals on nematodes and fungi, management of *R. reniformis* and *F. solani*, and screening of chickpea varieties against *R. reniformis, F. solani* and heavy metals.

2.1: EFFECT OF HEAVY METALS ON NEMATODES:

Several reports on the effects of pollutants on plant pathogens have been published, but relatively a few have concerned nematodes (Heagle, 1973; Parveen and Alam, 1998). Studies relating to the alteration in the course of development of plant diseases caused by nematodes are also quite meagre. Since very little is known about the effects of heavy metal pollutants on nematodes and since industrial and urban pollution is becoming more prevalent, plant pathologists should be concerned with this problem not only because of the possible effect on the biological pathogens *per se* in relation to disease development, but also because of the possible usefulness of these organisms as indicators of the source and extent of heavy metal pollution.

In the context of pollution impacts on nematodes, preliminary studies have been made on marine/free living forms and the work on plants parasitic nematodes is rather a recent interest of the scientists.

(a) PLANT-PARASITIC NEMATODES:

During the last two decades, research on plant parasitic nematodes in ecological and agricultural processes has gained more attention. There are various reports available on the effects of pollutants on plant parasitic nematodes (Shew *et al.*, 1982; Bisessar and Palmer, 1984; Singh, 1989; Parveen and Alam, 1998). The impact of heavy metals on plant-parasitic nematodes and the disease caused by them is being discussed here in detail.

In the course of attempts by Crosse and Pitcher (1953) to obtain a microbiologically sterile inoculum of *Aphelenchoides ritzemabosi* (Schwartz) Steiner & Buhrer, distilled water from a still fitted with a rhodium-plated condenser plate was found to be highly toxic to this nematode. This difficulty was overcome by using glass-distilled water, but, subsequently attempts were made to identify the toxic principle in the metal-distilled water. Tests of rhodium, both ionic and colloidal, proved negative and subsequent spectrographic analysis failed to detect any rhodium in residues obtained by evaporation of the metal-distilled water. However, silver residues equivalent to about 1ppm were found in the water and the examination of the still revealed that the rhodium plating had completely broken down, exposing an underlying silver plate. Test with solutions of silver salts showed toxicity comparable to that of the metal-distilled water at concentrations of 0.5 to 1.0 ppm silver ions (Ag+). A further test of the metal-distilled water showed *Ditylenchus dipsaci* (Kühn) Filipjev to be twice to four times more resistant than *A. ritzemabosi.*

Further evidence of interspecific difference in susceptibility to metallic contaminants was provided by observations of considerable mortality in *Xiphinema diversicaudatum* (Micoletzky)Thorne, extracted from soil, using phosphor-bronze 50 μ-aperture sieves in Petri dishes containing tap water for the final stage of the process (Seinhorst, 1956). Analysis showed that tap water in which such sieves had stood for 18-22 hrs contained 1-1.6 ppm cupric ions

(Cu^{++}), compared with 0.1 ppm for water kept in glass dishes. Subsequent tests showed that mortality would be considerably reduced by using plastic sieves and that mortality comparable to that occurring in the presence of phosphor-bronze sieves resulted from exposure to solutions of copper salts containing approximately 1 ppm Cu^{++}.

Van Gundy and Thomason (1962) pointed out that a concentration of 0.5 ppm copper killed *Trichodorus christiei* Allen within 24 h. Clarke and Shepherd (1966) indicated that several inorganic ions including Ni^{2+} and Cr^{3+} stimulated hatching of eggs of *Heterodera* spp. De Maeseneer (1967) obtained 80% control of three longidorid species within two months by the incorporation of 200 ppm Cu^{++} in the form of cupric sulphate, but, obtained no control of tylenchid or other dorylaimid nematodes. Moreover, Ibrahim and Hollis (1967) reported that dilute aluminum chloride and cadmium chloride attracted *Tylenchorhynchus martini* Fielding.

Some inorganic ions hatched several species of *Heterodera* and some only a few. For example, Zn^{2+} hatched as many as eight out of the nine species while others such as Sr^{2+} and Li^{+}, only two. Closely related ions sometimes had very different effects; Zn^{2+} hatched *H. glycines* Ichinohe well but Cd^{2+} inhibited hatching of this species. Only vanadate ions hatched *H. rostochiensis* Wollenweber as effectively as potato root diffusate (Shepherd and Clarke, 1971).

Hafkenscheid (1971) observed that during virus transmission many specimens of *Trichodorus pachydermus* Seinhorst, showed little or no mortality after extraction from the soil by the method of Oostenbrink (1960). These nematodes were useless for the experiments. The reason attributed to this was that the copper supported sieves released copper ions, which were toxic to the nematodes and caused death of nematodes. Cooper (1971) noted *Trichodorus* spp. to be most plentiful in soils with copper or manganese deficiencies, but, despite demonstrating *in vitro* toxicity from solutions containing 1 ppm Cu^{++}, he observed no change in *Trichodorus* population of such soils for sixteen

months after rectifying the deficiencies by applications of Cupric and Manganese sulphates. Pitcher and McNamara (1972) conducted *in vitro* tests with concentrations of 0.1 and 0.01 ppm Ag^{+} ions and of 10.0, 1.0 and 0.1 ppm Cu^{++} ions for three species of nematodes and showed *Pratylenchus penetrans* (Cobb) Chitwood & Oteifa to be the most susceptible to silver, and *Xiphinema diversicaudatum* the most susceptible to copper. *Aphelenchoides ritzemabosi* was the least affected by either silver or copper.

Bisessar *et al.* (1983) studied the effect of heavy metals and *Meloidogyne hapla* Chitwood on celery (*Apium graveolens* L.) which was grown on organic soil in the vicinity of a nickel refinery. The treatment consisted of nickel at 7500 ppm, copper at 800 ppm and cobalt at 100 ppm. It was found that nematode alone was responsible for an average shoot weight 12% less than the controls while heavy metals alone resulted in shoot weight 79% less than the controls. Their combined treatment (heavy metal + nematode) caused a shoot weight 85% less than the controls. Root galls were also much pronounced in the plants which received the treatment of nematode and heavy metal in comparison to those received the treatment of nematode only.

Sturhan (1986) evaluated the influence of heavy metals and other elements on soil nematodes by applying Be, Cd, Hg, Sn, Pb, V, Cr, Ni, Se, F, Br and As to soil at two different concentrations. In case of V, at higher concentration, there was significant reduction in nematode population. Fluorine and V appeared to be toxic to certain nematodes even at the lower concentration. *Trichodorus primitivus* (de Man) Micoletzky was affected by seven of twelve elements and *Scutylenchus tartuensis* (Krall) Siddiqi, *Criconemoides informis* (Micoletzky) Taylor by at least three and *Pratylenchus* spp. by two, while numbers of *Aphelenchoides* spp. and *Aphelenchus avenae* Bastian were not markedly reduced in any of the contaminated soils. Related species such as *Tylenchorhynchus dubius* (Butschli) Filipjev, *S. tartuensis* and *Merlinius microdorus* (Geraert) Siddiqi reacted differently to the same elements. He

further concluded that the soil nematodes may be suitable as bioindicators for certain environmental chemicals.

Alphey and Brown (1987) studied the effects of pollutants on plant parasitic nematodes viz. *Xiphinema diversicaudatum, Longidorus elongatus* (de Man) Thorne & Swanger, *Trichodorus primitivus and T. pachydermus*. They found that the presence of DBP (Dibutylphthalate) - contaminated glazing strip in the soil did not significantly decrease the numbers of migratory plant parasitic nematodes. They also observed the mean number of nematodes surviving after two months in Cu watered soil and found the different nematode counts as *Xiphinema* in fresh tap-water (34.1), Cu tap water (21.8) and $CuSO_4$ solution (13.7). Similarly, the different nematode counts of *Longidorus* were 233.0, 189.3 and 202.5 and trichodorids were 148.6, 141.7 and 117.1 recorded in the corresponding treatments.

Khan and Salam (1990) reported that the higher inoculum levels of *M. javanica* caused significant reduction in plant growth of pigeonpea (*Cajanus cajan* (L.) Millsp.). Similarly, the higher concentrations of Co and Ni also suppressed the plant growth. The reduced nodulation and complete inhibition of root galling caused by *M. javanica* were recorded in the presence of Ni. The interaction of Co with *M. javanica* increased root galling and reduced plant growth by a greater amount than the total reductions caused by the individual treatments. They also reported that Ni was inhibitory to the hatching of *M. javanica* at all concentrations except 9.71 mg/l and cobalt showed inhibitory effect at 1117 and 11170 mg/l. Cobalt was stimulatory at 11.7 and 111.7 mg/l. Both Ni and Co were toxic to the second stage juveniles. All juveniles were killed in 9710 mg/l of Ni and in Co at and above 111.7 mg/l.

The adverse effect of the heavy metals (Cd and Pb) on the growth of females of *M. incognita* as well as its pathogenic potential on tomato

(*Lycopersicon esculentum* Mill.) were found to have correlation with the protein profile of the nematode. Number of polypeptides were significantly reduced with the increased concentrations of heavy metals. Moreover, nematode infected plants showed higher concentration of heavy metals than nematode free plants (Parveen, 1995).

Khan *et al.* (1996) investigated the individual and combined effects of *Meloidogyne javanica* and nickel as $NiCl_2$ (10, 50, 100 and 200 ppm) on the plant growth, flowering, fruit setting, seed protein and root nodulation on chickpea. Nickel at 100 ppm and 200 ppm caused pigmentation of leaf margins and significantly suppressed plant growth, seed protein and nodulation. The nematode also caused similar negative effects. The infected and treated plants developed more Ni injury at all concentrations and root galling at 50 ppm. The eggmass production and fecundity however decreased in the treated plants except at 10 ppm. A synergistic relationship between *M. javanica* and Ni (50 ppm) was apparent resulting in a greater suppression of the variables considered. Nickel at 200 ppm reduced the negative effect of the nematode.

Parveen and Alam (1999a) reported that the hatching of *Meloidogyne incognita* was inhibited and mortality of second stage juveniles increased with an increase in the concentration of heavy metals and exposure time. Lead was found relatively more toxic to the nematode than cadmium and acute effective toxic values (EC_{50}) were 50.8 ppm at 96 hrs for Cd and 53.5 ppm at 72 hrs or 13.9 ppm at 96 hrs for Pb.

Parveen and Alam (1999b) evaluated the efficacy of organic additives in presence of heavy metal pollutants (Cd and Pb) for the management of root-knot nematode, *M. incognita* on tomato. The heavy metals were found to be more damaging to plant growth parameters than *M. incognita*, irrespective of the treatments. Even though Cd and Pb

showed nematode toxic effects, yet it was noted that both the heavy metals and nematode together caused more plant damage than that caused by either of them alone. It was interesting to note that barring the oil-seed cakes of neem and castor, the treatments could not check the synergistic effect of the metal-nematode combine. Therefore, it was concluded that these oil-cakes are beneficial to tomato plants under the attack of root-knot nematode, even in the soils polluted with Cd and Pb.

Parveen and Alam (2001) screened the different tomato cultivars against root-knot nematode, *M. incognita* in presence of lead and observed that the degree of susceptibility of tomato cultivars against root-knot increased in presence of lead. On the basis of observations of different parameters, tomato cv. Akra Vikas was found to be most suitable against the combined stress of the nematode infection and lead.

Bakonyi *et al.* (2003) studied the effect of Cd, Cr, Se and Zn at a maximum rate of 270 mg/Kg on a nematode assemblage after 6-10 years of application in a field where winter wheat *(Triticum aestivum* L.*),* sunflower (*Helianthus annus* L.), sorrel (*Rumex acetosa* L.), barley *(Hordeum vulgare* L.*)* and rape (*Brassica juncea* (L.) Czern. & Coss.) were grown. Cadmium showed a moderate effect on nematodes in spite of the fact that this element significantly decreased the plant biomass, Cr was harmful to plants only in the first year of study, but it decreased *Aporcelaimellus* Heyns density and maturity index but increased *Pratylenchus* Filipjev density. Selenium proved to be toxic at concentration of 11mg/Kg. Some advantageous effects of Zn were found in the first year which disappeared later on.

Nagy *et al.* (2004) investigated the long term effects of Cd, Cr, Cu, Se and Zn, seven years after artificially contaminating plots of an agricultural field on a calcareous chernozen soil. They found that nematode density was significantly reduced by 90 and 270 mg Kg^{-1} Se

as well as by 270 mg Kg^{-1} Cr, while 90 and 270 mg Kg^{-1} Se also reduced nematode generic richness. The maturity Index values consistently decreased with increasing Cr and Se concentrations and to a lesser extent in Zn plots as well. Structure index showed decreasing trends in increasing Cr and Se concentrations and to a lesser extent Zn treatments, while in Cd, it showed a moderate increase. Moreover, the proportion of the most sensitive omnivorous and predatory nematodes decreased clearly as a consequence of Cr and Se treatments.

Parveen (2004) evaluated the effect of cadmium at 7.5, 15.0, 30.0 and 60.0 ppm on the penetration of *M. incognita* and growth parameters of tomato cv. Pusa Ruby. The results revealed that Cd inhibited root penetration by second stage juveniles of *M. incognita*, which subsequently affected the development of root galls in tomato roots. Cadmium was highly injurious to tomato plants at all concentrations. These inhibitory effects on plant growth and other parameters viz. fresh and dry weight of plants, chlorophyll content of leaves, water absorption capability of roots significantly increased with increasing concentration of the metal and further increased in the presence of the nematode.

Nickel amendments at 200 or 400 mg/l caused browning and/or necrosis of foliage. Either Ni or *M. incognita* were able to decrease root and shoot growth, and carotenoid and chlorophyll contents of foliage. Root-knot nematode disease severity (i.e. degree of galling) increased when plants received a Ni amendment of 50 or 100 mg/l. Nematode reproduction (eggmasses/root system) and soil population increased when soils were amended with 50 mg Ni/l. However, higher concentrations of Ni amendments (200 and 400 mg/l) decreased the number of galls, eggmasses, fecundity and the soil population of *M. incognita*. Nickel contents of roots, stems and foliage were greater in nematode infected plants than uninfected plants grown in soils receiving no Ni amendments. The order of Ni accumulation was: root > leaves > stem, and

leaves > root > stem in infected and uninfected plants, respectively. A small increase in root Ni concentration (up to 24 µg Ni/g dry root) was associated with increased juvenile penetration and gall formation of nematodes, however, further increases of Ni in roots (due to 200 and 400 mg Ni treatments) suppressed nematode pathogenesis. This interaction between *M. incognita* and Ni on dry matter of plant organs was found to be concentration dependent, with the interaction being synergistic at 50 mg/l, but at 400 mg Ni the effect was antagonistic with regard to the effect of Ni on nematodes and plant growth (Khan *et al.* , 2006).

(b) FREE LIVING / MARINE NEMATODES:

Marine nematodes may prove to be sensitive biological indicators of pollution because they are very diverse taxonomically and occur everywhere, usually in great numbers, often exceeding other taxa by orders of magnitude (Platt *et al.*, 1984). Hodda and Nicholas (1986) found that polluted area were more diverse taxonomically than unpolluted areas. Quite a large number of reports are available on the impact of heavy metal pollutants on marine and free-living nematodes (Parveen and Alam, 1998). Feldmesser and Rebois (1966) studied the effect of Cd salts on nematodes and observed that 48 h-LC_{50} for mixed population of *Panagrellus* Thorne and *Rhabditis* Dujardin ranged from 35 to 45 mg/l which compares favourably with the mean adult LC_{50} value of 26.3 mg/l for *Panagrellus silusiae* (de Man) Goodey.

Popham and Webster (1979) observed that the growth of *Caenorhabditis elegans* (Maupas) Dougherty was inhibited at 400 µM Cd. Haight *et al.*(1982) evaluated the toxicity of seven heavy metals viz. Cr, Ni, Cd, Pb, Hg, Cu and Zn on a free living nematode, *P. silusiae*. Acute lethal toxicity values (LC_{50}) were obtained for each heavy metal and *P. silusiae* was found to be highly resistant. Juvenile worms were slightly more sensitive than adults but this trend was not significant. It

was found that Cu, Cr and Cd effectively blocked the growth of nematode at all stages of development. The highest soluble concentrations of Pb and Ni were without any effect on growth of the worms, high concentration of Zn (>500 mg/l) partially blocked growth and Hg was either lethal at 20 mg/l or ineffective in blocking growth at 10 mg/l.

Howell (1984) found that the shortening of lifespan of the marine nematode, *Enoplus brevis* Bastian was concentration-dependent in filter-sterilized sea water. The life span of *E. brevis*, at a concentration of 0.44 µM Cd was severely shortened compared to that of individuals exposed to 0.044 µM Cd.

Bogaert *et al.* (1984) showed that the marine nematode *Diplolaimelloides bruciei* Hopper was more sensitive than the *Panagrellus redivivus* (Linnaeus) Goodey for determining the toxicity of $HgCl_2$ and 2-acetamidofluorine but less sensitive for methylmercury chloride, phenacetin and 4-aminobiphenyl. The marine nematode, *Monhystera microphthalma* de Man was less sensitive to $HgCl_2$, selenium oxide and caesium chloride.

Vranken *et al.* (1985) observed a decreased success in attaining the adult in the marine nematode species of *Monhystera microphthalma*, *M. disjuncta* Bastian and *Pellioditis marina* (Bastian) Dougherty at concentration of 11, 43 and 219 µM $CdCl_2$, respectively. A significant prolongation of developmental time of *M. microphthalma* was observed at 44 µM Cd. Van Kessel *et al.* (1989) revealed that the growth of *C. elegans* was significantly reduced from a level of 1 µM $CdCl_2$. Reproduction of the nematodes was also reduced at 1 µM exposure level. The growth was retarded at the early juvenile stages at levels of 160 and 320 µM and did not reach the adult stage and therefore did not

reproduce. Williams and Dusenbery (1990) evaluated the toxicity of soluble forms of Ag, Hg, Cu, Be, Al, Pb, Cr, As, Ti, Zn, Cd, Ni and Sr to *C. elegans*. In all the cases LC_{50} value for 1-4 days of exposure were determined. It was observed that metals Pb, Cr and Cd had the lowest LC_{50} at 96 h (0.06mg/l) with *C. elegans* while Sr had the highest LC_{50} (465 mg/l). All the metals except As had a significantly lower LC_{50} at 96 h than at 24 h. Comparison with other invertebrates indicated that *C. elegans* was more sensitive to Pb, Cr and Be and less sensitive to As.

Anderson *et al.* (2001) carried out a study to characterize the sensitivity of movement, feeding, growth and reproduction as end points for heavy metal toxicity with *C. elegans*. Median effective concentrations (EC_{50}) for 24 h exposure to Pb, Cu and Cd were determined for movement, feeding and growth, and 72 h EC_{50} was derived for reproduction. The order of toxicity was Cu > Pb > Cd for each end-point including lethality and movement. No differences were found in sensitivity among end points for any metal. When exposed for 4 h at sublethal concentrations that were 14 times the 24 h EC_{50} value, Pb and Cu reduced feeding to the same extent while movement was reduced significantly more by Pb than Cu. Thus, the differences in sensitivity of end-points was more apparent at 4 h, which was not evident at 24 h, suggesting that potentially different mechanism of toxicity for 24 and 4 h tests.

Jonker *et al.* (2004) investigated that whether effects of mixtures of Cu-Cd and Cu-Carbenazium on *C. elegans* were similar to the effects of the individual compounds. More Cd in the mixture increased the toxicity and more copper decreased the toxicity. The effect of copper-carbendazen on reproduction was synergistic at low dose levels and antagonistic at high dose levels and independent of time. Mixture effects

on the juveniles and reproduction period were similar to single compound effects.

2.2: EFFECT OF HEAVY METALS ON FUNGI:

Several reports are available on the effect of heavy metals on growth and multiplication of fungi, which showed that its effect varied on different fungal genera.

Bisessar (1982) observed that the abnormally high concentrations of Pb, As, Cd, and Cu decreased, and population counts of fungi increased with increasing distance from the Pb smelter. The negative correlation coefficient between fungi and the level of Pb, As, Cd, and Cu in the soil was statistically significant. The microflora of the contaminated soil was also altered and the marked quantitative reduction or elimination of organisms was attributable to heavy metal pollution emission from the smelter. Nordgren *et al.* (1985) studied the microfungal species in coniferous forest soils surrounding a brass mill at Gussum in Southeast Sweden. The concentrations of Cu and Zn were 20,000 µg/g dry soil close to the mill and that of Pb was 100 µg/g dry soil and the pH was 2 units above the normal value 3.5-4. The heavy metal contamination strongly affected the microfungal species composition. There was a decrease in the isolation frequency of fungi common in coniferous soil e.g. *Penicillium spinulosum* Thom., *P. montanense* Christensen & Backus, *P. brevicompactum* Dierckx, *Oidiodendron tenuissium* (Perk) Hughes, *O. echinulatum* Barron *and O. maius* Barron, whereas, the isolation frequency of other fungi viz. *Paecilomyces farinosus* (Dickson ex Fr.) Brown & Smith, *Geomyces pannorum* (Link) Sigler & Carmichael, *Chalara constricta* Raj & Kendr., *C. longipes* (Preuss) Cooke ,less common or rare and sterile forms was increased. The fungi belonging to genus *Mortierella* Coemans were less affected by the heavy metal pollution.

The response of few test fungi was assessed by exposing them to known concentration of heavy metals. *Trichoderma harzianum* Rifai and *T. viride* Pers. showed tolerance to an increased level of heavy metals. The growth of *Drechslera halodes* (Drechs.) Subram. & Jain, *D. tetramera* (McKinney) Subram. & Jain and *Curvularia lunata* (Wakker) Boed. was supported by higher concentrations of Zn. The growth of *Apergillus versicolor* (Vuill.) Tirab. and *Colletotrichum dematium* (Berk.) Arx. was enhanced by lower concentration of Nickel. The colony growth of *C. dematium, Fusarium moniliformae* Sheld., *Cladosporium cladosporioides* (Fr.) de Vr. and *A. versicolor* was stimulated by the lower concentrations of Hg and Cu. In addition to *T. harzianum* and *T. viride, C. cladosporioides* also showed more resistance towards Ag. Intense yellow secretion by *T. viride* was observed in media amended with Co, whereas addition of Hg and Pb resulted in yellow pigmentation among the spores of *T. harzianum* (Lokesha and Somashekar, 1990).

Singh *et al.* (1992) found that the incorporation of Ni, Co, Cr, Pb and Cu into liquid or solid medium effectively suppressed the growth of *Paecilomyces lilacinus* (Thom.) Samson. The suppressive effect was concentration dependent and the metals also differed in their efficiency for suppressing the growth. Parveen and Alam (1993) exposed the nematode antagonistic fungus *Paecilomyces lilacinus* to known concentration of Cd *in vitro*. It was observed that with an increase in the concentration of Cd, the dry mycelial weight and sporulation of the fungus decreased.

Chandra and Muthumary (1993) reported that three species of *Aspergillus* Mich. ex Fr. viz. *A. niger* Van Tiegh, *A. terreus* Thom. and *A. flavus* Link ex Fr. were found to be tolerant to Cu. The biomass production indicated that lower concentration (0.1µg/ml) promotes the growth of these species. *Aspergillus flavus* exhibited greater efficiency

of Cu uptake and the efficiency increased with the increase in the concentration of Cu. Transmission Electron Microscopic studies in *A. niger* and *A. flavus* showed that Cu toxicity leads to change in conidial wall such as wall thickening and accumulation of melanin in the wall layers. In addition some membrane bound precipitated bodies found in between the outer and the inner layers of the conidial wall.

Narayana and Manoharachary (1994) reported that the fungi belonging to genera *Aspergillus, Penicillium* Link ex Fr. and *Trichoderma* Pers. ex Fr. were tolerant and highly adapted to the effluents containing heavy metals. However, some Mucorales and Ascomycetes were reported to be sensitive. It was concluded that the predominantly occurring fungi might have degraded the effluent residues and later multiplying with the help of some useful metabolites. Hashem and Bakali (1994) reported that *Fusarium solani* isolated from Saudi Arabian soil was able to grow on liquid medium containing upto 300µg/ml Co or Ni. The fungus was more tolerant to cobalt than nickel and accumulated the heavy metals in its mycelium.

The different concentrations of Pb were found to be inhibitory to the mycelial growth as well as the sporulation of nematode antagonistic fungus, *Paecilomyces lilacinus*. The percent inhibition increased with an increase in the dose of heavy metal (Parveen and Alam, 1997). Kalim *et al.* (1999) reported that root-rot of cowpea caused by *Rhizoctonia solani* and *R. bataticola* was controlled to the extent of 40% and 44.5% by the application of 5 and 10 ppm Cu and copper sulphate, respectively. Reduction in disease incidence was attributed to the increased activities of polyphenol oxidase and peroxidase along with higher amounts of total phenols.

Blaudez *et al.* (2000) tested thirty nine isolates of *Paxillus involutus* (Batsch) Fr., *Pisolithus tinctorius* (Pers.) Coker & Couch,

Suillus bovinus (L. ex Fr.) Kuntze, *S. luteus* (L.) Roussel and *S. variegates* (Fr.) Kuntze grown on Cd, Cu, Ni and Zn amended media to determine their *in vitro* tolerance measured as inhibition of biomass production. Twenty one isolates were from heavy metal polluted sites whereas the others were from non-contaminated sites. There was a strong interspecific variation in metal tolerance, *S. luteus, S. variegatus* and *P. tinctorius* were more tolerant to Cu, Cd and Zn as compared to *P. involutus*, whereas the reverse was true for Ni. The EC_{50} values for isolates originating from polluted sites were not statistically different from EC_{50} values for isolates originating from non-contaminated sites.

Kredics *et al.* (2001a) investigated the effects of ten heavy metals viz. Al, Cu, Ni, Co, Cd, Zn, Mn, Pb, Hg and Fe on the *in vitro* activities of β-glucosidase, cellobiohydrolase, β-xylosidase and endoxylanase enzymes for six strains of *Trichoderma* and to isolate and characterize heavy metal-resistant mutants. The results revealed that at a concentration of 1 mM, only Hg showed significant inhibitory effects on the *in vitro* enzyme activities and in all other cases, the enzymes remained active. A total of 177 heavy metal resistant mutants were isolated and tested for cross resistance to other heavy metals. Some mutants were effective antagonists of *Fusarium* Link ex Fr., *Pythium* Pringsh. and *Rhizoctonia* DC ex Fr. strains, even on media containing the respective heavy metals. They further suggested that *Trichoderma* mutants resistant to heavy metals might be of value for use with heavy metal contaminating pesticides, as a part of an integrated plant protection system.

Kredics *et al.* (2001b) found that mycelial growth of the six *Trichoderma* strains was significantly influenced by the heavy metals. The smallest variation of IC_{50} values among the six strains was found for nickel and cobalt, while IC_{50} values for iron showed significant variation. The lowest IC_{50} values were found for copper, while the

highest for Al. When all heavy metals were taken into consideration, *T. harzianum* T66 and *T. atroviride* Karst. T122 were the most resistant, while, *T. viride* strains T114 and T124 were the most sensitive.

Rajendran *et al.* (2002) tested the toxicity of $NiCl_2$ to *Aspergillus niger*. In contrast to 50% condial inhibition at 1.7 mM nickel, hyphal extension was affected even at a lower concentration (0.4mM) suggesting that hyphae are more sensitive than conidia to Ni. Reddy *et al.* (2002) investigated the influence of Al on growth and mineral nutrition of two ectomycorrhizal fungi, *Contharellus cibarius* Fr. and *Pisolithus* Alb. & Schwein. sp. *in vitro*. The mycelial biomass of both fungi decreased as the concentration of Al increased in the culture medium but *C. cibarius* was more resistant than *Pisolithus* sp. This growth inhibition was associated with impaired mineral nutrition. Increasing exogenous Al concentration caused Al accumulation in *Pisolithus* sp. upto 40 mg g^{-1} dry weight but this accumulation was much less with *C. cibarius*.

Yesil *et al.* (2004) collected and identified a group of 21 macrofungi from 29 different locations and these fungi were then analyzed for the heavy metal contents. The highest Pb and Cu levels were found in *Russula rubroalba* (Singer) Romagn while the highest Cd level was determined for the species of *Agaricus xanthodermus* Genev. Fe level was maximum from the species *Funalia trogii* (Berk.) Bondartsev & Singer, Mn for *Mycena personsii* Stev., Zn level was for *Morchella esculenta* (L.) Pers. and Co level was for *A. xanthodermus*. The lowest Cd, Cu, Mn and Zn contents were found in *Inotus hispidus* (Bull. ex Fr.).

2.3: MANAGEMENT OF *ROTYLENCHULUS RENIFORMIS* AND *FUSARIUM SOLANI*:

In today's scenario, the main aim of the agriculture is to produce maximum with the least involvement of land, water and manpower. The age-old environmental friendly disease management practices like, sanitation, fallowing, summer ploughing, crop rotation, mixed cropping, adjustment of date of sowing, green manuring etc. to combat plant pathogens and the problem of soil-sickness are gradually losing their acceptability. The pace of development and durability of resistant varieties has been slow and unreliable in spite of tremendous advancements made in the field of plant genetic engineering. The chemical control too has its own limitations such as high capital investment, non-remunerative, poor-availability, selectivity, temporary effect , efficacy affected by physico-chemical and biological factors, development of pest resistance, resurgence of pests, pollution of food and feeds, health hazards, toxicity towards plants and animals etc. All these have led the scientists to create awareness to develop such management practices, which alone or in integration with other practices could bring about a reasonably good degree of reduction of inoculum potential and / or disease potential and at the same time it may also ensure sustainability of production, cost effectiveness and healthy ecosystem. Use of bioagents, organic matters and resistant varieties for the management of fungal and nematode diseases are important approaches in this direction.

There are several reports on the use of *Trichoderma harzianum, Paecilomyces lilacinus* and oil-cakes for the management of fungal and nematode diseases. However, in the present review only those findings have been included in detail, which are related with management of *R. reniformis* and *F. solani* by the application of *T. harzianum*, *P. lilacinus* and oil cakes.

(a) BIOCONTROL AGENTS:

Though biocontrol agents include all classes / groups of organisms existing in an ecosystem, maximum emphasis for developing biocontrol programmes has invariably gone to fungal and bacterial biocontrol agents (Ibrahim *et al.*, 1978; Alam and Jairajpuri, 1990). The important genera of fungi studied as biocontrol agents are *Trichoderma, Gliocladium* Corda, *Aspergillus, Chaetomium* Kunze ex Fr., *Penicillium, Neurospora* Shear and Dodge, *Fusarium, Rhizoctonia, Dactylella* Grove, *Arthrobotrys* Corda, *Catenaria* Sorokin, *Paecilomyces, Glomus* Tul. etc. Among these species *Trichoderma* and *P. lilacinus* have been widely explored and recommended for the management of fungal and nematode diseases, respectively (Stirling, 1991).

(i): *PAECILOMYCES LILACINUS*:

The genus *Paecilomyces* was described by Bainier (1907) as a close relative of *Penicillium* Link ex Fr. Samson (1975) placed *Penicillium lilacinum* Thom. and some other fungi in the genus *Paecilomyces* and proposed *Paecilomyces lilacinus* (Thom.) Samson, Comb. Nov. In early 1978, *P. lilacinus* was isolated from eggmasses of *Meloidogyne incognita acrita* Chitwood infecting potato roots in a central Peruvian highland in the Huanuco Valley (Strattner, 1979; Jatala *et al.*, 1979; Jatala, 1982). A review on *P. lilacinus* as biocontrol agent for the control of nematode diseases of plants has been given by Jatala (1986), Alam (1990), Stirling (1991), Goswami and Tripathi (1998), Arif and Parveen (2003),Sheela *et al.*(2005) and Singh and Trivedi (2007).

Lysek (1966) reported perforation in nematode eggshell by *P. lilacinus*. Jatala *et al.* (1979) found the fungus frequently infecting the eggs and occasionally infecting the females of *M. incognita acrita*. The eggs within the cysts of *Globodera pallida* (Stone) Mulvey & Stone and eggmasses of *M. incognita acrita* were penetrated by the fungus within 10-12 days where the embryo was eventually destroyed. In a later study,

Jatala (1986) reported that the destruction of *M. incognita* embryo by *P. lilacinus* took within five days under laboratory condition. Mature females of the root knot nematode were generally penetrated by the fungus through the anus or vulva while cysts of *G. pallida* through the vulva and the broken or exposed neck region. About 70-90% eggs of these nematodes became infected within one month of inoculation (Jatala *et al.* 1979). The percentage of egg infection was found directly correlated with the length of fungal infection (Jatala, 1985).

In case of *Meloidogyne* Göldi, *Tylenchulus* Cobb and *Nacobbus* Thorne & Allen, the fungal hyphae first grow in the gelatinous matrix, then form a network around the eggs while proliferation hyphal branches penetrate the eggs (Jatala, 1986). In case of *Globodera* Skarbilovich cyst, the fungus first grows saprophytically on the mucilaginous body contents, then parasitizes the eggs where pre-gastrulation eggs are more prone to attack. Several ultra structural changes take place in the egg shell due to exogenous metabolites and chitinolytic activity of *P. lilacinus* (Jatala, 1986).

Dunn *et al.* (1982) reported the presence of an appressorium during colonization of eggs of root-knot nematode. But Jatala (1986) challenged their findings, and claimed that hyphal penetration of egg shell is brought about by mechanical pressure and / or enzymatic activity such as chitinase production. However, an infection peg is formed by swelling of the hyphal branch at the penetration point. The nature of egg shell has direct correlation with the rate of fungal penetration of the egg. A relatively simpler egg shell of *Meloidogyne* eggs allows the fungus to penetrate at much faster rate than it did in those of *Globodera* and *Nacobbus* where the egg shell shows more complexity (O'Hara and Jatala, 1985; Jatala, 1986).

Morgan-Jones *et al.* (1984) studied the parasitism of *M. arenaria* eggs and larvae by *P. lilacinus*. The fungal hyphae penetrate the egg shell through

small pores dissolved in the vitalline layer. The resulting changes in shell permeability cause the eggs to become swollen. The fungus then grows and crushes the chitin and lipid layers and destroys the contents of the egg, including the developing larvae whose cuticles are disrupted. The endogenous hyphae then emerge by rupturing the egg shell and produces conidiophores with chain of conidia. Several ultrastructural changes take place in the egg shell due to the fungus. The vetilline layer is separated into three distinct layers with uneven thickening, the chitin layer becomes vacuolated while the lipid layer disappears. Similarly, there is a disruption in the cuticle of the developing larvae which become necrotic.

Paecilomyces lilacinus causes egg deformation in *M. incognita* with the help of diffusible toxic metabolites (Jatala, 1985; 1986). The fungus causes alteration in egg's cuticular structure by enzymatic activity. This helps in hyphal penetration and it either changes egg shell permeability or causes perforations in the cuticle which allows seepage or free movement of diffusible metabolic compounds (Jatala, 1986).

Paecilomyces lilacinus exhibits chitinase activity when grown on chitin agar plates (Gintis *et al.*, 1983).Chitin constitutes the largest portion of the nematode egg shell, while the larval cuticle lacks it. This explains the effectiveness of the fungus as an egg destroyer, which however, becomes almost ineffective once the nematode larvae are formed. Bonants *et al.* (1995) observed proteolytic activity of the fungus. The serine protease produced by *P. lilacinus* possibly plays a role in the penetration of the fungus through egg shell of the nematode.

Cabanillas *et al.* (1988) studied the histopathology of the interaction of *P. lilacinus* with *M. incognita* on tomato. Root galling and gaint - cell formation were absent in roots inoculated with fungus - infected eggs of *M. incognita*. Almost similar condition was observed when tomato roots were dipped in spore suspension of *P. lilacinus* and inoculated with *M. incognita*. The fungus colonized the surface of

epidermal cells as well as the internal cells of epidermis and cortex. Thus the possibility of biological protection of plant surfaces with the fungus against *M. incognita* was suggested by the authors.

Paecilomyces lilacinus has given a good control of *Meloidogyne* spp. (Guevara *et al.*, 1985;Khan and Saxena,1996; Hazarika *et al.*, 2000); *M. incognita* (Jatala *et al.*, 1980;Khan, 1986; Ibrahim *et al.*, 1987,1999; Priya and Kumar, 2006); *M. javanica* (Croshier *et al.*, 1985;Walia *et al.*, 1991; Freitas *et al.*,1995); *M. arenaria* (Rodriguez-Kabana *et al.*,1984b; Siddiqi *et al.*, 2000); *M. hapla* (DoChul and SangChan, 2004; Kiewnick and Sikora, 2006); *Heterodera cajani* (Haseeb and Shukla,2004); *H. glycines* (Rodriguez-Kabana *et al.*, 1984a; Wang *et al.*, 1997); *H. avenae* Wollenweber (Khan *et al.*, 1997a); *Globodera pallida* (Jatala *et al.*, 1979;Franco *et al.*, 1981; Seenivasan *et al.*, 2007); *G. rostochiensis* (Wollenweber) Mulvey & Stone (Davide and Zorilla, 1983; Seenivasan *et al.*, 2007);*Rotylenchulus reniformis* (Khan ,1986; Reddy and Khan, 1988, 1989;Vicente *et al.*, 1991;Vicente and Acosta, 1992;Ashraf *et al.*, 2005); *Tylenchulus semipenetrans* Cobb (Herrera *et al.*,1984; Reddy *et al.*, 1996);*Radopholus similis* (Cobb) Thorne (Koshy *et al.*, 2003;Mendoza *et al.*, 2004); *Nacobbus aberrans* (Thorne) Thorne & Allen (De Sisler *et al.*,1985); *Hoplolaimus indicus, Helicotylenchus indicus* Siddiqi, *Tylenchorhynchus brassicae* Siddiqi , *Tylenchus filiformis* Butschli, *Hemicriconemoides mangiferae* Siddiqi, *Pratylenchus coffeae* (Zimmermann) Filipjev & Schuurmans Steckhoven, *Longidorus elongatus, Xiphinema basiri* and *Trichodorus mirzai* Siddiqi (Anver, 2003 and Tiyagi and Ajaz, 2004).

The literature survey revealed that there is a little information available on *P. lilacinus* as biocontrol agent against *R. reniformis* as compared to *Meloidogyne* spp., *Globodera* spp. and *Heterodera* spp. Reddy and Khan (1989) investigated the effect of *P. lilacinus* alone and in combination with carbofuran on the management of reniform

nematode, *R. reniformis* infecting brinjal (*Solanum melongena* L.). Inoculation of plants with *P. lilacinus* was found to be effective in increasing plant height and root weight and reducing the nematode population both in soil and roots. The fungus gave least reproduction factor of nematode, however, increased the percentage of males. *Paecilomyces lilacinus* infected both eggs and mature females of reniform nematode.

The reniform nematode was effectively controlled on watermelon (*Citrullus lanatus* (Thunb.) Mansf.), and chilli (*Capsicum annuum* L.) by applying *P. lilacinus* to the soil one or two weeks before planting (Vicente *et al.*, 1991; Vicente and Acosta, 1992).

Mahmood and Siddiqui (1993) found that the application of *P. lilacinus* significantly improved the plant growth and reduced the population buildup of reniform nematode on tomato. Walters and Barker (1994) indicated that *P. lilacinus* has detrimental effect on *R. reniformis* population development on tomato var. Rutgers under both greenhouse and field microplot conditions. The microplots treated with *P. lilacinus* showed that total number of reniform nematode on tomato was decreased by 59% at midseason and by 36% at harvest which consequently improved the shoot and fruit growth of the plants.

Anver and Alam (1997) reported that the *P. lilacinus* significantly improved the plant growth parameters of pigeonpea and reduced reproduction factor of nematodes when inoculated with *R. reniformis* and / or *M. incognita.*

Jayakumar *et al.* (2002) evaluated the efficacy of seed treatment (single dose) and soil application (split doses) of *P. lilacinus* against the reniform nematode (*R. reniformis*) infecting cotton (*Gossypium hirsutum* L.) cv. MCU 5. Seed treatment of cotton at 10 g *P. lilacinus* /kg seed + soil application in split doses of 100 and 50 kg/ha at the time of sowing and at 30 or 60 days thereafter

was the most effective in controlling the reniform nematode and also improved the growth and yield of cotton.

Senthamizh and Rajendran (2002) observed that the application of *P. lilacinus* @ 10 × 10^6 spores / Kg soil significantly improved the plant growth of cotton cv. MCU 5 and reduced the population buildup of *R. reniformis* both in roots and soil as compared to nematode inoculated control. Similarly, Mojumder *et al.* (2002) reported that the application of *P. lilacinus* caused significant reduction in soil population of *R. reniformis* on eggplant.

Visalakshi *et al.* (2002) reported that the application of *P. lilacinus* @ 10 ml (10 × 10^6 spores / Kg soil) significantly reduced the population of *R. reniformis* and improved the growth of seedlings of papaya (*Carica papaya* L.) var. CO.3 in terms of length and weight of shoot and root.

Ashraf *et al.* (2005) reported that the application of *P. lilacinus* @1 g mycelium+spores / Kg soil significantly suppressed the population build up of reniform nematode. Moreover, the integration of neem cake with *P. lilacinus* gave best results in controlling reniform nematode on okra (*Abelmoschus esculentus* (L.) Moench).

The nematicidal effect of culture filtrates of different isolates of *P. lilacinus* on hatching and mortality of *R. reniformis* were also observed by Khan and Husain (1989) and Ashraf and Khan (2005).Secondary metabolites from dried mycelia of *P. lilacinus* were isolated using various extraction solvent. Nematicidal tests of the crude extracts were conducted *in vitro* against freshly hatched juveniles of *R. reniformis* and menthol extract was found to be more active. The LC_{50} value against *R. reniformis* was found to be 91.4, 127.5 and 54.4 µgm l^{-1} at 24, 48 and 72 hrs, respectively (Sanyal and Prashad, 2005). The seed treatment with culture filtrate of *P. lilacinus* significantly increased the plant growth parameters of pigeonpea and chickpea and reduced the population buildup of plant parasitic nematodes including *R. reniformis* as compared to their respective controls (Anver, 2003 and Tiyagi and Ajaz, 2004).

Paecilomyces lilacinus not only reduced the population buildup of plant parasitic nematodes but it was also found to be antagonistic to some pathogenic fungi. This behaviour of *P. lilacinus* also helped in the control of disease complexes involving nematodes and fungi. *Paecilomyces lilacinus* was found to inhibit the growth of *Macrophomina phaseolina* (Tassi.) Goid. and *Rhizoctonia solani* in agar plates (Shahzad and Ghaffar, 1989). Khan and Husain (1990b) observed that the culture filtrates of most of the fungi including *P. lilacinus* at higher concentrations showed inhibitory effect on the growth of *R. solani in vitro*.

According to Siddiqui *et al.* (1998) the application of *P. lilacinus* showed better results in the control of root infecting fungi viz. *M. phaseolina*, *R. solani* and *F. solani* with enhancement in growth of sunflower and chickpea.

Khan and Husain (1988b; 1990a) evaluated the potential of *P. lilacinus* as biocontrol agent for controlling monopathogenic and multipathogenic infections on cowpea (*Vigna unguinculata* L.) involving *R. solani, M. incognita* and *R. reniformis*. They reported that the presence of *P. lilacinus* suppressed nematode multiplication which consequently reduced the plant damage caused by either individually or concomitance of above named pathogens. Considerable number of females and eggmasses were also found to be parasitized by the fungus. They, however, concluded that the *P. lilacinus* was more effective against monopathogenic than against multipathogenic infections.

Shahzad and Ghaffar (1989) studied the effectiveness of *P. lilacinus* as biocontrol agent of root-rot and root-knot disease complex of mungbean (*Phaseolus aureus* Roxb.) and okra. The results showed that *P. lilacinus* significantly reduced the *M. incognita* root-knot index on okra and mungbean as compared to their respective controls. *Paecilomyces lilacinus* reduced *M. phaseolina* colonization of roots by

33% on mungbean and 45% on okra, whereas, *R. solani* infection was reduced by 67% and 37% on mungbean and okra, respectively.

Khan *et al.* (1997b) found that the application of *P. lilacinus* in the seedlings of papaya inoculated with *F. solani* and *M. incognita* either individually or concomitantly showed significant improvement in plant growth and reduced the reproduction factor of root-knot nematode and root-rot caused by *F. solani* as compared to control.

Siddiqui *et al.* (1999;2000) reported that *P. lilacinus* not only reduced the root-knot nematode, *M. javanica* infection but also effectively suppressed the percentage of infection caused by soil-borne fungi viz. *M. phaseolina, F. oxysporum, F. solani* and *R. solani*, which consequently improved the plant growth of mungbean, mashbean (*Vigna mungo* (L.) Hepper) and tomato.

Haseeb *et al.* (2006) studied the effect of different bioinoculants in the management of *M. incognita - F. solani* disease complex on tomato cv. K 25. The results revealed that the application of *P. lilacinus* @ 50 kg / ha (2×10^8 cfu/g) significantly reduced the root-knot development, *M. incognita* reproduction rate and root infection by *F. solani*, which consequently increased the number of fruits, fruit weight and plant growth parameters viz. plant height, fresh and dry weight as compared to untreated – inoculated plants.

Khan and Husain (1988a) reported that cowpea seed treatment with culture filtrate of *P. lilacinus* was also found to be effective against *R. reniformis* and / or *R. solani* infections.

(ii): *TRICHODERMA HARZIANUM*:

Trichoderma spp. are the common soil inhabitants, especially in organic soil, which interact with other fungi including plant pathogenic species .The antagonistic nature of fungal species from the genus *Trichoderma* was first reported over 70 years ago by Weindling (1932) who observed the parasitic nature of *T. lignorum* (Tode) Harz. on several plant pathogens including

Rhizoctonia solani, *Phytophthora parasitica* Dastur, *Pythium* spp. and *Sclerotium rolfsii* Sacc. Wells *et al.* (1972) for the first time reported the use of *T. harzianum* in biocontrol experiments under natural field conditions. They suggested that 1-3 applications of *T. harzianum* inoculum were highly effective in reducing damage caused by *S. rolfsii* to tomato. Since then *Trichoderma* isolates have been shown to be very successful in controlling fungal foliar disease (Lo *et al.*, 1997; Perello *et al.*, 2006) and soil borne diseases (Upadhyay and Mukhopadhyay, 1986; Elad *et al.*, 1980; Sivan *et al.*, 1987; Jayalakshmi *et al.*, 2003). This led to the movement towards the commercialization of *Trichoderma* as a biopesticide for use in agriculture (Pathak *et al.*, 2007).

Information on the role of *Trichoderma* species as biocontrol agent for controlling various fungal and plant parasitic nematode diseases has been reviewed by Papavizas (1985), Chahal and Chahal (1994), Mukhopadhyay (1994), Spiegel and Chet (1998) and Pathak *et al.* (2007).

The first observable interaction between *Trichoderma* and its host is expressed by direct growth of the mycoparasite hyphae towards the host, initiated by a chemotropic reaction (Chet *et al.*, 1981). The hyphae upon contact, coil around the host (Benhamou and Chet, 1993), and penetrate its mycelium. This process involves the release of lytic enzymes by *Trichoderma* (Elad *et al.*, 1982) which serve to partially degrade the host cell wall. Several possible mechanisms have been suggested to be involved in *Trichoderma's* antagonism:

(a) The direct mycoparasitism, whereby the host-fungus cell wall is degraded by the lytic enzymes secreted by *Trichoderma* (Chet *et al.*, 1996).

(b) The production of volatile or nonvolatile antibiotics by the fungus (Baker and Griffin, 1995).

(c) Space or nutrient (carbon, nitrogen, iron, etc.) limiting factors that compete with the host (Sivan and Chet, 1989).

(d) *Trichoderma* species have been shown to induce phytoalexins production (Howell *et al.*, 2000) and systemic resistance (Yedidia *et al.*, 1999) in the plants.

Okhovat and Karampour (1996) evaluated the antagonistic fungi viz. *T. harzianum* (T1 and T2), *T. viride* (T3 and T4), *T. koningii* Oudem.(T5) and *Gliocladium virens* (G1 and G2) to control the root - rot of chickpea caused by *F. solani*. The results showed that the corresponding antagonistic fungi decreased the root-rot by 40, 56, 69, 44, 64, 36 and 32 % as compared to *F. solani* inoculated plants (control).

Selvarajan and Jeyarajan (1996) screened the *Trichoderma* spp. *in vitro* to select a suitable antagonistic for chickpea root rots caused by *F. solani* and *M. phaseolina*. They observed that the *T. viridi*, *T. hamatum* (Bonord.) Bain. and *T. harzianum* formed inhibition zones on potato dextrose agar against both the pathogens (*F. solani* and *M. phaseolina*) of chickpea. They further reported that these *Trichoderma* spp. reduced the sporulation of *F. solani* and sclerotial size, germination and germ tube number of *M. phaseolina*.

Ram *et al.* (1997) tested *T. harzianum* for the biological control of rhizome root-rot of ginger (*Zingiber officinale* Roscoe) caused by *F. solani* and *Pythium myriotylum* Drechs. The results showed that *T. harzianum* multiplied in the soil and rhizosphere and inhibited the growth of pathogens. Soil application of the biocontrol agent was more effective than treatment of the planting material.

Bohra and Lodha (1999) reported that disease of jojopa (*Simmondsia chinensis* (Link) Schneider) caused by soil borne fungi *Ganoderma lucidum* (Leyss.) Karst, *F. solani* and *M. phaseolina* in India were sucessusfully controlled by *T. harzianum*.

Ambikapathy *et al.* (2002) investigated the antagonistic interactions of *Aspergillus candidus* Link, *A. niger*, *A. sydowii* (Bain. & Sartory) Thom & Church, *A. sulphureus* (Fres.) Thom & Church, *Gliocladium* sp., *Penicillium citrinum* Thom, *T. harzianum* and *T. viride* against *F. solani*. The highest

inhibition of *F. solani* growth was obtained with *A. niger* followed by *T. viride, Gliocladium* sp., *T. harzianum* and *P. citrinum.*

Escobar *et al.* (2004) studied the response of *T. harzianum* to iron, salinity, pH, temperature and volatile and diffusible metabolites to determine the efficacy of the fungus as biocontrol agent against *R. solani* and *F. solani* infecting tomatoes. They found that *T. harzianum* strain Th 650 provided the highest control of both pathogens. Joshi and Raut (2005) reported that *T. harzianum* significantly inhibited the colony area and sporulation of *F. solani* in Petri plates.

El-Deeb and El-Naggar (2006) observed that *T. harzianum* inhibited the linear growth of root-rot fungi viz. *F. moniliforme, F. solani* and *R. solani.*

Besides, antagonistic effect of *T. harzianum* on the fungal diseases, it was also found to reduce the development and multiplication of plant parasitic nematodes. This behaviour also helped in the control of disease complexes caused by fungi and nematodes. *Trichoderma harzianum* has given a good control of *M. incognita* (Amin and Mostafa-Fatma ,2000; Nagesh *et al.*,2006; Mukhopadhyay *et al.*, 2006); *M. javanica* (Haggag and Amin, 2001; Sharon *et al.*, 2001; Mahdy *et al.*, 2006); *M. arenaria* (Windham *et al.*, 1989), *M. graminicola* Golden & Birchfield (Pathak *et al.*, 2005); *Heterodera cajani* (Haseeb and Shukla, 2004); G. *rostochiensis* (Saifullah and Thomas,1996); *Rotylenchulus reniformis* (Haggag and Amin,2001; Amin and Bochari ,2006; Shukla and Haseeb,2004); *Tylenchulus semipenetrans* (Reddy *et al.*,1996); *Pratylenchus zeae* Graham and *Helicotylenchus exallus* Sher (Ismail *et al.*, 2006).

Shukla and Haseeb (2004) evaluated the efficacy of *Trichoderma harzianum* (100 kg/ha) for the management of pre-transplant nematode populations of reniform nematode in the soil. They observed that the pre-transplant treatment with *T. harzianum* increased oil content of fresh herb and fresh and dry weights of spearmint (*Mentha spicata* L.) and suppressed the population of *R. reniformis.*

Haggag and Amin (2001) studied the effect of *Trichoderma* species in control of root- rot fungus, *F. solani,* root-knot nematode, *M. javanica* or reniform nematode, *R. reniformis* disease complex and on growth of sunflower plant under greenhouse conditions. Treating two weeks old sunflower seedlings cv. Giza 1 with *T. harzianum, T. viride, T. koningii, T. reesei* or *T. hamatum* gave highly significant effect in control of Fusarium-rot disease incidence and nematodes infection on sunflower roots. Infection of *F. solani* was highly increased in *M. javanica* infested soil than *R. reniformis.* Treatments of *Trichoderma* species led to decreasing of *Fusarium* cfu counts in soil infested with either *M. javanica* or *R. reniformis* and also significantly improved the plant growth parameters. *Trichoderma hamatum, T. harzianum* and *T. koningii* gave the greatest reduction in disease incidence caused by *F. solani, M. javanica* or *R. reniformis* infestation. Generally, there was highly significant reduction in Fusarium-wilt disease and nematode population which consequently increased plant growth parameters of sunflower when treated with *Trichoderma* species.

Antoon *et al.* (2006) evaluated the effect of *T. harzianum* for the management of nematode-disease complexes of the root-knot nematode *M. javanica* and two soil fungi *F. solani* and *M. phaseolina* on four tobacco (*Nicotiana tabaccum* L.) vars. Baghdad, Sumer, Rabeea and Sharqi Mahalli. The results indicated that *Trichoderma harzianum* successfully controlled the disease complex when it was applied 2 weeks before planting.

Haseeb *et al.* (2006) studied the effect of different bioinoculants in the management of *M. incognita-F. solani* disease complex on tomato cv. K 25. The results revealed that the application of *T. harzianum* @ 50 kg / ha (2×10^8 cfu/g) significantly reduced the root-knot development, *M. incognita* reproduction rate and root infection by *F. solani*, which consequently increased the number of fruits, fruit weight and plant growth parameters viz. plant height, fresh and dry weight as compared to untreated-inoculated plants.

Amin and Bochari (2006) studied the effect of culture filtrates of *T. harzianum*, *T. viride*, *T. koningii*, *T. reesei* and *T. hamatum* in controlling *R. reniformis in vitro* in 14 cm Petri-dishes after one week exposure and under greenhouse conditions on one month old eggplant cv. black beauty seedlings in 12 cm plastic pots containing 1 Kg sandy-loam (1:1, V/V) soil at 30±5 °C. The culture filtrates of *T. harzianum* was highly significant in controlling the reniform nematode on eggplant by decreasing the female and egg-masses of nematode. *Trichoderma harzianum* also inhibited the nematode activity and movements of the nematode juveniles *in vitro* after one week exposure. The role of *Trichoderma* in control of nematode was manifested by production of toxic metabolites and inhibition of nematode penetration and developments.

(b) ORGANIC AMENDMENTS:

Man has added organic and inorganic amendments to soil for centuries to improve soil fertility and increase crop yield. Soil organic amendments are potential alternatives to the harmful chemical control of plant pathogens. Linford *et al.* (1938) were the first to study the nematicidal effect of organic amendments by incorporating chopped pine apple (*Annas comosus* L.) leaves into soil for control of *Meloidogyne* spp. in cowpea. They noted that the population of free living nematodes increased while that of *Meloidogyne* spp. decreased, and suggested that increased organic matter supported microbial and animal species antagonistic to nematodes. Since then many different types of organic amendments have been applied to a variety of crops to reduce population of plant parasitic nematodes (Muller and Gooch 1982; Rodriguez-Kabana 1986; D'Addabbo 1995; Akhtar and Malik, 2000) and pathogenic fungi (Khan *et al.*, 1974b; Chattopadhyay and Rai, 2004) on different crops. It has been shown that the efficacy of the organic amendments against pathogens depends upon the type of organic matter, nematode, fungus, host plant species involved and the prevailing ecological conditions.

Among a wide variety of organic matters that have been tested as organic amendments to soil for managing plant parasitic nematodes and fungi, are oil seed cakes. Oil seed cakes are by-products of plant seed oils processing industries, and may suppress plant parasitic nematodes and fungi in economically important crops (Khan *et al.*, 1974b; Hafez and Sundararaj 1999; Sasanelli *et al.*, 2003; Jothi *et al.*, 2004; Radwan *et al.*, 2004; Tiyagi and Ajaz, 2004; Chattopadhyay and Rai, 2004). It is believed that with the liberal supply of water, oil-cakes decompose and release many compounds including ammonia, nitrates, phenols, aldehydes, amino acids, fatty acids, organic acids and carbohydrates (Hasan, 1977; Zakaria *et al.*, 1980; Alam *et al.*, 1982; Sitaramaiah and Singh, 1978; Yassin and Ismail, 1994). All these chemicals have been reported to be highly deleterious to many plant parasitic nematodes (Badra *et al.*, 1979; Alam and Jairajpuri, 1990) and pathogenic fungi (Zakaria *et al.*, 1980). Sayre (1980) and Mian and Rodriguez-Kabana (1982) suggested that the action of organic amendments against plant parasitic nematodes may also be due to the decomposing specific proteins or specific material that affects cuticle structures of nematodes. Besides, the roots of plants grown in soil amended with oil-cakes undergo physiological changes which make them unfavorable for fungal and nematode penetration and development, thus inducing certain degree of resistance against the pathogens. Moreover, soil amendments induce fungistasis in soil due to the activity of other micro-organisms and antifungal substances (Homma *et al.*, 1979; Chattopadhyay and Rai, 2004).

A number of oil-cakes such as cass (*Guizotia obyssinica* Cass.),castor (*Ricinus cummunis* L.), cotton , groundnut (*Arachis hypogaea* L.), karanj (*Pongamia pinnata* L.), linseed (*Linum usitatissimum* L.), mahua (*Madhuca indica* Gmel.), mustard (*Brassica campestris* L.), neem/margosa (*Azadirachta indica* Juss.), ramie (*Bochmeria tenacissima* (L.) Gandichr), rocket salad/duan (*Eruca sativa* L.), sal (*Shorea robusta* Gaertu.), simarouba (*Simarouba glauca* DC.) and sunflower have been found effective to a varying degree against a

number of plant parasitic nematodes viz. *Meloidogyne exigua* Goeldi, *M. incognita, M. arenaria, M. hapla, M. javanica , M. graminicola, Heterodera cajani, H. schachti* Schmidt., *H. avenae, Globodera rostochinensis, Rotylenchulus reniformis, Tylenchulus semipenetrans, Radopholus similis, Pratylenchus delattrei* Luc., *P. zeae, P. thornei* Sher & Allen , *Tylenchorhynchus elegans* Siddiqi, *T. brevilineatus* Williams, *T. brassicae, T. vulgaris Hoplolaimus indicus, Helicotylenchus* spp., *H. erythrinae* (Zimmerman) Golden, *H. dihystera* (Cobb) Sher, *H. indicus, Ditylenchus cypei* Mukherjee, *Hemicriconemoides mangiferae, Tylenchus filiformis, Aphelenchoides sacchari* Hooper, *Aphlenchus avenae, Xiphinema basiri, Longidorus elongatus* and *Trichodorus mirzai* (Lear, 1959;Rao and Prasad, 1969; Akhtar and Alam, 1993; Pandey *et al.*, 2003b; Begam and Sivakumar ,2005; Prasad *et al.*, 2005 and Anver, 2006).

The suppression in the populations of *Tylenchorhynchus* sp., *Hoplolaimus* sp., *Helicotylenchus* sp., *R. reniformis* and *M. incognita* has been observed around the roots of eggplant, guava (*Psidium guajava* L.) and citrus (*Citrus cinensis* L.) when the soil was amended with oil-cakes of margosa, mahua, groundnut and castor (Khan *et al.*, 1969).

Khan *et al.* (1974c) reported that the amendments of neem, mahua, groundnut and castor significantly suppressed the population of reniform nematode in the rhizosphere of eggplant. Mishra and Prasad (1974) while evaluating the effect of different soil amendments in the management of plant parasitic nematodes on wheat and mung reported that oil-cakes of neem, mahua, mustard, groundnut, til, cotton and linseed effectively reduced the population buildup of plant parasitic nematodes including *R. reniformis*, which consequently increased the plant growth parameters and yield of both the crops.

Alam *et al.* (1982) found that water soluble fractions of oilseed-cakes of mahua, castor, mustard, neem and peanut and their mixtures with unsterilized soil were highly deleterious to *R. reniformis*. Rao *et al.* (1987) reported that the application of oil cakes of neem, karanj and polang @ 1 ton / ha

significantly reduced the multiplication of *R. reniformis* and consequently increased the plant growth of okra. However, Khan and Husain (1988a) reported that seed treatment with neem cake was effective and groundnut cake was ineffective in the management of reniform nematode on cowpea.

Patel and Patel (1992) reported that the soil amended with oil cakes of piludi (*Salvadora persica* L.), mustard, karanj, castor and mahua significantly improved the growth of pigeonpea by reducing the population of *R. reniformis.*

Yassin and Ismail (1994) evaluated the effect of cotton, linseed, soybean and sunflower oilseed-cakes on the reproduction of *R. reniformis* and growth of cowpea plants under greenhouse conditions. Application of all tested oilseed cakes reduced the final population, rate of buildup and reproduction of nematode than those of untreated soil in all soil types. Moreover, the reduction in such values varied greatly according to the type of employed oilseed cakes and soil type. In general, cotton and sunflower proved to be the most effective oilseed cakes for controlling *R. reniformis* and gave the greatest growth of cowpea plants when, compared with the other tested oilseed cakes in sandy and both sandy loam and clayloam soil, respectively.

Mishra and Goswami (1996) reported that neem cake @1000 Kg/ ha and mustard cake@ 1000 Kg/ha significantly reduced the population of reniform nematode infesting chickpea in microplots.

The oil seed-cakes of neem, castor, linseed, groundnut, mahua, duan and soyabean were tested for their efficacious nature against plant parasitic nematodes infesting lentil and also on the subsequent crop, mungbean in field conditions. The soil population of plant parasitic nematodes including *R. reniformis* were significantly reduced on lentil (*Lens esculenta* Moench) as well as on the subsequent crop mungbean grown as next generation crop by the incorporation of oil seed cakes. A several fold improvement was noted in plant growth parameters such as plant weight, pollen fertility, pod numbers, chlorophyll content, nitrate reductase activity in the leaves and root nodulation on both the crops (Tiyagi *et al.*, 2001). Similarly, Tiyagi *et al.* (2002) studied

the biodegradable effect of oil seed cakes of neem, castor, linseed, groundnut, mahua and duan on plant parasitic nematodes infesting fenugreek (*Trigonella foenum-graecum* L.) and also on the subsequent crop mungbean under field conditions. The soil population of plant parasitic nematodes including *R. reniformis* were significantly reduced on fenugreek as well as on the subsequent crop mungbean by the incorporation of oil seed cakes. As a consequence, several fold improvement was noted in plant growth parameters such as height, pollen fertility, pod numbers, root nodulation, nitrate reductase activity in the leaves and chlorophyll content on both the crops.

Mojumder *et al.* (2002) reported that the soil amendment with neem cake caused significant reduction in soil population of *R. reniformis* infecting eggplant.

Patel *et al.* (2003) conducted an experiment to evaluate the efficacy of castor cake for the management of reniform nematode, *R. reniformis*, infesting cotton. The application of castor cake was effective in reducing the nematode population by 49% and increasing yield by 34% over the control.

Anver (2003) found that oil seed-cakes of neem, castor, mustard and duan were found to be highly effective in significantly reducing the multiplication of nematodes including *R. reniformis*, which consequently increased plant growth and bulk density of woody stem of pigeonpea.

Tiyagi and Ajaz (2004) found that the oil-cakes of neem, castor, groundnut, linseed, sunflower and soybean were highly effective in reducing the multiplication of soil nematodes including *R. reniformis* and subsequently increased the plant growth of chickpea.

Shukla and Haseeb (2004) evaluated the efficacy of neem cake (1000 kg/ha) for the management of pre-transplant reniform nematode populations in the soil. They found that pre-transplant treatment with neem cake increased plant fresh and dry weights, and oil content of fresh herb by suppressing the nematode populations.

Ashraf *et al.* (2005) evaluated the efficacy of oil-cakes of neem, mustard, castor, sunflower and linseed @ 15 g/ Kg soil for the management of *R. reniformis* infecting okra. All the treatments quite effectively suppressed the nematode population and kept the infection at significantly low level. Among oil-cakes neem cake gave best results for the management of *R. reniformis* followed by linseed, sunflower, castor and mustard cake.

Anver (2006) reported that oil seed-cakes of neem, groundnut, castor, mustard and duan were found to be highly effective in reducing the multiplication of nematodes including *R. reniformis*, which consequently increased plant growth and oil content of linseed.

Similarly, a number of oil-cakes such as castor, cotton, groundnut, karanj ,linseed, mahua, mustard, neem and rocket salad have also been found to be effective to a varying degree against a number of plant pathogenic fungi viz. *Alternaria alternata, Colletotrichum atramentarium* (Berk. & Br.) Taub., *C. falcatum* Went , *Fusarium* sp., *F. oxysporum, F. oxysporum f.* sp. *lycopersici* Snyd. & Hans., *F. oxysporum f.* sp. *ciceri, F. udum, Macrophomina phaseolina, Phyllosticta phaseolina* Sacc., *Phytium aphanidermatum* (Edson) Fitzp., *Rhizoctonia solani, Sclerotium rolfsii* and *Septoria leguminum* Desm. (Chauhan, 1963; Singh and Pandey, 1965; Khan *et al.*, 1974b; Matti and Sen, 1984; Sharma and Philip, 1997; Mathur and Gujar, 2002; Tiyagi *et al.*, 2002; Singh and Singh, 2003; Begam and Sivakumar, 2005; Raghuvanshi *et al.*, 2006).

Singh *et al.* (1987) found that ginger rhizome rot caused by *F. solani* was reduced by the amendments of oil-cakes viz. *Azadirachta indica, Calophyllum inophyllum* L. or *Pongamia glabra.*

Sharma and Philip (1997) evaluated the fungitoxic effects of oil-cake extracts of neem and karanj on the mycelial growth, spore production and spore germination of *F. solani* causing root-rot disease in mulberries. Both the

extracts inhibited the germination and growth of *F. solani* and neem extracts were most effective.

Sharma *et al.* (2004) reported that mustard and cotton cake were effective in managing the root-rot of *Prosopis cineraria* (L.) Druce caused by *F. solani.*

2.4: SCREENING TEST:

One of the most economical and effective ways to control plant diseases caused by fungi, bacteria and plant parasitic nematodes is through growing resistant plant cultivars which decrease yield losses , increase profits and result in more production of food and fiber. Unlike chemical methods, nematode management with resistant cultivars requires no special equipment or extra capital investment by growers. The recognition of strain / races within the species of fungi and nematodes opens new areas for research. Many, if not all of the cultivars already reported to have some resistance value will have to be reevaluated because previously reported resistance may be race specific.

There are several reports available on the screening of chickpea varieties against nematodes and fungal diseases. Moreover, there is no information available on the screening of chickpea varieties against the heavy metals. In the present review only those findings have been included which are related to the response of chickpea varieties against *R. reniformis* and *F. solani.*

Lakshminarayan *et al.* (1990) screened 20 chickpea varieties for their resistance against *R. reniformis* and *F. solani* and found that no variety was resistant or moderately resistant against either pathogen. However, only one variety (IC-4927) gave tolerant reaction against *F. solani* and nine varieties (IC-4918, IC-4919, IC-4921, IC-4924, IC-4926, IC-4929, IC-4931, IC-4932 and IC-4934) showed tolerant response against *R. reniformis.*

Anver and Alam (1995) screened 24 chickpea accessions for their reaction to reniform nematode and reported that accession GL-83119 as resistant against *R. reniformis.* The other varieties which showed varied

reaction of susceptibility have been classified as highly susceptible (BG -351, K-850, KPG-102), susceptible (BGM-413,BGM-426, BGM-466, Gn-581, PGD-84, RSG-509) and moderately resistant (AKG-35, BG-257, BGM-465, GCP-22, GCP-52, GL-769, GL-84125, GL-87227, CNG-585, H-86-100, ICCU-10, ICCU-19, RSG-2 and WDG-8608) to *R. reniformis.*

Ashraf and Khan (2004) evaluated 56 chickpea cultivars for their reaction against the reniform nematode, *R. reniformis*. The cultivars BG-1086, KPG-59 and Pusa-372 were resistant while BG-1072, BG-1108, ICC-88506 and Pusa-1003 were moderately resistant to *R. reniformis*. BG-276, BG-1100, BG-10863, BGD-112, BGD-117 and CIYTSL-2 showed tolerant response to *R. reniformis*. BG-376, BG-1032, BG-1087, BG-1095, BGD-72, BGD-98, BGD-1104, Biogreen, C-235, CSG-9505, EC-442507, GPF-2, ICC-88503, ICCV-5, L-550, Pusa-212, RSG-143, RTY-411 and SAKI-9303 were susceptible, while the remaining cultivars exhibited highly susceptible reactions to *R. reniformis.*

Materials & Methods

The most widely occurring and economically important pathogens viz. reniform nematode (*Rotylenchulus reniformis* Race-3) and root-rot fungus (*Fusarium solani*) in the area and an economically important legume host, chickpea (*Cicer arietinum* var. Kranti) were selected for the present study. The experiments embodying the thesis were conducted in the presence and absence of heavy metal pollutants viz. chromium and nickel. These heavy metals were used in the form of their chloride salts.

3.1: PREPARATION AND STERILIZATION OF SOIL MIXTURE:

Sandy loam soil(Table-A)collected from a fallow field near to the campus of Aligarh Muslim University was sieved through 16 mesh sieve and mixed with organic manure in the ratio of 4:1, respectively. Throughout the course of studies, unless stated otherwise, 6 inch pots were filled with this soil mixture @ 1 kg/pot. A little amount of water was poured in each pot to just wet the soil before transferring to an autoclave for sterilization at 20 PSI pressure for 20 minutes. Sterilized pots were allowed to cool down at room temperature before use for experiments.

3.2: RAISING AND MAINTENANCE OF TEST PLANTS:

Seeds of chickpea var. Kranti were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for two minutes, then washed thrice in sterilized distilled water and treated with chickpea strain of *Rhizobium* before sowing. Sucrose solution (5%) was used as a sticker for bacterization. The bacterised seeds, dried at room temperature, were sown @ 5 seeds / pot and after their germination seedlings were thinned to one plant per pot. One week old, well established and healthy seedlings were used for experimental purposes throughout the course of investigation.

Table-A: Physical and chemical properties of soil and tap water used for the experimental purposes.

Sandy loam soil= sand (69%) + slit (23%) + clay (8%)
pH value of soil = 7.0
Cation Exchange Capacity (CEC) of soil = 4.0
Carbon Nitrogen (C/N) ratio= 9.5
Initial concentration of chromium (Cr) in soil = 2 ppm
Initial concentration of nickel (Ni) in soil = 4 ppm
Initial concentration of chromium (Cr) in water = 1 ppm
Initial concentration of nickel (Ni) in water = 1.20 ppm

3.3: RAISING AND MAINTENANCE OF NEMATODE CULTURE:

The pure culture of reniform nematode, *Rotylenchulus reniformis* was raised on castor plants using a single eggmass collected from infected roots of chickpea. The eggmass was surface sterilized by treating it with 1:500 aqueous solution of chlorox (Calcium hypochlorite) for 5 minutes as described by den Ouden (1958). Treated eggmass was washed thrice with distilled water. The eggs, in egg mass were allowed to hatch out at 28±2°C in BOD incubator on a coarse sieve lined with double layered tissue paper and kept in a Petri dish containing sufficient amount of sterilized distilled water. Castor seedling growing in 12 inch earthen pots containing autoclaved soil was inoculated with the reniform nematodes so obtained. Reniform nematodes were extracted from the pot soil after two months through graded sieves of 16, 60 and 400 mesh according to modified Cobb's sieving and gravity method followed by Baermann funnel technique (Southey, 1986). Reniform nematodes so obtained were used for inoculating fresh castor seedlings grown in 12 inch earthen pots containing sterilized soil. The immature females of reniform nematode infested the roots and multiplied there in respective pots. After 6-8 weeks, a little of soil from the root zone was examined to confirm the establishment and multiplication of the nematode. After 2-3 months, the plants were cut at the ground level and soil was processed for the extraction of nematodes by the technique mentioned earlier. The roots were thoroughly washed under running tap water, cut into small pieces and transferred near the root zone of castor seedlings growing in large earthen pots locally called as "Nand" containing about 20 Kg sterilized soil. Separate soil suspension containing males and females (1:1) of *R. reniformis* was also transferred with the help of sterilized pipette to the root zone of castor seedling. Castor seedlings were inoculated from time to time to maintain the regular supply of nematode inoculum.

Culture of *R. reniformis* multiplied and maintained in this way was, thereafter used for obtaining required inoculum.

3.4: PREPARATION OF NEMATODE INOCULUM:

For preparing the inoculum of *R. reniformis* soil was collected from the root zone of heavily infected castor plants in which pure culture of nematode was raised. This soil was processed for extraction of immature females of reniform nematode using the technique mentioned earlier.

The water suspension of nematode was thoroughly stirred for making homogenous distribution of nematodes before taking 10 ml suspension in counting dish for counting the number of immature females of reniform nematode under the stereoscopic microscope. An average of three counts was taken to determine the density of nematodes in suspension.

Volume of water in the nematode suspension was so adjusted that each ml contained about 100 immature females. It was done by adding more water or decanting the excess amount of water so that 10 ml of this suspension contained 1000 immature females of *R. reniformis.* The male and female ratio of reniform nematode in the inoculum was 1:1, respectively.

3.5: ISOLATION OF FUNGUS FROM THE INFECTED CHICKPEA ROOTS:

The chickpea plants exhibiting root-rot symptoms were collected in polythene bags from the infected field at Mathura Road, Aligarh, where chickpea was cultivated on a large scale. Serial washing technique was employed to isolate *F. solani* from the infected root tissues (Harley and Waid, 1955). Infected pieces of roots were transferred to sterilized dish containing sterilized water and gently freed of soil particles. The root pieces were then transferred to another dish and the process was repeated till such time that all the adhering soil particles were removed. The root pieces were then cut into approximately 5 mm pieces and transferred to a 100 mm diameter Petri dish containing 0.1 % mercuric chloride solution. After about two minute, the root

pieces were given three successive washings in sterilized distilled water and soaked on filter paper. Five of these root pieces were plated in each of the 10 Petri dishes containing Potato Dextrose Agar (P.D.A.) with the help of sterilized forceps under aseptic conditions. These inoculated Petri dishes were incubated at 28 $\pm 2^0$ C for about 10 days. The fungus that developed on the root segments was examined and identified .On confirmation of its identity as *Fusarium solani*, its pure culture was prepared.

3.6: ISOLATION OF FUNGAL BIOCONTROL AGENTS FROM THE RHIZOSPHERE OF CHICKPEA:

About 25 chickpea plants were collected in polythene bags form the same field as mentioned above. Excess amount of soil adhering to the roots was removed by shaking the root system. The soil still left adhering to the roots was scrapped and collected over the butter paper with the help of sterilized needle. The soil thus obtained from each plant was thoroughly mixed and one microspatula of this soil was transferred to a Petri dish to which 15 – 20 ml of sterilized, melted and cooled potato dextrose agar was poured later. Ten plates were poured for each sample performing all operations under aseptic conditions. The inoculated Petri dishes were incubated at 28 $\pm 2^0$ C in BOD incubator. The fungi that developed were examined and identified after 10 days of inoculation. On confirmation of their identity as *Trichoderma harzianum* and *Paecilomyces lilacinus*, pure culture of these fungal biocontrol agents were prepared.

3.7: RAISING AND MAINTENANCE OF FUNGAL CULTURES:

The fungal inoculum of *F. solani, T. harzianum* and *P. lilacinus* was further raised on modified Richard's medium as used by Harman *et al.* (1991) , having following composition:

Potassium nitrate (KNO_3)	10.00 g
Potassium dihydrogen phosphate (KH_2PO_4)	5.00 g
Magnesium sulphate ($MgSO_4.7H_2O$)	1.30 g
Ferric chloride ($FeCl_3$)	0.02 g
Sucrose	8.00 g
Tomato juice	150.00 ml
Distilled Water	1000.00 ml

The prepared medium was sterilized in an autoclave at 15 PSI pressure for 15 minutes in 250 ml Erlenmeyer flasks each containing about 100 ml of medium. Small bits of the mycelium of *F. solani* were transferred to these conical flasks. Same procedure was repeated for raising and maintenance of *T. harzianum* and *P. lilacinus*. Inoculated flasks were incubated at 28±2°C in BOD incubator for about 20 days to allow the fungal growth to be used for further studies.

Pure culture of *F. solani, T. harzianum* and *P. lilacinus* was continuously maintained on P.D.A. contained in test tubes by inoculation of the respective fungus after every 20 days.

3.8: PREPARATION OF FUNGAL INOCULUM:

After incubating the flasks with the test fungi for about 20 days, the liquid medium was filtered through Whatman's filter paper No. 1, the mycelial mat was washed in distilled water to remove the traces of medium and gently pressed between the folds of blotting paper to remove the excess amount of water. Inoculum was prepared by mixing 10 g fungus (mycelial mat + spores) in 100 ml of sterilized distilled water and blending it for 30 seconds in a Waring blender (Stemerding, 1964). Thus each 10 ml of this suspension contained 1.0 g of fungus.

3.9: INOCULATION TECHNIQUES:

One week old chickpea seedlings were inoculated with either 1000 immature females of *R. reniformis* or 3g of *F. solani* throughout the course of investigations. Feeder roots of seedlings just before inoculations were exposed by removing the top layer of soil and the required quantity of nematode suspension / fungal inoculum was poured uniformly all around the exposed roots using sterilized pipette. Exposed roots were immediately covered after the inoculation by levelling the soil properly, followed by light irrigation.

Throughout the course of studies all the treatments were replicated three times. The pots were arranged in a randomized block design in an open field exposed to natural light and irrigated with tap water (Table-1) on alternate days.

3.10: PREPARATION OF STOCK SOLUTIONS OF HEAVY METALS:

Stock solution of chromium (1000 ppm) was prepared by using chromium chloride ($CrCl_3.6H_2O$), considering atomic weight of chromium as 51.9961 and molecular weight of the salt as 266.48. Similarly for nickel, the stock solution (1000 ppm) was prepared form Nickel chloride ($NiCl_2.\ 6H_2O$) taking into account atomic weight of Ni as 58.6934 and the molecular weight of the salt as 237.71. Appropriate amounts of stock solutions were added to distilled water so as to get the desired concentration of the heavy metals used in different experiments.

3.11: PREPARATION OF STOCK SOLUTION OF HEAVY METALS FOR EXPERIMENTS CONCERNING NEMATODE HATCHING AND MORTALITY:

Stock solution of chromium and Nickel were prepared in the same manner as described in 3.10. Desired dilutions viz. 25, 50, 100,200 and 400

ppm of heavy metals was obtained by adding appropriate quantities of stock solution to the nematode suspension.

3.12: PREPARATION OF STOCK SOLUTIONS OF HEAVY METALS FOR POT EXPERIMENT:

Stock solution of different concentration viz. 250, 500, 1000, 2000 and 4000 ppm of heavy metals were prepared in the same manner as described in 3.10. One hundred ml solution of each concentration was added to 1 Kg autoclaved soil per pot so as to get 25, 50, 100, 200 and 400ppm (on dry weight basis) of the metals in relation to known quantity of the soil used per pot.

3.13: EXPERIMENTS:

(i) RACE IDENTIFICATION OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS*:

The ten populations of reniform nematode, collected randomly from chickpea growing areas of Mathura Road and Khair Road locations of Aligarh were used for race identification. Among these populations five (MR-1, MR-2, MR-3, MR-4 and MR-5) were collected from Mathura Road and another five populations (KH-1, KH-2, KH-3, KH-4 and KH-5) were collected from Khair Road. These populations were cultured and maintained on castor grown in 15 inch earthen pots as described earlier.

Seeds of castor var. CH-1, cotton var. H-777, cowpea var. Pusa Komal, Bajra (*Pennisetum typhoides* var. Pusa 23) and mustard var. Pusa Bold were sown in 6 inch earthen pots containing sterilized soil. Soon after germination seedlings were thinned to one seedling per pot and one week well established seedlings were inoculated with 1000 immature females of reniform nematode / pot. All these crops except mustard which is a rabi season crop, were grown in kharif season.

For observations of eggmasses per root system in each treatment, plants were depotted after 5 weeks of inoculation, roots were washed free of soil and then observed directly under stereoscopic binocular. The roots were then stained with acid fuchsin (Byrd *et al.*, 1983) for recording number of females of *R. reniformis* per root system .Each treatment was replicated thrice.

Categorization of races of reniform nematode was done as suggested by Prasada Rao and Ganguly (1996).

(ii) STUDIES ON THE EFFECT OF HEAVY METALS ON THE HATCHING AND MORTALITY OF *ROTYLENCHULUS RENIFORMIS IN VITRO*:

The different concentration viz. 0, 25, 50, 100, 200 and 400 ppm of chromium and nickel were prepared in distilled water as described in 3.12.

(a) NEMATODE HATCHING:

For determining, the effect of heavy metals viz. Cr and Ni on the cumulative larval hatch of reniform nematode , five freshly picked eggmasses of nearby uniform size were sterilized and transferred to Petri dishes (40mm diameter) containing 5 ml solution of different concentration (25,50,100,200 and 400 ppm) of Cr or Ni. Eggmasses kept in distilled water served as control and all the treatments were replicated thrice. These Petri dishes were incubated at 25^0C in BOD incubator and the total number of hatched larvae were counted after 5 days with the help of nematode counting dish.

(b) NEMATODE MORTALITY:

For determining, the effect of heavy metals on mortality of *R. reniformis,* one hundred freshly hatched nematodes were transferred to Petri dishes (40 mm diameter) containing 5 ml solution of different concentrations (25, 50, 100, 200 and 400 ppm) of Cr or Ni, following the procedure described by Alam (1985).An aqueous suspension containing about 100 reniform

nematode was poured over a metallic sieve of 350 meshes (1.5 cm diameter and 1.0 cm height, fitted with a flat handle of 5 cm). Thus the nematodes remained over the mesh and the water was drained off. Then the sieve was inverted over a Petri dish and the nematodes were washed down with 5 ml solution of the heavy metal. In this way, the nematodes were transferred to the solutions without changing its concentration. The same procedure was repeated for different concentrations with washed sieve. Each treatment including distilled water as control was replicated thrice. After 12, 24, 48, 72 and 96 hours, the number of immobilized nematodes was first transferred to distilled water for an hour to ascertain their mortality. If they failed to regain mobility, they were considered as dead and then percent mortality was determined.

(iii) TO STUDY THE GROWTH AND SPORULTION OF ROOT-ROT FUNGUS, *FUSARIUM SOLANI* IN PRESENCE OF HEAVY METALS:

Desired amounts of Cr and Ni were added into the modified Richard's medium to obtain desired concentrations (25, 50, 100, 200 and 400 ppm) of each heavy metal in the medium. One hundred ml of modified Richard's medium containing heavy metal of desired concentration was poured into 250 ml Erlenmeyer flasks. Modified Richard's medium without heavy metal served as control and each treatment was replicated thrice. These flasks were then autoclaved at 15 PSI pressure for 15 minutes. After cooling, the flasks were inoculated with *F. solani* and incubated at 28 ± 2^0C.

After 15 days of inoculation the mycelial mats were gently taken out from the flasks. These mycelial mats were kept in Petri plates. For estimation of sporulation of *F. solani,* the conidia were harvested by adding 20 ml sterilized distilled water per Petri plate and carefully scrapping the conidia present on the surface of the mycelial mat with a soft paint brush. Finally the distilled water along with conidia was decanted from each Petri plate. The number of microconidia, macroconidia and chlamydospores / mycelial mat

were determined with the help of a Haemocytometer. For determining the dry mycelial weight of *F. solani*, the mycelial mats were kept in an oven for about 24 hours running at 60^0C temperature and dry mycelial weight was recorded.

In order to determine the uptake of heavy metals by *F. solani*, the dried and weighed mycelial mat was ashed in a muffle furnace at 450^0C for 12 hour and digested with 1 ml of mixture of analar grade nitric acid and hydrogen peroxide (1:1). Then it was centrifuged at 3000 rpm and the clear supernatant was made upto 10 ml with distilled water and analyzed for metal by Flame-atomic absorption spectrophotometer (Varian Techtran AA 1475).

(iv) DETERMINATION OF POTENTIAL PATHOGENIC LEVEL OF *ROTYLENCHULUS RENIFORMIS* AND *FUSARIUM SOLANI* ON CHICKPEA:

In order to determine the potential pathogenic level of *R. reniformis*, capable of causing significant damage , one week old seedlings were inoculated with 250, 500, 1000, 2000 , 4000 and 8000 immature females of reniform nematode. Similarly, for determination of potential pathogenic level of *F. solani*, the chickpea seedlings were inoculated with 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g of fungus. Since inoculation of plants with 1000 immature females of *R. reniformis* or with 3 g of *F. solani* caused significant reduction in plant growth, these inoculum levels were used in subsequent studies.

(v) EFFECT OF CHROMIUM AND NICKEL ON PATHOGENIC POTENTIAL OF *ROTYLENCHULUS RENIFORMIS* AND *FUSARIUM SOLANI* ON CHICKPEA:

In order to study the effect of heavy metals (Cr and Ni) on pathogenic potential of either *R. reniformis* or *F. solani,* appropriate amounts of stock solutions of Cr/Ni were added to the pots in such a way to get the different concentrations (25, 50, 100 and 400 ppm) as per procedure described earlier. The seedlings raised in these pots were later inoculated with either 1000 immature females of *R. reniformis* or 3.0 g of *F. solani.* Untreated and

uninoculated plants served as control. Each treatment was replicated three times. The schedule of the treatments was as follows:

1. Untreated – Uninoculated
2. Untreated – Inoculated with *R .reniformis*
3. Untreated – Inoculated with *F. solani*
4. Treated with Cr / Ni 25 ppm
5. Cr / Ni 25 ppm + *R. reniformis*
6. Cr / Ni 25 ppm + *F. solani*
7. Cr / Ni 50 ppm
8. Cr / Ni 50 ppm + *R. reniformis*
9. Cr / Ni 50 ppm + *F. solani*
10. Cr / Ni 100 ppm
11. Cr / Ni 100 ppm + *R. reniformis*
12. Cr / Ni 100 ppm + *F. solani*
13. Cr / Ni 200 ppm
14. Cr / Ni 200 ppm + *R. reniformis*
15. Cr / Ni 200 ppm + *F. solani*

(vi) ESTIMATION OF HEAVY METALS BY ATOMIC ABSORPTION SPECTROPHOTOMETERY (AAS) IN CHICKPEA PLANTS INFECTED WITH *ROTYLENCHULUS RENIFORMIS / FUSARIUM SOLANI*:

After determining the dry weight of shoot and root of plants related to the experiment number (v), these plants were further dried in an oven at 105^0C for 24 h. The dried material was grounded into the fine powder with the help of mortar and pestle. Five gram of the dried powder of each sample was digested in the 20 ml boiling Analar HNO_3 in a 50 ml Kjeldahl flask. Digestion usually completed within about half an hour. The digests were made up to 25 ml by adding required quantity of HNO_3. The prepared solution was used for the

estimation of heavy metals by using Atomic Absorption Spectrophotometer (Harding and Whitton, 1981).

The instrument (AAS) was calibrated by using a series of standards Cr solutions of varying concentrations (0.21 to 1.50 ppm). Absorbance of standards was measured at 358.2 nm wavelength using air- acetylene flame. The calibration curve was plotted and then concentration of Cr in the samples was determined.

Similarly, instrument (AAS) was calibrated by using a series of standards Ni solutions of varying concentrations (0.21 to 1.50 ppm). Absorbance of standards was measured at 232.3 nm wavelength using air-acetylene flame. The calibration curve was plotted and then concentration of Ni in the samples was determined.

(vii) EFFECT OF HEAVY METALS ON THE LIFE CYCLE OF *ROTYLENCHULUS RENIFORMIS* ON CHICKPEA:

In order to study the effect of heavy metals on the life cycle of reniform nematode, one week old and well established seedlings grown in the soil treated with 100 ppm of chromium or nickel were inoculated with 1000 immature females of *R. reniformis*. The seedlings grown in untreated soil and inoculated with *R. reniformis* served as control. Thus there were three set of treatments and each set having 120 plants.

Observations on the development of reniform nematode were recorded from three plants of each set after every 24 hours and continued upto the completion of life cycle. Roots were stained with 0.01% acid fuchsin in lactophenol to study unswollen, slightly swollen or fully swollen females with matrix and eggmasses. The eggmasses were picked up randomly and pressed under a cover slip on glass slide and the number of eggs per eggmass were counted. The soil was also processed to isolate the different stages of nematode present in the soil from the day of observation of egg laying females. The

temperature during the experimentation was in the range of 28 ± 2^0 C under glasshouse conditions.

(viii) EFFECT OF CHROMIUM AND NICKEL ON THE EFFICACY OF OIL-SEED CAKES, BIOCONTROL AGENTS AND BAVISTIN IN THE MANAGEMENT OF *ROTYLENCHULUS RENIFORMIS* AND *FUSARIUM SOLANI* INFECTING CHICKPEA:

The powdered oil cakes of neem, mustard, mahua, castor, linseed and sesame were added and thoroughly mixed with soil @ 20g oil-cake /Kg autoclaved soil. The biocontrol agents viz. *T. harzianum* and *P. lilacinus* @ 2 g mycelia mat + spores were also separately added and thoroughly mixed with 1 Kg autoclaved soil and kept in 6 inch earthen pots. Appropriate quantities of Cr/Ni were also added to the pots to obtain concentrations of 100 ppm of the heavy metal in the soil. These pots were kept moist for three weeks to facilitate the proper decomposition of the oil-cakes. After a waiting period of three weeks, seeds of chickpea (5 seeds/ pot) were sown in these pots and after their germination seedlings were thinned to one seedling per pot.

For comparison of effect of oil-cakes and biocontrol agents, a generally recommended fungicide viz. carbendazim / Bavistin (2-(methoxy-carbamoyl)-benzimidazole) was also used for the management of fungus and nematode diseases. Seeds of chickpea were treated separately with Bavistin prior to sowing. A slurry coating containing 0.2 g Bavistin and 15 g talc powder (used as inert material) was made in 20 ml synthetic neutral gum (used as a sticker). Seeds weighing 100 g were added and the container was shaken to have coating of slurry over the seeds. A care was taken to have a uniform coating over the seeds. The treated seeds were then spread in a tray and allowed to dry in shade at room temperature. These treated seeds were then sown @5 seeds per pot in earthen pots containing heavy metals (100 ppm) viz. Cr or Ni and were thinned to one seedling per pot soon after germination.

The seedlings were later inoculated with either 1000 immature females of *R. reniformis* or 3.0 g of *F. solani*. Untreated and uninoculated plants served as control. Each treatment was replicated three times. The schedule of the treatments was as follows:

1. Untreated-Uninoculated control
2. *R. reniformis* (Rr)
3. *F. solani* (Fs)
4. Chromium (Cr)
5. Nickel (Ni)
6. Rr + Cr
7. Fs + Cr
8. Rr + Ni
9. Fs + Ni
10. Mustard cake (Mc)
11. Mc + Rr
12. Mc + Fs
13. Mc + Cr
14. Mc + Ni
15. Mc + Rr + Cr
16. Mc + Fs + Cr
17. Mc + Rr + Ni
18. Mc + Fs + Ni
19. Castor cake (Cc)
20. Cc + Rr
21. Cc + Fs
22. Cc + Cr
23. Cc + Ni
24. Cc + Rr + Cr
25. Cc + Fs + Cr
26. Cc + Rr + Ni
27. Cc + Fs + Ni
28. Mahua cake (M)
29. M + Rr
30. M + Fs
31. M + Cr
32. M + Ni
33. M + Rr + Cr
34. M + Fs + Cr
35. M + Rr + Ni
36. M + Fs + Ni
37. Linseed cake (Lc)
38. Lc + Rr
39. Lc + Fs
40. Lc + Cr
41. Lc + Ni
42. Lc + Rr + Cr
43. Lc + Fs + Cr
44. Lc + Rr + Ni
45. Lc + Fs + Ni
46. Sesame cake (Sc)
47. Sc + Rr
48. Sc + Fs
49. Sc + Cr
50. Sc + Ni
51. Sc + Rr + Cr
52. Sc + Fs + Cr
53. Sc + Rr + Ni
54. Sc + Fs + Ni
55. Neem cake (Nc)
56. Nc + Rr
57. Nc + Fs
58. Nc + Cr
59. Nc + Ni
60. Nc + Rr + Cr
61. Nc + Fs + Cr
62. Nc + Rr + Ni
63. Nc + Fs + Ni
64. *Paecilomyces lilacinus* (Pl)
65. Pl + Rr
66. Pl + Fs
67. Pl + Cr
68. Pl + Ni
69. Pl + Rr + Cr
70. Pl + Fs + Cr
71. Pl + Rr + Ni
72. Pl + Fs + Ni
73. *Trichoderma harzianum* (Th)
74. Th + Rr
75. Th + Fs
76. Th + Cr
77. Th + Ni
78. Th + Rr + Cr
79. Th + Fs + Cr
80. Th + Rr + Ni
81. Th + Fs + Ni
82. Bavistin (B)
83. B + Rr
84. B + Fs
85. B + Cr
86. B + Ni
87. B + Rr + Cr
88. B + Fs + Cr
89. B + Rr + Ni
90. B + Fs + Ni

To determine the infection of biocontrol agents viz. *P. lilacinus* and *T. harzianum* in eggmasses, eggs and females of *R. reniformis* , fifty females and eggmasses of reniform nematode were collected from the roots of plants inoculated with either *P. lilacinus* or *T. harzianum* and *R. reniformis*. These females and eggmasses were transferred separately into Petri dishes containing 1.0 % water agar and inoculated at 28 ± 2^{0}C in BOD incubator. After 7 days incubation, the percentage of fungus infected females and eggmasses was calculated. For determining the percentage of infected eggs in each eggmass, the eggmasses were stained with cotton blue in lactophenol and gently pressed over a glass slide to separate the eggs. The numbers of eggs infected were counted under the microscopic fields and the percentage of infected eggs was calculated.

(ix) SCREENING OF DIFFERENT VARIETIES OF CHICKPEA AGAINST *ROTYLENCHULUS RENIFORMIS, FUSARIUM SOLANI,* CHROMIUM AND NICKEL:

Twenty five chickpea varieties viz. Annegiri-1, Avarodhi, CSJD, JG-74,Gauraw,Gaut, Gulab, K-850, KGD-1168, KUSCR-2, KWR-108, Pant 186, Phule-G 8602, Phule G 92028, Phule G 96020, Pragati, Pusa-1103, Pusa -120, Pusa-1060, Radhey , Sadabahar, Vardan, Vijay, WCG-2 (Surya) and XVSCR-2 were screened for resistance to reniform nematode (*R. reniformis*), root-rot fungus (*F. solani*) and heavy metals (Cr and Ni) . Five bacterised and sterilized seeds of each chickpea variety were sown in 6 inch earthen pots containing 1 kg autoclaved soil either untreated or treated with 100 ppm Cr or Ni. Soon after germination the seedlings were thinned to one plant per pot .One week well established seedlings were inoculated with either 1000 immature females of *R. reniformis* or 3g of *F. solani*. The untreated and uninoculated plants of each variety served as control and each treatment was replicated thrice. After three months the crop was terminated and plant dry weight (root and shoot), reproduction factor of nematode and disease index (%) of *F. solani* were determined. The degree of resistance and susceptibility of different chickpea

varieties to *R. reniformis*, *F. solani* and heavy metals was determined by using Resistance – Susceptibility Indices as given below.

Resistance – Susceptibility Indices:

(a) FOR RENIFORM NEMATODE:

(1) Reproduction factor of nematode < 1.0; no significant reduction in plant growth = Resistant (R).

(2) Reproduction factor of nematode 1.1-2.0; significant reduction in plant growth < 10.0 % = Moderately Resistant (MR).

(3) Reproduction factor of nematode 2.1-4.0; significant reduction in plant growth 10.1-15.0 % = Tolerant (T).

(4) Reproduction factor of nematode 4.1-7.0; significant reduction in plant growth 15.1-25.0 % = Susceptible (S).

(5) Reproduction factor of nematode >7.00; significant reduction in plant growth > 25 % = Highly Susceptible (HS).

(b) FOR ROOR-ROT FUNGUS:

(1) Disease index < 10.0%; no significant reduction in plant growth = Resistant (R).

(2) Disease index 10.1 – 20.0%; significant reduction in plant growth < 10.0 % = Moderately Resistant (MR).

(3) Disease index 20.1-30.0%; significant reduction in plant growth 10.1-15.0 % = Tolerant (T).

(4) Disease index 30.1-50.0%; significant reduction in plant growth 15.1-25.0 % = Susceptible (S).

(5) Disease index > 50.0%; significant reduction in plant growth > 25 % = Highly Susceptible (HS).

(c) FOR HEAVY METALS:

Resistance rating against heavy metals (Cr and Ni) were determined by taking into account the percentage reduction in plant growth only as follows:

(1) No significant reduction in plant growth = Resistant (R).

(2) Significant reduction in plant growth < 10.0 % = Moderately Resistant (MR).

(3) Significant reduction in plant growth 10.1-15.0 % = Tolerant (T).

(4) Significant reduction in plant growth 15.1-25.0 % = Susceptible (S).

(5) Significant reduction in plant growth > 25 % = Highly Susceptible (HS).

3.14: RECORDING OF OBSERVATIONS:

(i) PLANT GROWTH DETERMINATION:

Plants were uprooted after 90 days of inoculation. Roots were washed thoroughly in slow running tap water. Utmost care was taken to avoid loss or injury of root system during the entire operation. For measuring length and weight, the plants were cut with a sharp knife just above the base of root emergence. Length of shoot and root was recorded in centimeters from the cut end to the tip of first leaf and the longest root, respectively. The excess water of plants was removed by putting them between the two folds of blotting sheets for some time before recording the fresh weight of shoots and roots. For measuring dry weight, the shoot and root were kept in envelops separately for drying in an oven running at 80°C for 24 hours and after this the dry weight of both root and shoot was taken separately. For interpretation of results, the reduction in plant growth was calculated in terms of percentage dry weight reduction.

(ii) ROOT-NODULE ESTIMATION:

Nodulation was estimated by counting the number of nodules per root system.

(iii) SEED YIELD PER PLANT:

The pods from each plant, representing each treatment, were threshed and cleaned to assess the seeds weight per plant.

(iv) POPULATION ESTIMATION OF *ROTYLENCHULUS RENIFORMIS*:

For extraction of reniform nematode, the soil from each treatment was mixed thoroughly and a sub-sample of 200g soil was processed through sieves according to Cobb's sieving and gravity method followed by Baermann funnel technique. However, in the experiment pertaining to life cycle, the extraction of reniform nematode from the soil was done according to Centrifugal – Floatation technique (Jenkins, 1964).

The nematode suspension was collected in a beaker and volume made upto 100ml. For proper distribution of nematodes, the suspension was bubbled with the help of pipette and 10ml suspension of each sample was drawn and transferred to a counting dish. The number of nematodes were counted in three replicates for each sample. Mean of three such counting's was calculated and the final population of nematode / Kg soil was determined.

To estimate the nematode population in roots, 1.0g root from each replicate was macerated with enough water in an electrically operated waring blender for about 30-40 seconds. The macerate was collected in a beaker and volume made upto 100ml. The nematode population was counted as described above. Reproduction factor (R) of the nematode was calculated by the formula R = Pf / Pi, where Pf represented the final and Pi the initial population of the nematode.

(v) Estimation of Disease index (%):

In order to determine the disease index interms of *Fusarium solani* infection, washed roots of inoculated plants were cut into 1.0 cm pieces, then treated with 10% KOH solution and finally kept at 90 °C for 1 h. These root segments were washed again with distilled water, then acidified and stained with trypan blue (0.5% (*V/V*) in lactophenol) as described by Philips and Hayman (1970). Five stained pieces of each taproot were mounted on slides in lactophenol and presence of mycelium of the fungus was estimated. The disease index (%) was calculated by measuring the infected portion in relation to total length of root pieces.

(vi) DETERMINATION OF WATER ABSORPTION CAPACITY OF CHICKPEA ROOTS:

After 90 days of inoculation plants were uprooted and washed gently to avoid any damage to plant tissues. These plants were kept singly in 500 ml Erlenmeyer flasks containing a known amount of water (300 ml) with the support of cotton plugs. Flasks having cotton plugs and only water served as control. These flasks were kept on glasshouse bench with temperature ranging from 28±2°C. The plants were taken out of the flasks after 24 hours and the remaining quantity of water was measured. Amount of water lost from the unplanted flasks was deducted from the amount of water lost from the flasks having plants and the difference gave the actual amount of water lost by the plants or in other words amount of water absorbed by the roots.

(vii) ESTIMATION OF CHLOROPHYLL CONTENT:

The chlorophyll content in fresh leaf was estimated following the method worked out by Mackinney (1941).

One gram of finely cut fresh leaves and 20 ml of 80% acetone was grounded into a fine pulp using a mortar and pestle. The mixture was centrifuged at 5,000 rpm for 5 minutes. The supernatant was collected in 100

ml volumetric flask. The residue was washed three times, using 80% acetone. Each washing was collected in the same volumetric flask and volume was made upto the mark, using 80% acetone. The absorbance was read at 645 and 663 nm against the blank (80% acetone) on spectrophotometer. The chlorophyll content present in the extract (mg g^{-1} tissue) was calculated using the following equations:

$$\textbf{Chlorophyll a} = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times \frac{V}{1000 \times W}\ (\text{mg g}^{-1}\ \text{fresh mass})$$

$$\textbf{Chlorophyll b} = 22.9\ ((A_{645}) - 4.68\ (A_{663}) \times \frac{V}{1000 \times W}\ (\text{mg g}^{-1}\ \text{fresh mass})$$

Where

A = absorbance at specific wavelengths

V = final volume of chlorophyll extract in 80% acetone

W = fresh mass of tissue used for extraction

(viii) ESTIMATION OF PROTEIN CONTENT:

The total protein content in seeds was estimated by adopting the methodology of Lowry *et al.* (1951). Fifty milligram (50 mg) of the oven dried seed powder was transferred to a mortar. The sample was ground with the addition of 1 ml of 5% trichloroacetic acid. The pulp was transferred to a glass centrifuge tube with repeated washing with 5% TCA to make the final volume 5 ml. The mixture was centrifuged at 4000 rpm for 15 minutes and the supernatant was discarded. Five ml of 1N NaOH was added to the residue. The tube was kept in a water bath at 60° C for 30 minutes .After cooling for 15 minutes, the mixture was centrifuged at 4000 rpm for 15 minutes. The supernatant collected in 25 ml volumetric flask with repeated washings. Volume was made upto the mark by using 1N NaOH and used to estimate total protein content. One ml of the above extract was transferred to a test tube and 5 ml of reagent C (Table-B) was added to it. The solution was shaken well and

Table- B: Reagents for the estimation of protein content of seeds.

Reagent A	2% sodium carbonate (2 g dissolved in 100 cm^3 DDW) and 0.1N NaOH (4g NaOH dissolved in 1000 cm^3 DDW) were mixed in the ratio of 1:1.
Reagent B	0.5% copper sulphate (500 mg $CuSO_4$ dissolved in 100 cm^3 DDW) and 1% sodium tartarate (1g sodium tartarate dissolved in 100 cm^3 DDW), were mixed in the ratio of 1:1.
Reagent C	50 cm^3 of reagent A was mixed with 1cm^3 of reagent B, except omission of sodium hydroxide

allowed to stand at room temperature for 15 minutes. Folin phenol reagent (0.5 ml) was added rapidly with immediate mixing. The blue colour developed. The absorbance of this solution was read at 660 nm using spectrophotometer. A blank was run with each set of samples. The total protein content was calculated by comparing the absorbance of each sample with a calibration curve plotted by taking known graded concentrations of bovine albumin.

(ix) STATISTICAL ANALYSIS:

The experimental data was analyzed following the standard procedures described by Gomez and Gomez (1984). Each treatment was replicated three times. The 'F' test was applied to assess the significance of the data at 5% and 1% level of probabilities. Least Significant Difference (L.S.D.) was calculated to compare the effect of various components by putting the values on the following formula.

$$\text{L.S.D.}_{\text{(at 5\% level)}} = \sqrt{\frac{\text{Standard Error} \times 2}{\text{Replicates}}} \times \text{t (value) 5\%}$$

$$\text{L.S.D.}_{\text{(at 1\% level)}} = \sqrt{\frac{\text{Standard Error} \times 2}{\text{Replicates}}} \times \text{t (value) 1\%}$$

Results

4.1: IDENTIFICATION OF RACE OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* ASSOCIATED WITH CHICKPEA:

Since biological races are known to occur in *Rotylenchulus reniformis*, it was considered desirable to study the populations of reniform nematode in the present study and identify its race before initiating the research work so that the results obtained may be interpreted more scientifically and with authenticity.

The data presented in Table 1 and Figs 1 to 1.1 clearly showed that all the isolates of *R. reniformis* collected from chickpea attacked on host differential plants viz. castor var. CH-1, cotton var. H-777, cowpea var. Pusa Komal and mustard var. Pusa Bold. However, these isolates did not infect Bajra var. Pusa-23.The number of females and eggmasses per root system varied on the different differential host plants. The number of females / root system varied from 105-119 in castor, 64-80 in cowpea, 55-70 in mustard and 40-55 in cotton. Similar trend was also observed in the number of eggmasses per root system. The number of eggmasses per root system varied from 64-73 in castor, 47-56 in cowpea, 31-48 in mustard and 33-42 in cotton.

From the above results it may be concluded that all the isolates of *R. reniformis* were able to attack and multiply on castor, cowpea, cotton and mustard, but these populations were unable to infect bajra, therefore, the populations of *R. reniformis* collected from different locations belonged to the same race, which is designated as Race-3 as per the scheme suggested by Prasada Rao and Ganguly (1996).

4.2: EFFECT OF CHROMIUM AND NICKEL ON THE HATCHING AND MORTALITY OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS IN VITRO*:

It is evident from the data presented in Table 2 and Fig. 2 that the different concentrations of chromium significantly inhibited the hatching of reniform nematode, *Rotylenchulus reniformis*. The larval emergence was

Table-1: Identification of race of reniform nematode, *Rotylenchulus reniformis* associated with chickpea by using differential host plants.

Isolates	Differential host plants / No. of females and eggmasses per root system									
	Cotton var. H -777		Castor var. CH -1		Cowpea var. Pusa Komal		Bajra var. Pusa - 23		Mustard var. Pusa Bold	
	Females	Eggmasses	Females	Eggmasses	Females	Eggmasses	Females	Eggmasses	Females	Eggmasses
MR - 1	52	38	115	71	80	56	-	-	68	48
MR - 2	54	40	107	64	72	53	-	-	58	33
MR - 3	48	36	113	65	78	52	-	-	59	35
MR - 4	53	40	105	64	67	48	-	-	67	40
MR - 5	55	42	112	68	75	55	-	-	70	42
KR - 1	50	38	117	72	76	56	-	-	65	37
KR - 2	41	34	116	70	70	52	-	-	57	31
KR - 3	45	36	110	65	64	47	-	-	60	34
KR - 4	40	33	119	73	68	50	-	-	62	36
KR - 5	46	38	114	67	75	54	-	-	55	31
L.S.D. (at 5% level)	5.71	10.10	21.5	13.4	18.82	9.72	-	-	16.22	7.67
L.S.D.(at 1% level)	22.50	14.51	30.89	19.25	27.04	13.96	-	-	23.30	11.01

Fig.1: Identification of race of reniform nematode, *Rotylenchulus reniformis* associated with chickpea by using differential host plants.

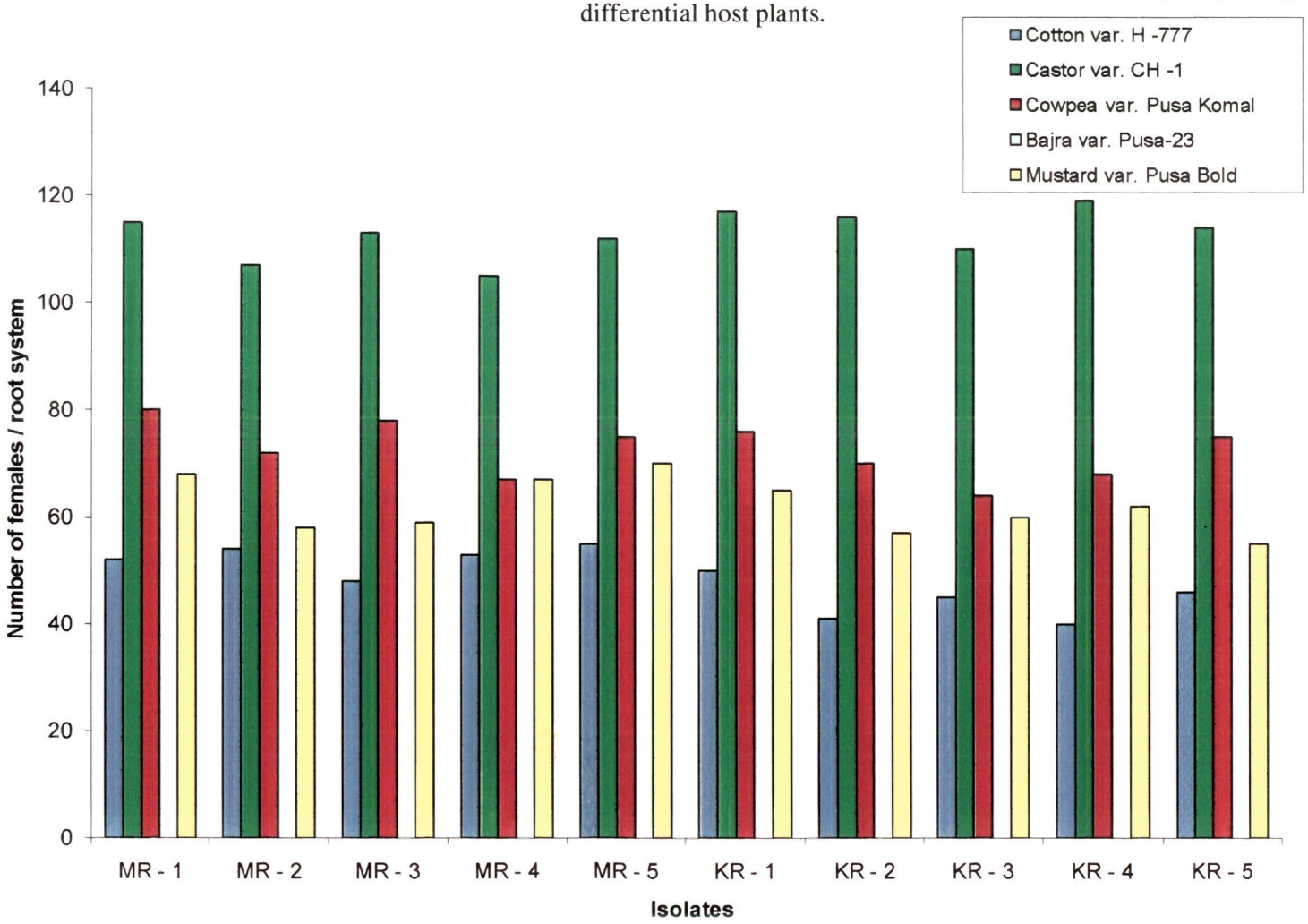


Fig. 1.1: Identification of race of reniform nematode, *Rotylenchulus reniformis* associated with chickpea by using differential host plants. .

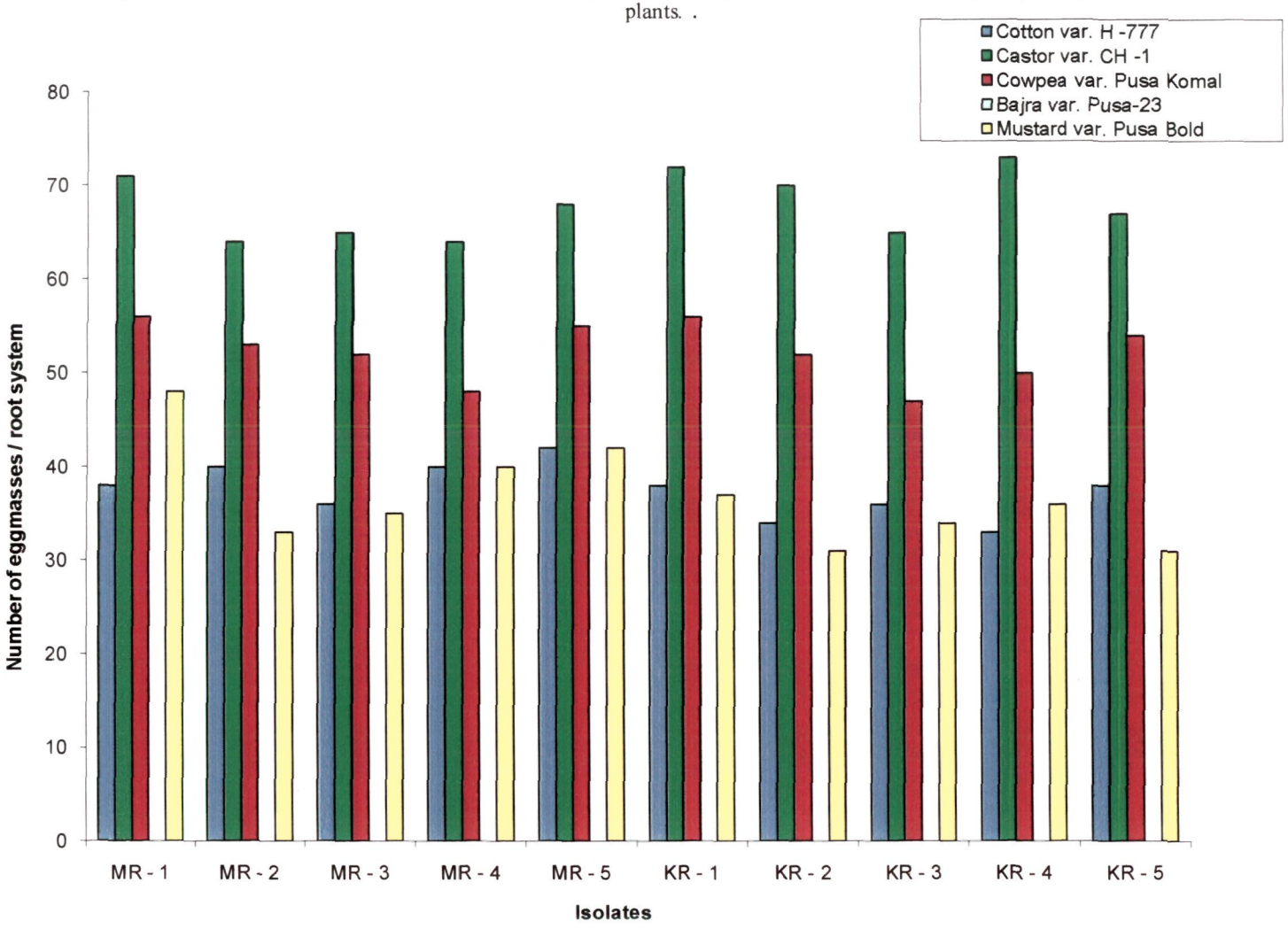


Table -2: Effect of chromium and nickel on the hatching of reniform nematode, *Rotylenchulus reniformis in vitro.*

Concentrations (ppm)	Heavy metals / Number of juveniles emerged after 5 days		L.S.D.	
	Chromium	Nickel	at 5% level	at 1% level
0	212.3	212.3	-	-
25	160.7	191.7	11.26	25.97
	(24.3)	(9.7)		
50	120.3	144.0	14.07	32.45
	(43.3)	(32.1)		
100	66.0	106.0	16.72	38.58
	(68.9)	(50.0)		
200	29.7	73.3	18.61	42.93
	(86.0)	(65.4)		
400	0	25.0	20.41	47.08
	(100)	(88.2)		
L.S.D. (at 5% level)	9.04	8.43		
L.S.D. (at 1% level)	14.18	13.23		

In parenthesis are given percentage reduction over control.

gradually decreased with an increase in the concentration of chromium from 25 to 400 ppm. However, the hatching of reniform nematode was completely suppressed at 400 ppm concentration of chromium. Inhibition in the larval emergence relative to control was 24.3, 43.3, 68.9 and 86.0 % at 25, 50, 100 and 200 ppm concentrations of chromium, respectively.

Similarly, the different concentrations of nickel also significantly inhibited the larval emergence of reniform nematode. There was a relative decrease in the larval emergence with the corresponding increase in the concentration of nickel. The percent inhibition on the hatching as compared to the control was 9.7, 32.1, 50.0, 65.4 and 88.2 at 25, 50, 100, 200 and 400 ppm concentration of Ni, respectively (Table 2 and Fig. 2).

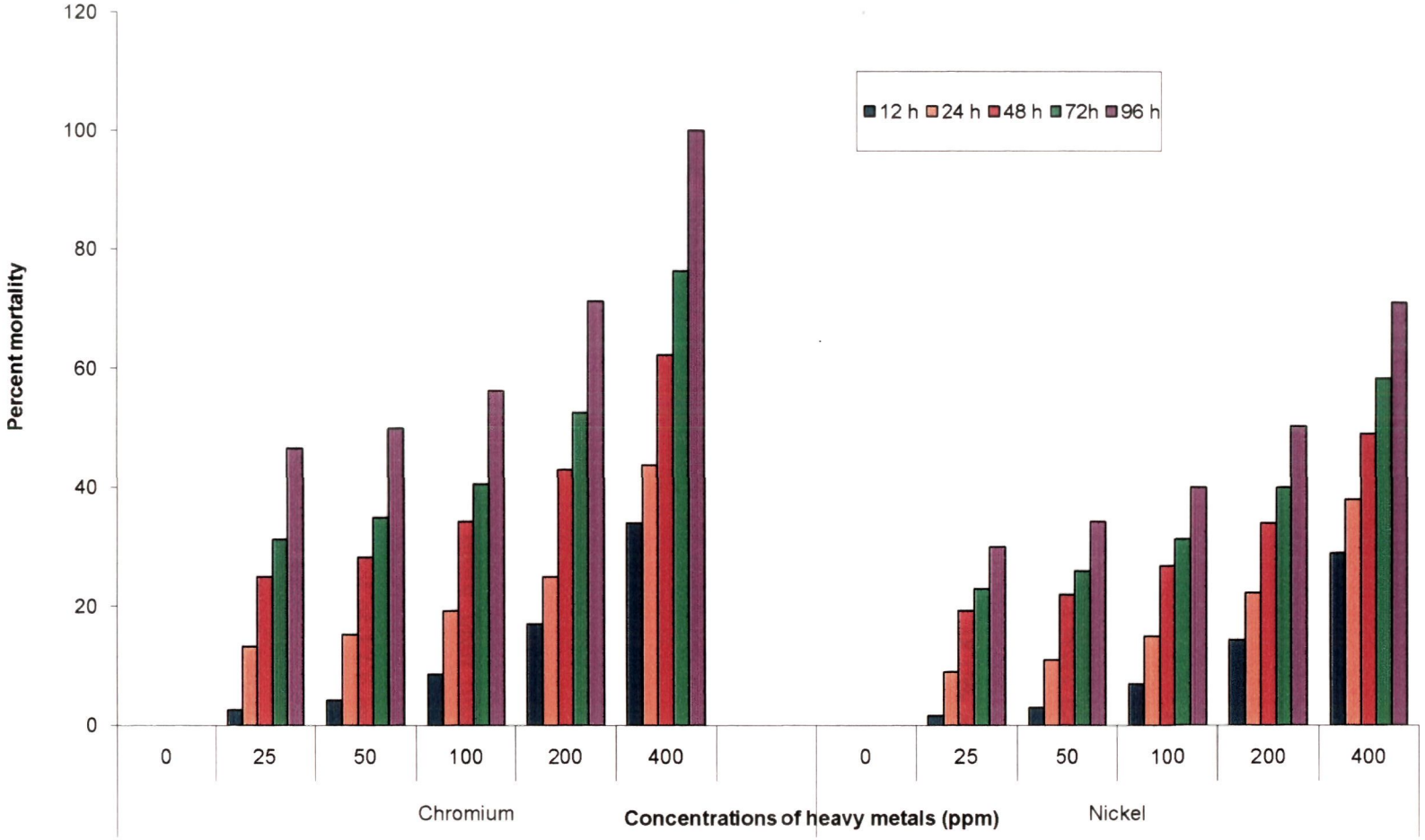
It was further noticed that chromium showed significantly greater toxic effect on the inhibition of hatching of reniform nematode as compared to nickel.

It is apparent from the data presented in Table 2.1 and Fig. 2.1 that different concentrations of chromium and nickel showed nematicidal effect of varying degree on reniform nematode, *R. reniformis*. The nematode mortality was directly proportional to the concentration of Cr and Ni. The rate of mortality was low in the beginning but an appreciable increase was recorded with an increase in the period for which reniform nematode was exposed to different concentrations of Cr and Ni. The highest mortality of reniform nematode was recorded in 400 ppm Cr after 96 hours of exposure. However, on the other hand, the lowest mortality was observed in 25 ppm Cr after 12 h exposure period. The percentage mortality of reniform nematode was 2.6, 4.3, 8.6, 17.0 and 34.0 in the concentrations of 25, 50,100, 200 and 400 ppm Cr, respectively when nematodes were exposed for 12 hours. Similarly, in the corresponding concentrations , the mortality was observed as 13.3, 15.3, 19.3, 25.0 and 43.8 % after 24h; 25.0, 28.3, 34.3, 43.0 and 62.3% after 48h; 31.3,

Table-2.1: Effect of chromium and nickel on the mortality of reniform nematode, *Rotylenchulus reniformis in vitro*.

Exposure period (h)	Heavy metals	Percentage mortality / concentrations of heavy metals (ppm)						L.S.D.	
		0	25	50	100	200	400	at 5% level	at 1% level
12	Cr	0.0	2.6	4.3	8.6	17.0	34.0	3.53	5.53
	Ni	0.0	1.6	3.0	7.0	14.3	29.0	3.28	5.14
24	Cr	0.0	13.3	15.3	19.3	25.0	43.8	3.83	6.01
	Ni	0.0	9.0	11.0	15.0	22.3	38.0	3.63	5.70
48	Cr	0.0	25.0	28.3	34.3	43.0	62.3	4.50	7.04
	Ni	0.0	19.3	22.0	26.8	34.0	49.0	4.00	6.26
72	Cr	0.0	31.3	35.0	40.6	52.6	76.3	5.00	7.78
	Ni	0.0	23.0	26.0	31.3	40.0	58.3	4.35	6.81
96	Cr	0.0	46.6	50.0	56.3	71.3	100	5.67	8.88
	Ni	0.0	30.0	34.3	40.0	50.3	71.0	4.79	7.51
L.S.D. (at 5% level)			3.57	3.68	3.76	4.06	5.50		
L.S.D. (at 1% level)			6.00	6.10	6.25	6.74	7.46		

Fig. 2.1: Effect of chromium and nickel on the mortality of reniform nematode, *Rotylenchulus reniformis in vitro.*



35.0, 40.6, 52.6 and 76.3% after 72h; and, 46.6, 50.0, 56.3, 71.3 and 100% after 96h exposure. Moreover, the percentage of mortality of reniform nematode was not significant at 25 ppm Cr at 12 hour exposure period and between 25 and 50 ppm concentrations of Cr in all the exposure periods.

It is evident from the data presented in Table 2.1 and Fig. 2.1 that the highest mortality of reniform nematode was recorded in 400 ppm Ni when reniform nematode was exposed for 96 hours, while, the lowest mortality was recorded in 25ppm Ni at 12 hour exposure period. The percentage mortality was found as 1.6, 3.0, 7.0, 14.3 and 29.0 in 25, 50, 100, 200 and 400 ppm concentrations of Ni, respectively when nematodes were exposed for 12 h. The rate of nematode mortality was further increased with an increase in exposure period. It was recorded as 9.0, 11.0, 15.0, 22.3 and 38.0% after 24h; 19.3, 22.0, 26.8, 34.0 and 49.0% after 48h; 23.0, 26.0, 31.3, 40.0 and 58.3 % after 72h; and 30.0, 34.3, 40.0, 50.3 and 71.0 % after 96h exposure in 25, 50, 100, 200 and 400 ppm concentrations of Ni, respectively. However, the percentage mortality of reniform nematode was not significant at 25 and 50ppm Ni at 12 h exposure period. Similarly, the mortality of reniform nematode was also not significant between 25 and 50 ppm concentrations of Ni in all the exposure periods.

It can be concluded from the above results that the nematicidal effect of chromium on the reniform nematode was more than nickel. A significantly different effect on the mortality of reniform nematode was observed when a comparison between Cr and Ni was made except at 12 h exposure period in all the concentrations and at 24 h exposure in 200 ppm.

4.3: EFFECT OF CHROMIUM AND NICKEL ON THE GROWTH, SPORULATION AND HEAVY METAL UPTAKE OF *FUSARIUM SOLANI IN VITRO*:

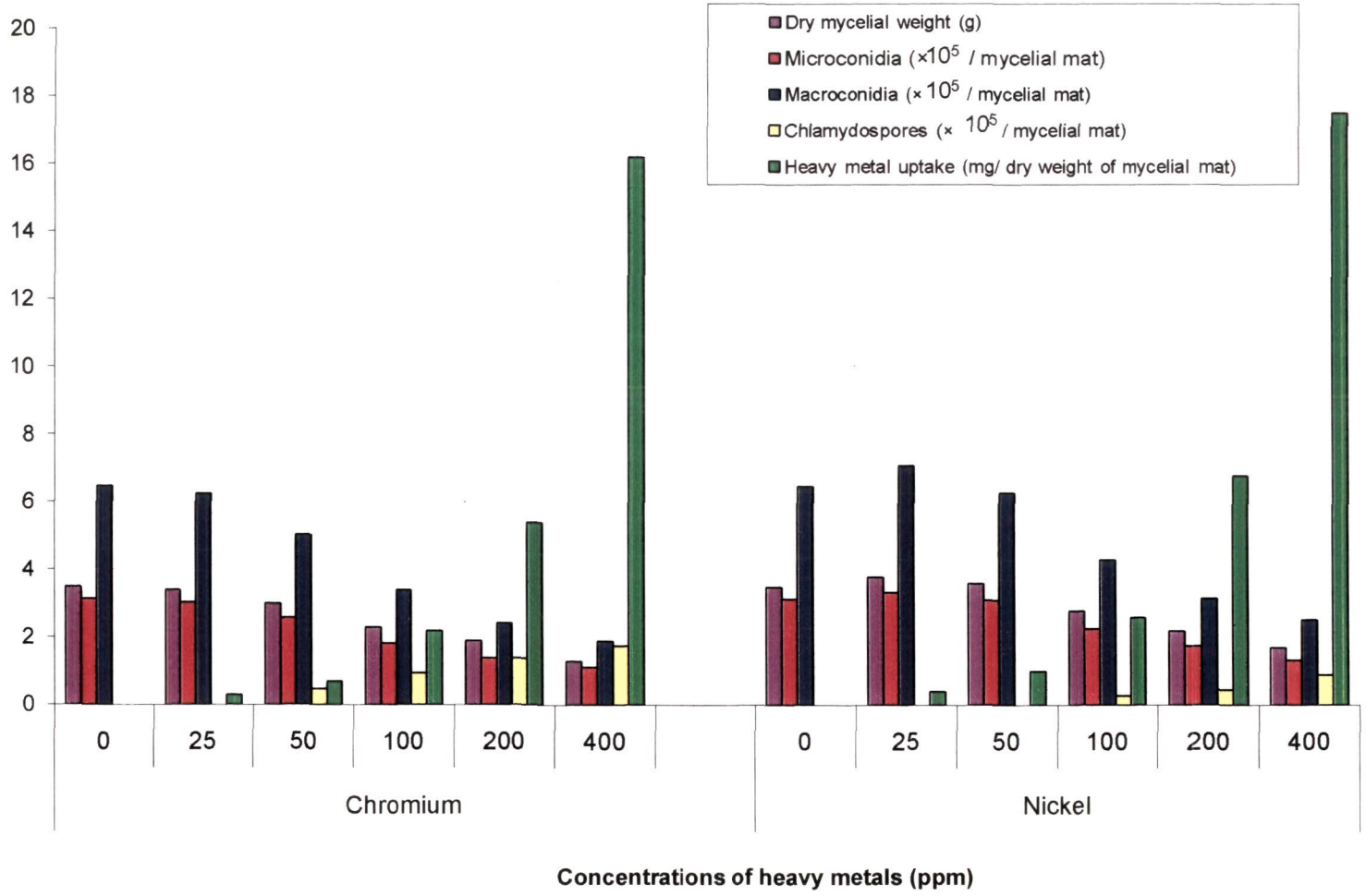
The data presented in Table 3 and Fig. 3 clearly showed that the effect of Cr and Ni on the growth and sporulation of *Fusarium solani*

Table-3: Effect of chromium and nickel on the growth, sporulation and heavy metal uptake of *Fusarium solani in vitro*.

Treatments (ppm)	Heavy metal	Dry mycelial weight (g)	Microconidia ($\times 10^5$/ mycelial mat)	Macroconidia ($\times 10^5$/ mycelial mat)	Chlamydospores ($\times 10^5$/ mycelial mat)	Heavy metal uptake (mg /g dry weight of mycelial mat)
0	-	3.5	3.14	6.48	0.0	0.0
25	Cr	3.4 (-2.8)	3.04 (-3.1)	6.25 (-3.5)	0.0	0.3
	Ni	3.8 (+8.5)	3.34 (+6.3)	7.10 (+9.5)	0.0	0.4
50	Cr	3.0 (-14.2)	2.58 (-17.8)	5.04 (-22.2)	0.47	0.7
	Ni	3.6 (+2.8)	3.11 (-1.0)	6.28 (-3.0)	0.0	1.0
100	Cr	2.3 (-34.2)	1.83 (-41.7)	3.41 (-47.3)	0.95	2.2
	Ni	2.8 (-20.0)	2.27 (-27.7)	4.30 (-33.6)	0.28	2.6
200	Cr	1.9 (-45.7)	1.39 (-55.7)	2.43 (-62.5)	1.38	5.4
	Ni	2.2 (-37.1)	1.75 (-44.2)	3.16 (-51.2)	0.46	6.8
400	Cr	1.3 (-62.8)	1.12 (-64.3)	1.89 (-70.8)	1.76	16.2
	Ni	1.7 (-51.4)	1.33 (-57.6)	2.52 (-61.1)	0.89	17.5
L.S.D. (at 5% level)		0.24	0.16	0.52	0.08	0.37
L.S.D. (at 1% level)		0.34	0.23	0.74	0.11	0.53

In parenthesis are given percent reduction (-) or stimulation (+) over control.

Fig. 3: Effect of chromium and nickel on the growth, sporulation and heavy metal uptake of *Fusarium solani in vitro.*



was concentration dependent and the toxicity was found to be directly proportional to the concentration of heavy metals. Moreover, it was observed that Cr was more toxic than Ni. The uptake / accumulation of heavy metals by *F. solani* increased with an increase in the concentration of heavy metals and the uptake of Ni was more than that of Cr.

A significant gradual reduction in the dry mycelial weight and sporulation of *F. solani* was observed with an increase in the concentration of Cr ranging from 25-400 ppm except in 25 ppm concentration which showed no significant variation with respect to control. The incorporation of 25, 50, 100, 200 and 400 ppm of Cr in the modified Richard's medium resulted in 2.8, 14.2, 34.2, 45.7 and 62.8 % reduction in dry mycelial weight, respectively as compared to control. Similarly, the sporulation of fungus was also adversely affected by Cr. Inhibitory effect of Cr on the formation of macroconidia was more than the microconidia. In the corresponding concentrations of Cr, the reduction in microconidia / mycelial mat was 3.1, 17.8, 41.7, 55.7 and 64.3%, and that of macroconidia / mycelial mat was 3.5, 22.2, 47.3, 62.5 and 70.8 %. However, on the other hand, the chlamydospores formation was recorded at and above 50 ppm Cr, which increased significantly with an increase in the concentration of Cr .The uptake of Cr by *F. solani* significantly increased with an increase in the concentration of Cr from 25-400 ppm. It was recorded as 0.3, 0.7, 2.2, 5.4 and 16.2 mg / g dry mycelial weight of the fungus, respectively.

Nickel was also inhibitory for the growth and sporulation of the fungus except at 25 and 50 ppm Ni. There was a significant gradual increase in reduction in dry mycelial weight and sporulation of *F. solani* with an increase in the concentration of Ni from 100-400 ppm as

compared to control. However, on the other hand there was a significant increase in dry mycelial weight (+8.5%) and sporulation of the fungus, i.e. microconidia (+6.3%) and macroconidia (+9.5%) in the treatment of 25ppm Ni as compared to control. Although, no significant variation in dry mycelial weight and sporulation of *F. solani* was recorded at 50 ppm concentration of Ni as compared to control. The incorporation of 100, 200 and 400 ppm of Ni in the medium resulted in 20.0, 37.1 and 51.4% reduction in dry mycelial weight, respectively as compared to control. Similarly, the sporulation of fungus was also adversely affected by Ni. Inhibitory effect of Ni on the formation of macroconidia was more than the microconidia. In the corresponding concentrations of Ni, the reduction in microconidia / mycelial mat was recorded as 27.7, 44.2 and 57.6%, and in macroconidia / mycelial mat was 33.6, 51.2 and 61.1%. It was also noted that at and above 100 ppm Ni, the chlamydospore formation began and increased significantly with an increase in the concentration of Ni.

The uptake of Ni by *F. solani* was also significantly increased with an increase in the concentration of Ni in the medium. It was recorded as 0.4, 1.0, 2.6, 6.8 and 17.5 mg / g dry mycelial weight of the fungus in the treatments of 25, 50, 100, 200 and 400 ppm of Ni, respectively.

It can be concluded from the above results that Cr showed significantly greater toxic effect on growth of mycelium and development of micro and macro conidia of *F. solani* than Ni. The inhibitory effect of both the heavy metals on the development of macroconidia was more than the microconidia. The development of chlamydospores was not recorded in control and lowest concentration of Cr. Similarly, the formation of chlamydospores was not observed up to the concentration of 50 ppm Ni. Moreover, the uptake of Ni by *F. solani*

was significantly higher than the Cr except at 25 and 50 ppm concentrations.

4.4: STUDIES ON POTENTIAL PATHOGENIC LEVEL OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* AND ROOT-ROT FUNGUS *FUSARIUM SOLANI* ON CHICKPEA:

It is evident from the data presented in Tables 4 to 4.1 and Fig. 4 that with an increase in the inoculum level of *Rotylenchulus reniformis,* there was a corresponding decrease in the plant growth, yield and nodulation of chickpea plants, except the inoculum level of 250 immature females per plant which slightly increased plant growth as compared to uninoculated plants (control). The inoculum levels upto 500 immature females of *R. reniformis* did not show significant variation in the plant growth, yield and nodulation of chickpea. However, the significant reduction in these parameters was recorded at and above 1000 immature females of *R. reniformis.* Inoculation of plants with 500, 1000, 2000, 4000 and 8000 immature females of *R. reniformis* resulted in 11.9, 28.5, 34.5, 36.9 and 40.4 % plant growth reduction, respectively over uninoculated control. The reduction in the yield was recorded as 4.7, 12.3, 30.5, 35.2, 38.8 and 41.1% in the plants inoculated with 250, 500, 1000, 2000, 4000 and 8000 immature females, respectively. In the corresponding inoculum levels, the reduction in nodulation was 3.6, 9.1, 27.5, 31.1, 33.0 and 37.3%.

Similarly, with an increase in the inoculum level from 250 to 8000 immature females of *R. reniformis,* there was a gradual decrease in chlorophyll content of leaves, protein content of seeds and water absorption capacity of roots. However, the significant reduction in these parameters was recorded at and above 1000 immature females of *R. reniformis* per plant as compared to control. The reduction in chlorophyll 'a' and chlorophyll 'b' ranged between 7.0 - 31.4 % and 9.8 - 33.5% respectively in inoculum level from 250 - 8000 immature females / plant. The inoculation of plants with 250, 500, 1000, 2000,

Table-4: Potential pathogenic level of reniform nematode, *Rotylenchulus reniformis* on chickpea.

Inoculum levels	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
0	38.8	27.5	66.3	15.7	10.3	26.0	5.4	3.0	8.4
250	39.3	27.5	66.8	15.9	10.6	26.5	5.4	3.1	8.5 (+1.1)
500	36.3	25.4	61.7	14.4	9.2	23.6	4.7	2.7	7.4 (-11.9)
1000	30.0	20.3	50.3	11.9	7.6	19.5	3.9	2.1	6.0 (-28.5)
2000	27.7	18.9	46.6	10.8	6.9	17.7	3.6	1.9	5.5 (-34.5)
4000	26.2	18.0	44.2	10.3	6.6	16.9	3.5	1.8	5.3 (-36.9)
8000	24.9	16.9	41.8	9.9	6.2	16.1	3.3	1.7	5.0 (-40.4)
L.S.D. (at 5% level)			10.45			3.25			1.21
L.S.D. (at 1% level)			14.65			4.55			1.69

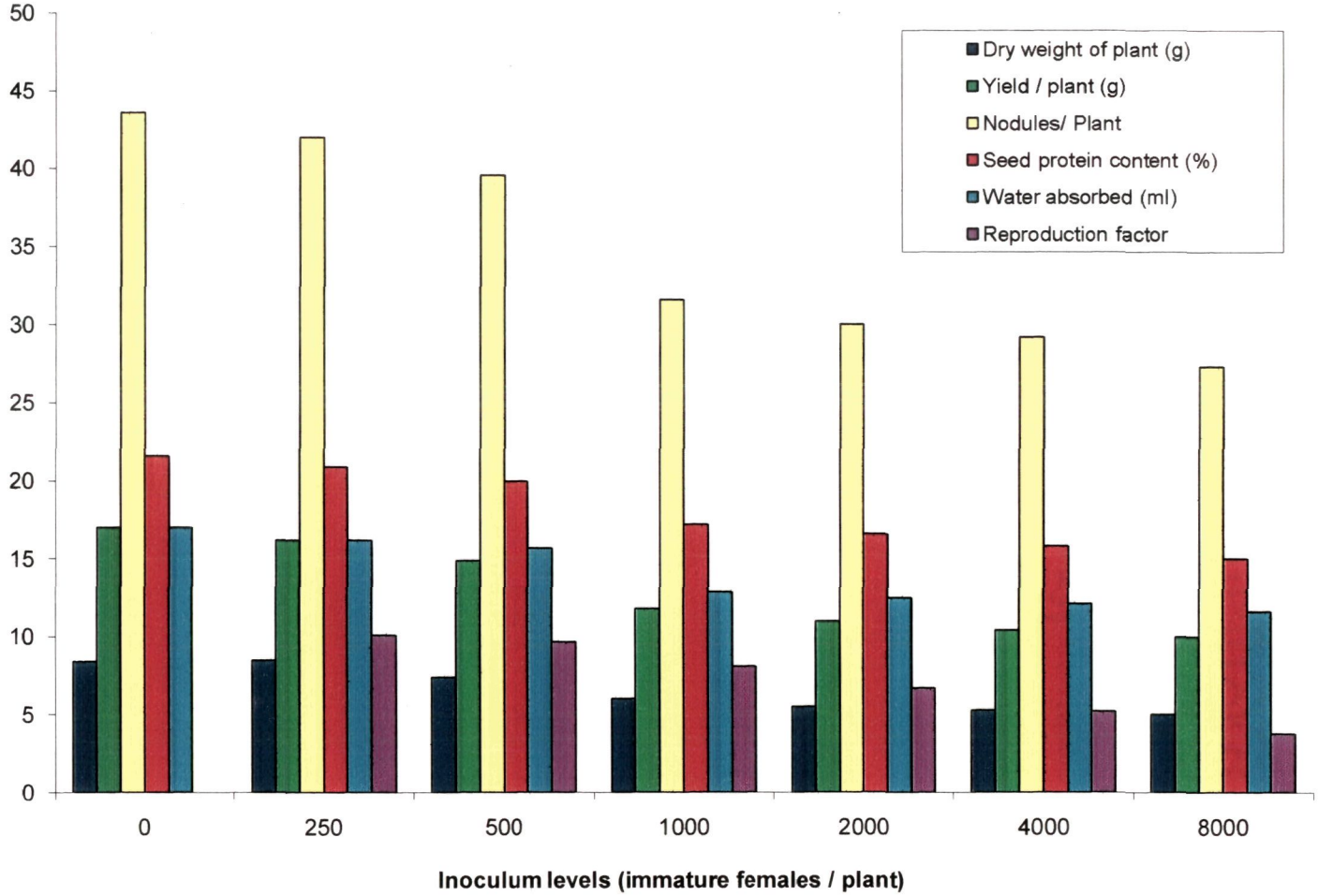
In parenthesis are given percent reduction (-) or stimulation (+) over control.

Table-4.1: Potential pathogenic level of reniform nematode, *Rotylenchulus reniformis* on chickpea.

Inoculum levels	Yield plant^{-1} (g)	Nodules plant^{-1}	Chlorophyll (mg/g)		Seed protein content (%)	Water absorbed (ml)	Nematode population pot^{-1}			R = Pf/Pi
			a	b			Nematodes / Kg soil	Females root system^{-1}	Total	
0	17.0	43.6	0.554	0.798	21.6	17.0	-	-	-	-
250	16.2	42.0	0.515	0.719	20.9	16.2	2423	120	2543	10.1
	(4.7)	(3.6)	(7.0)	(9.8)	(3.2)	(4.7)				
500	14.9	39.6	0.495	0.700	20.0	15.7	4665	230	4895	9.7
	(12.3)	(9.1)	(10.6)	(12.2)	(7.4)	(7.6)				
1000	11.8	31.6	0.415	0.588	17.2	12.9	7724	400	8124	8.1
	(30.5)	(27.5)	(25.0)	(26.3)	(20.3)	(24.1)				
2000	11.0	30.0	0.403	0.560	16.6	12.5	12882	535	13417	6.7
	(35.2)	(31.1)	(27.2)	(29.8)	(23.1)	(26.4)				
4000	10.4	29.2	0.390	0.550	15.8	12.1	20210	610	20820	5.2
	(38.8)	(33.0)	(29.6)	(31.0)	(26.8)	(28.8)				
8000	10.0	27.3	0.380	0.530	15.0	11.6	29747	687	30434	3.8
	(41.1)	(37.3)	(31.4)	(33.5)	(30.5)	(31.7)				
L.S.D. (at 5% level)	2.64	5.32	0.072	0.105	2.10	2.23				0.30
L.S.D. (at 1% level)	3.10	7.47	0.101	0.147	2.94	3.12				0.42

In parenthesis are given percentage reduction over control.

Fig. 4: Potential pathogenic level of reniform nematode, *Rotylenchulus reniformis* on chickpea.



4000 and 8000 immature females resulted in 3.2, 7.4, 20.3, 23.1, 26.8 and 30.5% reduction in the protein content of seeds, respectively. Similarly, when plants were inoculated with the corresponding inoculum levels of *R. reniformis*, the reduction in water absorption capacity was 4.7, 7.6, 24.1, 26.4, 28.8 and 31.7%, respectively (Table 4.1 and Figs 4 to 4.1).

The data presented in Table 4.1 and Fig. 4 clearly indicate that the population of reniform nematode was maximum at the highest inoculum level and minimum at the lowest inoculum level of immature females / plant. The reproduction factor of the nematode decreased significantly with an increase in the inoculum level from 250 - 8000 immature females, which revealed that the nematode multiplication showed a declined trend with an increase in the inoculum level suggesting it to be a density dependent phenomenon. The reproduction factor was highest (10.1) in plants inoculated with 250 immature females of *R. reniformis,* which was followed by 9.7, 8.1, 6.7, 5.2 and 3.8 in plants inoculated with 500, 1000, 2000, 4000, and 8000 immature females, respectively.

The plants inoculated with 1000 or above inoculum level showed unthrifty appearance. The leaves were chlorotic and small. Plants were less vigorous and smaller than the healthy plants. Moreover, the characteristic symptom of dirty roots was also observed upon gently uprooting the plants. Soil particles adhered to the sticky gelatinous matrix secreted by mature female causing the "dirty root" symptoms. Root injury is characterized by discolouration and epidermal necrosis. The root system of plant is reduced as compared to healthy plants.

It is clear from the data presented in Table 4.2 to 4.3 and Fig. 4.2 that with an increase in the inoculum level of *F. solani,* there was a corresponding decrease in the plant growth, yield and nodulation of chickpea plants as compared to uninoculated plants. The inoculum level upto 2.0 g of *F. solani*

Fig. 4.1: Effect of *Rotylenchulus reniformis* on the chlorophyll content of leaves of chickpea.

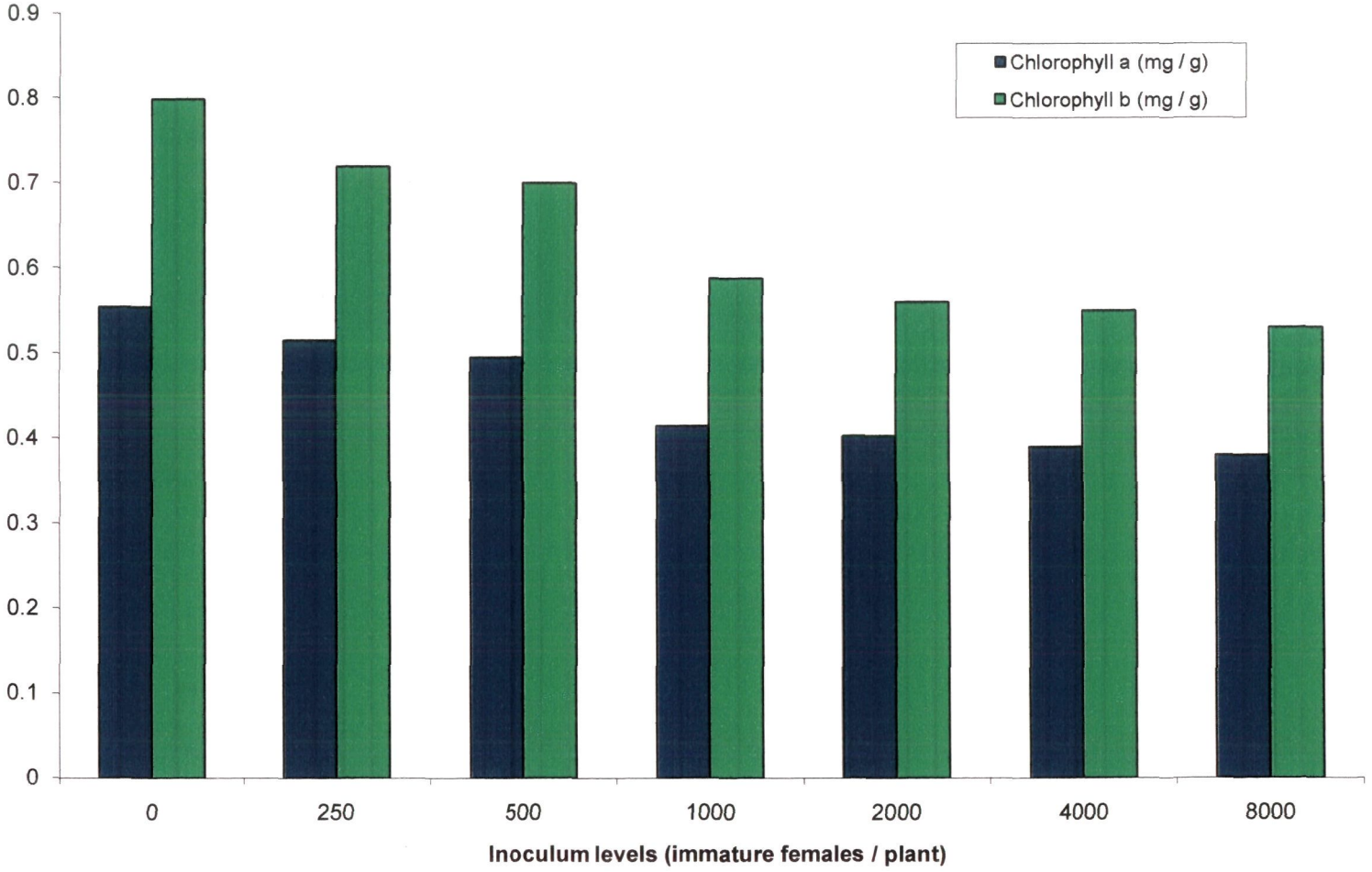


Table-4.2: Potential pathogenic level of root-rot fungus, *Fusarium solani* on chickpea.

Inoculum levels	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
0	38.8	27.5	66.3	15.7	10.3	26.0	5.4	3.0	8.4
0.5	38.1	26.2	64.3	15.3	9.7	25.0	5.2	2.7	7.9 (5.9)
1.0	37.3	25.5	62.8	14.8	9.4	24.2	4.9	2.6	7.5 (10.7)
2.0	35.0	24.2	59.2	14.2	8.7	22.9	4.8	2.5	7.3 (13.0)
3.0	28.2	19.5	47.7	11.0	6.9	17.9	3.7	2.0	5.7 (32.1)
4.0	27.9	19.0	46.9	10.8	6.8	17.6	3.5	1.9	5.4 (35.7)
5.0	26.9	18.1	45.0	10.2	6.4	16.6	3.4	1.8	5.2 (38.0)
L.S.D. (at 5% level)			8.85			4.50			1.42
L.S.D. (at 1% level)			12.41			6.30			2.00

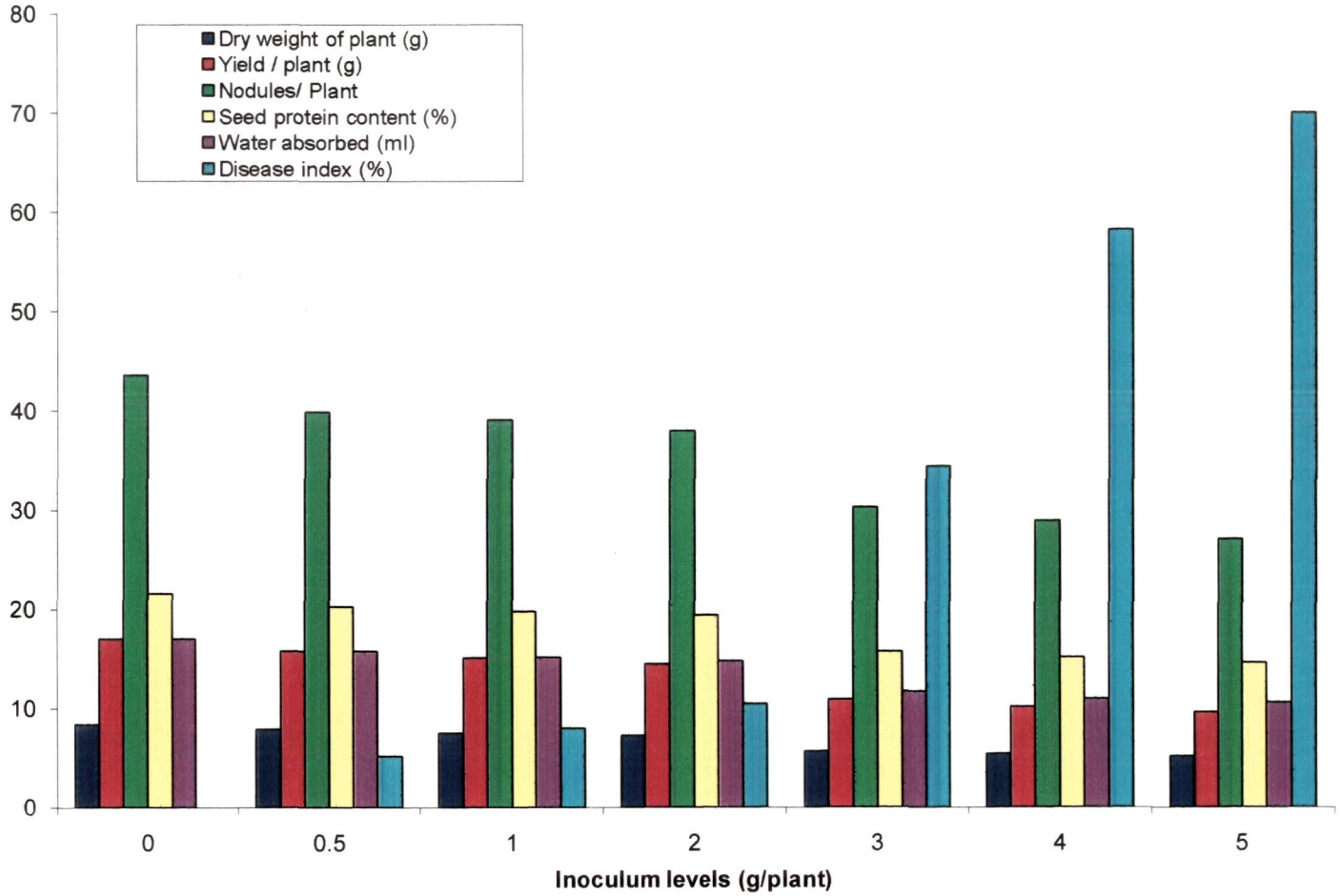
In parenthesis are given percentage reduction over control.

Table-4.3: Potential pathogenic level of root-rot fungus, *Fusarium solani* on chickpea.

Inoculum levels	Yield plant^{-1} (g)	Nodules plant^{-1}	Chlorophyll (mg/g)		Seed protein content (%)	Water absorbed (ml)	Disease index (%)
			a	b			
0	17.0	43.6	0.554	0.798	21.6	17.0	-
0.5	15.8 (7.0)	39.9 (8.4)	0.526 (5.0)	0.771 (3.3)	20.3 (6.0)	15.8 (7.0)	5.2
1.0	15.1 (11.1)	39.1 (10.3)	0.506 (8.6)	0.746 (6.5)	19.8 (8.3)	15.2 (10.5)	8.0
2.0	14.5 (14.7)	38.0 (12.8)	0.490 (11.5)	0.720 (9.7)	19.4 (10.1)	14.8 (12.9)	10.5
3.0	10.9 (35.8)	30.3 (30.5)	0.415 (25.0)	0.614 (23.0)	15.7 (27.3)	11.7 (31.1)	34.4
4.0	10.2 (40.0)	28.9 (33.7)	0.403 (27.2)	0.574 (28.0)	15.2 (29.6)	11.0 (35.2)	58.2
5.0	9.6 (43.5)	27.1 (37.8)	0.373 (32.6)	0.525 (34.2)	14.6 (32.4)	10.6 (37.6)	70.0
L.S.D. (at 5% level)	3.00	6.41	0.070	0. 096	3.15	2.61	11.0
L.S.D. (at 1% level)	4.20	9.00	0.098	0.134	4.41	3.65	17.24

In parenthesis are given percentage reduction over control.

Fig.4.2: Potential pathogenic level of root-rot fungus, *Fusarium solani* on chickpea.

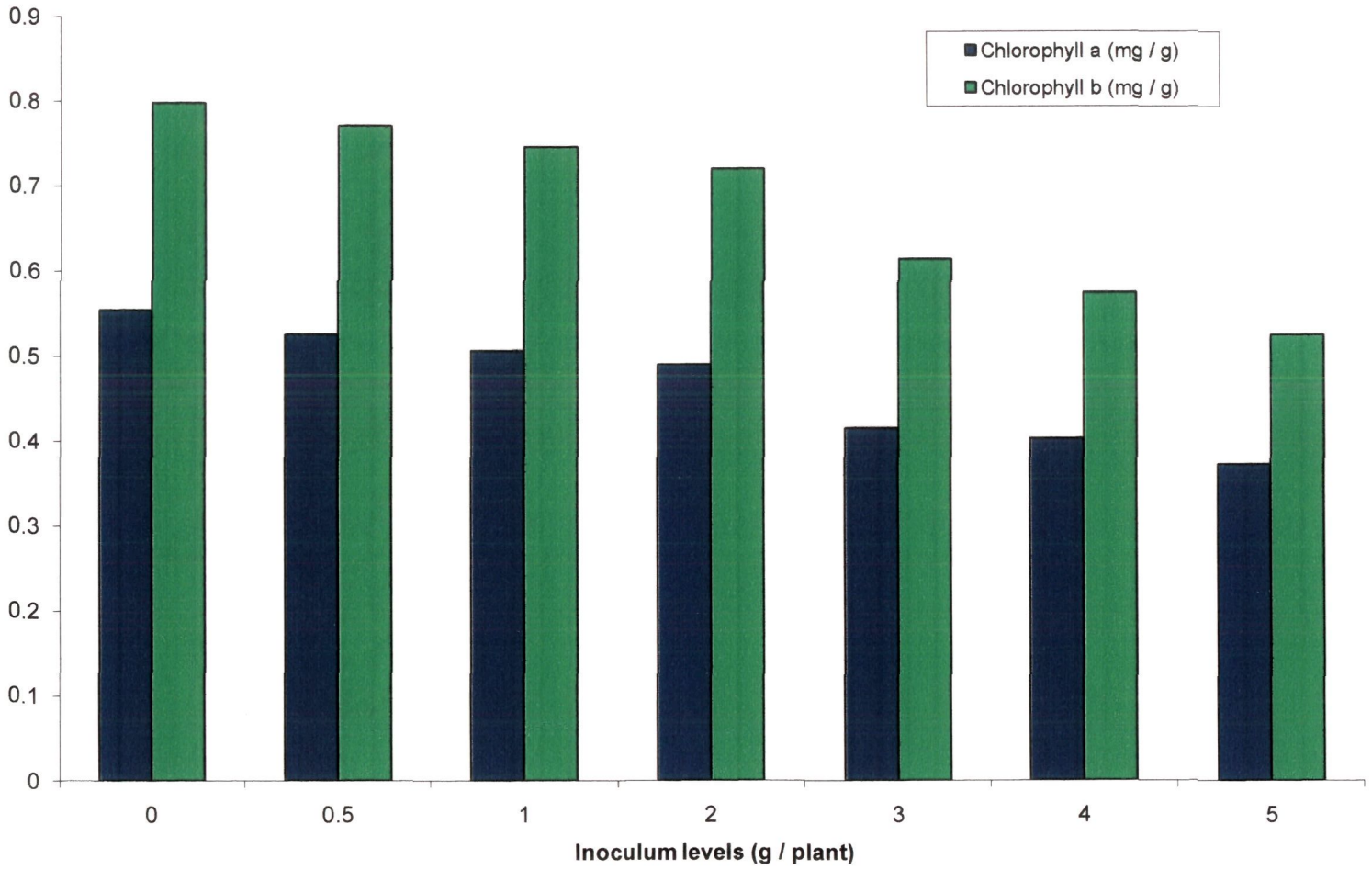


did not show significant variation in the plant growth, yield and nodulation of chickpea, as compared to control. However, the significant reduction in these parameters was recorded at and above 3.0g of *F. solani.* Inoculation of plants with 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g of *F. solani* resulted in 5.9, 10.7, 13.0, 32.1, 35.7 and 38.0% plant growth reduction of chickpea. In the corresponding inoculum levels, the reduction in nodulation was recorded as 8.4, 10.3, 12.8, 30.5, 33.7 and 37.8%, and the reduction in yield was recorded as 7.0, 11.1, 14.7, 35.8, 40.0 and 43.5%. Moreover, there was a corresponding increase in the disease index of *F. solani* with an increase of inoculum levels. The significant increase in disease index of *F. solani* was recorded at and above 3.0 g of *F. solani* per plant. The disease index was observed as 5.2, 8.0, 10.5, 34.4, 58.2 and 70.0% in the plants inoculated with 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g of *F. solani* per plant, respectively.

Similarly, with an increase in the inoculum level from 0.5 to 5.0 g of *F. solani,* there was also a gradual decrease in chlorophyll content of leaves, protein content of seeds and water absorption capacity of roots as compared to uninoculated plants (Table 4.3 and Fig. 4.2 to 4.3). However, the significant reduction in these parameters was recorded at and above 3.0g of *F. solani* / plant. The reduction in chlorophyll 'a' and chlorophyll 'b' ranged between 5.0-32.6% and 3.3-34.2 % , respectively in the inoculum levels from 0.5 - 5.0 g of *F. solani* / plant. The inoculation of plants with 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g of *F. solani* resulted in a reduction of 6.0, 8.3, 10.1, 27.3, 29.6 and 32.4% in the protein content, respectively (Table 4.3).

The reduction in water absorption capacity over uninoculated plants was also highest (37.6%) at the inoculum level of 5.0 g of *F. solani* / plant, followed by 35.2,31.1,12.9,10.5 and 7.0 %, in the plants inoculated with 4.0, 3.0, 2.0, 1.0 and 0.5 g *F. solani* / plant , respectively (Table 4.3).

Fig. 4.3 : Effect of different inoculum levels of *Fusarium solani* on chlorophyll content of chickpea leaves.



The chickpea plants inoculated at and above 3.0 g of *F. solani* showed stunting of plant growth and also yellowing of leaves, starting from the lower leaves and proceeding in each leaf and leaflet from tip and downwards. The leaf size was reduced individually as well as in totality giving an impression of reduced laminate condition. Sudden drooping of leaves during the day time was also recorded at highest inoculum level. Such plants showed very poor, discoloured and rotten root system.

In general, the severity of the disease increased with increase in the inoculum level of *R. reniformis* and *F. solani* at and above 1000 immature females of *R. reniformis* / plant and 3.0 g of *F. solani* / plant, respectively. It can be concluded from these results that the potential pathogenic level of *R. reniformis* and *F. solani* on chickpea was recorded as 1000 immature females of *R. reniformis* / plant and 3.0 g of *F. solani* / plant, respectively. Therefore, these inoculum levels of the test pathogens were used in the rest of experiments.

4.5: EFFECT OF CHROMIUM AND NICKEL ON PATHOGENIC POTENTIAL OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* AND ROOT-ROT FUNGUS, *FUSARIUM SOLANI* INFECTING CHICKPEA:

It is evident from the data presented in Tables 5 to 5.1 and Fig. 5 that the reduction in plant growth, yield and nodulation of chickpea was directly proportional to the concentration of Cr. However, the concentration of Cr upto 50 ppm did not show any significant reduction in the corresponding parameters of chickpea as compared to untreated-uninoculated plants (control). The reduction in plant growth was recorded as 2.6, 3.9, 11.8 and 15.7% in the plants grown in soil treated with 25, 50, 100 and 200 ppm Cr, respectively. Similarly, in the corresponding treatments the reduction in yield was found to be 3.3, 5.3, 14.6 and 19.3 %, and the reduction in nodulation was 2.1, 3.4, 14.4 and 16.3%.

Table-5: Effect of chromium on pathogenic potential of reniform nematode, *Rotylenchulus reniformis* (Rr) infecting chickpea.

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)		
Cr (ppm)/Rr	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
0	35.4	25.9	61.3	13.6	9.8	23.4	4.8	2.8	7.6
25	35.2	24.8	60.0	13.3	9.5	22.8	4.7	2.7	7.4 (2.6)
50	35.0	24.7	59.7	13.5	9.2	22.7	4.7	2.6	7.3 (3.9)
100	33.1	23.2	56.3	12.5	8.7	21.2	4.4	2.3	6.7 (11.8)
200	32.6	22.5	55.1	12.1	8.4	20.5	4.2	2.2	6.4 (15.7)
Rr	28.3	20.1	48.4	10.6	7.4	18.0	3.6	2.0	5.6 (26.3)
25 + Rr	28.2	20.1	48.3	10.4	7.3	17.7	3.6	1.9	5.5 (27.6)
50 + Rr	26.6	18.5	45.1	9.6	6.7	16.3	3.2	1.8	5.0 (34.2)
100 + Rr	24.3	16.8	41.1	8.8	5.8	14.6	2.9	1.5	4.4 (42.1)
200 + Rr	22.3	15.6	37.9	8.1	5.4	13.5	2.6	1.4	4.0 (47.3)
L.S.D. (at 5% level)			3.12			1.32			0.51
L.S.D. (at 1% level)			4.27			1.81			0.70

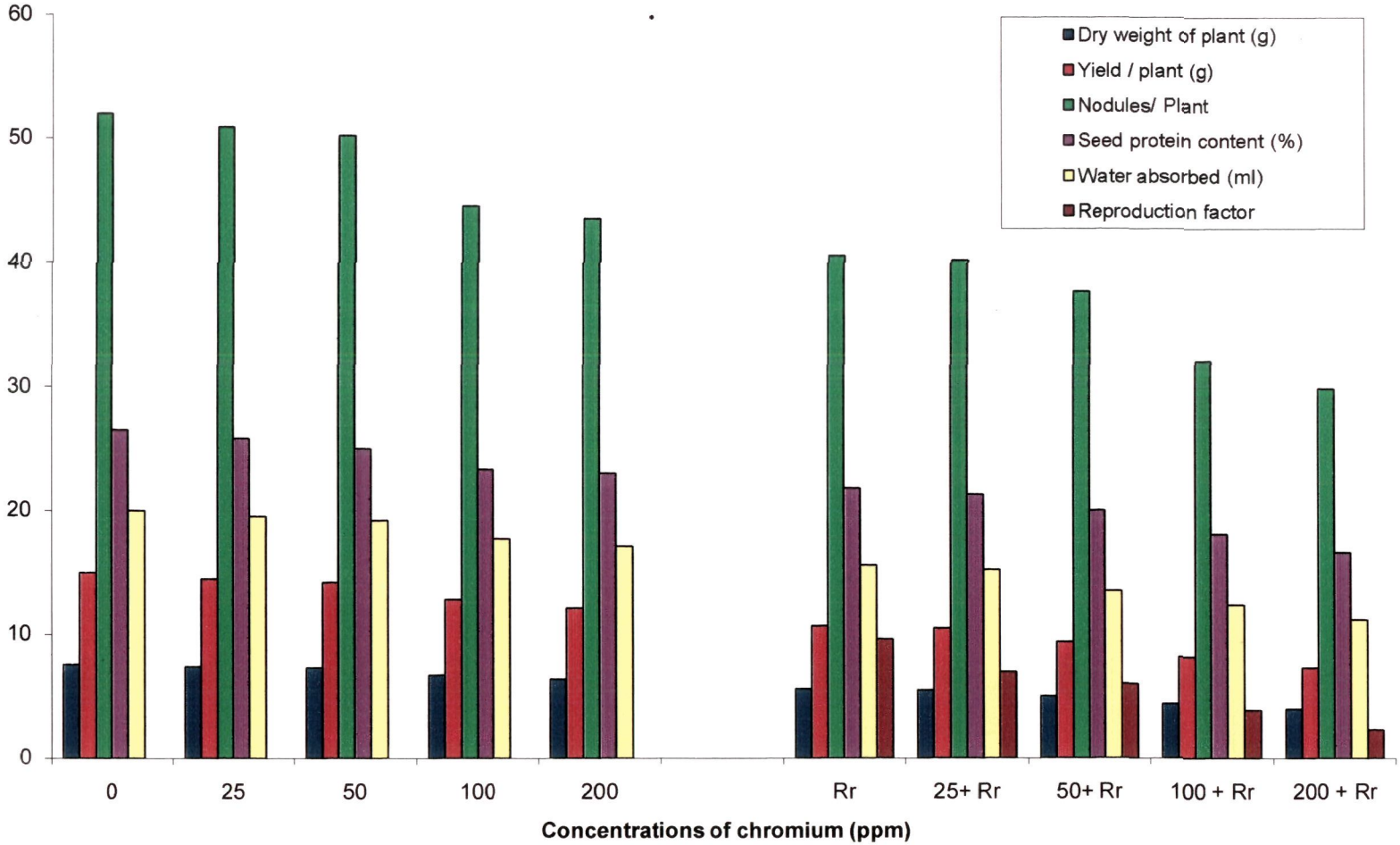
In parenthesis are given percentage reduction over control.

Table-5.1: Effect of chromium on pathogenic potential of reniform nematode, *Rotylenchulus reniformis* (Rr) infecting chickpea.

Treatments Cr (ppm)/Rr	Yield plant^{-1} (g)	Nodules plant^{-1}	Chlorophyll (mg/g) a	Chlorophyll (mg/g) b	Seed protein content (%)	Water absorbed (ml)	Nematode population pot^{-1} Nematodes / kg soil	Females root system^{-1}	Total	R=Pf/Pi
0	15.0	52.0	0.570	0.810	26.5	20.0	-	-	-	-
25	14.5 (3.3)	50.9 (2.1)	0.554 (2.8)	0.793 (2.0)	25.8 (2.6)	19.5 (2.5)	-	-	-	-
50	14.2 (5.3)	50.2 (3.4)	0.540 (5.2)	0.772 (4.6)	25.0 (5.6)	19.2 (4.0)	-	-	-	-
100	12.8 (14.6)	44.5 (14.4)	0.489 (14.2)	0.710 (12.3)	23.3 (12.0)	17.7 (11.5)	-	-	-	-
200	12.1 (19.3)	43.5 (16.3)	0.467 (18.0)	0.687 (15.1)	23.0 (13.2)	17.1 (14.5)	-	-	-	-
Rr	10.7 (28.6)	40.5 (22.1)	0.437 (23.3)	0.638 (21.2)	21.8 (17.7)	15.6 (22.0)	9199	410	9609	9.60
25 + Rr	10.5 (30.0)	40.1 (22.8)	0.432 (24.2)	0.617 (23.8)	21.3 (19.6)	15.2 (24.0)	6709	330	7039	7.00
50 + Rr	9.4 (37.3)	37.6 (27.6)	0. 397 (30.3)	0.585 (27.7)	20.0 (24.5)	13.5 (32.5)	5734	290	6024	6.00
100 + Rr	8.2 (45.3)	32.0 (38.4)	0.332 (41.7)	0.507 (37.4)	18.1 (31.6)	12.4 (38.0)	3793	137	3930	3.90
200 + Rr	7.3 (51.3)	29.8 (42.6)	0.304 (46.6)	0.481 (40.6)	16.6 (37.3)	11.2 (44.0)	2250	60	2310	2.3
L.S.D. (at 5% level)	1.27	4.22	0.044	0.057	1.64	1.31				0.64
L.S.D. (at 1% level)	1.74	5.78	0.060	0.078	2.24	1.79				0.93

In parenthesis are given percentage reduction over control.

Fig. 5: Effect of chromium on pathogenic potential of reniform nematode, *Rotylenchulus reniformis* (Rr) infecting chickpea.

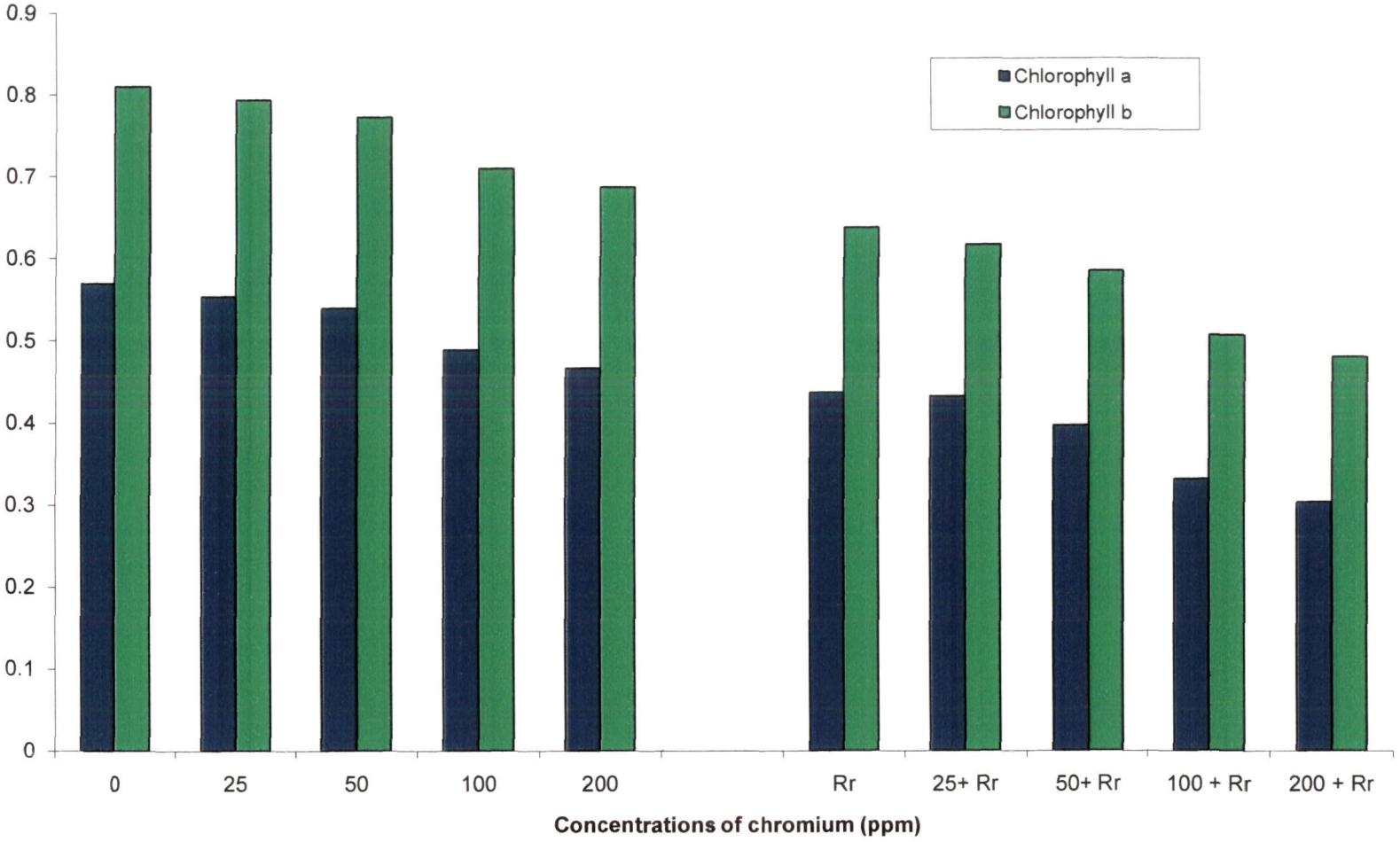


The data presented in Table 5.1 and Figs 5 to 5.1 clearly indicated that with an increase in the concentration of Cr from 25 to 200 ppm, there was also a gradual decrease in the chlorophyll content of leaves, protein content of seeds and water absorption capacity of roots as compared to control plants. However, the significant reduction in the corresponding parameters was observed at and above 100 ppm Cr. The reduction in chlorophyll 'a' content ranged between 2.8 to 18.0 % and chlorophyll 'b' ranged between 2.0 to 15.1% in plants treated with different concentrations of Cr . The treatment of the soil with 25, 50, 100 and 200 ppm Cr, resulted in 2.6, 5.6, 12.0 and 13.2 % reduction in protein content of chickpea seeds, respectively as compared to control. Similarly, in the corresponding treatments of Cr, the reduction in water absorption capacity of roots was 2.5, 4.0, 11.5 and 14.5% (Table 5.1).

The perusal of data presented in Tables 5 to 5.1 and 5.4 to 5.5 revealed that the inoculation of *R. reniformis* significantly reduced the plant growth (26.3%), yield (28.6%) and nodulation (22.1%) of chickpea as compared to control. However, the reduction in plant growth, yield and nodulation of chickpea plants was synergistically ameliorated in the plants inoculated with *R. reniformis* and treated with different concentrations of Cr except 25 ppm concentration which did not show synergistic effect. The reduction in plant growth was 27.6, 34.2, 42.1 and 47.3% in the plants inoculated with *R. reniformis* and treated with 25, 50, 100 and 200 ppm concentrations of Cr, respectively in comparison to control. Similarly, in the corresponding treatments, the reduction in yield was recorded as 30.0, 37.3, 45.3 and 51.3%, whereas the reduction in nodulation was found as 22.8, 27.6, 38.4 and 42.6% (Tables 5 to 5.1 and Fig. 5).

It is apparent from the data presented in Table 5.1 and Figs 5 to 5.1 that the inoculation of *R. reniformis* significantly reduced the chlorophyll content of leaves viz. chlorophyll 'a'(23.3%) and chlorophyll 'b' (21.2%), protein content

Fig.5.1: Effect of chromium on chlorophyll content of chickpea leaves in presence and absence of *Rotylenchlus reniformis* (Rr).



of seeds (17.7%) and water absorption capacity of roots (22.0%) as against control. Moreover, the reduction in the corresponding parameters of chickpea was synergistically increased in the plants inoculated with *R. reniformis* and grown in soil treated with different concentrations of Cr except at 25 ppm which was unable to show synergistic effect. The reduction in chlorophyll 'a' ranged between 24.2 to 46.6 % and chlorophyll 'b' ranged between 23.8 to 40.6 % in the different treatments of Cr (25,50,100 and 200 ppm). Similarly, the reduction in protein content was recorded as 19.6, 24.5, 31.6 and 37.3 % and water absorption capacity was 24.0, 32.5, 38.0 and 44.0% in plants inoculated with *R. reniformis* and soil treated with 25, 50, 100 and 200 ppm concentration of Cr, respectively as compared to control.

The population buildup of reniform nematode decreased significantly with an increase in the concentration of Cr as compared to untreated – inoculated plants (Table 5.1 and Fig 5.1).The reproduction factor was highest (9.6) in untreated-inoculated plants followed by 7.0, 6.0, 3.9 and 2.3 in plants inoculated with *R. reniformis* and soil treated with 25, 50, 100 and 200 ppm concentrations of Cr, respectively (Table 5.1).

The data presented in Table 5.2 to 5.3 and 5.6 to 5.7clearly indicated that the inoculation of *Fusarium solani* significantly reduced the plant growth (30.2%), yield (34.0%) and nodulation (28.2%) of chickpea plants as compared to control. Moreover, the reduction in plants growth, yield and nodulation of chickpea was ameliorated in the plants inoculated with *F. solani* and grown in soil treated with 25, 50, 100 and 200 ppm Cr. The reduction in plant growth was 31.5, 32.8, 39.4 and 42.1 % in the plants inoculated with *F. solani* and treated with 25, 50, 100 and 200 ppm Cr, respectively as against control. Similarly, in the corresponding treatments the reduction in yield was 36.0, 38.6, 46.6 and 50.6% and nodulation was 28.6, 34.4, 38.4 and 42.8% (Tables 5.2 to 5.3 and Fig. 5.2)

Table-5.2: Effect of chromium on pathogenic potential of root-rot fungus, *Fusarium solani* (Fs) infecting chickpea.

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)		
Cr(ppm)/ Fs	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
0	35.4	25.9	61.3	13.6	9.8	23.4	4.8	2.8	7.6
25	35.2	24.8	60.0	13.3	9.5	22.8	4.7	2.7	7.4 (2.6)
50	35.0	24.7	59.7	13.5	9.2	22.7	4.7	2.6	7.3 (3.9)
100	33.1	23.2	56.3	12.5	8.7	21.2	4.4	2.3	6.7 (11.8)
200	32.6	22.5	55.1	12.1	8.4	20.5	4.2	2.2	6.4 (15.7)
Fs	27.7	18.8	46.5	10.2	6.8	17.0	3.4	1.9	5.3 (30.2)
25 + Fs	26.2	18.6	44.8	9.9	6.7	16.6	3.4	1.8	5.2 (31.5)
50 + Fs	26.1	18.5	44.6	9.8	6.6	16.4	3.3	1.8	5.1 (32.8)
100 + Fs	25.3	17.1	42.4	9.2	6.0	15.2	3.0	1.6	4.6 (39.4)
200 + Fs	24.5	16.0	40.5	8.8	5.8	14.6	2.9	1.5	4.4 (42.1)
L.S.D. (at 5% level)			2.34			0.98			0.42
L.S.D. (at 1% level)			3.36			1.41			0.60

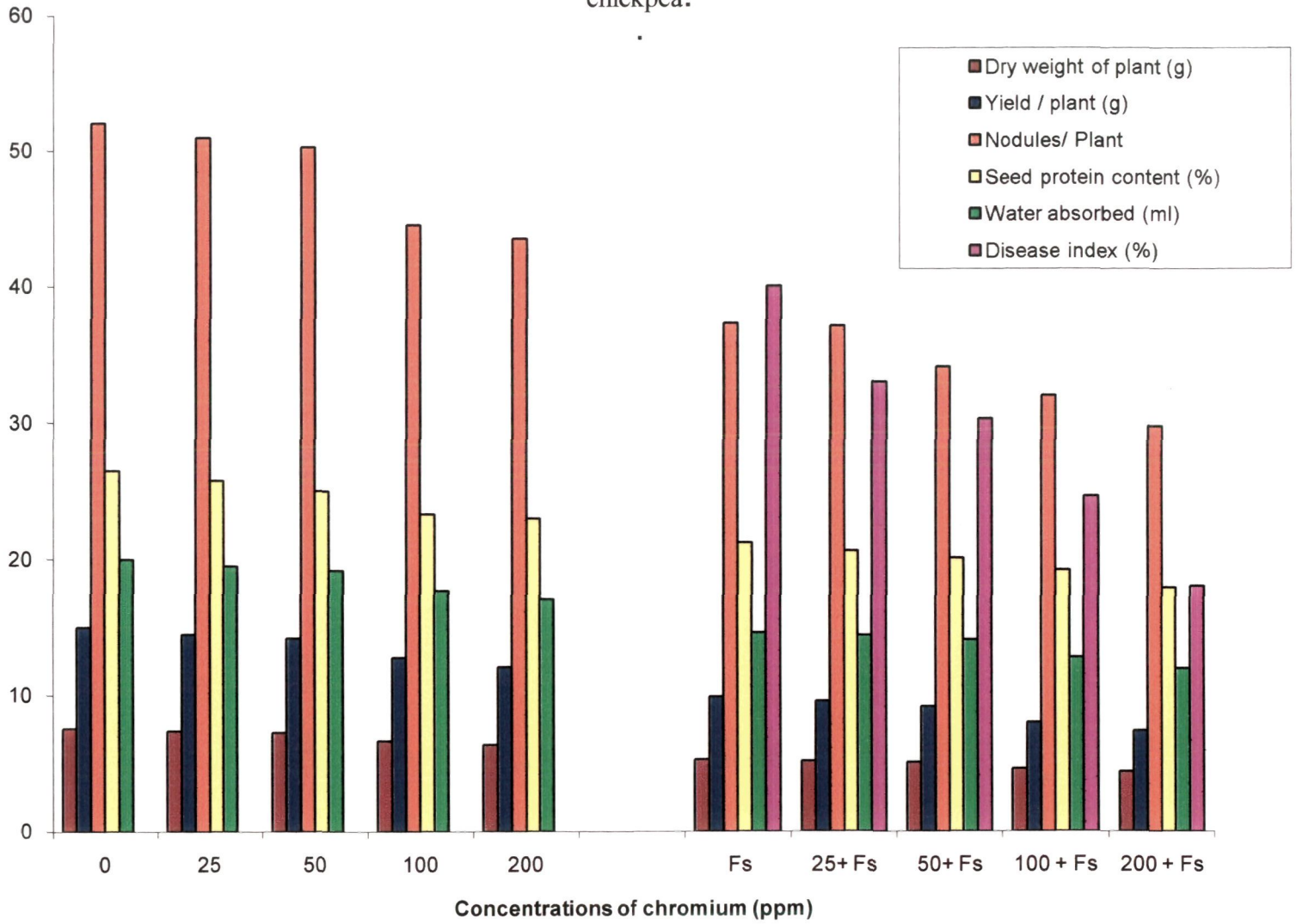
In parenthesis are given percentage reduction over control.

Table-5.3: Effect of chromium on pathogenic potential of root-rot, *Fusarium solani* (Fs) infecting chickpea.

Treatment Cr(ppm)/Fs	Yield plant^{-1} (g)	Nodules plant^{-1}	Chlorophyll (mg/g)		Seed protein content (%)	Water absorbed (ml)	Disease index (%)
			a	b			
0	15.0	52.0	0.570	0.810	26.5	20.0	-
25	14.5	50.9	0.554	0.793	25.8	19.5	-
	(3.3)	(2.1)	(2.8)	(2.0)	(2.6)	(2.5)	
50	14.2	50.2	0.540	0.772	25.0	19.2	-
	(5.3)	(3.4)	(5.2)	(4.6)	(5.6)	(4.0)	
100	12.8	44.5	0.489	0.710	23.3	17.7	-
	(14.6)	(14.4)	(14.2)	(12.3)	(12.1)	(11.5)	
200	12.1	43.5	0.467	0.687	23.0	17.1	-
	(19.3)	(16.3)	(18.0)	(15.1)	(13.2)	(14.5)	
Fs	9.9	37.3	0.412	0.609	21.2	14.6	40.0
	(34.0)	(28.2)	(27.7)	(24.8)	(20.0)	(27.0)	
25 + Fs	9.6	37.1	0.407	0.601	20.6	14.4	33.0
	(36.0)	(28.6)	(28.5)	(25.8)	(22.2)	(28.0)	
50 + Fs	9.2	34.1	0.389	0.578	20.1	14.1	30.3
	(38.6)	(34.4)	(31.7)	(28.6)	(24.1)	(29.5)	
100 + Fs	8.0	32.0	0.336	0.534	19.2	12.8	24.6
	(46.6)	(38.4)	(41.0)	(34.0)	(27.5)	(36.0)	
200 + Fs	7.4	29.7	0.323	0.506	17.9	12.0	18.0
	(50.6)	(42.8)	(43.3)	(37.5)	(32.4)	(40.0)	
L.S.D. (at 5% level)	1.12	3.87	0.038	0.045	1.60	1.05	6.21
L.S.D. (at1% level)	1.61	5.57	0.054	0.064	2.30	1.51	10.30

In parenthesis are given percentage reduction over control.

Fig.5.2: Effect of chromium on pathogenic potential of root-rot fungus *Fusarium solani* (Fs) infecting chickpea.



The data presented in Table 5.3 and Figs 5.2 to 5.3 indicated that the inoculation of *F. solani* significantly reduced the chlorophyll content of leaves viz. chlorophyll 'a' (27.7%) and chlorophyll 'b' (24.8%), protein content of seeds (20.0%) and water absorption capacity of roots (27.0%) as compared to control. Moreover, the reduction in the respective parameters of chickpea was ameliorated in the plants inoculated with *F. solani* and treated with 25, 50, 100 and 200 ppm Cr. The reduction in chlorophyll 'a' ranged between 28.5 to 43.3% and chlorophyll 'b' ranged between 25.8 to 37.5 % in the plants treated with 25, 50, 100 and 200 ppm Cr and inoculated with *F. solani.* Similarly, in the corresponding treatments, the reduction in protein content was observed as 22.2, 24.1, 27.5 and 32.4%, and in water absorption capacity of roots was 28.0, 29.5, 36.0 and 40.0 % as compared to control.

The disease index of *F. solani* gradually decreased with an increase in the concentration of Cr from 25 to 200 ppm as compared to untreated- inoculated plants. The disease index was highest (40.0 %) in the untreated-inoculated plants which was followed by 33.0, 30.3, 24.6 and 18.0% in the plants inoculated with *F. solani* and treated with 25, 50, 100 and 200 ppm Cr, respectively (Table 5.3 and Fig. 5.2).

It is evident from the data presented in Tables 5.4 to 5.7 and Fig. 5.4 that the reduction in plant growth, yield and nodulation of chickpea was directly proportional to the concentration of Ni. However, the concentration of Ni up to 50ppm did not show any significant reduction in the corresponding parameters of chickpea as compared to untreated-uninoculated plants. The reduction in plant growth was recorded to be 2.6, 5.2, 17.1 and 21.0% in the plants grown in soil treated with 25, 50, 100 and 200 ppm Ni, respectively. Similarly, in the corresponding treatments, the reduction in yield was found as 4.6, 7.3, 20.0 and 23.3 %, and reduction in nodulation was recorded as 3.0, 4.8, 15.3 and 19.6%.

Fig. 5.3: Effect of chromium on chlorophyll content of chickpea leaves in presence and absence of *Fusarium solani* (Fs).

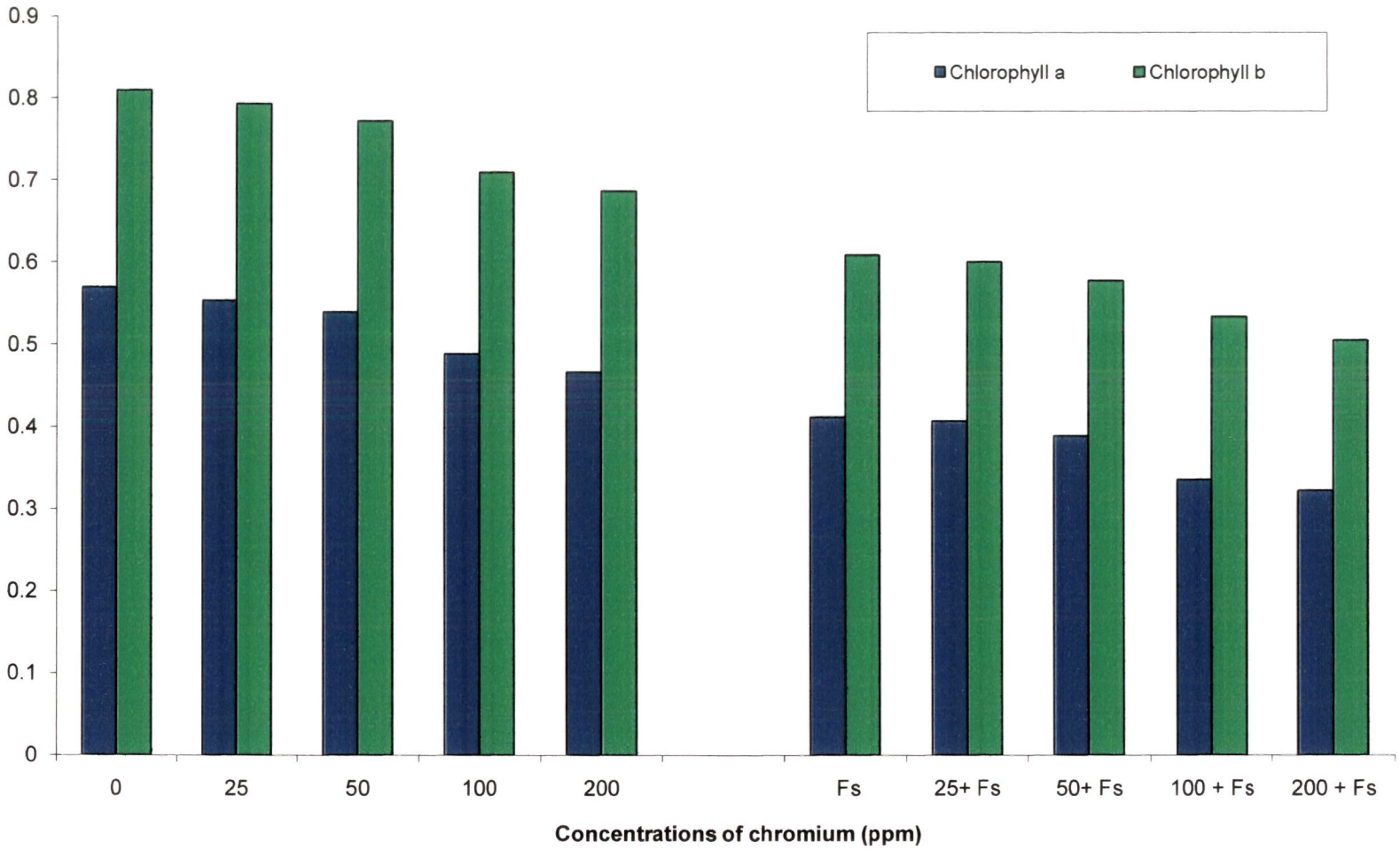
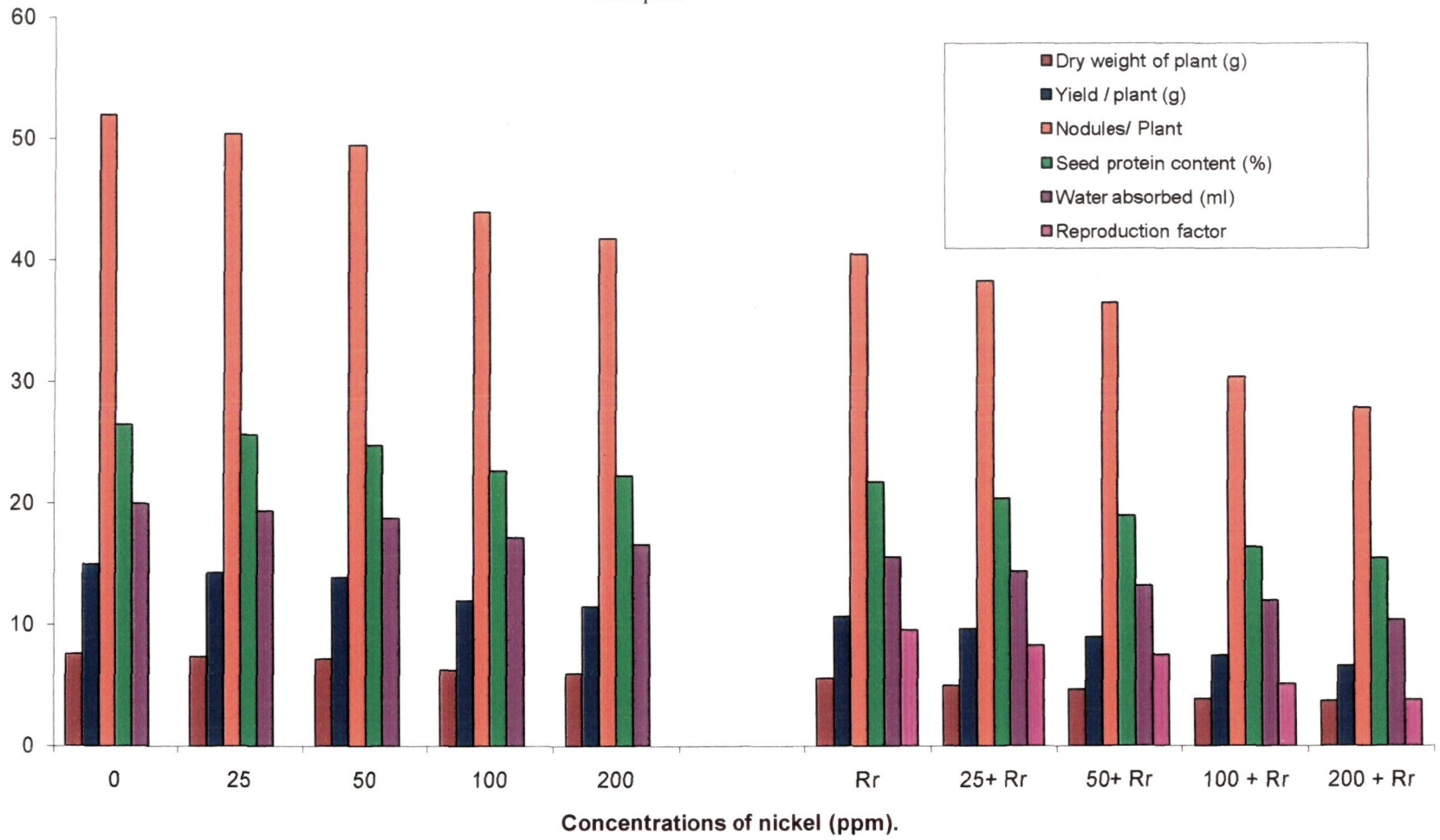


Table-5.4: Effect of nickel on pathogenic potential of reniform nematode, *Rotylenchulus reniformis* (Rr) infecting chickpea.

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)		
Ni (ppm)/ Rr	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
0	35.4	25.9	61.3	13.6	9.8	23.4	4.8	2.8	7.6
25	34.9	25.3	60.2	13.5	9.4	22.9	4.9	2.5	7.4 (2.6)
50	34.5	25.0	59.5	13.1	9.4	22.5	4.7	2.5	7.2 (5.2)
100	31.4	22.4	53.8	11.7	8.1	19.8	4.1	2.2	6.3 (17.1)
200	30.3	21.5	51.8	11.4	7.8	19.2	4.0	2.0	6.0 (21.0)
Rr	28.3	20.1	48.4	10.6	7.4	18.0	3.6	2.0	5.6 (26.3)
25 + Rr	26.2	18.3	44.5	9.7	6.6	16.3	3.3	1.7	5.0 (34.2)
50 + Rr	25.6	17.3	42.9	9.3	6.4	15.7	3.1	1.6	4.7 (38.1)
100 + Rr	23.1	14.5	37.6	8.0	5.2	13.2	2.6	1.3	3.9 (48.6)
200 + Rr	21.3	14.2	35.5	7.6	5.0	12.6	2.5	1.2	3.7 (51.3)
L.S.D. (at 5% level)			3.25			2.42			0.52
L.S.D. (at 1% level)			4.45			3.31			0.71

In parenthesis are given percentage reduction over control.

Fig.5.4: Effect of nickel on pathogenic potential of reniform nematode, *Rotylenchulus reniformis* (Rr) infecting chickpea.



The data presented in Tables 5.5 to 5.7 and Fig. 5.4 to 5.7 clearly indicated that with an increase in the concentration of Ni from 25 to 200 ppm, there was a gradual decrease in the chlorophyll content of leaves, protein content of seeds and water absorption capacity of roots as compared to control. However, the significant reduction in the corresponding parameters was observed at and above 100 ppm Ni. The reduction in chlorophyll 'a' content ranged between 3.1 to 21.0 % and chlorophyll 'b' ranged between 2.7 to 19.0 in plants treated with different concentrations of Ni. The treatments of soil with 25, 50, 100 and 200 ppm Ni, resulted in 3.0,6.4,14.3 and 15.8 % reduction in protein content of chickpea seeds, respectively as compared to control. Similarly, in the corresponding treatments of nickel, the reduction in water absorption capacity of roots was 3.0, 6.0, 14.0 and 17.0 %.

The data presented in Tables 5.4 to 5.5 and Fig. 5.4 revealed that the inoculation of *R. reniformis* significantly reduced the plant growth (26.3%), yield (28.6%) and nodulation (22.1%) of chickpea as compared to control. However, the reduction in plant growth, yield and nodulation of chickpea plants was synergistically ameliorated in the plants inoculated with *R. reniformis* and soil treated with different concentrations of Ni. The reduction in plant growth was 34.2, 38.1, 48.6 and 51.3% in the plants inoculated with *R. reniformis* and soil treated with 25, 50, 100 and 200 ppm concentrations of Ni, respectively over control. Similarly, in the corresponding treatments, the reduction in yield was found as 35.3, 40.0, 50.0 and 56.0%, whereas, the reduction in nodulation was recorded as 26.3, 29.8, 41.5 and 46.3%.

The data presented in Tables 5.5 and Figs 5.4 to 5.5 indicated that the inoculation of *R. reniformis* significantly reduced the chlorophyll content of leaves viz. chlorophyll 'a' (23.3%) and chlorophyll 'b' (21.2 %), protein content of seeds (17.7%) and water absorption capacity of roots (22.0%) as against control. Moreover, the reduction in the corresponding parameters of

Table-5.5: Effect of nickel on pathogenic potential of reniform nematode, *Rotylenchulus reniformis* (Rr) infecting chickpea.

Treatment Ni (ppm)/Rr	Yield plant^{-1} (g)	Nodules plant^{-1}	Chlorophyll (mg/g) a	Chlorophyll (mg/g) b	Seed protein content (%)	Water absorbed (ml)	Nematode population pot^{-1} Nematodes/ Kg soil	Nematode population pot^{-1} Females root system^{-1}	Nematode population pot^{-1} Total	R= Pf/pi
0	15.0	52.0	0.570	0.810	26.5	20.0	-	-	-	-
25	14.3 (4.6)	50.4 (3.0)	0.552 (3.1)	0.788 (2.7)	25.7 (3.0)	19.4 (3.0)	-	-	-	-
50	13.9 (7.3)	49.5 (4.8)	0.530 (7.0)	0.760 (6.1)	24.8 (6.4)	18.8 (6.0)	-	-	-	-
100	12.0 (20.0)	44.0 (15.3)	0.467 (18.0)	0.687 (15.1)	22.7 (14.3)	17.2 (14.0)	-	-	-	-
200	11.5 (23.3)	41.8 (19.6)	0.450 (21.0)	0.656 (19.0)	22.3 (15.8)	16.6 (17.0)	-	-	-	-
Rr	10.7 (28.6)	40.5 (22.1)	0.437 (23.3)	0.638 (21.2)	21.8 (17.7)	15.6 (22.0)	6199	410	9609	9.6
25 + Rr	9.7 (35.3)	38.3 (26.3)	0.402 (29.4)	0.588 (27.4)	20.4 (23.0)	14.4 (28.0)	7972	353	8325	8.3
50 + Rr	9.0 (40.0)	36.5 (29.8)	0.369 (35.2)	0.542 (33.0)	19.0 (28.3)	13.2 (34.0)	7212	305	7517	7.5
100 + Rr	7.5 (50.0)	30.4 (41.5)	0.319 (44.0)	0.481 (40.6)	16.4 (38.1)	12.0 (40.0)	4970	160	5130	5.1
200 + Rr	6.6 (56.0)	27.9 (46.3)	0.288 (49.4)	0.447 (44.8)	15.5 (41.5)	10.4 (48.0)	3682	120	3802	3.8
L.S.D. (at 5% level)	1.45	4.22	0.052	0.054	1.84	1.35				0.67
L.S.D. (at 1% level)	1.98	5.78	0.072	0.074	2.52	1.85				1.05

In parenthesis are given percentage reduction over control.

chickpea was synergistically ameliorated in the plants inoculated with *R. reniformis* and grown in soil treated with different concentrations (25, 50, 100 and 200 ppm) of Ni. The reduction in chlorophyll 'a' ranged between 29.4 to 49.4% and chlorophyll 'b' ranged between 27.4 to 44.8% in the respective treatments of Ni. Similarly, the reduction in protein content was found as 23.0, 28.3, 38.1 and 41.5% and in water absorption capacity was recorded as 28.0, 34.0, 40.0 and 48.0% in plants inoculated with *R. reniformis* and treated with 25, 50, 100 and 200 ppm concentration of Ni, respectively as compared to control (Table 5.5).

The population buildup of reniform nematode decreased significantly with an increase in the concentration of Ni as compared to untreated - inoculated plants. The reproduction factor was highest (9.6) in untreated-inoculated plants followed by 8.3,7.5,5.1 and 3.8 in plants inoculated with *R. reniformis* and treated with 25, 50, 100 and 200 ppm concentrations of Ni, respectively (Table 5.5).

The data presented in Tables 5.6 to 5.7 and Fig. 5.6 clearly indicated that the inoculation of *F. solani* significantly reduced the plant growth (30.2%), yield (34.0%) and nodulation (28.2%) of chickpea plants as compared to control. Moreover, the reduction in plant growth, yield and nodulation of chickpea was synergistically ameliorated in the plants inoculated with *F. solani* and treated with 25, 50, 100 and 200 ppm Ni. The reduction in plant growth was 36.8, 44.7, 53.9 and 59.2% in the plants inoculated with *F. solani* and grown in soil treated with 25, 50, 100 and 200 ppm Ni, respectively over control. Similarly, in the corresponding treatments the reduction in yield was observed as 40.0, 45.3, 58.0 and 62.0% and in nodulation was 34.0, 37.3, 46.1 and 48.2%.

The inoculation of *F. solani* significantly reduced the chlorophyll content of leaves viz. chlorophyll 'a' (27.7%) and chlorophyll 'b' (24.8%), protein

Table-5.6: Effect of nickel on pathogenic potential of root-rot fungus, *Fusarium solani* (Fs) infecting chickpea.

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)		
Ni (ppm) / Fs	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
0	35.4	25.9	61.3	13.6	9.8	23.4	4.8	2.8	7.6
25	34.9	25.3	60.2	13.5	9.4	22.9	4.9	2.4	7.4 (2.6)
50	34.5	25.0	59.5	13.1	9.4	22.5	4.7	2.5	7.2 (5.2)
100	31.4	22.4	53.8	11.7	8.1	19.8	4.1	2.2	6.3 (17.1)
200	30.3	21.5	51.8	11.4	7.8	19.2	4.0	2.0	6.0 (21.0)
Fs	27.7	18.8	46.5	10.2	6.8	17.0	3.4	1.9	5.3 (30.2)
25 + Fs	25.4	17.8	43.2	9.3	6.3	15.6	3.1	1.7	4.8 (36.8)
50 + Fs	23.8	16.5	40.3	8.8	5.8	14.6	2.7	1.5	4.2 (44.7)
100 + Fs	21.3	14.0	35.3	7.7	5.2	12.9	2.3	1.2	3.5 (53.9)
200 + Fs	19.1	12.5	31.6	6.7	4.3	11.0	2.1	1.0	3.1 (59.2)
L.S.D. (at 5% level)			4.62			1.57			0.64
L.S.D. (at 1% level)			6.64			2.25			0.92

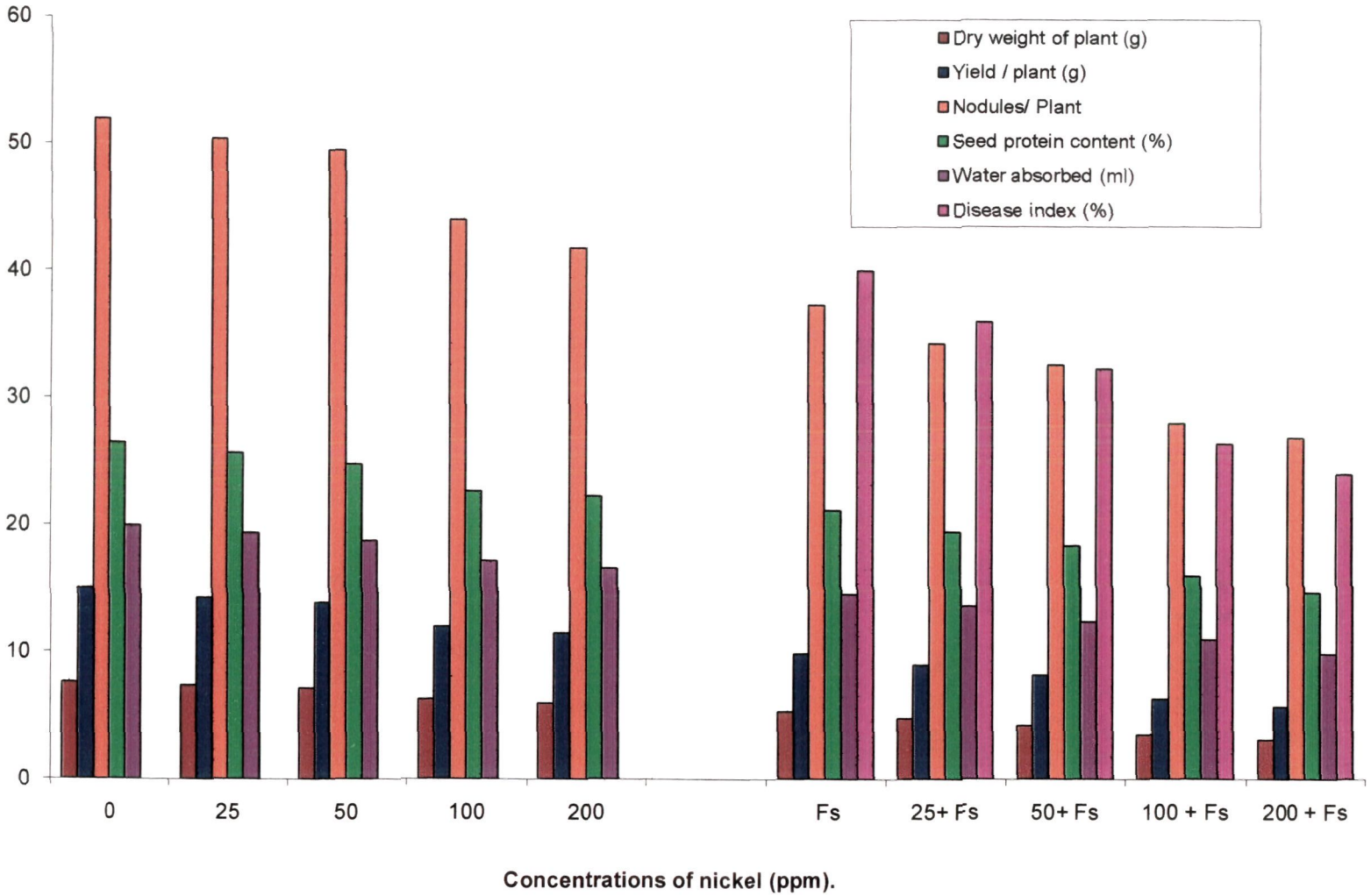
In parenthesis are given percentage reduction over control.

Table-5.7: Effect of nickel on pathogenic potential of root-rot fungus, *Fusarium solani* infecting chickpea.

Treatment Ni (ppm)/Fs	Yield plant^{-1} (g)	Nodules plant^{-1}	Chlorophyll (mg/g) a	Chlorophyll (mg/g) b	Seed protein content (%)	Water absorbed (ml)	Disease index (%)
0	15.0	52.0	0.570	0.810	26.5	20.0	-
25	14.3	50.4	0.552	0.788	25.7	19.4	-
	(4.6)	(3.0)	(3.1)	(2.7)	(3.0)	(3.0)	
50	13.9	49.5	0.530	0.760	24.8	18.8	-
	(7.3)	(4.8)	(7.0)	(6.1)	(6.4)	(6.0)	
100	12.0	44.0	0.467	0.687	22.7	17.2	-
	(20.0)	(15.3)	(18.0)	(15.1)	(14.3)	(14.0)	
200	11.5	41.8	0.450	0.656	22.3	16.6	-
	(23.3)	(19.6)	(21.0)	(19.0)	(15.8)	(17.0)	
Fs	9.9	37.3	0.412	0.609	21.2	14.6	40.0
	(34.0)	(28.2)	(27.7)	(24.8)	(20.0)	(27.0)	
25 + Fs	9.0	34.3	0.385	0.567	19.5	13.6	36.0
	(40.0)	(34.0)	(32.4)	(30.0)	(26.5)	(32.0)	
50 + Fs	8.2	32.6	0.353	0.528	18.4	12.4	32.3
	(45.3)	(37.3)	(38.0)	(34.8)	(30.5)	(38.0)	
100 + Fs	6.3	28.0	0.295	0.465	16.0	11.0	26.4
	(58.0)	(46.1)	(48.2)	(42.5)	(39.6)	(45.0)	
200 + Fs	5.7	26.9	0.267	0.422	14.7	9.9	24.0
	(62.0)	(48.2)	(53.1)	(47.9)	(44.5)	(50.5)	
L.S.D. (at 5% level)	1.20	5.03	0.060	0.067	1.98	1.48	5.50
L.S.D. (at 1% level)	1.72	7.23	0.086	0.096	2.84	2.13	9.12

In parenthesis are given percentage reduction over control.

Fig. 5.6: Effect of nickel on pathogenic potential of root-rot fungus, *Fusarium solani* (Fs) infecting chickpea.

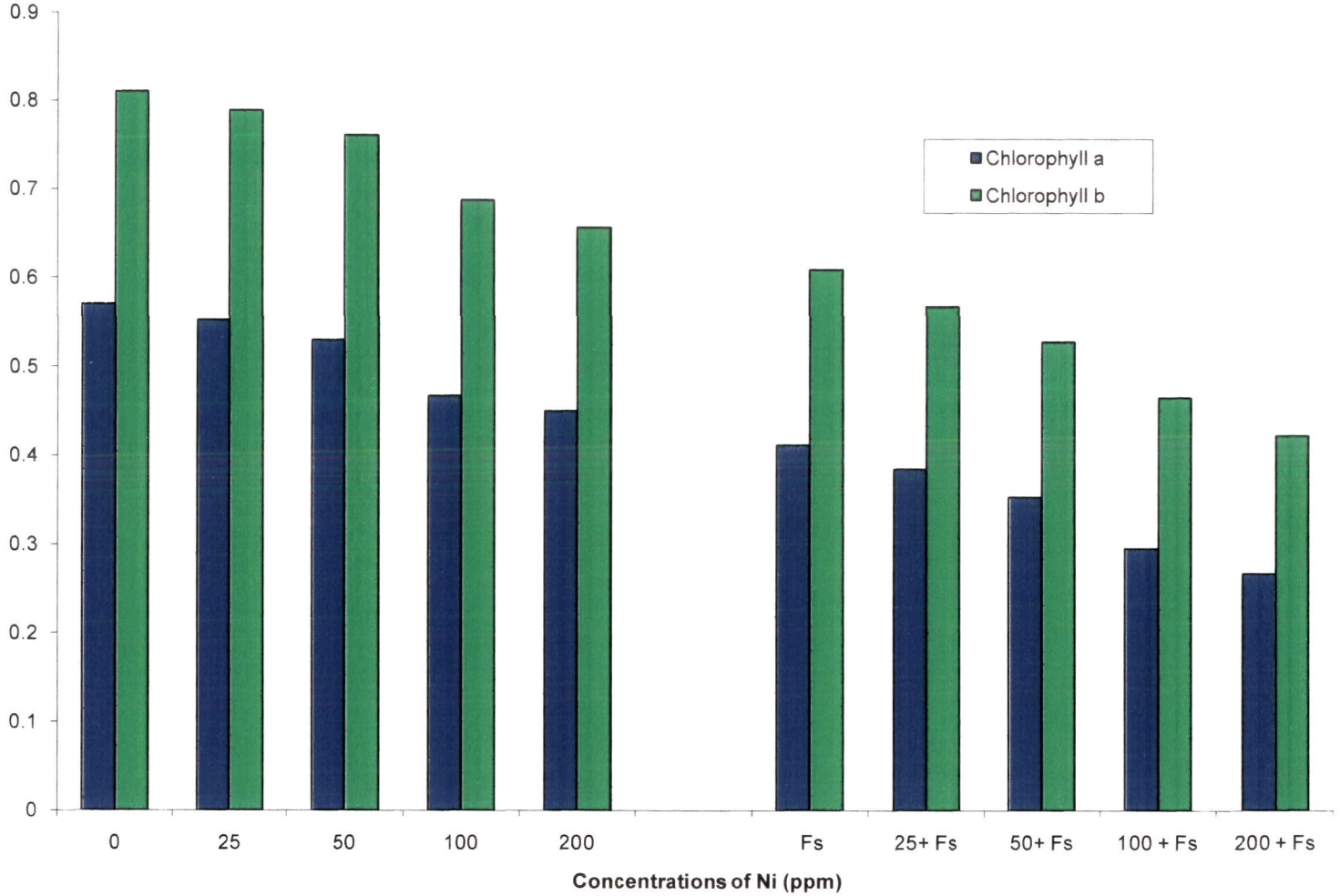


content of seeds (20.0%) and water absorption capacity of roots (27.0%) as against control. Moreover, the reduction in the above parameters of chickpea was synergistically ameliorated in the plants inoculated with *F. solani* and treated with 25, 50, 100 and 200 ppm Ni. The reduction in chlorophyll 'a' ranged between 32.4 to 53.1 and chlorophyll 'b' ranged between 30.0 to 47.9% in the plants grown in soil treated with 25, 50, 100 and 200 ppm Ni and inoculated with *F. solani.* Similarly, in the corresponding treatments, the reduction in protein content was observed to be 26.5, 30.5, 39.6 and 44.5%, and in water absorption capacity of roots was 32.0, 38.0, 45.0 and 50.5 % (Table 5.7 and Figs. 5.6 to 5.7).

The disease index of *F. solani* decreased gradually with an increase in the concentration of Ni from 25 to 200 ppm as compared to untreated- inoculated plants. The percent disease index was highest (40.0) in the untreated-inoculated plants which was followed by 36.0,32.3,26.4 and 24.0 in the plants inoculated with *F. solani* and treated with 25, 50, 100 and 200 ppm Ni (Table 5.7).

The symptoms of chlorosis and necrosis of leaves were observed in chickpea plants treated with either highest concentration of Cr (200 ppm) or higher concentrations of Ni (100 and 200 ppm). The plants inoculated with *R. reniformis* or *F. solani* exhibited the symptoms of chlorosis only. The plants inoculated with either *R. reniformis* or *F. solani* and grown in soil treated with different concentrations of heavy metals (Cr / Ni), the symptoms of chlorosis and necrosis were observed in all concentrations and the degree of severity of the symptoms was directly proportional to the concentration of heavy metals. It was further noticed that the severity of these symptoms was more pronounced in heavy metal treated plants in presence of *F. solani* than *R. reniformis.* Moreover, when comparison was made between the two heavy metals viz. Cr and Ni, Ni was found more toxic than Cr, when used either alone or in combination with the fungus or nematode.

Fig. 5.7: Effect of nickel on chlorophyll content of chickpea leaves in presence and absence of *Fusarium solani* (Fs).



It could be inferred from the above results that chromium and nickel were toxic to plants and reduced the plant growth and other parameters of chickpea at and above 100 ppm concentration. Therefore, 100 ppm concentration of Cr and Ni was used for the treatment of soil in rest of the experiments except the experiment pertaining to the accumulation of Cr and Ni in chickpea plants infected with *R. reniformis* and *F. solani*. Moreover, Ni was more toxic to plants than Cr and, Cr was more toxic towards *R. reniformis* and *F. solani* than Ni as indicated by reproduction factor of nematode and percentage of disease index of the fungus. It was also found that plant growth and other parameters of chickpea were significantly reduced in presence of either nematode or fungus. This reduction in plant growth and other parameters of chickpea was synergistically ameliorated when plants were inoculated with either nematode or fungus and soil treated with different concentrations of either Cr or Ni, except the treatment of 25 ppm Cr with nematode and all concentrations of Cr with fungus, which were unable to show synergistic effect.

4.6: ACCUMULATION OF CHROMIUM AND NICKEL IN CHICKPEA PLANTS INFECTED WITH *ROTYLENCHULUS RENIFORMIS* AND *FUSARIUM SOLANI*:

It is clear from the data presented in Tables 6 to 6.1 and Figs 6 to 6.1 that with an increase in the concentration of Cr from 25 to 200 ppm, the amount of Cr accumulated by plants increased. The amount of Cr accumulated in shoots of chickpea plants grown in soil treated with 0, 25, 50, 100 and 200 ppm Cr was recorded as 0.0, 0.0, 1.50, 4.50 and 9.00µg/g, respectively. In the corresponding treatments the amount of Cr accumulated in root was 0.20, 2.70, 5.00, 10.00 and 12.50µg/g. However, on the other hand in the plants inoculated with either *R. reniformis* or *Fusarium solani* and soil treated with different concentrations of Cr, the amount of Cr accumulation further increased as compared to uninoculated-treated plants. In the plants inoculated with *R.*

Table- 6: Accumulation of chromium in chickpea plants infected with *Rotylenchulus reniformis*.

Treatments (ppm)	Amount of chromium in plant material on dry weight basis (µg/g)					
	Uninoculated			Inoculated		
	Shoot	Root	Average	Shoot	Root	Average
0	0.0	0.20	0.1	0.15	0.35	0.25
25	0.0	2.70	1.35	1.00	3.00	2.00
50	1.50	5.00	3.25	3.50	7.50	5.5
100	4.50	10.00	7.25	10.25	11.25	10.75
200	9.00	12.50	10.75	11.70	14.50	13.1
L.S.D. (at 5% level)	0.85	1.35		0.76	1.05	
L.S.D. (at 1% level)	1.56	2.48		1.39	1.92	

Table- 6.1: Accumulation of chromium in chickpea plants infected *with Fusarium solani.*

Treatments (ppm)	Amount of chromium in plant material on dry weight basis (µg/g)					
	Uninoculated			Inoculated		
	Shoot	Root	Average	Shoot	Root	Average
0	-	0.20	0.10	0.25	0.40	0.32
25	-	2.70	1.35	2.25	3.25	2.75
50	1.50	5.00	3.25	6.50	8.00	7.25
100	4.50	10.00	7.25	8.20	10.30	9.25
200	9.00	12.50	10.75	10.00	12.00	11.00
L.S.D. (at 5% level)	0.85	1.35		1.22	1.54	
L.S.D. (at 1% level)	1.56	2.48		2.23	2.82	

Fig. 6: Accumulation of chromium in chickpea plants infected with *Rotylenchulus reniformis* (Rr).

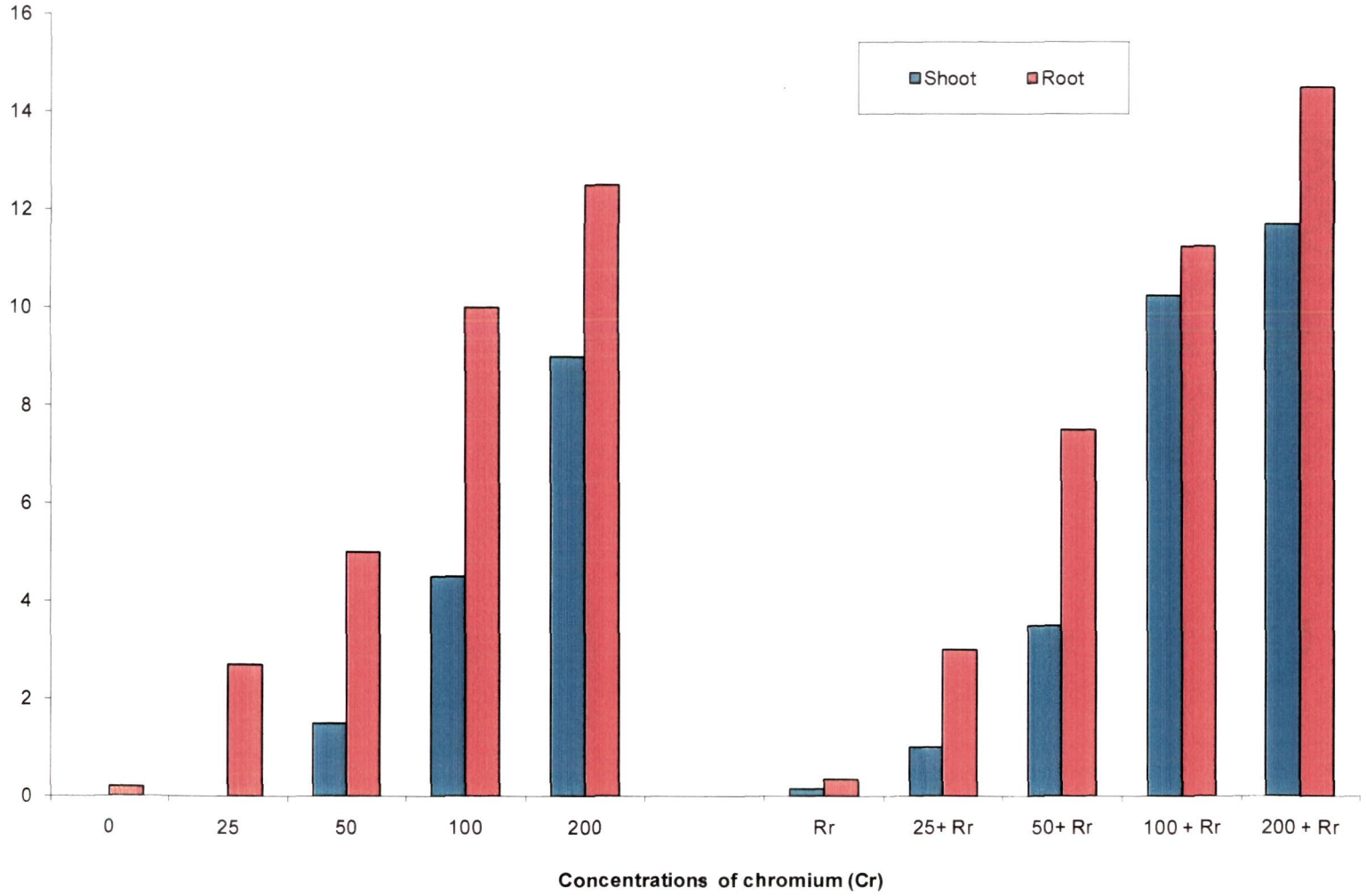
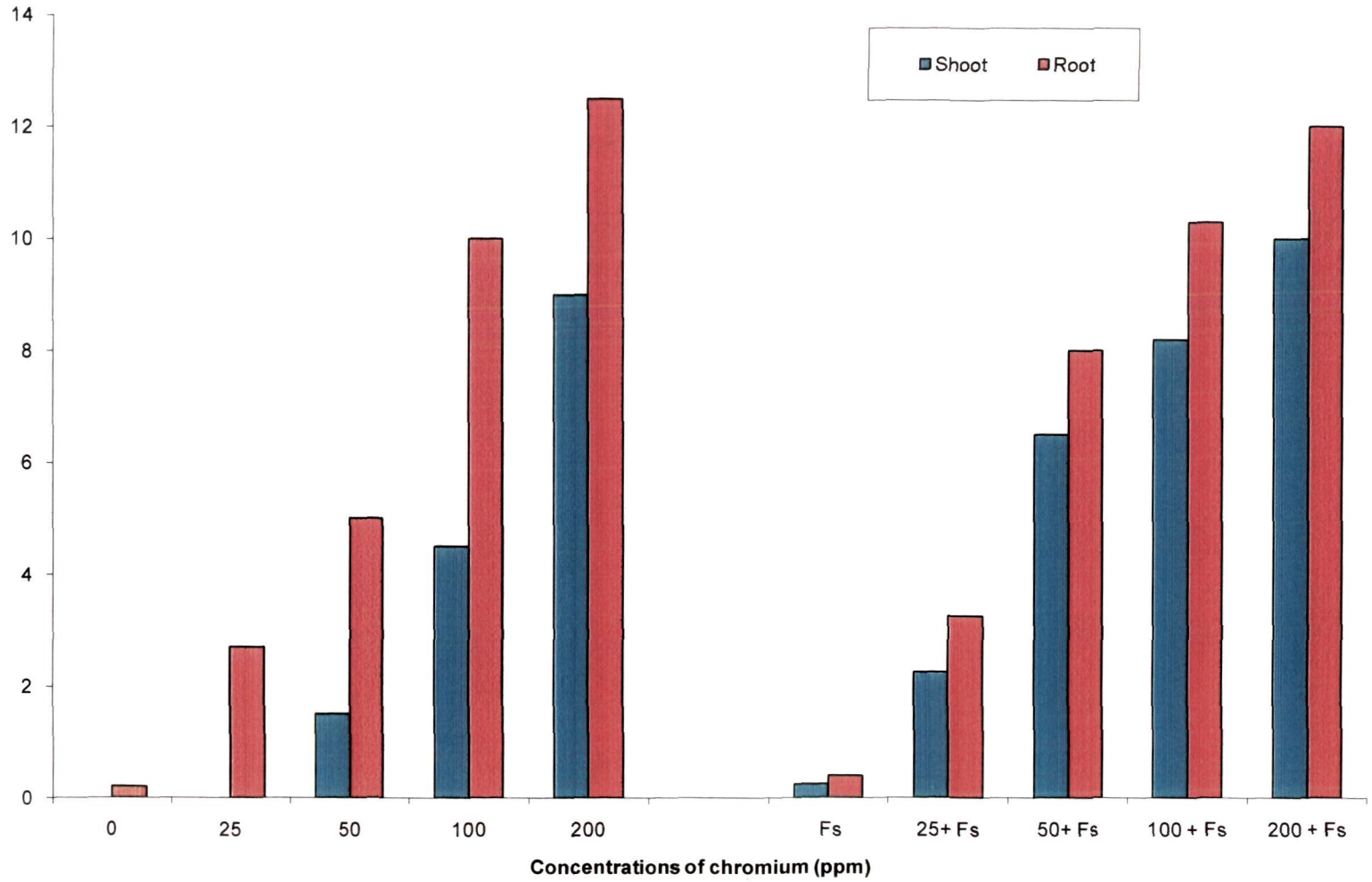


Fig. 6.1:Accumulation of chromium in chickpea plants infected with *Fusarium solani.* (Fs).



reniformis and grown in soil treated with 0, 25, 50, 100 and 200 ppm Cr, the amount of heavy metal found in shoots was 0.15, 1.0, 3.50, 10.25 and 11.70 µg/g, whereas in roots it was 3.00, 7.50, 11.25 and 14.50 µg/g (Table-6). Similarly, in the plants inoculated with *F. solani* and grown in the soil treated with the corresponding concentrations of Cr, the amount of heavy metal accumulated in shoots was 0.25, 2.25, 6.50, 8.20 and 10.00µg/g, while in roots it was found to be 0.40, 3.25, 8.00, 10.30 and 12.00 µg/g (Table 6.1).

The data presented in Tables 6.2 to 6.3 and Figs 6.2 to 6.3 clearly showed that with an increase in the concentration of Ni from 25-200 ppm, there was an increase in the amount of Ni accumulation in the chickpea plants. In the plants grown in soil treated with 0, 25, 50, 100 and 200 ppm Ni, the amount of Ni accumulated in shoots was 0.10, 2.00, 5.00, 11.70 and 15.35 µg/g, whereas, in roots it was recorded as 0.25, 5.00, 11.00, 13.30 and 14.15 µg/g .Moreover, the amount of Ni accumulation in plants was further increased when plants were inoculated with either *R. reniformis* or *F. solani* and grown in soil treated with different concentrations of Ni as compared to plants treated with Ni only. In the plants inoculated with *R. reniformis* and soil treated with 0, 25, 50, 100 and 200 ppm Ni, the amount of Ni accumulated in shoots was 0.15, 4.20, 11.00 13.30 and 15.10 µg/g and in roots it was 6.30, 13.00, 14.20 and 17.40 µg/g, respectively(Table 6.2). Similarly, in plants inoculated with *F. solani* and treated with corresponding concentrations of Ni, the amount of Ni accumulation in shoots was 0.35, 3.10, 8.35, 11.45 and 13.10 µg/g ,whereas, in roots it was found as 0.75, 4.40, 11.15, 13.05 and 15.40 µg/g (Table 6.3).

It can be concluded from above results that Cr was accumulated by plants in lesser amount than Ni. However, on the other hand, the concentration of heavy metal accumulation was more in inoculated plants than found in the uninoculated plants. The accumulation of heavy metals was more in fungus

Table- 6.2: Accumulation of nickel in chickpea plants infected with *Rotylenchulus reniformis*.

Treatments (ppm)	Amount of nickel in plant material on dry weight basis (µg/g)					
	Uninoculated			Inoculated		
	Shoot	Root	Average	Shoot	Root	Average
0	0.10	0.25	0.17	0.15	0.35	0.25
25	2.00	5.00	3.50	4.20	6.30	5.25
50	5.00	11.00	8.00	11.00	13.00	12.0
100	11.70	13.30	12.5	13.30	14.20	13.75
200	15.35	14.15	14.75	15.10	17.40	16.25
L.S.D. (at 5% level)	2.32	3.15		1.24	1.35	
L.S.D. (at 1% level)	4.25	5.78		2.28	2.48	

Table- 6.3: Accumulation of nickel in chickpea plants infected with *Fusarium solani*.

Treatments (ppm)	Amount of Nickel in plant material on dry weight basis (µg/g)					
	Uninoculated			Inoculated		
	Shoot	Root	Average	Shoot	Root	Average
0	0.10	0.25	0.17	0.35	0.75	0.55
25	2.00	5.00	3.50	3.10	4.40	3.75
50	5.00	11.00	8.00	8.35	11.15	9.75
100	11.7	13.3	12.5	11.45	13.05	12.25
200	15.35	14.15	14.75	13.10	15.40	14.25
L.S.D. (at 5% level)	2.32	3.15		1.24	1.35	
L.S.D. (at 1% level)	4.25	5.78		2.28	2.48	

Fig. 6.2: Accumulation of nickel in chickpea plants infected with *Rotylenchulus reniformis* (Rr).

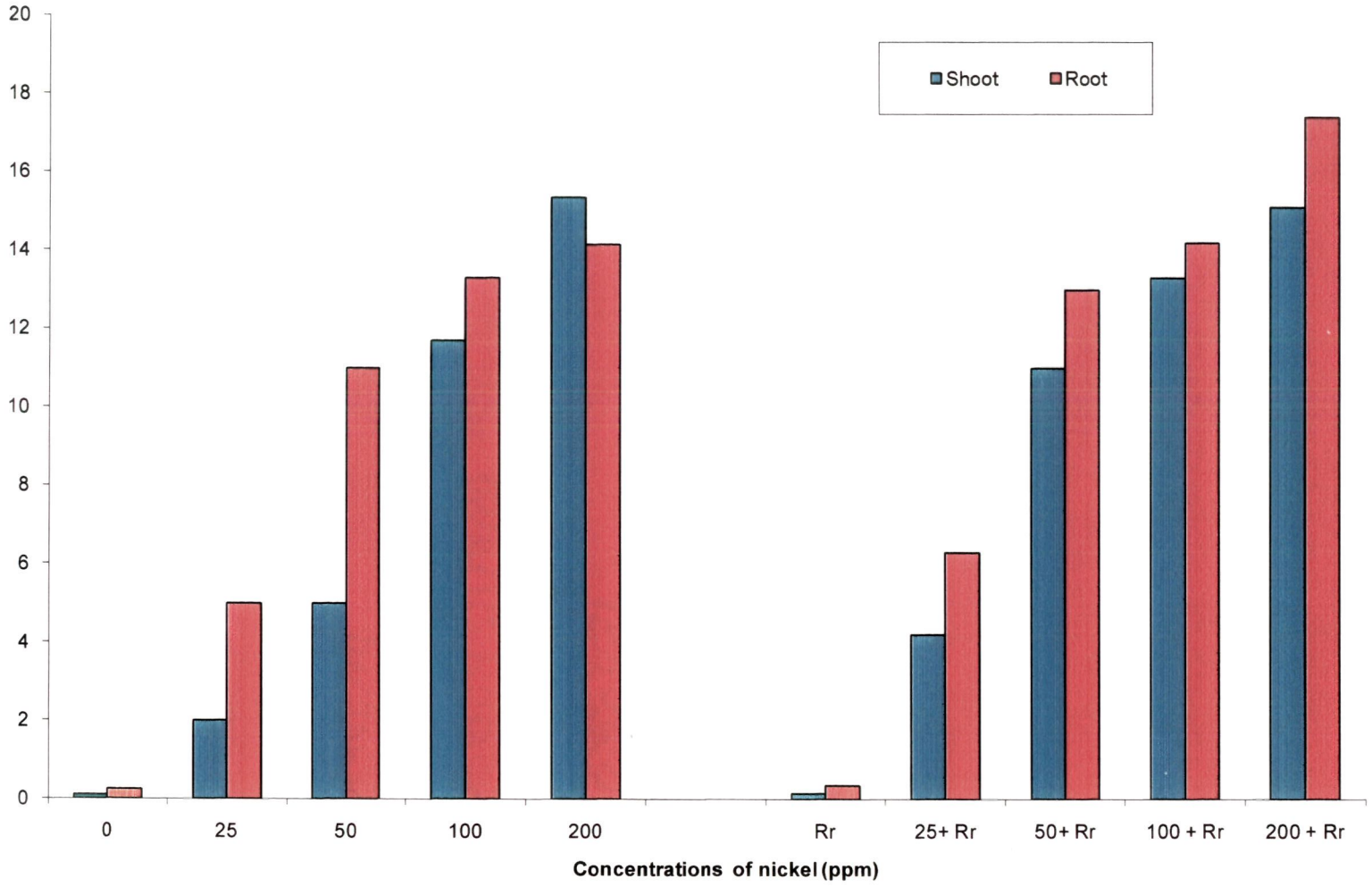
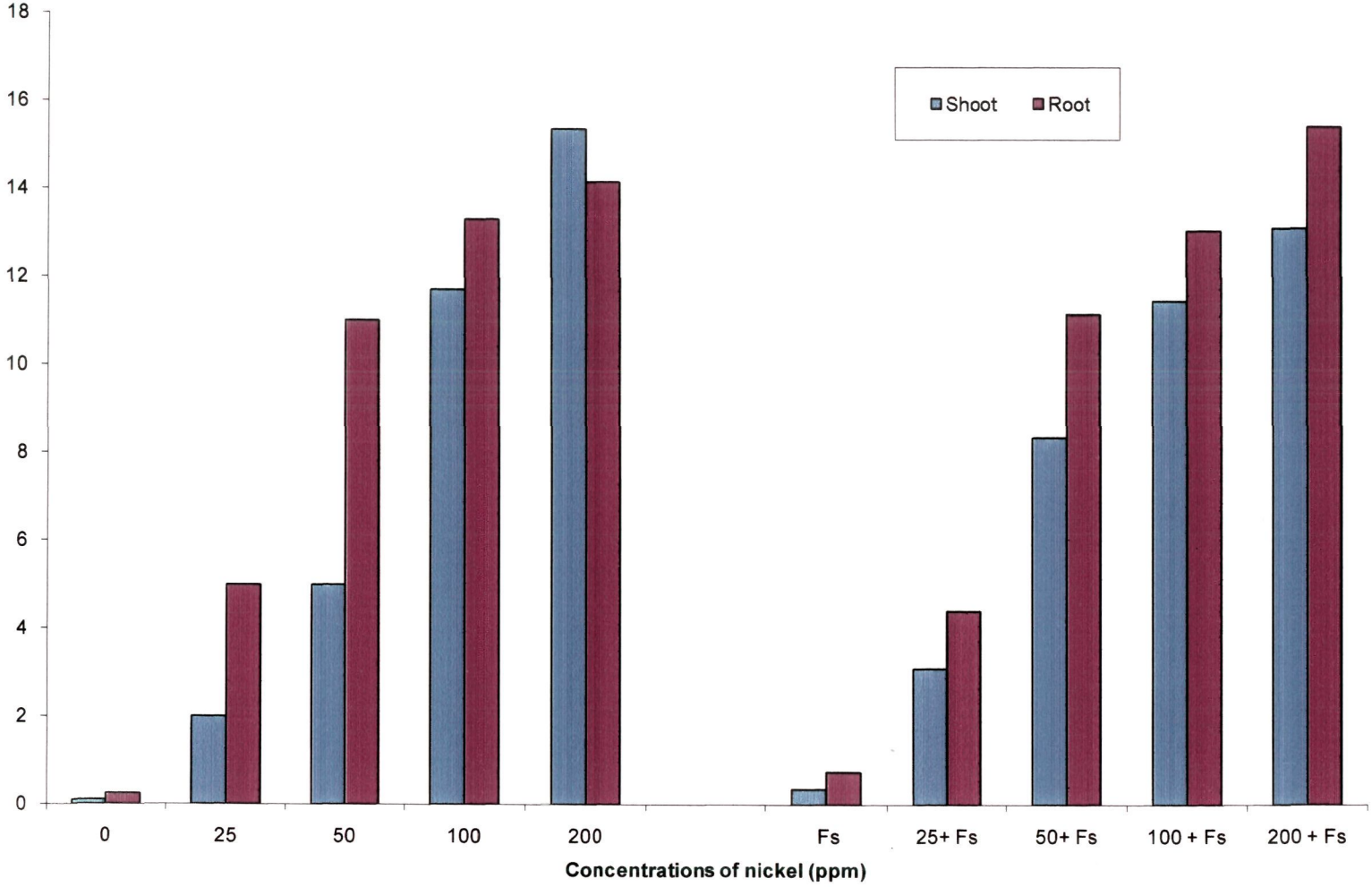


Fig. 6.3 : Accumulation of nickel in chickpea plants infected with *Fusarium solani* (Fs).



infected plants than in nematode infected plants. It was further observed that the amount of heavy metals was more in roots than in the shoots.

4.7: EFFECT OF CHROMIUM AND NICKEL ON THE LIFE CYCLE OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* INFECTING CHICKPEA:

It was observed that the penetration of immature females of reniform nematode (*Rotylenchulus reniformis*) in roots of chickpea grown in untreated soil (control) started on 1st day after the inoculation. The penetration of nematodes increased progressively upto 5th day with most of the penetration being achieved on 4th day (30.6%) and on 5th day (33.3%). The slight swelling at the posterior end of immature females (3.7%) was observed on 5th day of inoculation and continued upto 10 days. The maximum number of slight swollen females (32.0%) was recorded on 7th day. The fully swollen females (33.3%) were first recorded on the 10th day and maximum number (38.8%) of fully swollen females was achieved on 14th day. The adult females with matrix (15.1%) were also observed on 14th day. The females with eggmasses (64.0%) were first observed on 16th day. The total recovery of females was highest (64.0%) on 16th day, followed by 53.8, 42.6, 41.0, 38.5, 37.0, 30.6, 19.0, 15.2 and 9.3% on 14th ,10th,7th ,6th,5th,4th, 3rd ,2nd and 1st day of inoculation, respectively. The average number of 69 eggs per eggmass was recorded on 16th day (Table 7).

The eggs took four days to hatch into second stage juveniles which were first recorded on 20th day of inoculation while the third stage female and male juveniles were recorded on 23rd day of inoculation. The fourth stage female and male juveniles were recorded on 25th day of inoculation. The adult males and immature females were observed on 28th day of inoculation (Table 7.1).

The number of immature females and adult males per pot were 175 and 160, respectively on 28th day of inoculation. Similarly, on 28th day of inoculation the number of second stage juveniles, third stage female juveniles,

Table -7: Effect of chromium and nickel on the life cycle of reniform nematode, *Rotylenchulus reniformis* infecting chickpea.

Days after inoculation			Developmental stages of reniform nematode, *Rotylenchulus reniformis* (%).																		L.S.D.	
			Females unswollen			Females with slight swelling			Females fully swollen			Females with matrix			Females with eggmasses			Total recovery of females			at 5% level	at 1% level
*C	Cr	Ni	C	Cr	Ni	C	Cr	Ni	C	Cr	Ni	C	Cr	Ni	C	Cr	Ni	C	Cr	Ni		
1	1	1	9.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.3	-	-	1.01	1.38
2	2	2	15.2	4.7	6.00	-	-	-	-	-	-	-	-	-	-	-	-	15.2	4.7	6.3	1.28	1.74
3	3	3	19.0	6.7	12.0	-	-	-	-	-	-	-	-	-	-	-	-	19.0	6.7	12.0	1.45	1.97
4	4	4	30.6	16.0	19.3	-	-	-	-	-	-	-	-	-	-	-	-	30.6	16.0	19.3	1.97	2.68
5	5	5	33.3	22.0	24.0	3.7	-	-	-	-	-	-	-	-	-	-	-	37.0	22.0	24.0	2.14	2.93
6	6	6	14.5	25.3	28.0	24.0	-	-	-	-	-	-	-	-	-	-	-	38.5	25.3	28.0	1.96	2.67
7	8	7	9.0	13.3	8.3	32.0	14.8	23.7	-	-	-	-	-	-	-	-	-	41.0	28.1	32.0	2.15	2.94
10	12	11	-	6.0	13.0	9.3	17.0	11.3	33.3	7.9	19.0	-	-	-	-	-	-	42.6	30.9	33.8	2.22	3.03
14	18	16	-	-	-	-	-	-	38.8	28.6	31.0	15.1	3.4	8.2	-	-	-	53.8	32.0	39.2	2.47	3.37
16	20	18	-	-	-	-	-	-	-	-	-	-	-	-	64.0	40.7	47.1	64.0	40.7	47.1	2.78	3.79
																		(69)	(48)	(52)	6.84	15.78

*C= control

In parenthesis are given average number of eggs/ egg mass

Table- 7.1: Effect of chromium and nickel on the development of *Rotylenchulus reniformis* in soil.

Developmental stages	Treatments / Days after inoculation		
	Control (C)	**Chromium (Cr)**	**Nickel (Ni)**
Second stage juveniles	20^{th}	25^{th}	22^{nd}
Third stage female juveniles	23^{rd}	29^{th}	25^{th}
Third stage male juveniles	23^{rd}	29^{th}	25^{th}
Fourth stage female juveniles	25^{th}	33^{rd}	28^{th}
Fourth stage male juveniles	25^{th}	33^{rd}	28^{th}
Immature females	28^{th}	39^{th}	33^{rd}
Adult males	28^{th}	39^{th}	33^{rd}

third stage male juveniles, fourth stage female juveniles, fourth stage male juveniles and total population of nematode per pot was 823,210,184,189,145 and 1886, respectively(Table 7.2).

It was observed that the penetration of immature females of reniform nematode, *Rotylenchulus reniformis* in the roots of chickpea grown in chromium (100 ppm) treated soil started on 2nd day after inoculation. The penetration of nematode increased progressively upto 6th days with most of the penetration being achieved on 5th day (22.0%) and on 6th day (25.3%). The slight swelling at the posterior end of immature females (14.8%) was observed on 7th day of inoculation and continued upto 12 days (17.0%). The fully swollen females (7.9%) were first recorded on 12th day with maximum number of fully swollen females (28.6%) being achieved on 18th day. The adult females with matrix (3.4%) were first observed on 18th day and the females with eggmasses (40.7%) were observed on 20th day. The total recovery of females was highest (40.7%) on 20th day, followed by 32.0, 30.9, 28.1, 25.3, 22.0, 16.0, 6.7% and 4.7 % on 18th,12th,8th,6th,5th, 4th,3rd and 2nd day of inoculation, respectively. The average number of 48 eggs per eggmass was recorded on 20th day (Table 7).

The eggs took five days to hatch into second stage juveniles which were first recorded on 25th day of inoculation. The third stage female and male juveniles were recorded on 29th day of inoculation. The fourth stage female and male juveniles were recorded on 33rd day of inoculation. The adult males and immature females were observed on 39th day of inoculation (Table 7.1).

The number of immature females and adult males per pot were 93 and 118, respectively on 39th day of inoculation. Similarly, on the same day the number of second stage juveniles, third stage female juveniles, third stage male juveniles, fourth stage female juveniles, fourth stage male juveniles and total

Table-7.2: Effect of chromium and nickel on the population buildup and sex ratio of *Rotylenchulus reniformis* on chickpea.

Observation	Control (28^{th} *DAI)	Chromium (39^{th} DAI)	Nickel (33^{rd}DAI)	L.S.D. at 5% level	L.S.D. at 1% level
Second stage juveniles	823	458	511	26.63	61.43
3^{rd} stage female juveniles	210	109	128	13.81	31.85
3^{rd} stage male juveniles	184	136	152	9.29	21.44
4^{th} stage female juveniles	189	90	110	13.64	31.46
4^{th} stage male juveniles	145	127	146	7.70	17.76
Immature females	175	93	107	12.48	28.78
Adult males	160	118	134	8.52	19.65
Total females	574	292	345	23.11	53.29
Total males	489	381	432	13.73	31.66
Total population / pot	1886	1131	1288	37.73	87.04
Sex ratio (female : male)	1.17 : 1.00	1.00 : 1.30	1.00 : 1.21		

*DAI = Days after inoculation.

population of nematodes per pot was 458, 109,136,90,127 and 1131, respectively (Table 7.2).

Similarly, it was observed that the penetration of immature females of reniform nematode, *Rotylenchulus reniformis* in the roots of chickpea grown in nickel (100 ppm) treated soil was also started on 2nd day of inoculation. The penetration of nematodes increased progressively upto 6 days with most of the penetration (24.0%) being achieved on 5th day and on 6th day (28.0%). The slight swelling at the posterior end of immature females (23.7%) was observed on 7th day of inoculation and continued upto 11th days (11.3%). The fully swollen females (19.0%) were first recorded on 11th day with maximum number (31.0%) of fully swollen females being achieved on 16th day. The adult females with matrix (8.2%) were also first observed on 16th day. The females with eggmasses (47.1%) were recorded on 18th day of inoculation .The total recovery of females was highest (47.1%) on 18th day, followed by 39.2, 33.8, 32.0, 28.0, 24.0, 19.3, 12.0 and 6.3% on 16th, 11th, 7th, 6th, 5th, 4th , 3rd and 2nd day of inoculation, respectively. The average number of 52 eggs per eggmass was recorded on 18th day (Table 7).

The eggs took 4 days to hatch into second stage juveniles which were first recorded on 22nd day of inoculation. The third stage female and male juveniles were recorded on 25th day of inoculation. The fourth stage female and male juveniles were recorded on 28th day of inoculation. The adult males and immature females were observed on 33rd day of inoculation (Table 7.1).

The number of immature females and adult males per pot was 107 and 134, respectively on 33rd day of inoculation. Similarly, the number of second stage juveniles, third stage female juveniles, third stage male juveniles, fourth stage female juveniles, fourth stage male juveniles and total population of nematodes per pot was 511, 128, 152, 110, 146 and 1288 , respectively (Table 7.2).

It is clear from the above results that the penetration, development and multiplication of reniform nematode were inhibited and delayed by the presence of heavy metals viz., chromium and nickel as compared to control. The penetration of nematodes in control started after 1st day of inoculation which was however delayed by one day in plants grown in pots treated with either Cr or Ni. Females with slight swelling were first observed on 5th day in control whereas such females were recorded on 8th and 7th day in plants treated with Cr and Ni, respectively. The fully swollen females, females with matrix and females with eggmasses were first recorded on 10th, 14th and 16th day of inoculation in the control plants, respectively. The corresponding stages of development of reniform nematode were first recorded on 12th, 18thand 20th day, and on 11th, 16th and 18th day of inoculation in plants treated with Cr and Ni, respectively. The average number of eggs per eggmass was significantly reduced in both Cr (48) and Ni (52) treated plants as compared to control (69). The eggs took 4 days to hatch into second stage juveniles in control, while in Cr and Ni treated soil, eggs hatched in 5 and 4 days respectively. The second stage juveniles were recorded on 20th day in control as against 25th day in Cr and 22nd day in Ni treated soil, respectively. The third stage female and male juveniles were recorded on 23rd day in control, but, these stages of development were recorded on 29th and 25th day of inoculation in Cr and Ni treated soil, respectively. Similarly, fourth stage female and male juveniles were recorded on 25th day in control, while in Cr and Ni treated soil these stages were recorded on 33rd and 28th day after inoculation, respectively. The immature females and adult males were recorded on 28th, 39th and 33rd day, respectively in the corresponding treatments. In this way the life cycle of *R. reniformis* on chickpea was delayed by 11 days and 5 days in the presence of Cr and Ni respectively as compared to control. The number of immature females and adult males was also significantly reduced in plants treated with either Cr or Ni

as compared to control. The total population of the reniform nematode was also reduced in Cr (1131 nematodes / pot) and Ni (1288 nematodes / pot) treated pots as compared to control (1886 nematodes / pot) on the day of recovery of immature females. The female and male ratio (female: male) was 1.17:1.00 in control as against 1.00:1.30 and 1.00:1.21 in Cr and Ni treated soil, respectively.

4.8: EFFECT OF CHROMIUM AND NICKEL ON THE EFFICACY OF OIL-CAKES, BIOCONTROL AGENTS AND BAVISTIN IN THE MANAGEMENT OF *ROTYLENCHULUS RENIFORMIS* AND *FUSARIUM SOLANI* INFECTING CHICKPEA:

In the present experiment, as also established in my other experiments *R. reniformis*, *Fusarium solani* and both the heavy metals viz. Cr and Ni, when present singly or concomitantly, exhibited significant adverse effect on plant growth and yield of chickpea. The data presented in Tables 8 to 8.3 revealed that *R. reniformis* and *F. solani* caused significant reduction in plant growth (33.0 and 37.0%, respectively) and yield (37.0 and 40.0%, respectively) of chickpea as compared to control (uninoculated-untreated) plants. Similarly, the treatments of Cr and Ni in the soil significantly reduced plant growth (10.0 and 16.3%, respectively) and yield (12.0 and 19.4%, respectively) of chickpea. Moreover, the plant growth and yield of chickpea plants was synergistically reduced in the plants inoculated with *R. reniformis* and grown in soil treated with either Cr or Ni. However, the synergistic reduction in plant growth and yield of chickpea was observed only when *F. solani* was inoculated in the Ni-treated soil and no such reduction was found when the fungus was inoculated in Cr-treated soil.

Soil application with oil-cakes of neem, mustard, castor, mahua and sesame, and biocontrol agent *Trichoderma harzianum* significantly improved the plant growth and yield of chickpea as compared to untreated-uninoculated

Table-8: Effect of chromium on the efficacy of oil-cakes, biocontrol agents and bavistin in the management of *Rotylenchulus reniformis* infecting chickpea.

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant $^{-1}$ (g)	Nematode population/pot			
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		Nematodes/Kg soil	Females/ root system	Total	R=Pf/Pi
Untreated - uninoculated	38.8	29.6	68.4	16.8	12.2	29.0	5.9	3.3	9.2	17.5	-	-	-	-
R. reniformis (Rr)	27.3	19.8	47.1	11.6	8.1	19.7	4.0	2.1	6.1 (33.6)	11.0 (37.1)	11105	630	11735	11.7
Chromium (Cr)	37.0	26.6	63.6	15.5	10.8	26.3	5.3	2.9	8.2 (10.8)	15.4 (12.0)	-	-	-	-
Rr + Cr	21.3	15.3	36.6	8.7	6.6	15.3	3.2	1.6	4.8 (47.8)	8.1 (53.7)	8573	480	9017	9.0
Mustard cake (Mc)	51.3	36.7	88.0	21.5	15.1	36.6	7.4	4.1	11.5	21.3	-	-	-	-
Mc + Rr	43.7	29.3	73.0	17.9	11.7	29.6	5.9	3.1	9.0 (21.7)	16.2 (23.9)	7110	300	7410	7.4
Mc + Cr	49.6	34.8	84.4	20.9	14.1	35.0	7.1	3.8	10.9 (5.2)	19.6 (7.9)	-	-	-	-
Mc + Rr + Cr	40.2	27.5	67.7	16.7	11.0	27.7	5.7	2.9	8.6 (25.2)	14.9 (30.0)	2883	150	3033	3.0
L.S.D. (at 5% level)			4.24			2.40			0.71	1.38				2.30
L.S.D. (at 1% level)			6.27			3.55			1.04	2.03				4.22
Castor cake (Cc)	49.4	36.6	86.0	20.8	15.0	35.8	7.2	4.0	11.2	20.9	-	-	-	-
Cc + Rr	39.7	27.8	67.5	16.2	11.0	27.2	6.1	2.2	8.3 (25.8)	15.1 (27.7)	4584	236	8145	8.1
Cc + Cr	48.0	34.0	82.0	19.7	13.8	33.5	6.8	3.5	10.3 (8.0)	18.9 (9.5)	-	-	-	-
Cc + Rr + Cr	35.5	24.2	59.7	14.4	9.7	24.1	4.8	2.5	7.3 (34.8)	13.0 (37.7)	4584	236	4820	4.8
L.S.D. (at 5% level)			4.41			2.40			0.79	1.60				1.95
L.S.D. (at 1% level)			6.43			3.55			1.17	2.36				3.58
Mahua cake (M)	46.1	32.9	79.0	19.1	14.0	33.1	6.6	3.7	10.3	19.1	-	-	-	-
M + Rr	35.5	24.5	60.0	14.4	9.9	24.3	4.9	2.5	7.4 (28.1)	13.3 (30.3)	8375	465	8840	8.8
M + Cr	44.3	31.5	75.8	18.3	13.1	31.4	6.3	3.3	9.6 (6.7)	17.4 (8.9)	-	-	-	-
M + Rr + Cr	30.4	20.7	51.1	12.2	8.5	20.7	4.2	2.2	6.4 (37.8)	11.1 (41.8)	4320	210	4530	4.5
L.S.D. (at 5% level)			4.00			2.22			0.62	1.25				2.44
L.S.D. (at 1% level)			5.91			3.28			0.92	1.84				4.48

Contd.....

Table-8: (Contd.) Page 2

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant $^{-1}$ (g)	Nematode population / pot			R=Pf/Pi
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		Nematodes/Kg soil	Females/ root system	Total	
Linseed cake (Lc)	41.0	31.0	72.0	17.6	12.6	30.2	6.1	3.4	9.5	17.8	-	-	-	-
Lc + Rr	28.5	20.5	49.0	12.0	8.4	20.4	4.1	2.2	6.3 (33.6)	10.9 (38.7)	11580	620	12200	12.0
Lc + Cr	38.7	27.5	66.2	16.2	11.2	27.4	5.6	2.9	8.5 (10.5)	15.7 (11.7)	-	-	-	-
Lc + Rr + Cr	24.0	16.3	40.3	9.9	6.7	16.6	3.4	1.7	5.1 (46.3)	8.7 (51.1)	8780	440	9220	9.2
L.S.D. (at 5% level)			4.43			2.50			0.43	1.48				2.62
L.S.D. (at 1% level)			6.55			3.70			0.63	2.19				4.81
Sesame (Sc)	44.6	33.6	78.2	19.1	13.5	32.6	6.5	3.6	10.1	19.0	-	-	-	-
Sc + Rr	30.4	21.4	51.8	12.9	8.7	21.6	4.3	2.2	6.5 (35.6)	12.0 (36.8)	10595	615	11210	11.2
Sc + Cr	40.0	28.2	68.2	17.7	11.2	28.9	5.9	3.1	9.0 (10.8)	16.7 (12.1)	-	-	-	-
Sc + Rr + Cr	25.0	15.0	40.0	10.4	7.0	17.4	3.6	1.6	5.2 (48.5)	9.1 (52.1)	8420	420	8840	8.8
L.S.D. (at 5% level)			4.73			2.61			0.82	1.40				2.50
L.S.D. (at 1% level)			4.00			3.85			1.21	2.07				4.60
Neem cake (Nc)	58.4	32.7	91.1	22.2	15.8	38.0	7.6	4.3	11.9	22.2	-	-	-	-
Nc + Rr	50.3	27.1	77.4	18.8	13.0	31.8	6.4	3.4	9.8 (17.6)	17.5 (21.1)	6614	206	6820	6.8
Nc + Cr	57.8	31.7	89.5	22.1	15.1	37.2	7.5	4.1	11.6 (2.5)	20.8 (6.3)	-	-	-	-
Nc + Rr + Cr	49.3	26.1	75.4	18.5	12.6	31.1	6.3	3.3	9.6 (19.3)	16.7 (24.7)	3025	185	3210	3.2
L.S.D. (at 5% level)			3.65			1.72			0.57	1.53				2.36
L.S.D. (at 1% level)			5.40			2.54			0.84	2.26				4.33

Contd.....

Table-8: (Contd.) Page 3

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant^{-1} (g)	Nematode population / pot			R=Pf/Pi
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		Nematodes/Kg soil	Females/ root system	Total	
T. harzianum (Th)	47.9	35.1	83.0	20.3	14.6	34.9	7.0	3.9	10.9	20.4	-	-	-	-
Th + Rr	38.0	26.7	64.7	15.6	10.8	26.4	5.2	2.8	8.0 (26.6)	14.8 (27.4)	8109	400	8509	8.5
Th + Cr	46.3	32.5	78.8	19.4	13.1	32.5	6.6	3.4	10.0 (8.2)	18.4 (9.8)	-	-	-	-
Th + Rr + Cr	34.6	23.5	58.1	14.2	9.7	23.9	4.8	2.5	7.3 (33.0)	13.1 (35.7)	4873	247	5120	5.0
L.S.D. (at 5% level)			4.30			2.15			0.76	1.47				1.20
L.S.D. (at 1% level)			6.36			3.18			1.12	2.17				2.20
P. lilacinus (Pl)	41.0	30.0	71.0	17.3	12.5	29.8	6.1	3.3	9.4	17.7	-	-	-	-
Pl + Rr	37.8	26.1	63.9	15.5	11.0	26.5	5.4	2.8	8.2 (12.7)	14.7 (16.9)	3706	118	3824	3.8
Pl + Cr	38.7	27.3	66.0	16.0	11.2	27.2	5.5	2.9	8.4 (10.6)	15.7 (11.2)	-	-	-	-
Pl + Rr + Cr	32.0	21.6	53.6	12.9	8.8	21.7	4.4	2.3	6.7 (28.7)	11.8 (33.3)	3489	138	3627	3.6
L.S.D. (at 5% level)			3.12			1.48			0.43	1.82				2.47
L.S.D. (at 1% level)			4.61			2.19			0.63	2.69				4.53
Bavistin (B)	40.8	30.4	71.2	17.5	12.5	30.0	6.4	3.0	9.4	18.0	-	-	-	-
B + Rr	36.6	26.4	63.0	15.5	10.6	26.1	5.5	2.5	8.00 (14.8)	14.6 (18.8)	5092	150	5242	5.2
B + Cr	38.0	26.7	64.7	15.9	11.0	26.9	5.7	2.6	8.3 (11.7)	15.7 (12.7)	-	-	-	-
B + Rr + Cr	30.7	21.2	51.9	12.8	8.3	21.1	4.5	1.9	6.4 (31.9)	11.8 (34.4)	4762	255	5017	5.0
L.S.D. (at 5% level)			3.42			1.26			0.25	1.77				1.85
L.S.D. (at 1% level)			5.06			1.86			0.37	2.61				3.39

In parenthesis are given percentage reduction over control.

Table-8.1: Effect of nickel on the efficacy of oil-cakes, biocontrol agents and bavistin in the management of *Rotylenchulus reniformis* infecting chickpea.

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant $^{-1}$ (g)	Nematode population / pot			R=Pf/Pi
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		Nematodes/Kg soil	Females/ root system	Total	
Untreated - uninoculated	38.8	29.6	68.4	16.8	12.2	29.0	5.9	3.3	9.2	17.5	-	-	-	-
R. reniformis (Rr)	27.3	19.8	47.1	11.6	8.1	19.7	4.0	2.1	6.1 (33.6)	11.0 (37.1)	11105	630	11735	11.7
Nickel (Ni)	34.3	25.0	59.3	14.6	10.0	24.6	5.0	2.7	7.7 (16.3)	14.0 (20.0)	-	-	-	-
Rr + Ni	18.9	13.2	32.1	7.8	5.2	13.0	2.7	1.3	4.0 (56.5)	7.0 (60.0)	9330	480	9810	9.8
Mustard cake (Mc)	51.3	36.7	88.0	21.5	15.1	36.6	7.4	4.1	11.5	21.3	-	-	-	-
Mc + Rr	43.7	29.3	73.0	17.9	11.7	29.6	5.9	3.1	9.0 (21.7)	16.2 (23.9)	7110	300	7410	7.4
Mc + Ni	49.9	34.4	84.3	20.9	13.8	34.7	7.1	3.7	10.8 (6.0)	19.1 (10.3)	-	-	-	-
Mc + Rr + Ni	60.6	27.8	68.4	16.9	11.1	28.0	5.8	2.9	8.7 (24.3)	15.1 (29.1)	4273	250	4523	4.5
L.S.D. (at 5% level)			7.10			3.60			1.20	2.80				1.43
L.S.D. (at 1% level)			10.49			5.32			1.77	4.14				2.62
Castor cake (Cc)	49.4	36.6	86.0	20.8	15.0	35.8	7.2	4.0	11.2	20.9	-	-	-	-
Cc + Rr	39.7	27.8	67.5	16.2	11.0	27.2	6.1	2.2	8.3 (25.8)	15.1 (27.7)	4584	236	8145	8.1
Cc + Ni	45.4	31.4	76.8	18.8	12.9	31.7	6.3	3.4	9.7 (13.3)	17.3 (17.2)	-	-	-	-
Cc + Rr + Ni	39.4	15.6	55.0	12.5	9.0	22.5	4.6	2.3	6.9 (38.3)	11.9 (43.0)	5460	340	5800	5.8
L.S.D. (at 5% level)			8.22			4.35			1.05	2.64				1.48
L.S.D. (at 1% level)			12.16			6.43			1.55	3.90				2.72
Mahua cake (M)	46.1	32.9	79.0	19.1	14.0	33.1	6.6	3.7	10.3	19.1	-	-	-	-
M + Rr	35.5	24.5	60.0	14.4	9.9	24.3	4.9	2.5	7.4 (28.1)	13.3 (30.3)	8375	465	8840	8.8
M + Ni	42.8	29.0	71.8	17.5	12.0	29.5	5.9	3.1	9.0 (12.6)	16.3 (14.6)	-	-	-	-
M + Rr + Ni	31.7	21.3	53.0	12.9	8.9	21.8	4.3	2.3	6.6 (35.9)	11.4 (40.3)	5316	315	5631	5.6
L.S.D. (at 5% level)			6.15			3.62			0.75	1.43				1.52
L.S.D. (at 1% level)			9.10			5.35			1.11	2.12				2.79

Contd.....

Table -8.1: (Contd.) Page 2

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant $^{-1}$ (g)	Nematode population / pot			R=Pf/Pi
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		Nematodes/Kg soil	Females/ root system	Total	
Linseed cake (Lc)	41.0	31.0	72.0	17.6	12.6	30.2	6.1	3.4	9.5 (+3.2)	17.8 (+1.7)	-	-	-	-
Lc + Rr	28.5	20.5	49.0	12.0	8.4	20.4	4.1	2.2	6.3 (33.6)	10.9 (38.7)	11580	620	12200	12.0
Lc + Ni	36.6	26.0	62.6	15.3	10.3	25.6	5.2	2.7	7.9 (16.8)	14.3 (19.6)	-	-	-	-
Lc + Rr + Ni	20.6	13.9	34.5	8.8	5.6	14.4	2.9	1.4	4.3 (54.7)	7.4 (58.4)	8190	450	8640	8.6
L.S.D. (at 5% level)			7.00			4.00			1.38	2.35				1.58
L.S.D. (at 1% level)			10.36			5.91			2.04	3.48				3.00
Sesame (Sc)	44.6	33.6	78.2	19.1	13.5	32.6	6.5	3.6	10.1	19.0	-	-	-	-
Sc + Rr	30.4	21.4	51.8	12.9	8.7	21.6	4.3	2.2	6.5 (35.6)	12.0 (36.8)	10595	615	11210	11.2
Sc + Ni	39.8	28.2	68.0	16.8	11.2	28.0	5.6	2.9	8.5 (15.8)	15.4 (18.9)	-	-	-	-
Sc + Rr + Ni	21.7	15.0	36.7	8.8	5.8	14.6	2.9	1.5	4.4 (56.4)	7.6 (60.0)	8647	475	9122	9.1
L.S.D. (at 5% level)			8.88			3.50			0.86	1.42				1.00
L.S.D. (at 1% level)			13.14			5.17			1.27	2.01				1.83
Neem cake (Nc)	58.4	32.7	91.1	22.2	15.8	38.0	7.6	4.3	11.9	22.2	-	-	-	-
Nc + Rr	50.3	27.1	77.4	18.8	13.0	31.8	6.4	3.4	9.8 (17.6)	17.5 (21.1)	6614	206	6820	6.8
Nc + Ni	55.3	29.4	84.7	20.0	14.8	34.8	7.0	3.7	10.7 (10.0)	19.5 (12.1)	-	-	-	-
Nc + Rr + Ni	45.4	23.8	69.2	17.1	11.4	28.5	5.7	3.0	8.7 (26.8)	15.1 (31.9)	4050	300	4350	4.3
L.S.D. (at 5% level)			6.47			3.28			0.82	3.12				1.25
L.S.D. (at 1% level)			9.57			4.85			1.21	4.62				2.29

Contd.....

Table-8.1: (Contd.) Page 3

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant^{-1} (g)	Nematode population / pot			R=Pf/Pi
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		Nematodes/Kg soil	Females/ root system	Total	
T. harzianum (Th)	47.9	35.1	83.0	20.3	14.6	34.9	7.0	3.9	10.9	20.4	-	-	-	-
Th + Rr	38.0	26.7	64.7	15.6	10.8	26.4	5.2	2.8	8.0 (26.6)	14.8 (27.4)	8109	400	8509	8.5
Th + Ni	43.7	30.1	73.8	18.2	12.4	30.6	6.2	3.2	9.4 (13.7)	17.1 (16.1)	-	-	-	-
Th + Rr + Ni	30.6	20.0	50.6	12.4	8.3	20.7	4.2	2.1	6.3 (42.2)	11.3 (44.6)	5226	310	5536	5.5
L.S.D. (at 5% level)			7.24			3.85			0.47	2.50				1.12
L.S.D. (at 1% level)			10.71			5.70			0.70	3.70				2.10
P .lilacinus (Pl)	41.0	30.0	71.0	17.3	12.5	29.8	6.1	3.3	9.4	17.7	-	-	-	-
Pl + Rr	37.8	26.1	63.9	15.5	11.0	26.5	5.4	2.8	8.2 (12.7)	14.7 (16.9)	3706	118	3824	3.8
Pl + Ni	36.1	25.4	61.5	14.9	10.3	25.2	5.2	2.6	7.8 (17.0)	14.1 (20.3)	-	-	-	-
Pl + Rr + Ni	28.7	19.5	48.2	11.8	8.2	20.0	4.1	2.1	6.2 (34.0)	10.7 (39.5)	3313	102	3235	3.2
L.S.D. (at 5% level)			4.25			2.22			1.76	2.70				1.66
L.S.D. (at 1% level)			6.70			3.28			2.61	4.00				3.05
Bavistin (B)	40.8	30.4	71.2	17.5	12.5	30.0	6.4	3.0	9.4	18.0	-	-	-	-
B + Rr	36.6	26.4	63.0	15.5	10.6	26.1	5.5	2.5	8.00 (14.8)	14.6 (18.8)	5092	150	5242	5.2
B + Ni	35.5	25.5	61.0	15.1	10.1	25.2	5.4	2.3	7.7 (18.0)	14.4 (20.0)	-	-	-	-
B + Rr + Ni	29.0	19.4	48.4	11.8	8.0	19.8	4.1	1.8	5.9 (37.2)	11.1 (38.3)	3074	133	3407	3.4
L.S.D. (at 5% level)			6.00			2.65			0.76	2.20				1.56
L.S.D. (at 1% level)			8.88			3.92			1.12	3.25				2.86

In parenthesis are given percentage reduction over control.

plants. The highest improvement in the plant growth and yield was recorded in neem cake treated plants followed by mustard cake, castor cake, *T. harzianum*, mahua cake and sesame cake. Moreover, soil application with *Paecilomyces lilacinus*, linseed cake and Bavistin did not show any significant effect on the improvement of plant growth and yield (Tables 8 to 8.3 and Fig.7).

Soil application with biocontrol agents (*T. harzianum* and *P. lilacinus*), Bavistin and most of the tested oil-cakes viz. neem, mustard, castor and mahua significantly reduced the reproduction factor of reniform nematode, which consequently increased the plant growth and yield as compared to unamended and *R. reniformis* inoculated chickpea plants. However, on the other hand, the amendments of linseed cake and sesame cake did not show significant differences in plant growth, yield and reproduction factor of reniform nematode. The greatest improvement in plant growth and yield of chickpea and reduction in population buildup of reniform nematode were recorded in soil treated with *P. lilacinus* followed by Bavistin, neem cake, mustard cake, castor cake, *T. harzianum* and mahua cake (Tables 8 to 8.1 and Fig.7.1).

Soil application with biocontrol agent (*T. harzianum*), Bavistin and most of the oil-cakes viz. neem, mustard, linseed, castor and mahua significantly reduced the disease index of *F. solani*, which consequently increased the plant growth and yield of chickpea. However, on the other hand, the application of *P. lilacinus* and sesame cake did not show significant differences in plant growth, yield and disease index of *F. solani* as against unamended and *F. solani* inoculated plants. The greatest improvement in plant growth and yield of chickpea and reduction in disease index of *F. solani* were recorded in soil treated with Bavistin followed by *T. harzianum,* neem cake, linseed cake, mustard cake ,castor cake and mahua cake (Tables 8.2 to 8.3 and Fig. 7.2).

Soil application with biocontrol agent (*T. harzianum*) and most of the tested oil-cakes viz. neem, mustard, mahua and castor significantly reduced the

Table -8.2: Effect of chromium on the efficacy of oil- cakes, biocontrol agents and bavistin in the management of *Fusarium solani* infecting chickpea.

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant $^{-1}$ (g)	Disease index (%)
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		
Untreated -Uninoculated	38.8	29.6	68.4	16.8	12.2	29.0	5.9	3.3	9.2	17.5	-
F. solani (Fs)	26.8	18.9	45.7	9.1	9.7	18.8	3.8	1.9	5.7 (38.0)	10.5 (40.0)	64.0
Chromium(Cr)	37.0	26.6	63.6	15.5	10.8	26.3	5.3	2.9	8.2 (10.8)	15.4 (12.0)	-
Fs + Cr	23.4	16.2	39.6	10.0	6.5	16.5	3.4	1.7	5.1 (44.5)	9.1 (48.0)	47.0
Mustard cake (Mc)	51.3	36.7	88.0	21.5	15.1	36.6	7.4	4.1	11.5	21.3	-
Mc + Fs	39.1	26.7	65.8	15.9	10.7	26.6	5.4	2.8	8.2 (28.6)	14.3 (32.8)	40.2
Mc + Cr	49.6	34.8	84.4	20.9	14.1	35.0	7.1	3.8	10.9 (5.2)	19.6 (7.9)	-
Mc + Fs + Cr	38.2	26.0	64.2	15.7	10.2	25.9	5.3	2.7	8.0 (30.4)	13.8 (35.2)	30.4
L.S.D. (at 5% level)			3.65			1.72			0.57	3.00	12.27
L.S.D. (at 1% level)			5.40			2.54			0.84	4.44	22.52
Castor cake (Cc)	49.8	36.6	86.0	20.8	15.0	35.8	7.2	4.0	11.2	20.9	-
Cc + Fs	35.6	24.9	60.5	14.7	9.9	24.6	4.9	2.6	7.5 (33.0)	13.5 (35.4)	45.0
Cc + Cr	48.0	34.0	82.0	19.7	13.8	33.5	6.8	3.5	10.3 (8.0)	18.9 (9.5)	-
Cc + Fs + Cr	34.3	23.1	57.4	12.0	11.1	23.2	4.7	2.3	7.0 (37.5)	12.7 (39.2)	33.0
L.S.D. (at 5% level)			4.61			2.44			0.70	1.71	10.58
L.S.D. (at 1% level)			6.82			3.61			1.03	2.53	19.42
Mahua cake (M)	46.1	32.9	79.0	19.1	14.0	33.1	6.6	3.7	10.3	19.1	-
M + Fs	30.6	20.7	51.3	12.3	8.5	20.8	4.1	2.2	6.3 (38.8)	11.6 (39.2)	60.3
M + Cr	44.3	31.5	75.8	18.3	13.1	31.4	6.3	3.3	9.6 (6.7)	17.4 (8.9)	-
M + Fs + Cr	29.3	19.6	48.9	11.7	8.1	19.8	4.0	2.0	6.0 (41.7)	10.8 (43.4)	40.7
L.S.D. (at 5% level)			6.44			2.45			0.82	1.30	8.98
L.S.D. (at 1% level)			9.53			3.62			1.21	1.91	16.48

Contd.....

Table -8.2: (Contd.) Page 2

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant $^{-1}$ (g)	Disease index (%)
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		
Linseed cake (Lc)	41.0	31.0	72.0	17.6	12.6	30.2	6.1	3.4	9.5	17.8	-
Lc + Fs	31.4	23.0	54.4	13.3	9.3	22.6	4.6	2.4	7.0 (26.3)	12.5 (29.7)	36.2
Lc + Cr	38.7	27.5	66.2	16.2	11.2	27.4	5.6	2.9	8.5 (10.5)	15.7 (11.7)	-
Lc + Fs + Cr	29.6	21.2	50.8	12.5	8.3	20.8	4.2	2.2	6.4 (32.6)	11.3 (36.5)	33.2
L.S.D. (at 5% level)			4.15			2.20			0.61	1.57	8.70
L.S.D. (at 1% level)			6.14			3.25			0.90	2.32	15.97
Sesame (Sc)	44.6	33.6	78.2	19.1	13.5	32.6	6.5	3.6	10.1	19.0	-
Sc + Fs	29.3	20.4	49.7	12.6	8.2	20.8	4.2	2.1	6.3 (37.6)	11.2 (41.0)	63.7
Sc + Cr	40.0	28.2	68.2	17.7	11.2	28.9	5.9	3.1	9.0 (10.8)	16.7 (12.1)	-
Sc + Fs + Cr	25.0	16.9	44.9	11.4	7.6	19.0	3.7	2.0	5.7 (43.5)	10.3 (45.7)	47.8
L.S.D. (at 5% level)			4.71			2.63			0.85	1.40	7.82
L.S.D. (at 1% level)			6.97			3.89			1.24	2.07	14.35
Neem cake (Nc)	58.4	32.7	91.1	22.2	15.8	38.0	7.6	4.3	11.9	22.2	-
Nc + Fs	47.1	24.8	71.9	17.4	11.8	29.2	5.8	3.1	8.9 (25.2)	16.0 (27.9)	25.7
Nc + Cr	57.8	31.7	89.5	22.1	15.1	37.2	7.5	4.1	11.6 (2.5)	20.8 (6.3)	-
Nc + Fs + Cr	46.5	24.5	71.0	17.2	11.6	28.8	5.8	3.0	8.8 (26.0)	15.9 (28.3)	15.2
L.S.D. (at 5% level)			4.51			2.41			0.87	2.13	13.00
L.S.D. (at 1% level)			6.67			3.56			1.29	3.15	23.86

Contd.....

Table-8.2: (Contd.) Page 3

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant $^{-1}$ (g)	Disease index (%)
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		
T. harzianum (Th)	47.9	35.1	83.0	20.3	14.6	34.9	7.0	3.9	10.9	20.4	-
Th + Fs	40.6	27.4	68.0	15.8	11.2	27.0	5.6	3.0	8.6 (21.1)	15.7 (23.0)	21.0
Th + Cr	46.3	32.5	78.8	19.4	13.1	32.5	6.6	3.4	**10.0 (8.2)**	18.4 (9.8)	-
Th + Fs + Cr	38.0	26.3	64.3	15.1	11.1	26.2	5.2	2.7	7.9 (27.5)	14.4 (29.4)	12.1
L.S.D. (at 5% level)			4.42			2.40			0.65	2.33	10.62
L.S.D. (at 1% level)			6.53			3.55			0.96	3.45	19.50
P. lilacinus (Pl)	41.0	30.0	71.0	17.3	12.5	29.8	6.1	3.3	9.4	17.7	-
Pl + Fs	28.4	19.8	48.2	11.6	8.0	19.6	4.0	2.0	6.0 (36.1)	10.9 (38.4)	58.8
Pl + Cr	38.7	27.3	66.0	16.0	11.2	27.2	5.5	2.9	8.4 (10.6)	15.7 (11.2)	-
Pl + Fs + Cr	24.7	17.1	41.8	10.1	6.8	16.9	3.5	1.7	5.2 (44.6)	9.5 (46.3)	50.0
L.S.D. (at 5% level)			4.46			1.48			0.43	1.67	9.00
L.S.D. (at 1% level)			6.86			2.19			0.63	2.46	16.52
Bavistin (B)	40.8	30.4	71.2	17.5	12.5	30.0	6.4	3.0	9.4	18.0	-
B + Fs	35.2	25.1	60.3	15.0	10.1	25.1	5.3	2.4	7.7 (18.0)	14.1 (21.6)	15.0
B + Cr	38.0	26.7	64.7	15.9	11.0	26.9	5.7	2.6	8.3 (11.7)	15.7 (12.7)	-
B + Fs + Cr	32.4	22.4	54.8	13.2	9.3	22.5	4.8	2.1	6.9 (26.5)	12.6 (30.0)	8.7
L.S.D. (at 5% level)			3.41			1.26			0.25	1.70	12.08
L.S.D. (at 1% level)			5.00			1.86			0.37	2.51	22.17

In parenthesis are given percentage reduction over control.

Fig. 7: Effect of oil-cakes, biocontrol agents and bavistin on the plant growth and yield of chickpea.

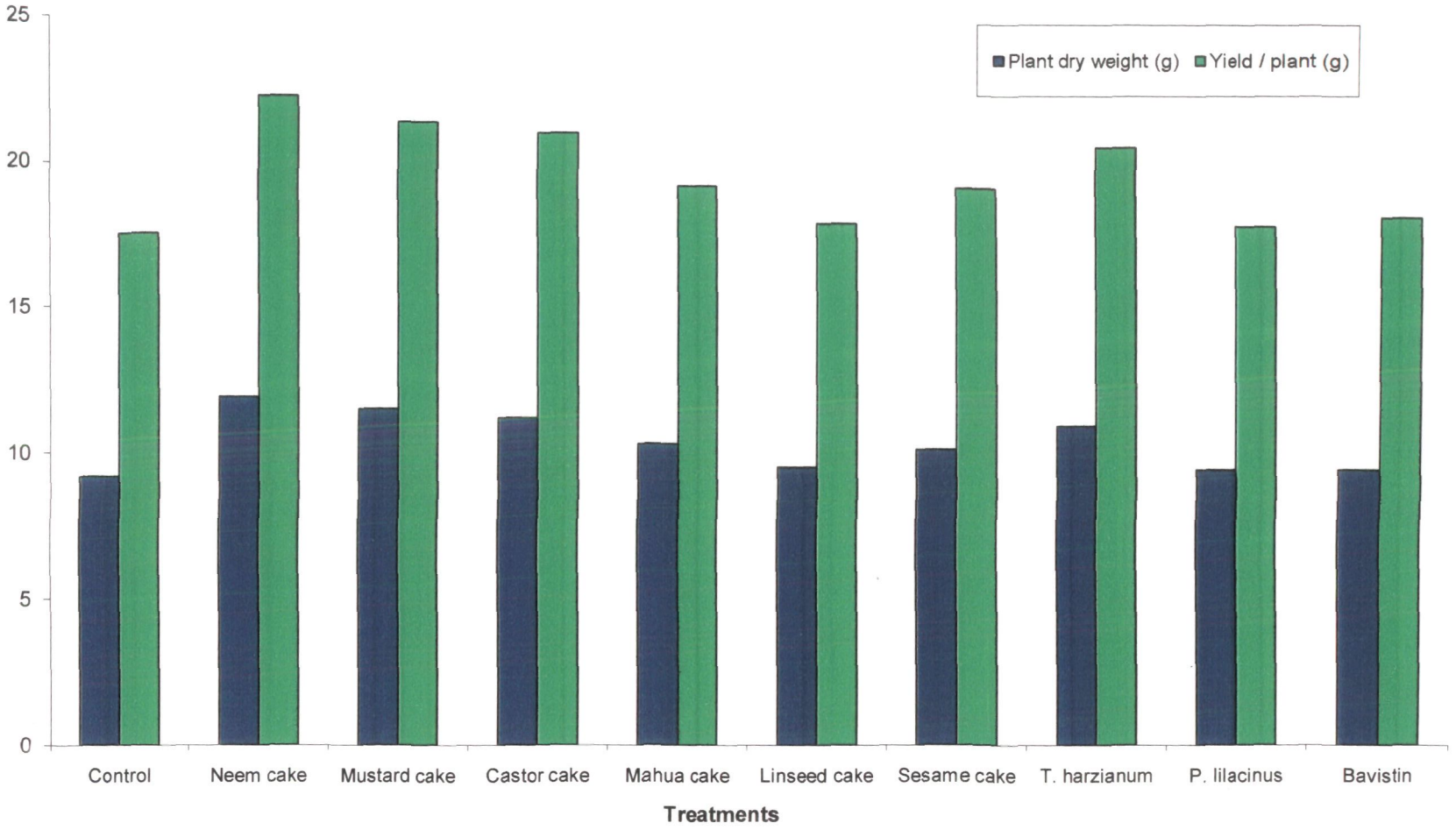


Fig. 7.1: Efficacy of oil-cakes, biocontrol agents and bavistin in the management of *Rotylenchulus reniformis* infecting chickpea.

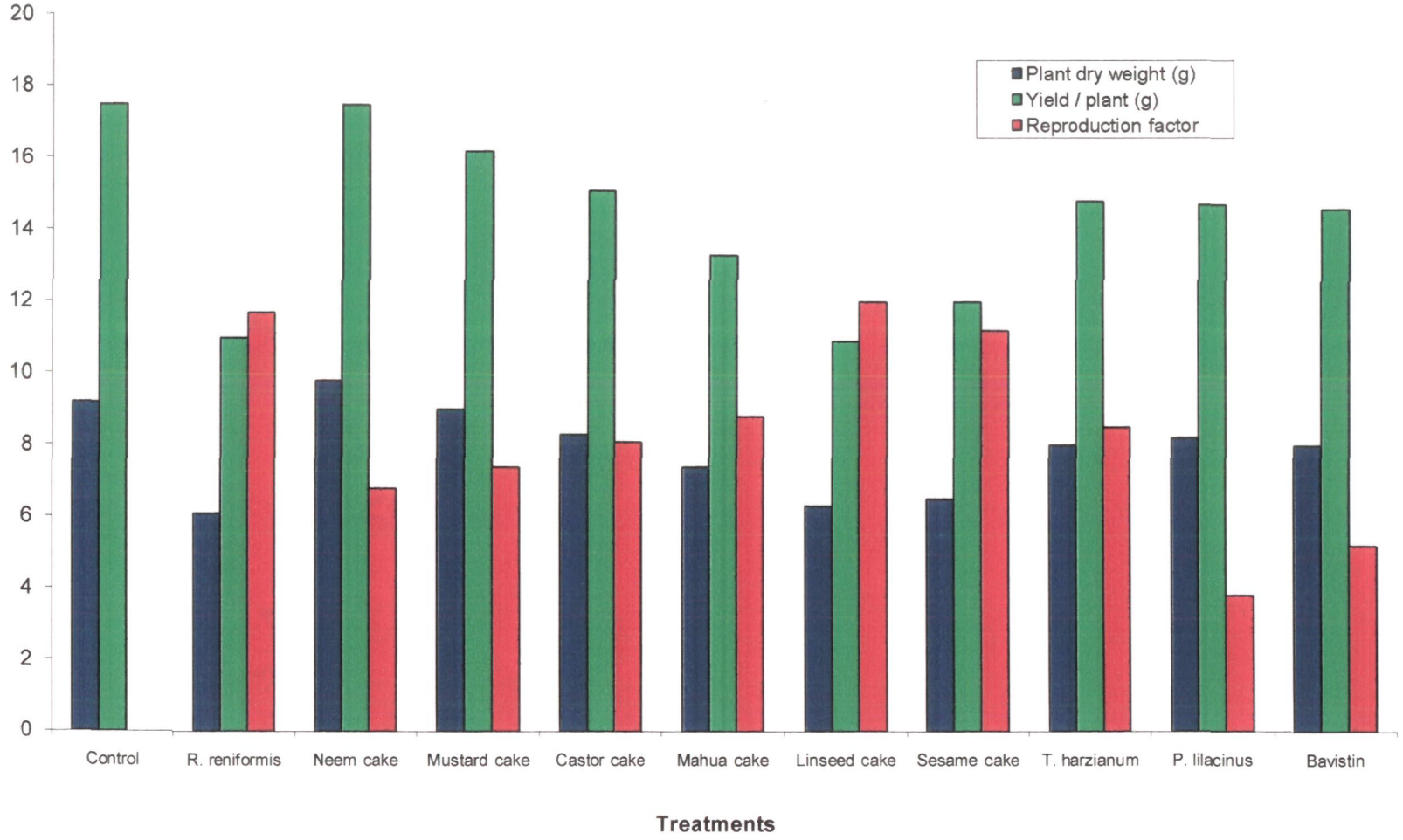
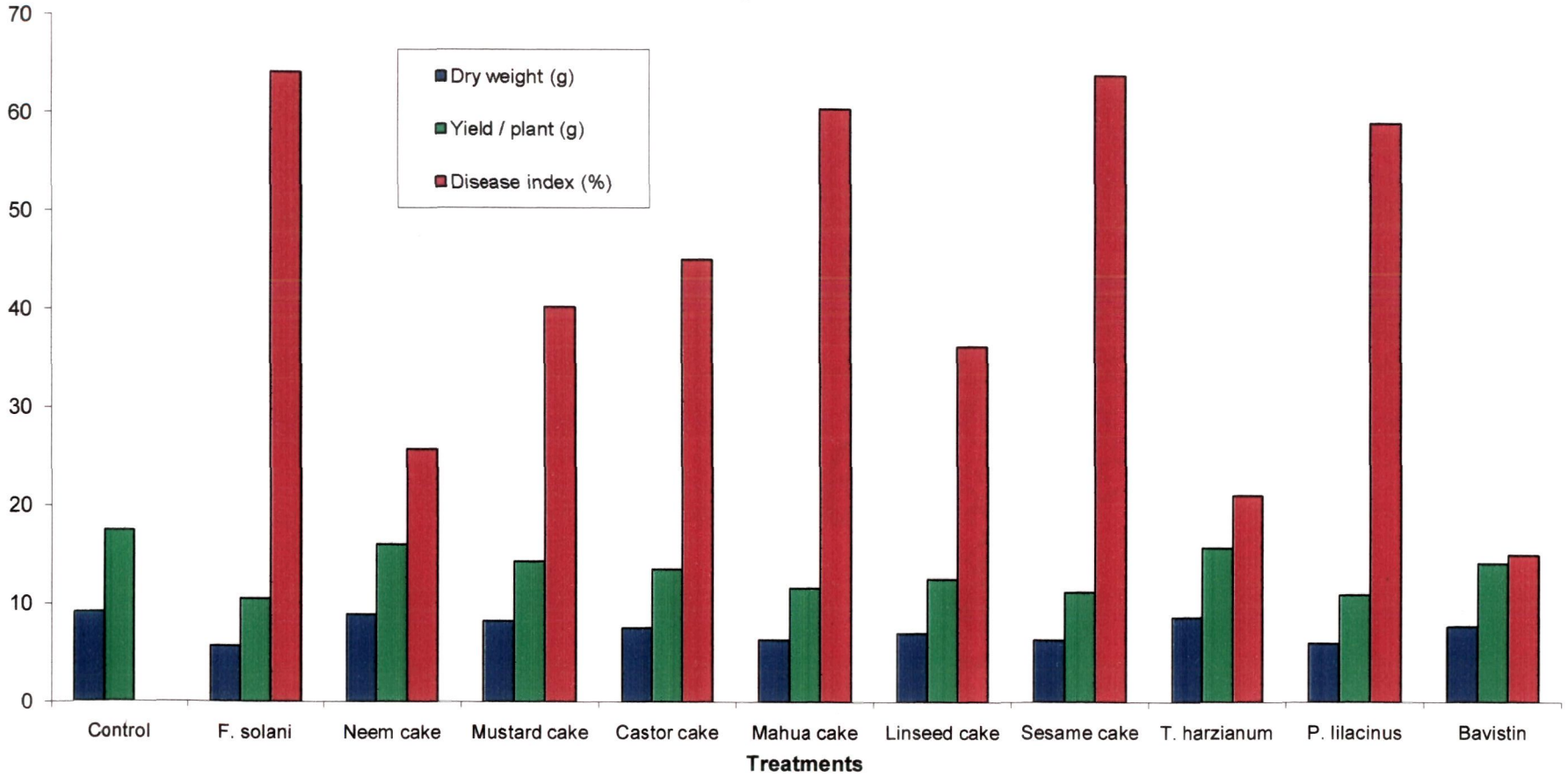


Fig. 7.2 : Efficacy of oil-cakes, biocontrol agents and bavistin in the management of *Fusarium solani* infecting chickpea.



damage caused by the heavy metal (Cr), which ultimately increased the plant growth and yield of chickpea as against unamended and Cr-treated plants. The greatest improvement in plant growth and yield of chickpea was recorded in soil treated with neem cake followed by mustard cake, mahua cake, castor cake and *T. harzianum*. However, on the other hand, application of Bavistin, *P. lilacinus,* linseed cake and sesame cake did not showed any significant differences in reducing the damage caused by the application of Cr. (Tables 8 and 8.2, and Fig. 7.3).

Soil application with biocontrol agent (*T. harzianum*) and tested oil-cakes viz. mustard cake, neem cake, mahua cake and castor cake significantly reduced the damage caused by Ni, which ultimately increased the plant growth and yield of chickpea as compared to unamended and Ni-treated plants. The greatest improvement in plant growth and yield of chickpea was recorded in soil treated with mustard cake followed by neem cake, mahua cake, castor cake and *T. harzianum* .Moreover, the application of Bavistin, *P. lilacinus*, linseed cake and sesame cake did not show any significant differences in reducing the damage caused by Ni. (Tables 8.1 and 8.3, and Fig. 7.4).

The data presented in Table 8 and Fig. 7.5 clearly revealed that the growth and yield of chickpea was synergistically reduced in the chickpea plants inoculated with *R. reniformis* and grown in soil treated with Cr. Moreover, this damage was significantly reduced by the application of *T. harzianum, P. lilacinus*, neem cake, mustard cake, castor cake, mahua cake and Bavistin. These treatments also significantly reduced the reproduction factor of reniform nematode. The highest improvement in the plant growth and yield of chickpea was recorded by the application of neem cake followed mustard cake, *T. harzianum,* castor cake, mahua cake, *P. lilacinus* and Bavistin as compared to those plants treated with Cr and *R. reniformis* only. However, the application of

Table -8.3: Effect of nickel on the efficacy of oil-cakes, biocontrol agents and Bavistin in the management of *Fusarium solani* infecting chickpea.

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant $^{-1}$ (g)	Disease index (%)
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		
Untreated -uninoculated	38.8	29.6	68.4	16.8	12.2	29.0	5.9	3.3	9.2	17.5	-
F. solani (Fs)	26.8	18.9	45.7	9.1	9.7	18.8	3.8	1.9	5.7 (38.0)	10.5 (40.0)	64.0
Nickel (Ni)	34.3	25.0	59.3	14.6	10.0	24.6	5.0	2.7	7.7 (16.3)	14.0 (20.0)	-
Fs + Ni	18.0	12.7	30.7	7.6	4.8	12.4	2.5	1.3	3.8 (58.6)	6.6 (62.2)	51.5
Mustard cake (Mc)	51.3	36.7	88.0	21.5	15.1	36.6	7.4	4.1	11.5	21.3	-
Mc + Fs	39.1	26.7	65.8	15.9	10.7	26.6	5.4	2.8	8.2 (28.6)	14.3 (32.8)	40.2
Mc + Ni	49.9	34.4	84.3	20.9	13.8	34.7	7.1	3.7	10.8 (6.0)	19.1 (10.3)	-
Mc + Fs + Ni	38.9	27.1	66.0	16.3	10.8	27.1	5.5	2.9	8.4 (26.9)	14.2 (33.3)	32.7
L.S.D. (at 5% level)			6.00			2.65			0.82	2.85	10.74
L.S.D. (at 1% level)			8.88			3.92			1.21	4.21	19.70
Castor cake (Cc)	49.8	36.2	86.0	20.8	15.0	35.8	7.2	4.0	11.2	20.9	-
Cc + Fs	35.6	24.9	60.5	14.7	9.9	24.6	4.9	2.6	7.5 (33.0)	13.5 (35.4)	45.0
Cc + Ni	45.4	31.4	76.8	18.8	12.9	31.7	6.3	3.4	9.7 (13.3)	17.3 (17.2)	-
Cc + Fs + Ni	29.9	19.9	49.8	8.9	11.1	20.0	4.0	2.1	6.1 (45.5)	10.6 (49.2)	38.6
L.S.D. (at 5% level)			8.90			4.33			1.44	2.40	10.01
L.S.D. (at 1% level)			13.16			6.40			2.13	3.55	18.37
Mahua cake (M)	46.1	32.9	79.0	19.1	14.0	33.1	6.6	3.7	10.3	19.1	-
M + Fs	30.6	20.7	51.3	12.3	8.5	20.8	4.1	2.2	6.3 (38.8)	11.6 (39.2)	60.3
M + Ni	42.8	29.0	71.8	17.5	12.0	29.5	5.9	3.1	9.0 (12.6)	16.3 (14.6)	-
M + Fs + Ni	27.8	18.0	45.8	11.0	7.5	18.5	3.7	1.9	5.6 (45.6)	9.8 (48.6)	45.0
L.S.D. (at 5% level)			6.47			3.28			1.01	1.34	9.22
L.S.D. (at 1% level)			9.57			4.85			1.49	1.98	16.92

Contd.....

Table-8.3: (Contd.) Page 2

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant $^{-1}$ (g)	Root – rot (%)
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		
Linseed cake (Lc)	41.0	31.0	72.0	17.6	12.6	30.2	6.1	3.4	9.5	17.8	-
Lc + Fs	31.4	23.0	54.4	13.3	9.3	22.6	4.6	2.4	7.0 (26.3)	12.5 (29.7)	36.2
Lc + Ni	36.6	26.0	62.6	15.3	10.3	25.6	5.2	2.7	7.9 (16.8)	14.3 (19.6)	-
Lc + Fs + Ni	26.7	18.6	45.3	11.3	7.4	18.7	3.8	2.0	5.8 (38.9)	10.0 (43.8)	36.2
L.S.D. (at 5% level)			8.30			3.25			0.62	1.37	8.00
L.S.D. (at 1% level)			12.28			4.81			0.92	2.02	14.68
Sesame (Sc)	44.6	33.6	78.2	19.1	13.5	32.6	6.5	3.6	10.1	19.0	-
Sc + Fs	29.3	20.4	49.7	12.6	8.2	20.8	4.2	2.1	6.3 (37.6)	11.2 (41.0)	63.7
Sc + Ni	39.8	28.2	68.0	16.8	11.2	28.0	5.6	2.9	8.5 (15.8)	15.4 (18.9)	-
Sc + Fs + Ni	20.1	15.0	35.1	8.5	5.8	14.3	2.9	1.4	4.3 (57.4)	7.3 (61.5)	48.0
L.S.D. (at 5% level)			8.85			3.52			0.83	1.45	5.75
L.S.D. (at 1% level)			13.09			5.20			1.22	2.14	10.55
Neem cake (Nc)	58.4	32.7	91.1	22.2	15.8	38.0	7.6	4.3	11.9	22.2	-
Nc + Fs	47.1	24.8	71.9	17.4	11.8	29.2	5.8	3.1	8.9 (25.2)	16.0 (27.9)	25.7
Nc + Ni	55.3	29.4	84.7	20.0	14.8	34.8	7.0	3.7	10.7 (10.0)	19.5 (12.1)	-
Nc + Fs + Ni	43.2	22.8	66.0	16.3	11.0	27.3	5.5	2.9	8.4 (29.4)	14.2 (36.0)	18.0
L.S.D. (at 5% level)			7.10			3.60			1.20	3.06	9.77
L.S.D. (at 1% level)			10.50			5.33			1.77	4.52	17.93

Contd.....

Table-8.3: (Contd.) Page 3

Treatments	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Yeild Plant $^{-1}$ (g)	Disease index (%)
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total		
T. harzianum (Th)	47.9	35.1	83.0	20.3	14.6	34.9	7.0	3.9	10.9	20.4	-
Th + Fs	40.6	27.4	68.0	15.7	11.2	27.0	5.6	3.0	8.6 (21.1)	15.7 (23.0)	21.0
Th + Ni	43.7	30.1	73.8	18.2	12.4	30.6	6.2	3.2	9.4 (13.7)	17.1 (16.1)	-
Th + Fs + Ni	34.6	24.3	58.9	14.5	9.9	24.4	5.0	2.5	7.5 (31.1)	13.3 (34.8)	15.2
L.S.D. (at 5% level)			8.00			2.77			6.00	2.40	8.62
L.S.D. (at 1% level)			11.84			4.10			8.88	3.55	15.80
P .lilacinus (Pl)	41.0	30.0	71.0	17.3	12.5	29.8	6.1	3.3	9.4	17.7	-
Pl + Fs	28.4	19.8	48.2	11.6	8.0	19.6	4.0	2.0	6.0 (36.1)	10.9 (38.4)	58.8
Pl + Ni	36.1	25.4	61.5	14.9	10.3	25.2	5.2	2.6	7.8 (17.0)	14.1 (18.6)	-
Pl + Fs + Ni	19.2	13.4	32.6	8.0	5.6	13.6	2.9	1.3	4.2 (55.3)	6.5 (63.2)	51.2
L.S.D. (at 5% level)			5.76			2.15			0.75	1.80	9.72
L.S.D. (at 1% level)			8.52			3.18			1.11	2.66	17.84
Bavistan (B)	40.8	30.4	71.2	17.5	12.5	30.0	6.4	3.0	9.4	18.0	-
B + Fs	35.2	25.1	60.3	15.0	10.1	25.1	5.3	2.4	7.7 (18.0)	14.1 (21.6)	15.0
B + Ni	35.5	25.5	61.0	15.1	10.1	25.2	5.4	2.3	7.7 (18.0)	14.4 (20.0)	-
B + Fs + Ni	29.0	20.3	49.3	12.3	8.1	20.4	4.3	1.9	6.2 (34.0)	11.2 (37.7)	10.1
L.S.D. (at 5% level)			3.30			1.21			0.33	3.13	11.00
L.S.D. (at 1% level)			4.48			1.92			0.49	4.63	20.19

In parenthesis are given percentage reduction over control.

Fig. 7.3: Efficacy of oil-cakes, bioagents and Bavistin in the management of damage caused by chromium on chickpea.

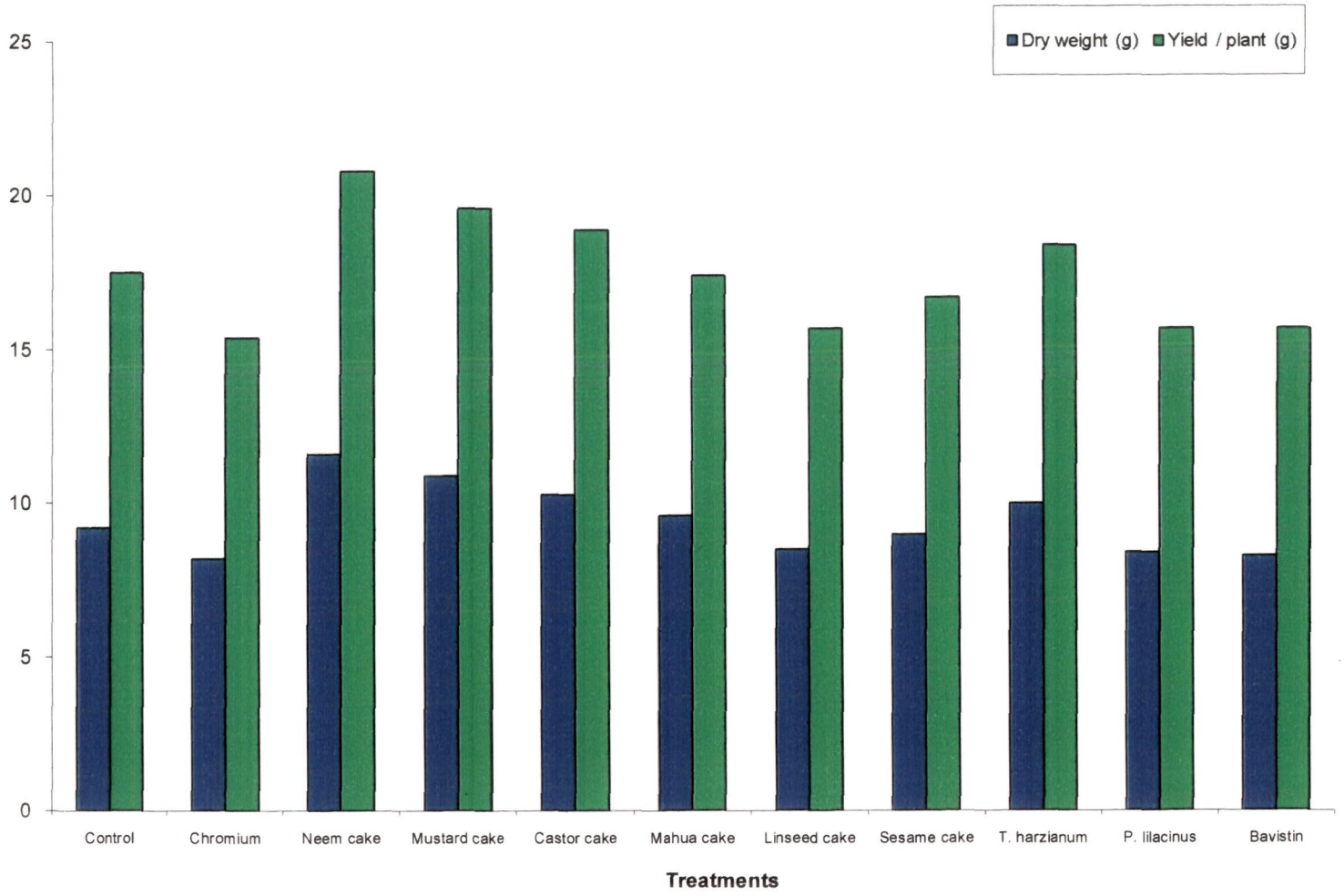


Fig.7.4: Efficacy of oil-cakes, bioagents and bavistin in the management of damage caused by nickel in chickpea.

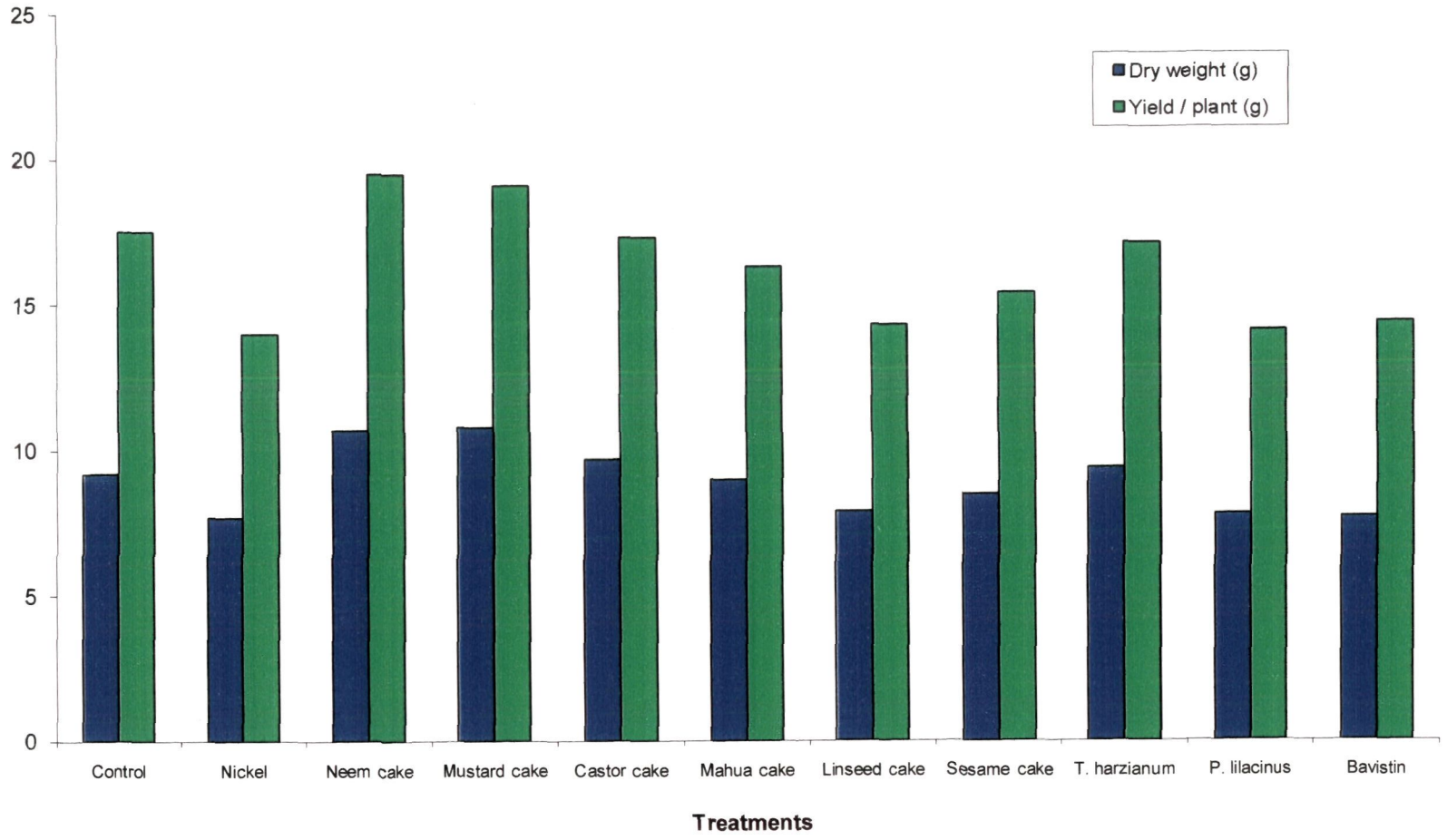
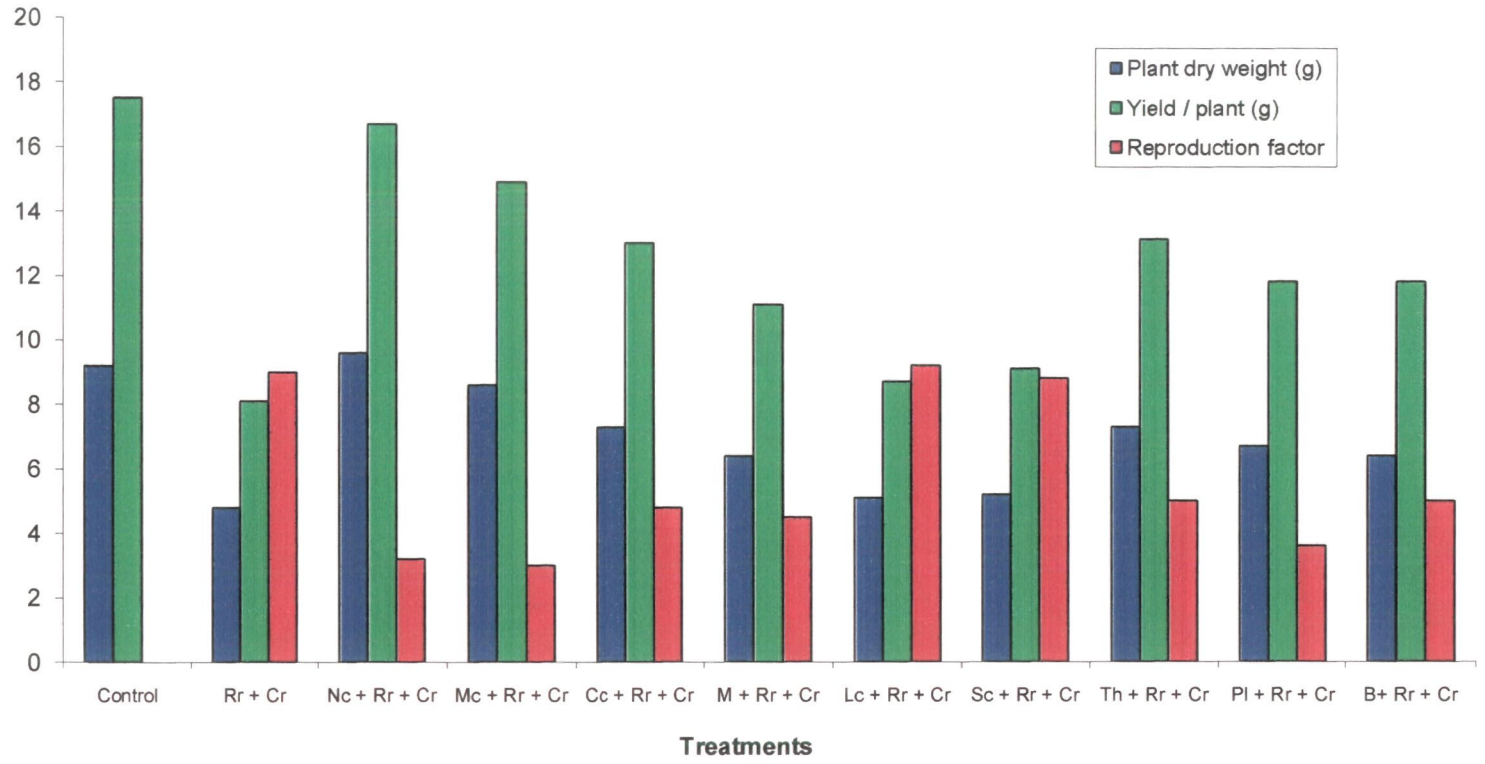


Fig. 7.5: Effect of chromium on the efficacy of oil-cakes , bioagents and bavistin in the management of *Rotylenchulus reniformis* (Rr) infecting chickpea.

Nc = Neem cake Mc = Mustard cake Cc = Castor cake M = mahua cake Lc = linseed cake
Sc = sesame cake Th = *T. harzianum* Pl = *P. lilacinus* B = Bavistin



linseed cake and sesame cake showed no significant role in reducing the combined damage of *R. reniformis* and Cr.

Similarly, the data presented in Table 8.2 and Fig 7.6 clearly revealed that the growth and yield of chickpea was significantly reduced in the plants inoculated with *F. solani* and grown in soil treated with Cr. Moreover, this damage was significantly reduced by the application of *T. harzianum,* neem cake, mustard cake, castor cake, mahua cake, linseed cake and Bavistin. These treatments also significantly reduced the disease index of *F. solani.* The highest improvement in the plant growth and yield of chickpea was recorded by the application of neem cake followed *T. harzianum* ,mustard cake, castor cake, mahua cake, linseed cake and Bavistin as compared to those plants treated with Cr and *F. solani* only. However, the application of *P. lilacinus* and sesame cake showed no significant role in reducing the combined damage of *R. reniformis* and Cr.

The data presented in Table 8.1 and Fig 7.7 clearly revealed that the growth and yield of chickpea was synergistically reduced in the plants inoculated with *R. reniformis* and grown in soil treated with Ni. Moreover, this damage was significantly reduced by the application of *T. harzianum, P. lilacinus*, neem cake, mustard cake, castor cake, mahua cake and Bavistin. These treatments also significantly reduced the reproduction factor of reniform nematode. The highest improvement in the plant growth and yield of chickpea was recorded by the application of mustard cake followed neem cake, mahua cake, castor cake, *T. harzianum, P. lilacinus* and Bavistin as compared to those plants treated with Ni and *R. reniformis* only. However, the application of linseed cake and sesame cake did not significantly reduce the damage caused by *R. reniformis* and Ni in chickpea.

Similarly, the data presented in Table 8.3 and Fig 7.8 clearly revealed that the growth and yield of chickpea was synergistically reduced in the plants

Fig. 7.6: Effect of chromium on the efficacy of oil-cakes, bioagents and bavistin in the management of *Fusarium solani* infecting chickpea.

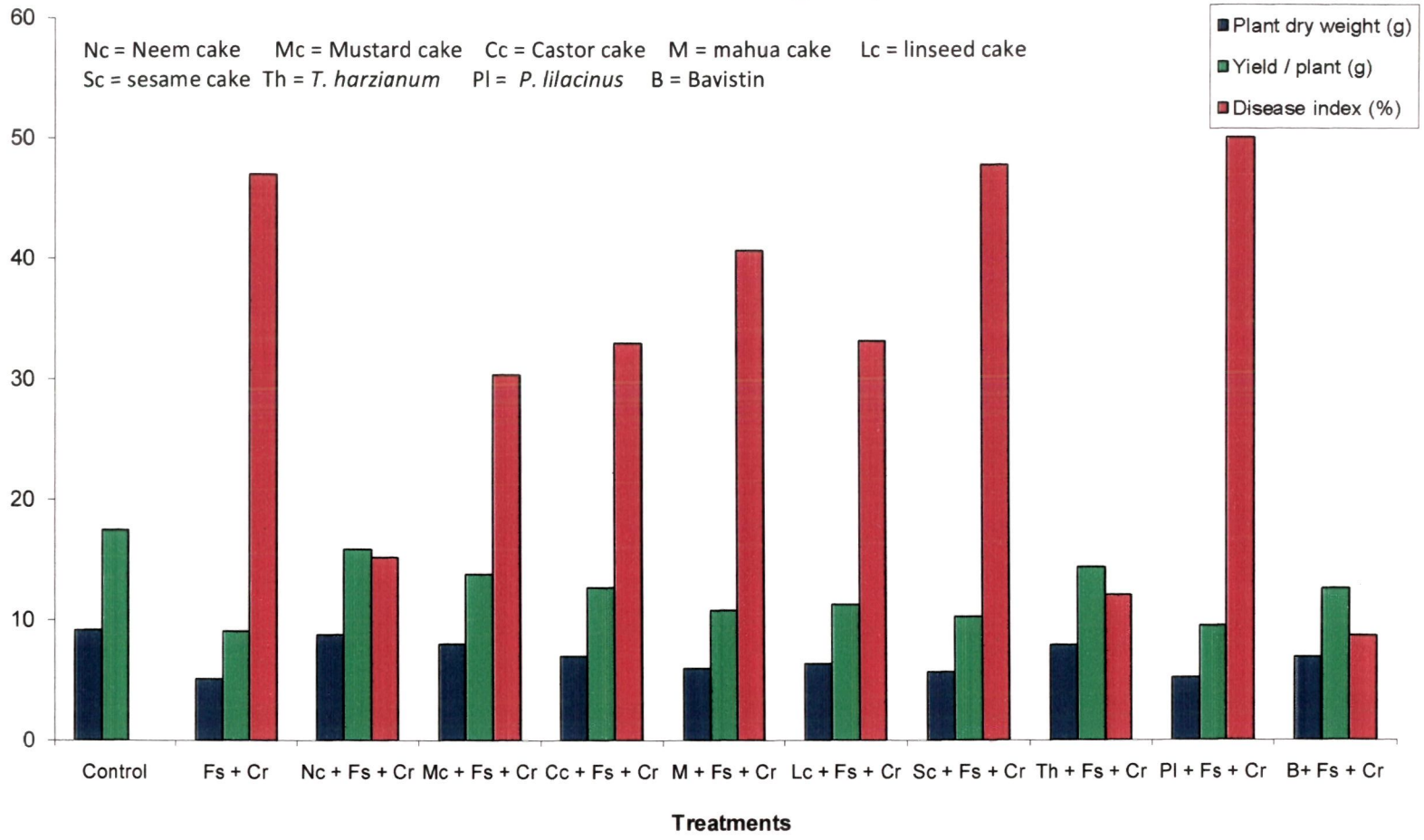


Fig.7.7: Effect of nickel on the efficacy of oil-cakes, bioagents and bavistin in the management of *Rotylenchulus reniformis* (Rr) infecting chickpea.

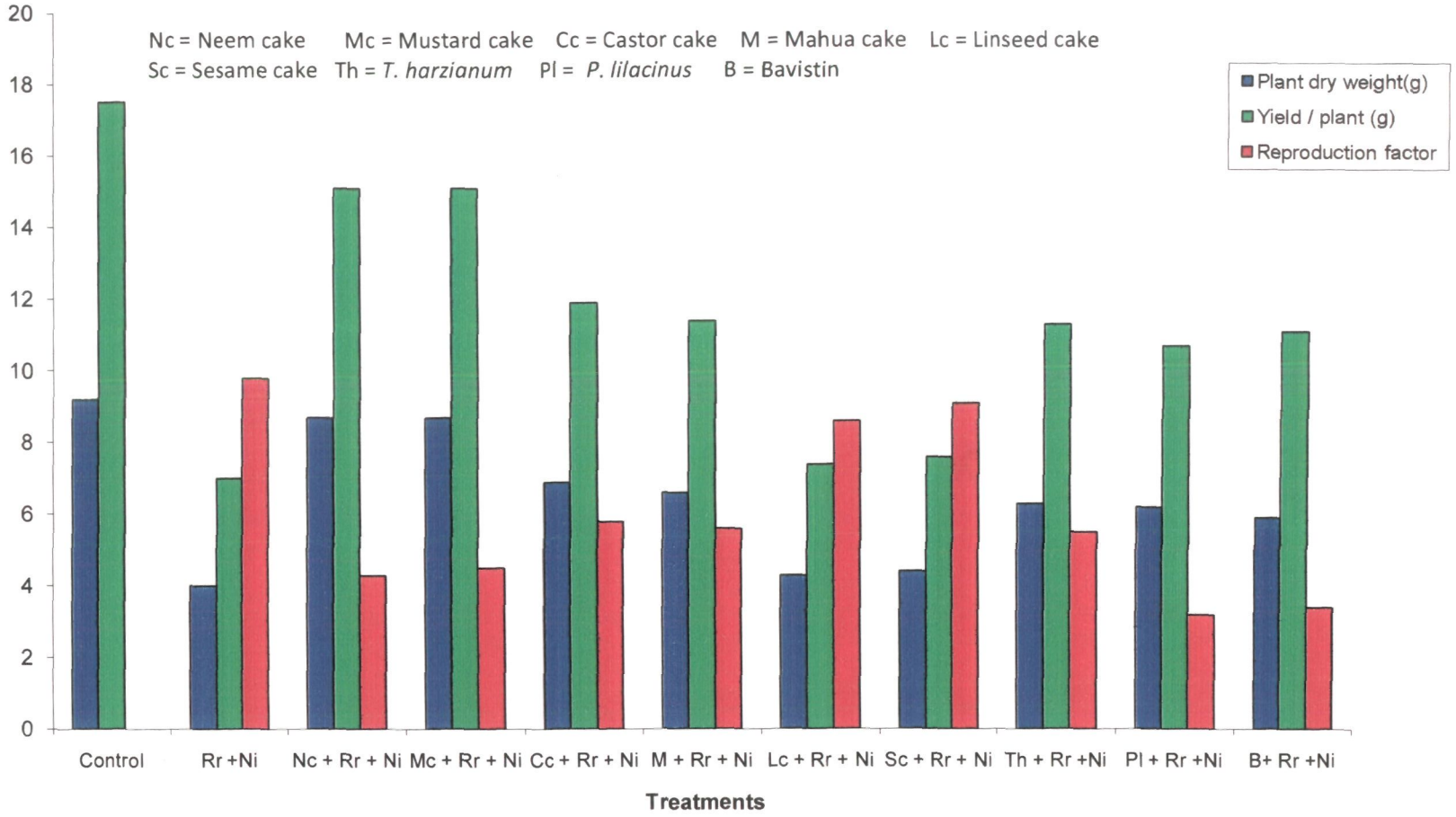


Fig. 7.8:Effect of nickel on the efficacy of oil-cakes, bioagents and bavistin in the management of *Fusarium solani* infecting chickpea

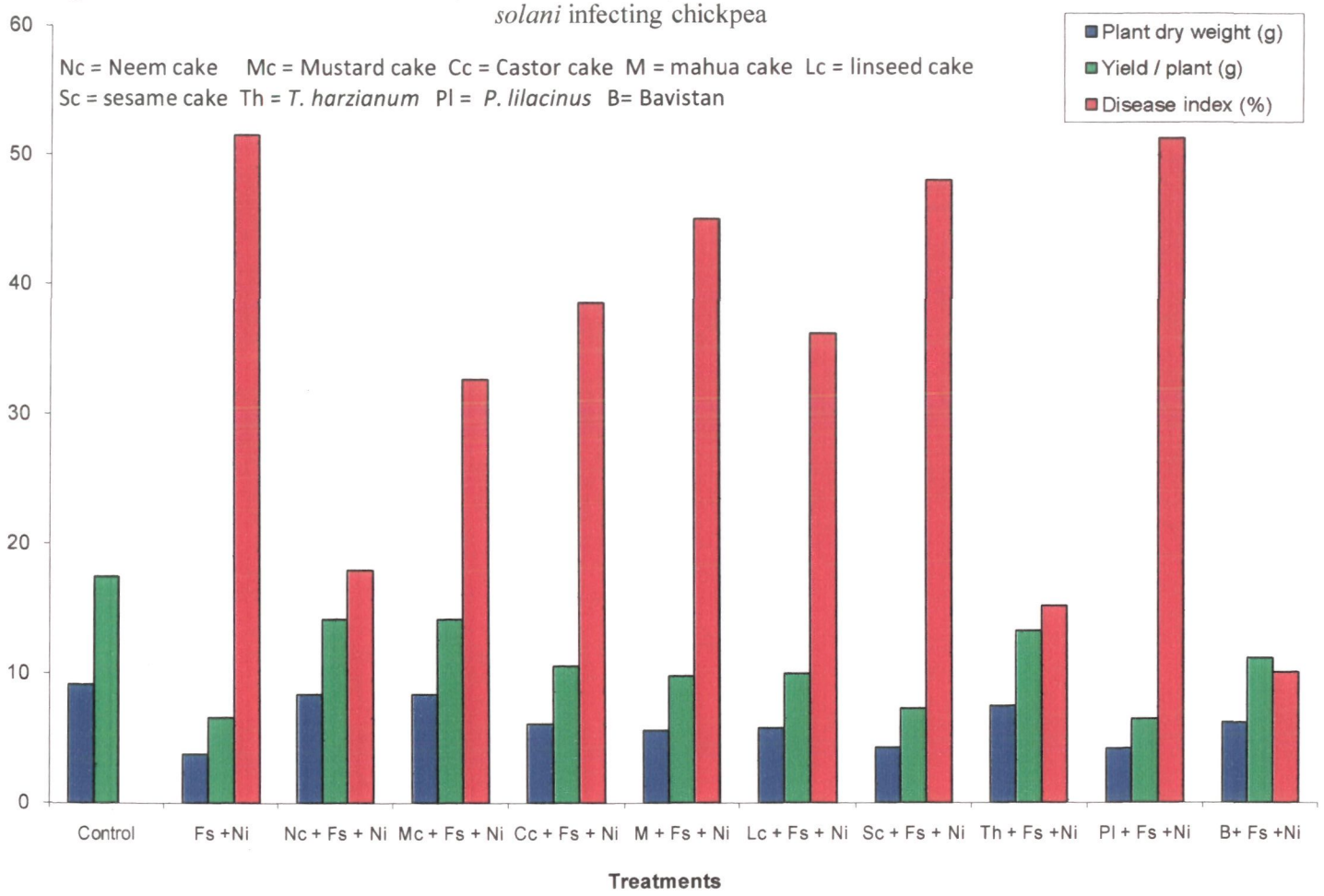


Table- 8.4: Parasitism of *Paecilomyces lilacinus* and *Trichoderma harzianum* on females, eggmasses and eggs of *Rotylenchulus reniformis* infecting chickpea.

Treatments	Fungal infection (%)		
	Females	Eggmasses	Eggs
P.lilacinus(Pl) + *R. reniformis*(Rr)	41	68	59
Pl +Rr +Cr	28	45	38
Pl +Rr +Ni	33	53	47
T. harzianum(Th) + Rr	-	46	39
Th +Rr+Cr	-	29	26
Th +Rr+Ni	-	37	30
L.S.D. (at 5% level)	4.00	6.50	3.43
L.S.D. (at 1% level)	9.22	10.10	5.37

inoculated with *F. solani* and grown in soil treated with Ni. Moreover, this damage was significantly reduced by the application of *T. harzianum,* neem cake, mustard cake, castor cake, mahua cake, linseed cake and Bavistin. These treatments also significantly reduced the disease index of *F. solani.* The highest improvement in the plant growth and yield of chickpea was recorded by the application of mustard cake followed neem cake, *T. harzianum,* castor cake, mahua cake, linseed cake and Bavistin as compared to those plants treated with Ni and *F. solani* only. However, the application of *P. lilacinus* and sesame cake showed no significant role in reducing the damage caused by the combined effect of *F. solani* and Ni.

It was also observed that the fungal biocontrol agents viz. *P. lilacinus* and *T. harzianum* parasitized the eggmasses, eggs and females of *R. reniformis* except *T. harzianum* which was unable to parasitize the females of *R. reniformis*. Percentage of infection caused by *P. lilacinus* was more on eggmasses followed by eggs and females. Similarly, in case of *T. harzianum*, the infection on eggmasses was more than eggs. The percentage of infection on *R. reniformis* caused by *P. lilacinus* was more than that of *T. harzianum.* Moreover, the parasitism of *P. lilacinus* and *T. harzianum* decreased in the presence of heavy metals (Cr/Ni).The reduction in parasitism of biocontrol agents was more in Cr treated pots than the Ni (Table 8.4).

4.9: SCREENING OF CHICKPEA VARIETIES FOR RESISTANCE AGAINST *ROTYLENCHULUS RENIFORMIS, FUSARIUM SOLANI,* CHROMIUM AND NICKEL:

It is evident from the data presented in Table 9 and Figs 8 to 8.3 that different chickpea varieties responded differently to the *R. reniformis*, *F. solani* and heavy metals viz. Cr and Ni. There was an adverse effect of each test pathogen and heavy metal on the growth of most of the chickpea varieties, irrespective the level of its resistance against pathogen or heavy metal.

Table-9: Reaction of different chickpea varieties to *Rotylenchulus reniformis* (Rr), *Fusarium solani* (Fs), chromium (Cr) and Nickel (Ni).

Variety	Treatments	Plant dry weight (g)			% dry weight reduction over control	R =Pf/Pi	Disease index (%)	Reaction
		Shoot	Root	Total				
	Control	5.5	4.5	10.0	-	-	-	-
Annegiri -1	Rr	4.0	3.0	7.0	30.0	8.2		HS
	Fs	3.9	2.9	6.8	32.0	-	50.6	HS
	Cr	4.7	3.5	8.2	18.0	-	-	S
	Ni	4.7	3.7	8.4	16.0			S
L.S.D. (at 5% level)				0.57				
L.S.D. (at 1% level)				0.94				
	Control	6.4	5.8	12.2	-	-	-	-
	Rr	5.3	4.7	10.0	18.0	5.7	-	S
Avarodhi	Fs	5.2	4.1	9.3	23.7	-	40.1	S
	Cr	5.8	5.0	10.8	11.4	-	-	T
	Ni	6.0	4.2	10.2	16.3	-	-	S
L.S.D. (at 5% level)				1.25				
L.S.D. (at 1% level)				2.07				
	Control	4.5	3.0	7.5	-	-	-	-
	Rr	4.0	2.5	6.5	13.3	3.1	-	T
CSJD	Fs	3.6	2.2	5.8	22.6	-	32.0	S
	Cr	3.9	2.4	6.3	16.0	-	-	S
	Ni	3.7	2.3	6.0	20.0	-	-	S
L.S.D. (at 5% level)				1.00				
L.S.D. (at 1% level)				1.65				
	Control	3.6	2.4	6.0	-	-	-	-
	Rr	3.4	1.9	5.3	11.7	2.8	-	T
JG - 74	Fs	3.3	2.0	5.3	11.6	-	20.7	T
	Cr	3.1	1.9	5.0	16.6	-	-	S
	Ni	3.0	1.7	4.7	21.6	-	-	S
L.S.D. (at 5% level)				0.45				
L.S.D. (at 1% level)				0.74				
	Control	5.5	4.0	9.5	-	-	-	-
	Rr	5.3	3.7	9.0	5.2	1.3	-	MR
Gauraw	Fs	4.7	3.3	8.0	15.7	-	42.5	S
	Cr	4.5	3.0	7.5	21.0	-	-	S
	Ni	4.8	3.3	8.1	14.7			T
L.S.D. (at 5% level)				1.15				
L.S.D. (at 1% level)				1.90				
	Control	3.9	2.5	6.4	-	-	-	-
	Rr	3.2	2.0	5.2	18.7	5.0	-	S
Gaut	Fs	3.1	1.9	5.0	21.7	-	35.3	S
	Cr	3.5	2.0	5.5	14.0	-	-	T
	Ni	3.6	1.8	5.4	15.6			S
L.S.D. (at 5% level)				0.66				
L.S.D. (at 1% level)				1.09				

Contd.....

Table-9: (Contd.) Page 2

Variety	Treatments	Plant dry weight (g)			% dry weight reduction over control	R=Pf/Pi	Disease index (%)	Reaction
		Shoot	Root	Total				
	Control	4.2	2.3	6.5	-	-	-	-
	Rr	3.3	1.8	5.1	21.5	4.8	-	S
Gulab	Fs	3.6	1.8	5.4	16.9	-	38.3	S
	Cr	4.0	2.0	6.0	7.6	-	-	T
	Ni	3.2	1.7	4.9	24.6			S
L.S.D. (at 5% level)				0.32				
L.S.D. (at 1% level)				0.53				
	Control	5.7	5.0	10.7	-	-	-	-
	Rr	4.7	3.6	8.3	22.4	4.3	-	S
K – 850	Fs	4.7	3.9	8.6	19.6	-	37.0	S
	Cr	5.2	4.2	9.4	12.1	-	-	T
	Ni	4.7	4.0	8.7	18.6			S
L.S.D. (at 5% level)				1.00				
L.S.D. (at 1% level)				1.65				
	Control	5.2	3.1	8.3	-	-	-	-
	Rr	4.9	2.7	7.6	8.4	1.8	-	MR
KGD – 1168	Fs	4.7	2.7	7.4	10.8	-	22.0	T
	Cr	4.4	2.5	6.9	16.8	-	-	S
	Ni	4.3	2.3	6.6	20.4			S
L.S.D. (at 5% level)				1.36				
L.S.D. (at 1% level)				2.25				
	Control	3.8	2.7	6.5	-	-	-	-
	Rr	2.5	1.6	4.1	36.9	9.2	-	HS
KUSCR- 2	Fs	2.4	1.6	4.0	38.4	-	57.5	HS
	Cr	3.3	2.2	5.5	15.3	-	-	S
	Ni	3.1	2.0	5.1	21.5			S
L.S.D. (at 5% level)				0.82				
L.S.D. (at 1% level)				1.35				
	Control	5.5	3.5	9.0	-	-	-	-
	Rr	5.2	3.2	8.4	6.6	1.5	-	MR
KWR - 108	Fs	5.3	3.3	8.6	4.4	-	3.2	R
	Cr	4.4	2.6	7.0	22.2	-	-	S
	Ni	4.7	3.1	7.8	13.3			T
L.S.D. (at 5% level)				0.95				
L.S.D. (at 1% level)				1.57				
	Control	3.6	2.2	5.8	-	-	-	-
	Rr	2.0	1.0	3.0	48.2	9.7	-	HS
Pant – 186	Fs	3.1	1.7	4.8	17.2	-	45.0	S
	Cr	3.4	1.9	5.3	8.6	-	-	MR
	Ni	3.3	1.8	5.1	12.0			S
L.S.D. (at 5% level)				0.50				
L.S.D. (at 1% level)				0.85				

Contd.....

Table-9: (Contd.) Page 3

Variety	Treatments	Plant dry weight (g)			% dry weight reduction over control	R=Pf/Pi	Disease index (%)	Reaction
		Shoot	Root	Total				
	Control	4.7	2.8	7.5	-	-	-	-
	Rr	4.6	2.7	7.3	2.6	0.6	-	R
Phule – G 8602	Fs	4.7	2.5	7.2	4.0	-	2.0	R
	Cr	4.7	2.7	7.4	1.3	-	-	R
	Ni	4.6	2.7	7.3	2.6			R
L.S.D. (at 5% level)				0.82				
L.S.D. (at 1% level)				1.35				
	Control	4.1	3.5	7.6	-	-	-	-
	Rr	3.8	3.0	6.8	10.5	2.1		T
Phule G 92028	Fs	3.0	2.8	5.8	23.6	-	46.8	S
	Cr	3.7	2.6	6.3	17.1	-	-	S
	Ni	3.4	2.8	6.2	18.4			S
L.S.D. (at 5% level)				0.62				
L.S.D. (at 1% level)				1.03				
	Control	5.4	4.0	9.4	-	-	-	-
	Rr	4.8	3.0	7.8	17.0	6.8		S
Phule G 96020	Fs	5.0	2.9	7.9	15.9	-	30.7	S
	Cr	4.7	2.5	7.2	23.4	-	-	S
	Ni	4.6	2.6	7.2	23.4			S
L.S.D. (at 5% level)				0.70				
L.S.D. (at 1% level)				1.32				
	Control	6.3	4.2	10.5	-	-	-	-
	Rr	4.0	2.4	6.4	39.0	10.2	-	HS
Pragati	Fs	5.0	3.2	8.2	21.9	-	48.0	S
	Cr	4.9	3.1	8.0	23.8	-	-	S
	Ni	4.6	2.9	7.5	28.5			HS
L.S.D. (at 5% level)				1.88				
L.S.D. (at 1% level)				3.12				
	Control	4.7	3.3	8.0	-	-	-	-
	Rr	3.0	2.0	5.0	37.5	11.5	-	HS
Pusa - 1103	Fs	2.7	2.1	4.8	40.0	-	60.1	HS
	Cr	4.8	2.2	7.0	12.5	-	-	T
	Ni	3.9	2.6	6.5	18.7			S
L.S.D. (at 5% level)				0.73				
L.S.D. (at 1% level)				1.21				
	Control	6.6	5.4	12.0	-	-	-	-
	Rr	4.5	2.5	7.0	41.6	11.0	-	HS
Pusa – 120	Fs	5.2	4.5	9.7	19.1	-	43.5	S
	Cr	5.3	4.3	9.6	20.0	-	-	S
	Ni	5.5	4.2	9.7	19.1			
L.S.D. (at 5% level)				1.48				
L.S.D. (at 1% level)				2.45				

Contd.....

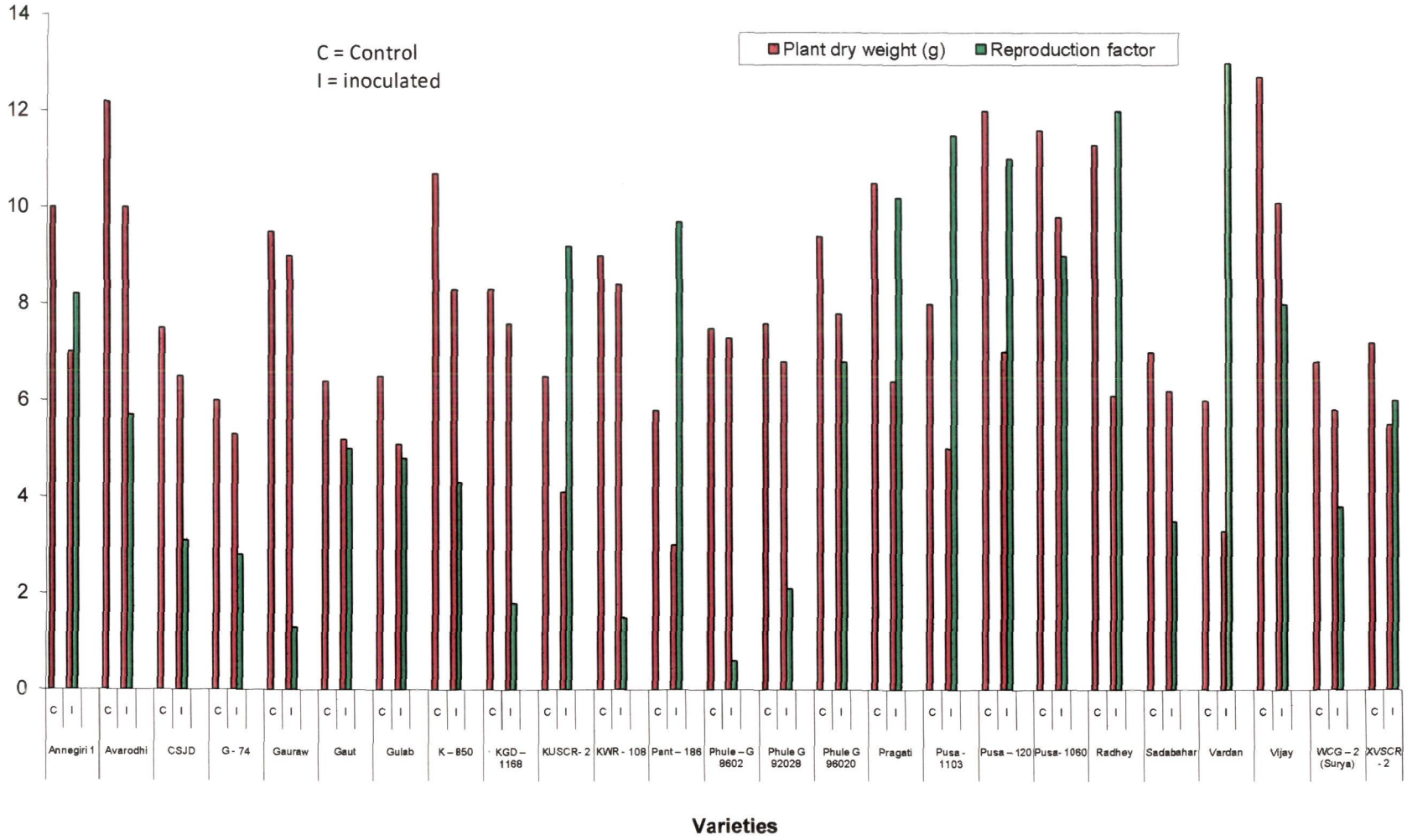
Table-9: (Contd.) Page 4

Variety	Treatments	Plant dry weight (g)			% dry weight reduction over control	R=Pf/Pi	Disease index (%)	Reaction
		Shoot	Root	Total				
	Control	6.0	5.6	11.6	-	-	-	-
	Rr	5.8	3.9	9.8	15.5	9.0	-	S
Pusa-	Fs	5.9	3.3	9.2	20.6	-	34.0	S
1060	Cr	5.0	5.0	10.0	13.7	-	-	T
	Ni	4.8	4.9	9.7	16.3			S
L.S.D. (at 5% level)				1.42				
L.S.D. (at 1% level)				2.35				
	Control	6.0	5.3	11.3	-	-	-	-
	Rr	4.7	1.4	6.1	46.0	12.0	-	HS
Radhey	Fs	4.8	2.5	7.3	35.3	-	53.2	HS
	Cr	6.3	4.2	10.5	7.0	-	-	MR
	Ni	5.1	4.2	9.3	17.6			S
L.S.D. (at 5% level)				1.52				
L.S.D. (at 1% level)				2.52				
	Control	4.5	2.5	7.0	-	-	-	-
	Rr	4.1	2.1	6.2	11.4	3.5	-	T
Sadabahar	Fs	4.0	2.0	6.0	14.2	-	24.4	T
	Cr	3.9	1.9	5.8	17.1	-	-	S
	Ni	3.6	1.8	5.4	22.8			S
L.S.D. (at 5% level)				0.70				
L.S.D. (at 1% level)				1.16				
	Control	3.7	2.3	6.0	-	-	-	-
Vardan	Rr	2.0	1.3	3.3	45.0	13.0	-	HS
	Fs	2.0	1.1	3.1	48.3	-	70.0	HS
	Cr	3.4	1.4	4.8	20.0	-	-	S
	Ni	3.2	1.8	5.0	16.6			S
L.S.D. (at 5% level)				0.62				
L.S.D. (at 1% level)				1.03				
	Control	7.1	5.6	12.7	-	-	-	-
	Rr	5.7	4.4	10.1	20.4	8.0	-	S
Vijay	Fs	4.0	2.9	6.9	45.6	-	62.0	HS
	Cr	6.2	4.0	10.2	19.6	-	-	S
	Ni	5.6	4.0	9.6	24.4			S
L.S.D. (at 5% level)				1.54				
L.S.D. (at 1% level)				2.55				
	Control	4.4	2.4	6.8	-	-	-	-
WCG – 2	Rr	3.5	2.3	5.8	14.7	3.8	-	T
(Surya)	Fs	4.2	2.1	6.3	7.3	-	12.5	MR
	Cr	4.6	1.4	6.0	11.7	-	-	T
	Ni	4.0	1.4	5.4	20.5			S
L.S.D. (at 5% level)				0.57				
L.S.D. (at 1% level)				0.94				
	Control	4.2	3.0	7.2	-	-	-	-
	Rr	3.7	1.8	5.5	23.6	6.0	-	S
XVSCR -	Fs	2.6	1.6	4.2	41.6	-	65.1	HS
2	Cr	3.6	2.5	6.1	15.3	-	-	S
	Ni	3.6	1.7	5.3	26.3			HS
L.S.D. (at 5% level)				1.35				
L.S.D. (at 1% level)				2.24				

The data presented in Table 9 and Fig. 8 revealed that out of 25 chickpea varieties, 8 were highly susceptible, 8 susceptible, 5 tolerant , 3 moderately resistant and one resistant against the reniform nematode , *R. reniformis* . Chickpea varieties viz. Annegiri-1, KUSCR-2, Pant-186, Pragati, Pusa-1103, Pusa-120, Radhey and Vardan were found highly susceptible to *R. reniformis* on the basis of reduction in plant growth (30.0, 36.9, 48.2, 39.0, 37.5, 41.6, 46.0 and 45.0%, respectively) and reproduction factor (8.2, 9.2, 9.7, 10.2, 11.5, 11.0, 12.0 and 13.0, respectively).The varieties viz. Avarodhi, Gaut, Gulab, K-850, Phule G 96020, Pusa-1060, Vijay and XVSCR-2 were recorded as susceptible to *R. reniformis* on the basis of plant growth reduction (18.0, 18.7, 21.5, 22.4, 17.0, 15.5, 20.4 and 23.6%,respectively) and reproduction factor (5.7, 5.0, 4.8, 4.3, 6.8, 9.0, 8.0 and 6.0, respectively). The varieties, CSJD, JG-74, Phule G 92028, Sadabahar and WCG-2 (Surya) exhibited tolerant reaction towards *R. reniformis* with reduction in plant growth (13.3, 1.7, 10.5, 11.4 and 14.7 %, respectively) and reproduction factor (3.1, 2.8, 2.1, 3.5 and 3.8, respectively) of the nematode. Three varieties (Gauraw, KGD-1168 and KWR-108) on the basis of reduction in plant growth (5.2, 8.4 and 6.6%, respectively) and reproduction factor (1.3, 1.8 and 1.5, respectively) were rated as moderately resistant. Only one variety (Phule G 8602) was found to be resistant against *R. reniformis* with 2.6% reduction in plant growth and 0.6 reproduction factor.

Out of 25 chickpea varieties, 7 were highly susceptible, 12 susceptible, 3 tolerant, 1 moderately resistant and 2 resistant against the root- rot fungus, *F. solani*. Chickpea varieties viz. Annegiri-1, KUSCR-2, Pusa-1103, Radhey, Vardan, Vijay and XVSCR-2 were found highly susceptible to *R. reniformis* on the basis of reduction in plant growth (32.0, 38.4, 40.0, 35.3, 48.3, 45.6 and 41.6%, respectively) and disease index of *F. solani* (50.6, 57.5, 60.1, 53.2,

Fig. 8: Screening of chickpea varieties for resistance against reniform nematode, *Rotylenchulus reniformis* (Rr).



70.0, 62.0 and 65.1 %, respectively). The varieties viz. Avarodhi, CSJD, Gauraw, Gaut, Gulab, K-850, Pant-186 , Phule G 92028, Phule G 96020, Pragati, Pusa-120 and Pusa-1060 were recorded as susceptible to *F. solani* on the basis of plant growth reduction (23.7, 22.6, 15.7, 21.8, 16.9, 19.6, 17.2, 23.6, 15.9, 21.9, 19.1 and 20.6 %, respectively) and disease index of *F. solani* (40.1, 32.0, 42.5, 35.3, 38.3, 37.0, 45.0, 46.8, 30.7, 48.0, 43.5 and 34.0 %, respectively). The varieties JG-74, KGD-1168 and Sadabahar exhibited tolerant reaction towards *F. solani* on the basis of reduction in plant growth (11.6, 10.8 and 14.2%, respectively) and disease index (20.7, 22.0 and 24.4 %, respectively). Only one variety WCG-2 (Surya) with reduction in plant growth (7.3%) and disease index (12.5%) was rated as moderately resistant. Two chickpea varieties viz. KWR-108 and Phule- G 8602 were found to be resistant against *F. solani* on the basis of reduction in plant growth (4.4 and 4.0%, respectively) and disease index (3.2 and 2.0%, respectively) caused by the fungus (Table 9 and Fig. 8.1).

The data presented in Table 9 and Fig. 8.2 revealed that out of 25 chickpea varieties, 15 were susceptible, 7 tolerant, two moderately resistant and 1 resistant against the heavy metal, chromium. Chickpea varieties viz. Annegiri-1, CSJD, JG-74, Gauraw , KGD-1168, KUSCR-2, KWR-108, Phule G 92028, Phule G 96020, Pragati, Pusa-120 , Sadabahar , Vardan, Vijay, and XVSCR-2 were found highly susceptible to chromium on the basis of percent reduction in growth of treated plants (18.0, 16.0, 16.6, 21.0, 16.8, 15.3, 22.2, 17.1, 23.4, 23.8, 20.0, 17.1, 20.0, 19.6 and 15.2%). The varieties namely Avarodhi, Gaut, Gulab, K-850, Pusa-1103, Pusa-1060 and WCG-2 (Surya) showed tolerant response towards chromium on the basis of plant growth reduction (11.4, 14.0, 7.6, 12.1, 12.5, 13.7 and 11.7%, respectively). Two varieties (Pant-186 and Radhey) were rated as moderately resistant to Cr on the basis of reduction in plant growth (8.6 and 7.0 %, respectively). Only one

Fig. 8.1: Screening of chickpea varieties for resistance against root-rot fungus, *Fusarium solani* (Fs).

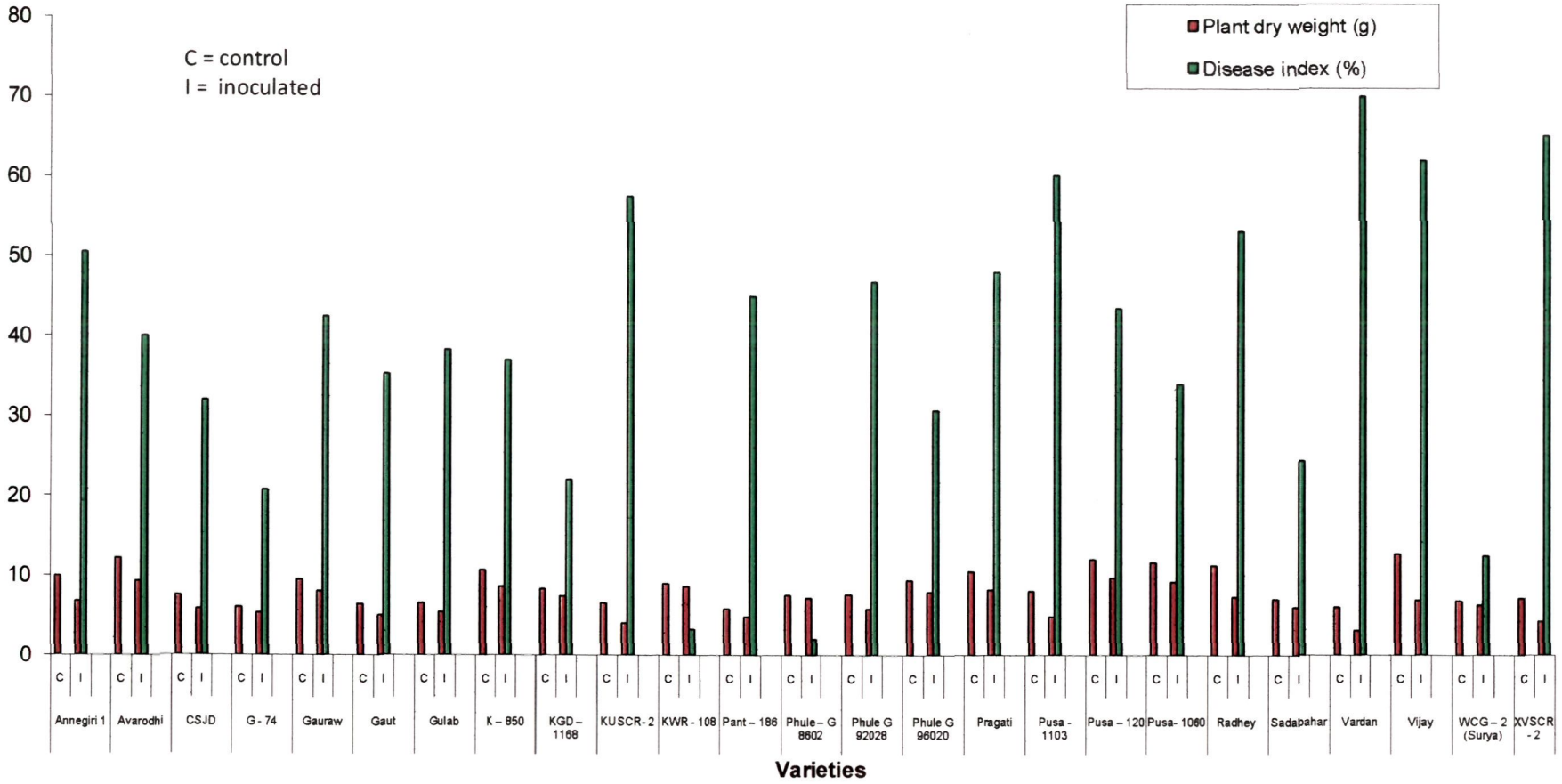
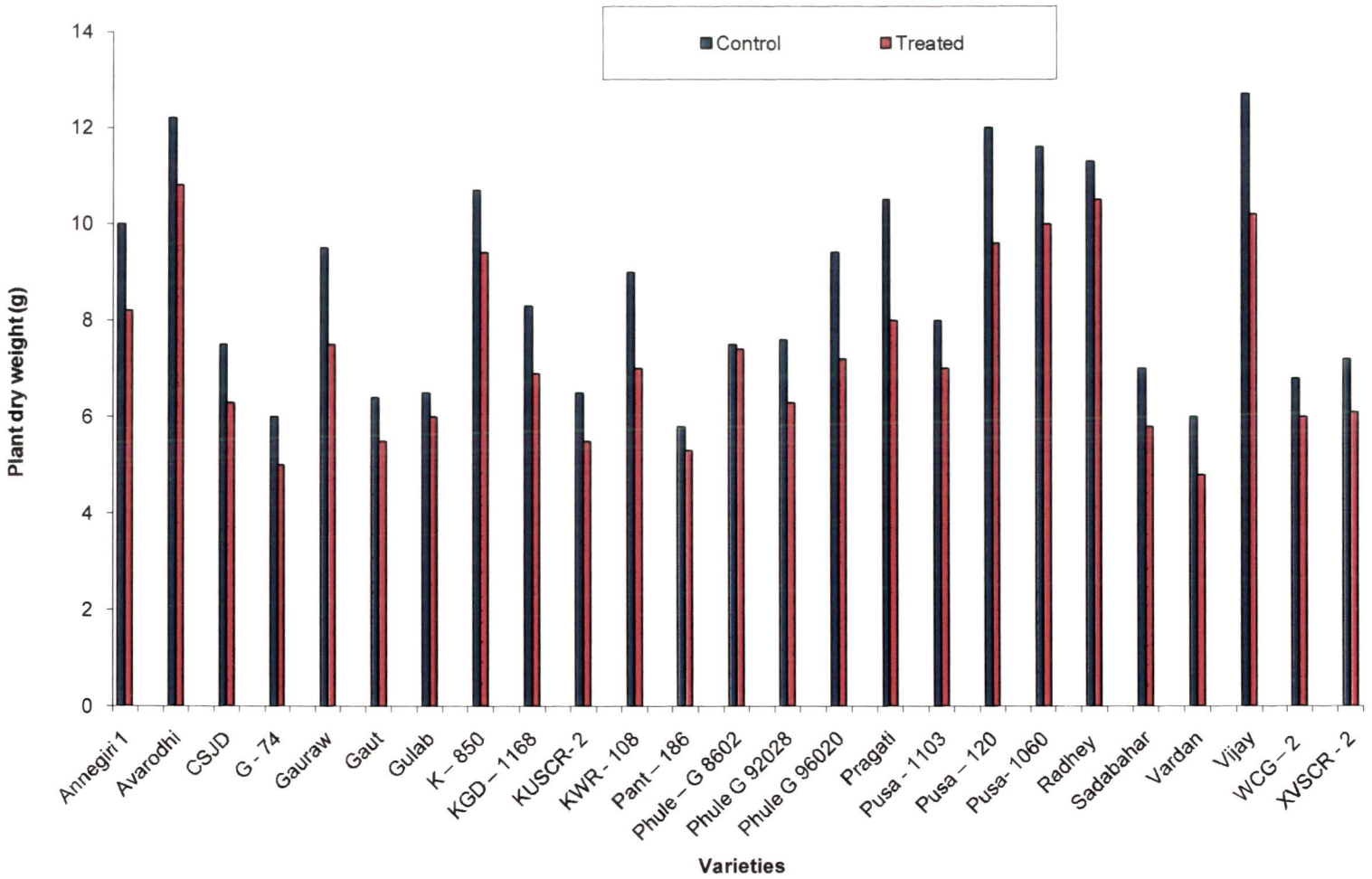


Fig. 8.2: Screening of chickpea varieties for resistance against heavy metal , chromium.



variety (Phule G 8602) was recorded as resistant to chromium with only 1.3 % reduction in plant growth.

Out of 25 chickpea varieties, 2 were highly susceptible, 19 susceptible, 3 tolerant and one resistant against the nickel .Two varieties namely Pragati and XVSCR-2 with 28.5% and 26.3% reduction in plant growth respectively were rated as highly susceptible to Ni. Varieties viz. Annegiri-1, Avarodhi, CSJD, JG-74, Gaut, Gulab, K-850, KGD-1168, KUSCR-2, Phule G 92028, Phule G 96020, Pusa-1103, Pusa-120, Pusa-1060, Radhey , Sadabahar, Vardan, Vijay and WCG-2 (Surya) were found susceptible to nickel on the basis of percent reduction in growth of treated plants (16.0, 16.3, 20.0, 21.6, 15.6, 24.6, 18.6, 20.4, 21.5, 18.4, 23.4, 18.7, 19.1, 16.3, 17.6, 22.8, 16.6, 24.4 and 20.5, respectively). Three varieties viz. Gauraw, KWR-108 and Pant-186 showed tolerant response towards nickel on the basis of reduction in plant growth (14.7, 13.3 and 12.0%, respectively). Only one variety (Phule G 8602) was recorded as resistant towards nickel with only 2.6 % reduction in plant growth (Table 9 and Fig. 8.3).

It was clear from the above results that the chickpea variety Phule G 8602 showed resistant reaction towards both the pathogens (*R. reniformis* and *F. solani*) and heavy metals (Cr and Ni). Therefore, the chickpea variety Phule G 8602 was again tested for resistance to check whether the resistance persisted in this variety if it is grown in soil concomitantly treated with heavy metal (Cr / Ni) and test pathogen (*R. reniformis* / *F. solani*).

The results presented in Table 9.1 and Fig. 8.4 clearly indicated that the variety Phule G 8602 showed resistant response towards *R. reniformis*, *F. solani*, Cr and Ni when the same chickpea variety grown in soil treated with either Cr or Ni even in the presence of either *R. reniformis* or *F. solani*. Inoculation of this variety with *R. reniformis* and *F. solani* caused 3.1 and 5.2

Fig. 8.3: Screening of chickpea varieties against heavy metal , nickel.

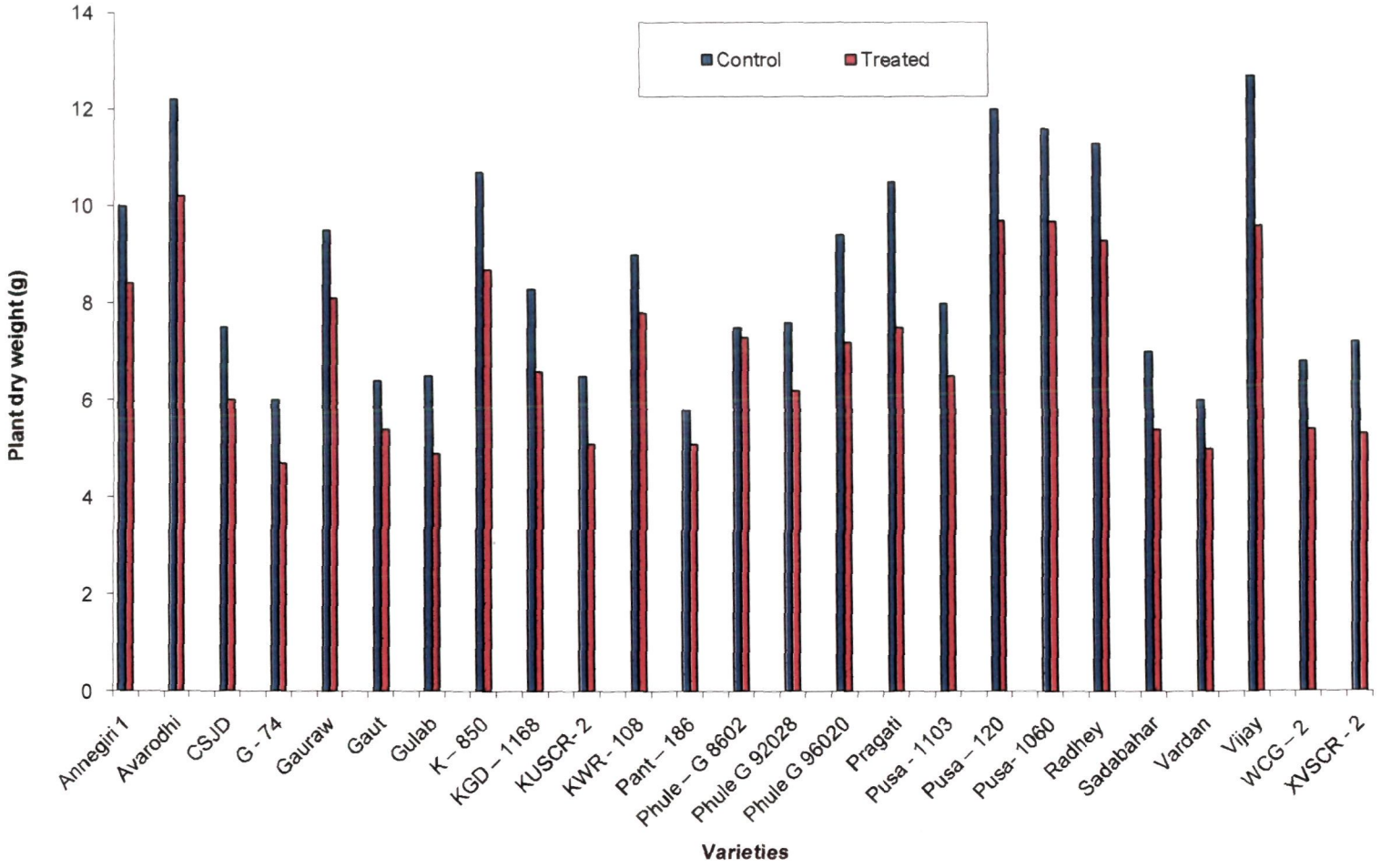
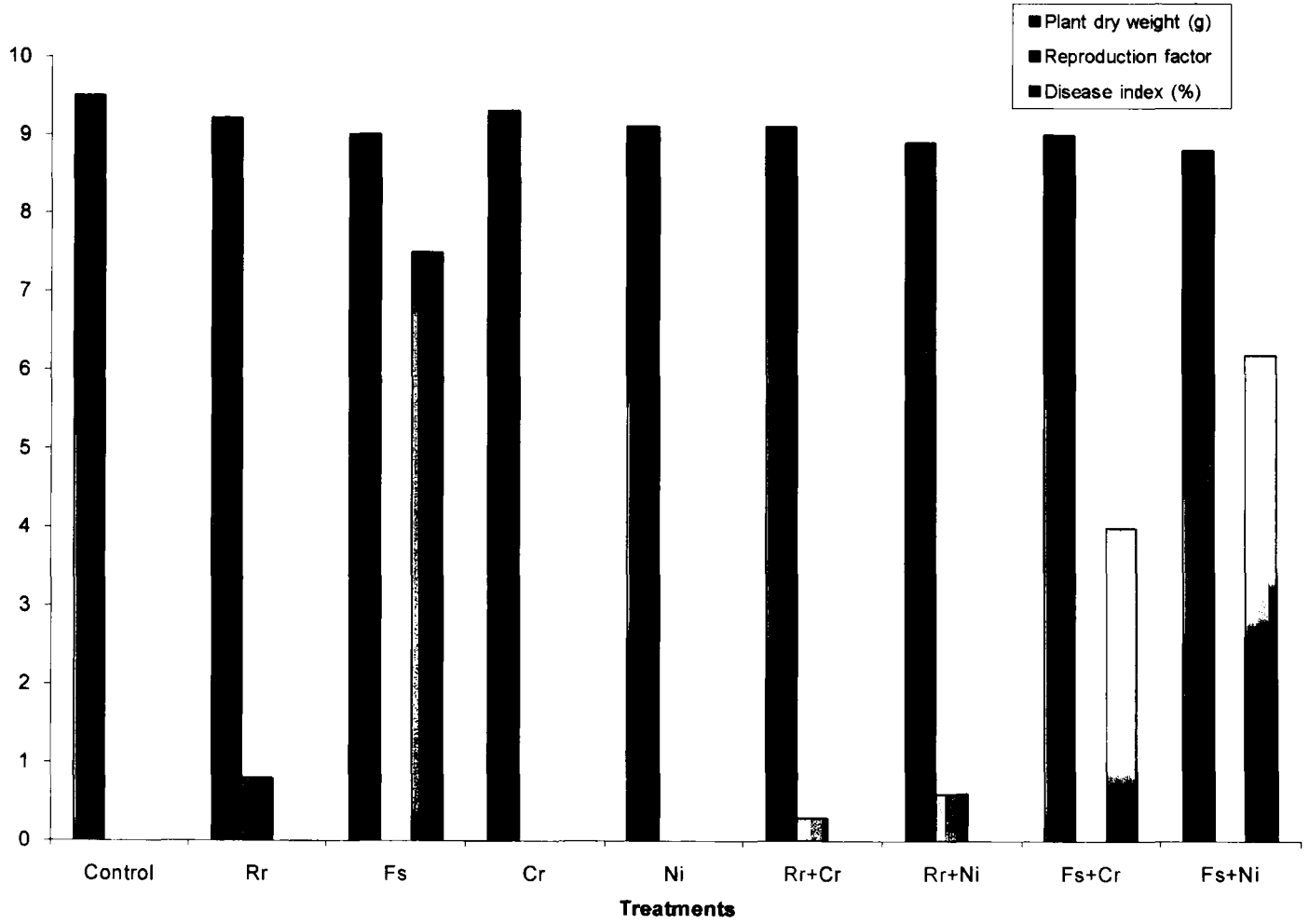


Table-9.1: Screening of chickpea variety Phule G 8602 against the test pathogens viz. *Rotylenchulus reniformis* (Rr) / *Fusarium solani* (Fs) and / or heavy metals viz. chromium (Cr) / Nickel (Ni).

Treatments	Plant dry weight (g)			% dry weight reduction over control	R=Pf/Pi	Disease index (%)	Reaction
	Shoot	Root	Total				
Control	6.2	3.3	9.5	-	-	-	-
Rr	6.1	3.1	9.2	3.1	0.8	-	R
Fs	6.0	3.0	9.0	5.2	-	7.5	R
Cr	6.1	3.2	9.3	2.1	-	-	R
Ni	6.1	3.0	9.1	4.2	-	-	R
Rr+Cr	6.0	3.1	9.1	4.2	0.3	-	R
Rr+Ni	6.1	2.8	8.9	6.3	0.6	-	R
Fs+Cr	5.8	3.2	9.0	5.2	-	4.0	R
Fs+Ni	5.7	3.1	8.8	7.3	-	6.2	R
L.S.D. (at 5% level)			1.00		0.62	5.00	
L.S.D. (at 1% level)			1.45		1.42	11.52	

Fig. 8.4: Screening of chickpea variety Phule G 8602 against the test pathogens viz. *Rotylenchulus reniformis* (Rr) / *Fusarium solani* (Fs) and / or heavy metals viz. chromium (Cr) / nickel (Ni).



% reduction in plant growth as compared to control. The reproduction factor of reniform nematode and percent disease index of root-rot fungus was recorded as 0.8 and 7.5, respectively. Similarly, the treatments of Cr and Ni resulted in 2.1 and 4.2 % reduction in plant growth of chickpea variety Phule G 8602. The reduction in plant growth was found as 4.2 and 6.3 % in the plants inoculated with *R. reniformis* and soil treated with either Cr or Ni, respectively. The reproduction factor of *R. reniformis* was recorded as 0.3 and 0.6 in the corresponding treatments. Similarly, the reduction in plant growth was observed as 5.2 and 7.3 % in plants inoculated with *F. solani* and treated with either Cr or Ni, receptively. The disease index of *F. solani* was recorded as 4.0 and 6.2% in the corresponding treatments. Therefore, it was concluded from these results that chickpea variety Phule G 8602 did not lose its resistance even in concomitant presence of test pathogen and heavy metal.

Discussion

Man is aware of the problems of maintaining himself and his descendants on the planet of fixed size. Not only the problem of food supply is becoming critical, but the waste and the end products of man's existence must be disposed off or utilized in such a way that the quality of his environment is not impaired for this or future generations. However, the lavish life styles and comforts have been achieved at the cost of many environmental problems, the pollution being the foremost. Pollution may be defined as an undesirable accumulation of residues of various organic and inorganic materials in soils and water as a result of man's activities. The problem of pollution would appear to have three phases-uncontrolled release of materials into ecosystems, release of pollutants from disposal systems because of improper or insufficient treatment or lack of knowledge concerning possible hazards and use of materials for specific purposes on land and vegetation which may have unrecognized pollutant hazards due to other properties of materials.

Heavy metals like lead, cadmium, chromium, nickel, mercury etc. have significantly been detected in soils due to industrial effluents, organic wastes, refuse burning, transport, power generation, smoke release from domestic and industrial chimneys etc. and are also found to depress the plant growth and yield at their different levels of application. Similarly, microorganisms including plant parasitic nematodes and plant-pathogenic fungi may also be greatly influenced by heavy metal contamination in the soil. Information with respect to the impact of heavy metals on reniform nematode, *Rotylenchulus reniformis* and root-rot fungus *Fusarium solani* on chickpea is not available. The focal theme of the present study is to assess the effect of heavy metals viz. Cr and Ni on pathogenic potential and management of *R. reniformis* and *F. solani* infecting chickpea. The results of different experiments are discussed in detail in the present chapter.

5.1: IDENTIFICATION OF RACE OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* ASSOCIATED WITH CHICKPEA:

Since biological races are known to occur in *Rotylenchulus reniformis*, it was considered desirable to study the populations of reniform nematode in the present study and identify its race before initiating the research work so that the results obtained may be interpreted more scientifically and with authenticity. The results (Table 1 and Figs 1 to 1.1) clearly showed that all the isolates of *R. reniformis* were able to attack and multiply on castor, cowpea, cotton and mustard, but these populations were unable to infect bajra, therefore, the populations of *R. reniformis* collected from different locations belonged to the same race, which is designated as Race-3.Hence *R. reniformis* Race-3 was used for the experimental purposes. So far there is no information regarding the races of reniform nematode associated with chickpea. Moreover, there are some reports which showed the occurance of different races of reniform nematode on different plants (Dasgupta and Seshadri, 1971; Nakasona, 1983; Khan, 1986; Prasada Rao and Ganguly, 1996).

5.2: EFFECT OF CHROMIUM AND NICKEL ON THE HATCHING AND MORTALITY OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS IN VITRO*:

It is evident from the results that both the heavy metals (Cr and Ni) were inhibitory to nematode hatching, Cr being more toxic than Ni. The percentage mortality of *R. reniformis* was correlated with the concentrations of heavy metals and exposure period. There was a gradual increase in the nematode mortality with an increase in the exposure period and the concentration of either Cr or Ni (Tables 2 to 2.1 and Figs 2 to 2.1).

In the present study, increase in the concentration of both Cr and Ni resulted in the increased inhibition in the hatching of *R. reniformis*. Khan and Salam (1990) in conformity with the present findings recorded that Ni was

inhibitory to the hatching of *Meloidogyne javanica* at all concentrations except 9.71 mg / l. Similar findings with different heavy metals on plant parasitic nematodes have also been reported by Robinson and Neal (1959), Khan *et al.* (1994) and Parveen and Alam (1999a). Robinson and Neal (1959) reported that adding microgram amounts of zinc sulphate and cadmium chloride to dithizone - extracted potato root diffusate inhibited hatching of *Heterodera rostochiensis* and they suggested that the metal ions were responsible for the inhibition of hatching. Khan *et al.* (1994) reported that the hatching of *M. incognita* was greatly suppressed by all the concentrations of cobalt used. Parveen and Alam (1999a) reported that with an increase in the concentration of Pb and Cd, there was a gradual increase in the inhibition of hatching of *M. incognita*, however, the former was reported to be more toxic. Moreover, my findings are contradictory with Clarke and Shepherd (1965, 1966) who indicated that several ions including Ni^{2+} and Cr^{3+} stimulated the hatching of *Heterodera* spp.

In the present study, the mortality of the nematode increased with an increase in the concentrations of heavy metals and the exposure periods. Adverse effect of the heavy metals on mortality of plant parasitic nematodes has been reported by others also (Khan and Salam, 1990; Khan *et al.* 1994; Parveen and Alam, 1999a). Khan *et al.* (1994) reported that all the concentrations of cobalt were effective in inducing mortality of second stage larvae of *M. incognita*. Khan and Salam (1990) found that Ni and Co were toxic to the second stage juveniles of *M. javanica* and all the juveniles were killed in 9710 mg/l of Ni and Co at and above 1111.7 mg/l. However, on the other hand, Jaworska *et al.* (1997) reported that several metals including Cr and Ni (except Pb II) even at naturally unrealistic concentrations did not cause mortality of *Heterorhabditis bacteriophora.*

According to Clarke and Shepherd (1966), it seems more probable that heavy metal ions are taken up by some constituent of egg or larvae, which alter the structure and function of the binding materials viz. nucleic acids and

proteins that provide many suitable ligands. The binding of ions by either nucleic acid or protein might alter their secondary and tertiary structure enough to change their behavior within the biological system, where they occur, which might be responsible for the mortality and inhibition of hatching of nematode. The variable effect of heavy metals on hatching and mortality of nematode could be due to the differences in the relative toxicity of heavy metals.

5.3: EFFECT OF CHROMIUM AND NICKEL ON THE GROWTH, SPORULATION AND HEAVY METAL UPTAKE OF *FUSARIUM SOLANI IN VITRO*:

The effect of heavy metals viz. Cr and Ni on the growth and sporulation of *Fusarium solani* and, uptake of these metals by *F. solani* was assessed *in vitro*. The results revealed that the growth and sporulation of *F. solani* significantly decreased with an increase in the concentration of Cr or Ni (except at 25 ppm Cr and, 25 and 50 ppm Ni). Moreover, the growth and sporulation of the fungus was significantly enhanced when it was grown in medium added with 25 ppm Ni. As far as the uptake of the heavy metal in the fungus was concerned, it increased with the increasing concentration of the heavy metals. However, the uptake of Ni by *F. solani* was more than that of Cr. Chlamydospore formation initiated at and above 50 ppm of Cr and 100 ppm of Ni, which increased further with an increase in the concentration of the heavy metals (Table 3 and Fig. 3).

The above results are in consonance with the findings of Singh *et al.* (1992), who reported that with an increase in the concentration of Cr and Ni, there was a corresponding decrease in the growth of fungus *Paecilomyces lilacinus* irrespective of the fact whether the fungus was raised either in liquid or solid medium. Similarly, Parveen and Alam (1993, 1997) reported that Cd and Pb were inhibitory to the growth of *P. lilacinus* and Pb was more toxic than Cd. The effect of heavy metals on the growth of different fungi have also been reported by various workers

from time to time (Babich and Stotzky, 1979; Lokesha and Somashekar, 1990; Kredics *et al.*, 2001b; Levinskaite, 2001). Moreover, according to Lokesha and Somashekar (1990) *Aspergillus versicolor* and *Colletotrichum dematium* showed good growth on Ni amended medium with 100mg/l as compared to control, which was found to be against my findings. This difference might be attributed to the use of different genera of fungi used in the experiments.

The sporulation of *F. solani* decreased with an increase in the concentration of Cr or Ni (except at 25ppm Ni), which holds true with the findings of Levinskaite (2001) who reported that conidiogenisis of *Penicillium* was affected by Ni. Rajendran *et al.* (2002) also tested the toxicity of $NiCl_2$ to *Aspergillus niger* and found that 1.7 mM nickel caused 50% conidial inhibition. Similar effects of Pb and Cd on the sporulation of *P. lilacinus* has also been reported by Parveen and Alam (1993, 1997). The inhibition in the growth and sporulation of *F. solani* in the presence of Cr and Ni may be due to the toxicity of these heavy metals. Another probable reason may be the interference of these metals in different metabolic activities such as enzyme synthesis and other biochemical reactions etc. (Kredics *et al.*, 2001a). The heavy metals had more toxic effects on the development of macroconidia as compared to that of microconidia which could be probably due to the reason that macroconidia had more absorption and accumulation of heavy metals because of their bigger size and greater surface area. It was interesting to note that there was no chlamydospore formation by *F. solani* in the medium either free from heavy metals or medium incorporated with 25ppm Cr and, 25 and 50ppm Ni. Moreover, the higher concentrations of either Cr or Ni lead to the formation of chlamydospores by *F. solani*, which might be due to the survival of the fungus under stress conditions created by these heavy metals. It is commonly known that *F. solani*

produces chlamydospores under unfavorable conditions. According to Booth (1971), this fungus abundantly produces only chlamydospores in nutrient deficient medium, however, it produces conidia when the nutrient status of the medium is raised by the addition of glucose (Alexander, 1965).

In the present study the heavy metals viz. Cr and Ni were taken up by the *F. solani* and it was increased with an increase in the concentration of heavy metals. Similar results on the uptake of copper by *Aspergillus* species has also been reported by Chandra and Muthumary (1993).

Narayana and Manoharachary (1994) reported that the fungi belonging to genera *Aspergillus, Penicillium* and *Trichoderma* were tolerant and highly adapted to the effluents containing heavy metals. However, some Mucorales and Ascomycetes were reported to be sensitive, towards heavy metals. It was concluded that the predominantly occurring fungi might have degraded the effluent residues and later multiplying with the help of some useful metabolites. Nordgren *et al.* (1985) also found that the fungal community was strongly affected by the heavy metal pollution.

The differences in the effect of Cr and Ni on growth, sporulation and their uptake by *F. solani* may be due to their variable toxicity.

5.4: STUDIES ON POTENTIAL PATHOGENIC LEVEL OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* AND ROOT-ROT FUNGUS *FUSARIUM SOLANI* ON CHICKPEA:

To determine the potential pathogenic level of reniform nematode and root-rot fungus, the seedlings of chickpea were separately inoculated with different inoculum level of *Rotylenc* ıd 8000 immature females per plant) and *F. solani* (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 g

per plant). There was a gradual increase in the reduction of plant growth, nodulation, yield, chlorophyll content, protein content and water absorption capacity of roots of chickpea with increase in inoculum level of *R. reniformis* except at the inoculum level of 250 immature females per plant which slightly increased plant growth as compared to uninoculated plants. However, the significant reduction in the above mentioned parameters of chickpea plants was recorded at and above 1000 immature females of reniform nematode.

The rate of nematode multiplication of *R. reniformis* decreased with an increase in the inoculum level. Since the root surface area for both, the lower and higher inoculum levels remained the same, crowding of reniform nematode at higher inoculum densities created competition among the nematodes, which resulted in their natural death and reduced multiplication. The high rate of nematode multiplication at low levels of inocula, on the other hand, could possibly be due to the positive factors like abundance of food, lack of competition and the ability of host to support these population levels. According to Oostenbrink (1966), the increase in the nematode populations and subsequent reductions in the yield of crop are directly influenced by the initial density of nematodes in the soil. His view holds true with the present findings wherein plant growth and yield of chickpea was proportionately affected with an increase in the initial inoculum levels of nematode. The progressive decrease in the plant growth parameters and nematode multiplication with increasing inoculum of *R. reniformis* on chickpea has also been reported by various workers (Mahapatra and Padhi, 1986; Ahmad *et al.*, 1987; Daraker and Jagdale, 1987; Zaidi *et al.*, 1988; Tiyagi and Alam, 1987; 1990).

In present study, the percentage of disease index of *F. solani* increased with increase in the inoculum level. Similarly, a direct correlation between increasing initial inoculum level with decreasing plant growth, yield, nodulation, chlorophyll content, protein content and water absorption capacity of roots was observed for the root-rot fungus, *F. solani.* However, the

significant reduction in respective parameters of chickpea was observed at and above 3.0 g of *F. solani* / plant. These results are in agreement with those of Mani (1982) and Khan and Husain (1991) who reported that with an increase in the inoculum level of *F. solani*, there was a corresponding increase in the plant growth reduction and the percentage of root-rot of chickpea and papaya, respectively.

The reduction in plant growth parameters may be due to physiological and structural alterations caused by the pathogens. The infection caused by reniform nematode and root-rot fungus might disrupt the root system and interferes with the physiological process involved in water and nutrient relations and the phytohormones originating in the roots (primary factors) , thereby creating a cascade effect on chlorophyll synthesis, photosynthesis and respiration in shoot (secondary factors) . The combination of these primary and secondary effects may lead to the reduction in plant growth, yield, protein content and chlorophyll content as compared to uninfected plants (Melakeberhan, 2004).My results are also in agreement with those of Tiyagi and Alam (1990) and Murukumar and Chavan, (1985) who reported that chlorophyll content in the leaves of chickpea decreased in plants infected with *R. reniformis* and Fusarium wilt fungus, respectively. Similarly, my results are in accordance with Khan *et al.* (1996) who reported that protein content of chickpea seeds decreased in plants infected with *Meloidogyne incognita.*

The reduction in the number of nodules in chickpea plants infected with either *R. reniformis* or *F. solani* might be due to the adverse effect of toxic substances from nematode and fungus infected roots on the rhizobium itself and / or due to nutritional interference particularly carbohydrates (Nutman, 1958). It is well known that rhizobial infection takes place through the root hairs into the cortex where the sites of nodulation exist. As the nematode and fungus infection depletes the root hairs, the rhizobial infection is inhibited. Reduction in nodulation may also be attributed to some changes in the host

metabolism due to nematode / fungus infection which makes it unsuitable or less preferred by the rhizobium. The reduction in number of nodules in the plants infected with *R. reniformis* was also reported by Taha and Raski (1969), Darekar and Jagdale (1987) and Tiyagi and Alam (1987). Similarly, these results are in agreement with the findings of Mani (1982) who reported that number of nodules decreased in chickpea plants infected with *F. solani.*

With an increase in the inoculum level of either *R. reniformis* or *F. solani*, there was a corresponding decrease in the water absorption capacity of chickpea roots, which is in agreement with the findings of Tiyagi and Alam (1990) who reported that inhibition in water absorption of chickpea plants was directly proportional to the inoculum level of *R. reniformis*. Khan (1986) also reported that the water absorption capability of cowpea roots was adversely affected by the infection of *R. reniformis, M. incognita* and *Rhizoctonia solani.* Subramanian and Saraswathidevi (1959) pointed that poor water absorption in diseased plants may be due to injury to roots as a result of infection with bacteria and viruses or due to the deformation , chocking and disturbance in the arrangement of tracheary elements. These possibilities cannot be ruled out in fungus or nematode infected plants as *F. solani* is an endoparasite known to damage, deform and disrupt the cortical and the conducting tissues of roots (Ren *et al.*, 2008), while , *R. reniformis* a semiendoparasite causes aberrations in the internal tissues (Agudelo *et al.*, 2005).Infection of either *R. reniformis* or *F. solani* inhibited the root growth and thus reduced total surface area of roots resulting in the poor absorption of water by the infected roots. Alternatively, the reduction in shoot weight (or leaf surface area) due to nematode or fungal infection might have also resulted in reduced transpiration pull which in turn retarded the water absorption capacity.

From the present investigations, it could be inferred that the potential pathogenic level of *R. reniformis* on chickpea is 1000 immature females / plant, which is in agreement with the finding of Tiyagi and Alam (1987) and Zaidi *et*

al. (1988) on chickpea. However, the results are contradictory with the finding of Mahaptra and Padhi (1986) who reported that an initial inoculum level of 500 nematodes / plant as pathogenic level on chickpea. According to Daraker and Jagdale (1987) and Sharma and McDonald (1990) the damage threshold level of *R. reniformis* on chickpea range from 1.0 to 2.0 nematodes per gram of soil. This variation in the inoculum threshold level of *R. reniformis* might due to different experimental conditions, races / strains of reniform nematode and chickpea variety used in their study.

Similarly, the potential pathogenic level of *F. solani* on chickpea was recorded as 3.0 g of *F. solani* per plant which is against the findings of mani (1982) and Khan and Husain (1991) who reported 4.0 g and 2.5 g of *F. solani* as pathogenic level in chickpea and papaya, respectively. This variation may also be attributed to the different experimental conditions, host status and different strains of *F. solani* used in the study.

The information gathered from the present study may provide a baseline for further research to develop appropriate strategies for the management of reniform nematode and root-rot fungus infecting chickpea.

5.5: EFFECT OF CHROMIUM AND NICKEL ON PATHOGENIC POTENTIAL OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* AND ROOT-ROT FUNGUS, *FUSARIUM SOLANI* INFECTING CHICKPEA:

An experiment was conducted to assess the effect of heavy metals (chromium and nickel) on the pathogenic potential of *Rotylenchulus reniformis* and *Fusarium solani* infecting chickpea. The results presented in Tables 5 to 5.7 clearly showed that with an increase in the concentration of heavy metal (Cr or Ni) from 25 to 200 ppm, there was a significant gradual increase in the reduction of plant growth, yield, nodulation, chlorophyll content, protein content and water absorption capacity of chickpea only at 100 and 200 ppm of Cr or Ni. Several studies have also revealed the adverse effects of chromium on

plant growth (Ottabbong, 1989; Sharma and Sharma, 1993; Shanker *et al.*,2005); yield (Sharma and Sharma, 1993; Panda and Choudhury,2005), nodulation (Wani *et al.*,2007), chlorophyll content (Rai *et al.*, 1992; Panda and Choudhury,2005), protein content (Rai *et al.*, 1992; Panda and Choudhury, 2005), and water absorption capacity of roots (Shanker *et al.*, 2005) of different plants. The reduction in plant growth parameters of chickpea in presence of Cr might be attributed to the deleterious effects of chromium on various physiological processes such as photosynthesis, water relations and mineral nutrition etc. (Cervantes *et al.*, 2001; Shanker *et al.* 2005). The reduction in the chlorophyll content in the Cr treated plants might be due to inhibition of chlorophyll synthesis and also an increase in chlorophyll degradation by heavy metal (Cervantes *et al.*, 2001; Shanker *et al.*, 2005).

The increase in the concentration of Ni in soil proved to be potentially toxic to chickpea plants, causing chlorosis and necrosis of leaves and reduced plant growth. Similar symptoms of Ni toxicity have also been reported on lettuce (Temple and Bisessar, 1981), celery (Bisessar *et al.*, 1983) and tomato (Khan *et al.*, 2006). Several studies have also revealed that nickel reduced the plant growth (Hagemeyer, 1999); yield (Hagemeyer,1999); nodulation (Wheeler *et al.*,2001); chlorophyll content (Mysliwa- Kurdziel *et al.*,1999) and water absorption capacity of roots (Poschenrieder and Barcelo, 1999) of different plants .It has been reported that the excess of nickel inhibits the chlorophyll biosynthesis and induces degradation (Krupa and Baszynski,1995; Abdel-Basset *et al.*, 1995) with a subsequent decrease in chlorophyll concentration (Molas, 2002) that is manifested as chlorosis and / or necrosis of foliage. There are considerable evidences that the excess of Ni acts as a potent inhibitor of growth, development and various metabolic processes in plants (Van Assche and Clijesters, 1990; Hagemeyer, 1999) which induces visible symptoms of phytotoxicity, depressing growth and dry matter of plants (Agarwala *et al.*, 1977; Austenfield, 1979; Hagmeyer *et al.*, 1999).

With an increase in the concentration of heavy metals (Cr and Ni), there was a corresponding decrease in the number of nodules per root system. This might be because the heavy metal pollutants reduce the population of symbiotic nitrogen fixing organisms (McGrath, 1994).

It is well known fact that heavy metals can act at different sites to inhibit a large number of enzymes having functional sulphydryl groups resulting in the disruption in the pathways of protein synthesis (Valle and Ulmer, 1972).According to Hampp *et al.* (1976) the relatively strong affinities of ligands of protein indicate that enzymes and other functional proteins are one of the prime targets of metal toxicity. These reasons might be responsible for the decrease of protein content with an increase in the concentration of Cr and Ni in the present study.

It is well known that the heavy metals may enter the xylem cells where they may form complexes with the elements of xylem cell sap and get deposited on the cell walls causing a hindrance in the supply of water to the different parts of plants (Panda and Patra, 2000). This might be the reason for reduction of water absorption capacity with an increase in the concentration of heavy metals (Cr and Ni) in the present study.

The inoculation of chickpea plants separately with *R. reniformis* and *F. solani* significantly reduced the plant growth, yield, nodulation, chlorophyll content, protein content and water absorption capacity of roots. However, the reniform nematode was less damaging than root-rot fungus.

In the plants inoculated with *R .reniformis* and grown in soil treated with 25, 50, 100 and 200 ppm concentrations of Cr and Ni, a synergistic reduction in plant growth, yield, nodulation, chlorophyll content, protein content and water absorption capacity of roots of chickpea was recorded except at 25ppm Cr. Moreover, the effect of interaction between *R. reniformis* with either of the heavy metals (Cr / Ni) on growth and other parameters was directly proportional to the concentrations of heavy metals. Khan *et al.* (1996) and

Khan *et al.* (2006) reported that the combined effect of Ni and root- knot nematodes viz. *M. javanica* and *M. incognita* on chickpea and tomato was synergistic at 50 ppm Ni, which is in agreement with my results also. At the same time they reported the combined effect of Ni and root-knot nematode on chickpea and tomato was not synergistic at 100 and 200 ppm Ni which is against my findings.

In the present study, the reproduction factor of the reniform nematode was significantly reduced with an increase in the concentration (25-200 ppm) of Ni / Cr. This might be due to the toxic effects of Cr / Ni to *R. reniformis*. However, Khan *et al.* (1996) and Khan *et al.* (2006) reported the increased number of galls, eggmasses, fecundity and soil population of *M. incognita* and *M. javanica* infecting chickpea and tomato, respectively at the 50 ppm Ni while it decreased with further increase in the concentration of heavy metal (100 and 200 ppm Ni). My observations are also in contrast with another study where even the higher concentration of Ni (7500 Ni / Kg soil) significantly increased the number of galls on celery roots (Bisessar *et al.*, 1983).But, the reason given to it was that the study was conducted in the soil contaminated with other metals (80 mg Cu and 100 mg Co / Kg soil). These differences in the results may also be due to the different nematodes and / or plants used in the experiments.

The plant growth, yield, nodulation, chlorophyll content, protein content and water absorption capacity of roots was synergistically reduced in the plants inoculated with *F. solani* and grown in soil treated with 25, 50, 100 and 200 ppm concentrations of Ni. However, on the other hand plants inoculated with *F. solani* and grown in soil treated with 25, 50, 100 and 200 ppm concentrations of Cr did not show synergistic effect on these parameters. Moreover, the effect of interaction between *F. solani* with either of the heavy metals on plant growth and other parameters was directly proportional to the concentration of heavy metal. Khan and Salam (1990) reported that the

interactive effect of *F. udum* and heavy metals on plant growth reduction was directly proportional to the concentration of heavy metals, which is in accordance with my results. In the present study, the disease index of *F. solani* showed a declining trend with increasing the concentration of heavy metals (Cr / Ni), which might be due to the toxic effects of Ni and Cr to *F. solani*. According to Khan and Salam (1990) nickel inhibited wilting caused by *F. udum* on pigeonpea, which is in confirmity with my findings. The inhibitory effect of Ni has also been reported against rust diseases on wheat (Chatrath *et al.*, 1974), groundnut (Seshadri, 1976) and sugarcane (Bachchhav *et al.*, 1978).

The present study demonstrates a concentration dependent relationship of heavy metals (Cr/Ni) in the soil with reniform nematode and root-rot fungus. The nematode or fungal infection can increase plant sensitivity to heavy metals in the soil which is also supported by my findings in which the accumulation of heavy metals increased in the plants grown in soil treated with heavy metals (Cr or Ni) and inoculated with either *R. reniformis* or *F. solani*. It can be concluded from the above study that the heavy metals in the soil can enhance the reduction in plant growth and yield of chickpea in presence of *R. reniformis* / *F. solani*.

5.6: ACCUMULATION OF CHROMIUM AND NICKEL IN CHICKPEA PLANTS INFECTED WITH *ROTYLENCHULUS RENIFORMIS* AND *FUSARIUM SOLANI*:

The amount of heavy metals viz. Cr and Ni in chickpea was estimated on dry weight basis by Atomic Absorption Spectrophotometery (AAS). It was interesting to note that Cr was accumulated by plants in a lesser amount than Ni. However, the concentration of the heavy metal accumulation was more in inoculated plants than the uninoculated plants. The heavy metals were accumulated in greater amounts by plants inoculated with fungus than the plants inoculated with the nematode. It was further noted that the amount of

heavy metals was more in roots than in the shoots (Tables 6 to 6.3). It was also observed that heavy metal accumulation in plants increased with an increase in the concentration of Cr or Ni.

The heavy metals accumulated in different parts of both inoculated and uninoculated plants but accumulation was greater in the former. In the nematode or fungus infected plants the heavy metals accumulation was greater in roots than in the shoots. These findings are in accordance with those of Bisessar *et al.* (1983) and Khan *et al.* (2006) who found similar accumulation of Ni in celery plants infected with *M. hapla* and tomato plants infected with *M. incognita*, respectively. Similar observations on the accumulation of Cd and Pb have also been reported by Parveen (1995) in tomato plants infected with *M. incognita*.

The induced accumulation of heavy metals in roots of nematode or fungus infected plants as compared to uninoculated plants was not clear, however, the nematode or fungal infection might be potentially disrupt the translocation of heavy metals from roots to aerial organs thus causes their greater accumulation in the roots (Wilcox-Lee and Loria, 1987).Similarly, Koeppe (1977) also reported that the translocation of heavy metals from roots to aerial parts is highly dependent on the physiological status of the plant.

5.7: EFFECT OF CHROMIUM AND NICKEL ON THE LIFE CYCLE OF RENIFORM NEMATODE, *ROTYLENCHULUS RENIFORMIS* ON CHICKPEA:

It is clear from the Tables 7 to 7.2 that the penetration, development and multiplication of reniform nematode, *R. reniformis* on chickpea was affected by the presence of heavy metals viz. Cr and Ni. *Rotylenchulus reniformis* required 28 days to complete the life cycle in chickpea, however, it was delayed by 11 and 5 days in the presence of Cr and Ni, respectively as compared to control. Several studies on the life cycle of reniform nematode were conducted by various workers on different pulse crops, which revealed that the duration of

life cycle of reniform nematode varied from 15-31 days. Moreover, no information is available regarding the duration of life cycle of reniform nematode in chickpea. Life cycle of reniform nematode was worked out initially by Linford and Oliveira (1940), according to him it takes about 25 days from egg to egg on cowpea. However, on the other hand, Peacock (1956) and Sharma and Haque (1993) reported that the life cycle of reniform nematode on cowpea was completed either in 15 days or 30-31 days. Similarly, the life cycle duration is reported to be either 25 days or 19 days on soybean (Peacock, 1956; Rebois, 1973) and 31 days on cluster bean (Bishnoi and Yadav, 1989). The number of eggs per eggmass also varied from host to host and on an average it ranges from 39 -120 eggs per eggmass (Swarup *et al.*, 1989). The variation in time required to complete its life cycle and number of eggs laid by single female might be due to various ecological factors, especially host status, temperature, moisture, pH etc. (Swarup and Dasgupta, 1986; Bhatti and Walia, 1992).

As far as the delay in the life cycle of reniform nematode is concerned, the reason may be due to the delay in the penetration and development of different stages of nematode in the presence of Cr and Ni. The delay in the penetration of root-knot nematode in the presence of different heavy metals on different crops has also been worked out by Khan *et al.* (1994) and Parveen (2004). Moulting is an important phase in the life cycle of nematode in which nematode undergoes structural changes (Bird and Bird, 1991). Cuticle changes with each moult and each new cuticle has a distinct composition of proteins (Blaxter and Robertson, 1998). The binding of heavy metal ions with proteins which might alter their secondary and tertiary structure enough to change their behavior within the biological system, where they occur (Sampson *et al.*, 1965). These reasons may also be responsible for the delay of life cycle and reduced population of reniform nematode in presence of Cr and Ni. Another

reason for this might be due to toxicological effects of Cr and Ni on reniform nematode.

The production of more males in Cr or Ni treated plants could be due to nutrition stress resulting due to various toxicological effects of heavy metals on plants. It is well established that the host nutrition is an important factor in altering sex ratios, usually with a shift towards males as nutrition becomes more unfavourable or when less food in the host tissue is available for nematode. These facts have been revealed by Triantaphyllou (1960) and Trudgill (1967).

5.8: EFFECT OF CHROMIUM AND NICKEL ON THE EFFICACY OF OIL-CAKES, BIOCONTROL AGENTS AND BAVISTIN IN THE MANAGEMENT OF *ROTYLENCHULUS RENIFORMIS* AND *FUSARIUM SOLANI* INFECTING CHICKPEA:

The effect of heavy metal (Cr and Ni) on the efficacy of oil-cakes (neem, mustard, mahua, castor, linseed and sesame), fungal biocontrol agents (*Paecilomyces lilacinus* and *Trichoderma harzianum*) and fungicide (Bavistin) in the management of *R. reniformis* and *F. solani* infecting chickpea was studied. The results presented in Tables 8 to 8.3 revealed that the inoculation of *R. reniformis* or *F. solani* significantly reduced the plant growth and yield of chickpea. Similarly, the treatments of both the heavy metals (Cr and Ni) also caused significant reduction in plant growth and yield of chickpea. Moreover, the plant growth and yield of chickpea plants was synergistically reduced in the plants inoculated with *R. reniformis* and grown in soil treated with either Cr or Ni. However, the synergistic reduction in plant growth and yield of chickpea was observed only when *F. solani* was inoculated in the Ni-treated soil and no such reduction was found when the fungus was inoculated in Cr-treated soil.

In the present study the amendments of oil-cakes viz. neem cake, mustard cake, castor cake, sesame cake and mahua cake significantly improved

the plant growth and yield of chickpea as compared to unamended-uninoculated plants, which may be due to their beneficial effects. The improvement in the growth of chickpea plants achieved by application of oil - cakes was attributed directly to the increase in nutrient status of soil, serving as manure. According to McConnell *et al.* (1993), the organic matter contributes to cation exchange capacity, water holding capacity and aggregate stability that leads to increase in crop yield (Muller and Gooch, 1982; Bryan and Lance, 1991).

It is well known fact that organic materials are good suppressants of plant-parasitic nematodes and plant pathogenic fungi, and the diseases they cause. Oil-cakes of different plants have consistently shown their efficacy against a variety of plant-parasitic nematodes (Singh and Sitaramaiah, 1971; Khan *et al.*, 1974a; Khan and Husain, 1988b; Pandey *et al.*, 2003b) and plant-pathogenic fungi (Khan *et al.*, 1974c; Zakaria *et al.*, 1980; Chattopadhyay and Rai, 2004) on many crops. In the present study, oil-cakes of neem, mustard, castor and mahua were found effective in the management of *R. reniformis* by reducing the reproduction factor of nematode which consequently increased plant growth and yield of chickpea. These results on the efficiency of oil-cakes for the control of reniform nematode are also in agreement with those reported by Khan *et al* (1974); Mishra and Prasad (1974), Yasssin and Ismail (1994), Mishra and Goswami (1996) and Ashraf *et al.* (2005) on different crops. Similarly, the amendments of oil-cakes viz. neem, mustard, castor, mahua and linseed were effective in reducing disease index of *F. solani*, which consequently increased the plant growth and yield of chickpea. The effectiveness of these oil-cakes in the management of diseases caused by *Fusarium* spp. have also been reported by several workers on different crops (Zakaria *et al.*, 1980;Mukhopadhyay and Gupta, 1991; Raj and Kapoor, 1996;Pamodaya and Reddy, 1999;Chattopadhyay and Rai, 2004), which is in agreement with the present findings. The results on the inefficiency of sesame

cake in managing *F. solani* are against the findings of Khan *et al.* (1974c), Matti and Sen (1984), Singh *et al.* (1980) who reported this cake to be effective in controlling *F. solani* on different crops.

It is believed that oil-cakes release some nematotoxic and fungitoxic substances during their degradation in the soil (Singh and Pandey, 1965; Khan *et al.*, 1973; Khan *et al.*, 1974c). Alam and Khan (1974) reported that with the liberal supply of water, oil-seed cakes decomposed and released many compounds including ammonia, phenols, and aldehydes. These compounds have a nematicidal nature which has been proved by many workers (Khan *et al.*, 1974a; Whitehead, 1976; Alam *et al.*, 1978; 1979). These compounds have also been reported to possess fungicidal nature (Krishnamurthy *et al.*, 1959; Zakaria *et al.*, 1980). Decomposition of oil-cakes also produces water soluble fractions which are highly toxic to nematodes (Alam *et al.*, 1982). Sayre (1980) and Mian and Rodriguez-Kabana (1982) suggested that the action of organic amendments against plant parasitic nematodes may be due to the decomposing specific proteins or specific material that affects cuticle structures of nematodes. Sitararmaiah and Singh (1978) also reported release of fatty acids, while Khan (1969) and Hasan (1977) have indicated the release of amino acids and carbohydrates during the decomposition of organic matter. All these chemicals have been reported to be highly deleterious to many plant parasitic nematodes (Khan, 1969; Alam *et al.*, 1979; Badra *et al.*, 1979) and pathogenic fungi (Zakaria *et al.*, 1980). The efficacy of organic amendments in controlling nematodes and fungi may also be due to the release of organic acids, ammonia and nitrates during decomposition of the organic material, which are toxic to nematodes and fungi (Zakaria *et al.*, 1980; Yassin and Ismail 1994). Better growth in oil - cake amended plants appears to be due to the reduction in nematode population and disease index of fungus as well as due to the effect of oil-cakes as manure. Besides, the roots of plants grown in soil amended with oil-cakes undergo physiological changes which make them unfavorable for

nematode penetration and feeding, thus inducing certain degree of resistance against the nematode attack (Alam *et al.*, 1980) and this may be also be true in case of fungus. The ineffectiveness of linseed cake and sesame cake in the management of *R. reniform* and, that of sesame cake in the management of *F. solani* may be due to small quantity of these oil-cakes used or possibly because of their active principles being diluted due to the frequent watering.

The present study revealed that the application of fungal biocontrol agent *P. lilacinus* significantly improved the plant growth and yield of chickpea by reducing the population of *R. reniformis*. My result are also in confirmity with the findings of Khan and Husain (1988,89) Khan and Saxena (1996), Vicente *et al.* (1991), Vicente and Acosta (1992), Walters and Barker (1994) and Ashraf *et al.* (2005) who reported the effectiveness of *P. lilacinus* in the management of reniform nematode on different crops. *Paecilomyces lilacinus* has been reported to reduce population densities of different plant parasitic nematodes and is considered as the most promising and practicable biocontrol agent (Jatala, 1986; Morgan-Jones *et al.*, 1984; Siddiqui *et al.*, 2000; Arif and Parveen, 2003). The inhibitory effect of *P. lilacinus* on the multiplication of reniform nematodes might be due to the parasitism of females, eggs and eggmasses by *P. lilacinus* and / or toxic metabolites produced by the fungus. The *P. lilacinus* has been reported to produce peptidal antibiotics viz. P 168, lilacinin, leucinonastatin and paecilotoxin (Arai *et al.*, 1973; Isogai *et al.*, 1980; Mikami *et al.*, 1989). These chemicals might be responsible for the killing of reniform nematode. Acetic acid has also been identified from culture filtrate of this fungus, which affects the movement of nematodes (Djian *et al.*, 1991). The mortality and inhibition in the hatching of reniform nematode in culture filtrates of *P. lilacinus* has also been reported by Khan and Husain (1989) and Ashraf and Khan (2005). Moreover, Lara *et al.* (1996) found that *P. lilacinus* did not affect the population levels of *R. reniformis*, which is against my findings. The differences in the results could be due to the

differences in experimental conditions, different strain of *P. lilacinus* and / or race of reniform nematode.

Paecilomyces lilacinus exhibits proteolytic and chitinolytic activity (Qkafor, 1967; Gintis *et al.*, 1983; Jatala, 1986; Khan and Saxena, 1997). This feature is of some significance because the eggshell of nematodes is mostly made up of protein and chitin (Bird and McClure, 1976). Moreover, it has been reported that the fungal hyphae penetrate the eggshell through small pores formed by the chitinase activity in the vitelline layer. The fungus then grows inside the eggs, crushes the chitin and lipid shell layers, and destroys the contents of the eggs including the developing larva whose cuticle are disrupted (Morgan-Jones *et al.*, 1984). It has been observed that the mycelial proliferation on the body of females of reniform nematode might result in probable biosynthesis of destructive metabolites endogenously. This endopathic activity of the fungus leads to the ultimate mortality of reniform nematode. In the present study *P. lilacinus* was unable to reduce the damage caused by *F. solani* infecting chickpea, which is against the findings of Siddiqui *et al.* (1999; 2000) and Shahzad and Ghaffar (1989) who reported that *P. lilacinus* controls *Macrophomina phaseolina, Rhizoctonia solani, F. oxysporum* and *F. solani* infection on sunflower, chickpea, mungbean, mashbean and tomato. The differences in the results could be due to the varied experimental conditions, different strains of *P. lilacinus* and *F. solani* used.

In the present study *T. harzianum* significantly improved the plant growth and yield of chickpea as compared to unamended-uniniculated plants which is also in confirmity with the findings of Paulitz *et al.* (1986), Windham (1989), Harman and Bjorkman (1998) and Harman *et al.* (2004) who reported enhanced growth of many plants induced by *T. harzianum*. The increase in plant growth and yield of chickpea plants might be due to the root colonization of *T. harzianum* which improved mineral uptake, mineral release from the soil

and organic matter and enhanced the production of plant growth hormones (Windham *et al.*, 1986; Beyrle, 1995).

Similarly, the soil application of *T. harzianum* also significantly improved the plant growth and yield of chickpea infected with reniform nematode by reducing the reproduction factor of *R. reniformis*. The reduction in reproduction factor of reniform nematode may be attributed to the parasitism of eggs and egg masses of reniform nematode by *T. harzianum* as observed in the present study. *Trichoderma harzianum* has also been reported as nematophagous fungus on eggs, juveniles and females of cyst nematode (Susan *et al.*, 1990), *Globodera rostochiensis* (Saiffullah and Thomas, 1996) and *Meloidogyne javanica* (Sharon *et al.*, 2007). Besides parasitism of eggs and eggmasses of reniform nematode as observed in the present study it is also hypothesized that the production of nematicidal compounds by *T. harzianum* (Suarez *et al.*, 2004) directly affected the nematode multiplication or made the roots less attractive and thus reduced nematode penetration which might have resulted in the reduction of nematode population. *Trichoderma harzianum* has also been found effective in the management of plant parasitic nematodes on many crops (Windham, *et al.*, 1989; Rao *et al.*, 1996; Siddique *et al.*, 1999; Sharon *et al.*, 2001; Haseeb *et al.*, 2005; Pandey *et al.*, 2007). Haggag and Amin (2001) reported that *T. harzianum* significantly reduced the infection of reniform nematode on sunflower which is also in accordance with present findings. Biocontrol activity of *T. asperellum* -203 and *T. atrovivide* IMI 206040 (both fungi, previously defined as strains of *T. harzianum*) have been reported antagonistic to *M. javanica* in soil (Sharon *et al.*, 2001). The effect of *Trichoderma* metabolites on root knot nematodes was demonstrated by implementing root – dip treatments with the fungal culture filtrate (Khan and Saxena, 1997a). Sharon *et al.* (2007) suggested that improved proteolytic activity of *Trichoderma* may also be important for the control of nematodes.

In the present study the application of *T. harzianum* significantly improved the plant growth and yield of chickpea inoculated with *F. solani* by reducing the disease index. My results are also in conformity with the findings of Okhovat and Karampour (1996), who reported the effectiveness of *T. harzianum* in controlling root-rot of chickpea caused by *F. solani*. Similarly, *T. harzianum* effectively managed *F. solani* on sunflower (Haggag and Amin, 2001) and ginger (Ram *et al.*, 1997).The inhibitory effect of *T. harzianum* against *F. solani* was probably due to mycoparasitism, competition for space and nutritional sources and antagonistic chemicals produced and released into the environment. Mukerji and Garg (1988) reported that *Trichoderma* spp. produced the antibiotic compounds (Trichodermin, Acetaldehyde), extra cellular enzymes (Chitinase, Cellulase, (1-3) -β Glucanase), unsaturated monobasic acid (Dermadine) and peptides (Alamethicine, Sugukacillin). Enzymes such as chitinases, glucanases and proteases seem to be very important in the mycoparsitic process (Harman *et al.*, 2004). According to Howell (2003) the production of chitinases may have direct significance in the parasitism of *Fusarium* spp. as these enzymes function by breaking down the polysaccharides, chitin and β-glucan that are responsible for the rigidity of the fungal cell walls thereby destroying cell wall integrity.

Moreover, *T. harzianum* may also induce systemic resistance mechanisms (Yedidia *et al.*, 1999) in chickpea plants that might have provided the protection against *R. reniformis* and *F. solani*.

In the present study Bavistin was also found to be effective in controlling the damage caused by *R. reniformis* and *F. solani* in chickpea by reducing the reproduction factor and disease index, respectively. This can be due to the persistent and systemic antinematode and antifungal effects of Bavistin. The effectiveness of Bavistin in controlling plant-parasitic nematodes on different crops has also been documented by Haseeb *et al.* (2005) and Khan

and Husain (1988a). Bavistin was found to be most effective in reducing the disease index of fungus which might be due to its antimitotic effect by forming a complex with sub-unit of microtubuli and preventing the normal assembly of microtubulin units into spindle fiber. Therefore, mitotic spindle is distorted and daughter nuclei fails to separate, resulting in cell death of fungi (Hewitt, 1998). Moreover, it has also been reported that Bavistin inhibited the synthesis of DNA and other related processes of fungi due to its antimetabolic nature (Vyas, 1993). The effectiveness of Bavistin in the management of *Fusarium* spp. infecting different plants has also been reported by Etebarian, (1992), Haseeb and Shukla (2002) and Haseeb *et al.* (2005).

The effect of heavy metals (Cr and Ni) on the efficacy of oil-cakes in management of *R. reniformis* and *F. solani* infecting chickpea revealed that only neem cake, mustard cake, castor cake and mahua cake proved to be effective in managing *R. reniformis* and *F. solani* both in presence and absence of heavy metals. The reason attributed to this may be that these oil-cakes might adsorb heavy metals, thus limiting their uptake by plants. According to Ajmal *et al.* (2005), the oil-cakes of mustard adsorb Cr and Ni. Parveen and Alam (1999b) found that neem cake was effective in the management of *M. incognita* on tomato in presence and absence of Pb and Cd. Since on one side these oil-cakes reduced nematode / fungal damage to chickpea and on other side they adsorbed heavy metals (Cr and Ni), thus reducing their uptake capacity in plants, and hence also reduced the combined effect of pathogens and heavy metals. The ability of *T. harzianum* in reducing the damage caused by *R. reniformis* or *F. solani* in the presence and absence of heavy metals (Cr and Ni) could be because of its ability to accumulate heavy metals (Ledin *et al.*, 1996) and its resistant nature towards heavy metals as reported by (Kredics *et al.*, 2001a;b).

It can be concluded from the present study that the application of neem cake, mustard cake, mahua cake, castor cake and *T. harzianum* seem to be best

options for managing *R. reniformis* and *F. solani* on chickpea in presence and absence of heavy metals (Cr and Ni).

4.9: SCREENING OF CHICKPEA VARIETIES FOR RESISTANCE AGAINST *ROTYLENCHULUS RENIFORMIS, FUSARIUM SOLANI,* CHROMIUM AND NICKEL:

In nature performance of pathogens/ parasites vary with changes in the host as well as environmental conditions, including pollutants. Crop cultivars, in addition to possessing gradation in resistance to parasitic or pathogenic agencies may also differ in their reaction to their pollutants. So it was considered worthwhile to work out that whether the observed disease response of a particular variety was a matter of resistance to the pathogen or to the pollutant. It was observed that different chickpea varieties responded differently to *R. reniformis*, *F. solani* and heavy metals viz. Cr and Ni. There was an adverse effect of each test pathogen and heavy metal on the growth of chickpea varieties, irrespective the level of its resistance against heavy metal or pathogen. These results are in conformity with those of earlier workers (Parveen and Alam, 1998; Ashraf and Khan, 2003).

Out of 25 chickpea varieties screened, 8 were highly susceptible (Annegiri-1, KUSCR-2, Pant-186, Pragati, Pusa-1103, Pusa-120, Radhey and Vardan), 8 susceptible (Avarodhi, Gaut, Gulab, K-850, Phule G 96020, Pusa-1060, Vijay and XVSCR-2), 5 tolerant (CSJD, JG-74, Phule G 92028, Sadabahar and WCG-2 (Surya)), 3 moderately resistant (Gauraw, KGD-1168 and KWR-108) and one resistant (Phule G 8602) against the reniform nematode, *R. reniformis*. Chickpea varieties namely Annegiri-1 and Radhey were found highly susceptible to *R. reniformis*. However, variety Annegiri-1 has been reported as susceptible to *M. incognita* (Krishna Rao and Krishnappa, 1995) and tolerant to *M. javanica* (Sharma *et al.*, 1995). Similarly, chickpea

variety Radhey has been reported as susceptible to *M. incognita* (Mani and Sethi, 1985; Pandey and Singh, 1990; Krishna Rao and Krishnappa, 1995).In the present study the chickpea variety Avrodhi was rated as susceptible to *R. reniformis*. Similarly, this variety has also been reported as susceptible and moderately susceptible to *M. incognita* by Krishna Rao and Krishnappa (1995) and Jaisani (1991), respectively. The chickpea variety Vijay was found as susceptible to *R. reniformis*, but, it was reported as resistant (Pandey *et al.*, 2003a) and tolerant (Ashraf *et al.*, 2003) to *M. incognita*. Pandey and Singh (1990) and Shelke *et al.* (1995) also reported the chickpea varieties viz. Anupam and Gaurav as susceptible against *M. incognita*.

Out of 25 chickpea varieties screened in the present study, 7 were highly susceptible (Annegiri-1, KUSCR-2, Pusa-1103, Radhey, Vardan, Vijay and XVSCR-2), 12 susceptible (Avarodhi, CSJD, Gauraw, Gaut, Gulab, K-850, Pant-186 , Phule G 92028, Phule G 96020, Pragati, Pusa-120 and Pusa-1060), 3 tolerant (JG-74, KGD-1168 and Sadabahar), 1 moderately resistant (WCG-2 (Surya)) and 2 resistant (KWR-108 and Phule- G 8602) against the root- rot fungus, *F. solani*. In the present findings two varieties viz. Pragati and Gaurav were susceptible to *F. solani* which are in confirmity with those of Yadav and Narain (1993) who also reported these varieties as susceptible against *Altenaria alternata*. Similarly, the chickpea variety Vijay was found highly susceptible and KWR- 108 as resistant to *F. solani*, these varieties also showed similar reaction to *R. bataticola* (Gangwar, 2002) and *F. oxysporum* (Shukla and Haseeb, 2001; Mishra *et al.*, 2001). In the present results, chickpea varieties Avrodhi and Radhey were rated as susceptible and highly susceptible to *F. solani*, respectively but the variety Avrodhi was reported as resistant to *F. oxysporum* (Mishra *et al.*, 2001) and variety Radhay as susceptible to *Altenaria alternata* (Yadav and Narain, 1993) and *R. bataticola* (Gangwar, 2002).Similarly, the varieties viz. Phule G 92028 and Phule G 96020 were

rated as susceptible to *F. solani* in the present experiment, but Gangwar (2002) observed these varieties as susceptible and moderately resistant to *R. bataticola*, respectively.

Similarly, out of 25 chickpea varieties, 15 were susceptible (Annegiri-1, CSJD, JG-74, Gauraw , KGD-1168, KUSCR-2, KWR-108, Phule G 92028, Phule G 96020, Pragati, Pusa-120 , Sadabahar , Vardan, Vijay, and XVSCR-2), 7 tolerant (Avarodhi, Gaut, Gulab, K-850, Pusa-1103, Pusa-1060 and WCG-2 (Surya)), two moderately resistant (Pant-186 and Radhey) and 1 resistant (Phule G 8602) against chromium, while, 2 were highly susceptible (Pragati and XVSCR-2), 19 susceptible (Annegiri-1, Avarodhi, CSJD, JG-74, Gaut, Gulab, K-850, KGD-1168, KUSCR-2, Phule G 92028, Phule G 96020, Pusa-1103, Pusa-120, Pusa-1060, Radhey , Sadabahar, Vardan, Vijay and WCG-2 (Surya)), 3 tolerant (Gauraw, KWR-108 and Pant-186) and one resistant (Phule G 8602) against the nickel.

It was interesting to note that the chickpea variety, Phule-G 8602 showed resistance against both the pathogens and heavy metals. Therefore, this variety was once again tested for its resistance to check whether the resistance persisted if the plants were grown in the soil treated with either Cr or Ni even in the presence of either *R. reniformis* or *F. solani*. The results indicated that the variety Phule-G 8602 showed resistance towards *R. reniformis, F. solani* and both the heavy metals (Cr and Ni) even when the same chickpea variety was grown in soil infested with the test pathogen (*R. reniformis/F. solani*) and contaminated with heavy metal (Cr/Ni).Therefore, this variety may be recommended to farmers to grow in the fields infested with reniform nematode and root-rot fungus and contaminated with Cr and Ni after making field trials.

These results indicate that there are good possibilities for finding resistant chickpea varieties against test pathogens and heavy metals and the replacement

of susceptible varieties with resistant ones appears to be the most economic and suitable method for control of plant diseases.

Resistance may be used as a key component in the integrated management programme for the management of *R. reniformis* and *F. solani* infecting chickpea and also to reduce the adverse effect of heavy metals (Cr and Ni). Moreover, the variety identified as resistant, can be used to introgress the genes for resistance against *R. reniformis* , *F. solani* and heavy metals (Cr and Ni) on chickpea, because resistant varieties give low cost, have no adverse effect on natural enemies or non- target organisms, do not show toxicity or residue problems, no special skill necessary for farmers. Similarly, the use of resistant cultivars could provide a way to maintain or increase crop production without increased land demands or adverse environmental consequences.

Summary

SUMMARY

The present study deals with the effect of two important heavy metal pollutants, viz. chromium and nickel on the pathogenic potential and management of reniform nematode, *Rotylenchulus reniformis* and the root-rot fungus, *Fusarium solani* infecting chickpea, *Cicer arietinum* var. Kranti. Aspectwise summary of the results is presented below:

Since biological races are known to occur in *Rotylenchulus reniformis*, it was considered desirable to study the populations of reniform nematode in the present study and identify its race before initiating the research work so that the results obtained may be interpreted more scientifically and with authenticity. The results revealed that all the isolates of *R. reniformis* collected from chickpea fields on both sides of Mathura Road and Khair Road, Aligarh were able to attack and multiply on castor, cowpea, cotton and mustard, but these populations were unable to infect bajra, therefore the populations of *R. reniformis* collected from different locations belonged to Race-3. Therefore, *R. reniformis* Race -3 was used for the experimental purposes.

In vitro studies were carried out to assess the effect of two heavy metals i.e. Cr and Ni on the hatching and mortality of *R. reniformis*. The results clearly indicated that both the heavy metals adversely affected the hatching of *R. reniformis*, Cr being more toxic than Ni. Not only the hatching of the nematode was inhibited but the heavy metals also caused significant mortality of the nematode. Both, hatching and mortality were found to be directly proportional to the concentration of heavy metals. Inhibition in the hatching was minimum at the lowest concentration (25 ppm) and maximum at the highest concentration (400 ppm) for Cr and Ni. Hundred percent inhibition in the hatching was observed at 400 ppm of Cr. Similarly, the nematode mortality was also increased with an increase in the concentration of the heavy metals as well as the exposure period. The lowest mortality was recorded at 25 ppm of Cr at 12 h exposure period and 100% mortality was obtained in 400 ppm

concentration of Cr at 96 h. Similarly, the lowest mortality of nematode was recorded in 25 ppm Ni at 12 h and it increased to 71.0% in 400 ppm Ni at 96 h exposure period.

The effect of heavy metals viz. Cr and Ni on the growth and sporulation of *F. solani*, and also uptake of these metals by *F. solani* was assessed *in vitro*. The results revealed that the growth and sporulation of *F. solani* significantly decreased with an increase in the concentration of Cr or Ni except at 25ppm Cr and, 25 and 50 ppm Ni. Moreover, the growth and sporulation was significantly enhanced when the fungus was grown in the medium added with 25 ppm Ni. The chlamydospore formation initiated at and above 50 ppm Cr and 100ppm Ni, which increased further with an increase in the concentration of heavy metals. Similarly, the uptake of heavy metals by *F. solani* was increased with an increase in the concentration of Cr / Ni. Overall, it was observed that Cr was more toxic to the fungus than Ni.

The potential pathogenic level of reniform nematode and root-rot fungus, was determined by inoculating the seedlings of chickpea separately with different inoculum levels of *R. reniformis* (250,500, 1000, 4000 and 8000 immature females per plant) and *F. solani* (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0g mycelium + spores per plant). There was a gradual increase in the reduction of plant growth, nodulation, yield, chlorophyll content, protein content and water absorption capacity of roots of chickpea with increase in inoculum level of *R. reniformis* except at the inoculum level of 250 immature females per plant which slightly increased plant growth as compared to uninoculated plants. However, the significant reduction in the above mentioned parameters of chickpea plants was recorded at and above 1000 immature females of reniform nematode. The rate of nematode multiplication of *R. reniformis* decreased with an increase in the inoculum level. The percentage of disease index of *F. solani* increased with increase in the inoculum level. Similarly, a direct correlation

between increasing initial inoculum level with decreasing plant growth, yield, nodulation, chlorophyll content, protein content and water absorption capacity of roots was observed for the root-rot fungus, *F. solani*. However, the significant reduction in respective parameters of chickpea was observed at and above 3.0 g of *F. solani* / plant. Hence, the potential pathogenic level of *R. reniformis* and *F. solani* on chickpea was recorded as 1000 immature females of *R. reniformis* / plant and 3.0 g of *F. solani* / plant, respectively.

A pot experiment was conducted to assess the effect of chromium / nickel on the pathogenic potential of *R. reniformis* / *F. solani* infecting chickpea. The results clearly showed that the plant growth, yield, nodulation, chlorophyll content of leaves, protein content of seeds and water absorption capacity of roots of chickpea decreased in the plants grown in soil treated with heavy metals either alone or in combination with the test pathogens. Moreover, significant toxic effects of either Cr or Ni on plant growth parameters were observed at and above 100 ppm concentrations of heavy metal. Chromium was found to be less toxic to chickpea plants and more toxic to nematode and fungus, whereas, nickel was less toxic to nematode and fungus, and more toxic to chickpea plant. With an increase in the concentration of heavy metals (Cr or Ni) from 25 to 200 ppm, there was a corresponding decrease in plant growth, yield, nodulation, chlorophyll content, protein content and water absorption capacity, which was further ameliorated in the presence of either nematode or fungus. This reduction in plant growth and other parameters of chickpea was synergistically ameliorated when plants were inoculated with either nematode or fungus and grown in soil treated with different concentrations of either Cr or Ni, except the treatment of 25 ppm Cr with nematode and all concentrations of Cr with fungus, which were unable to show synergistic effect. It can be concluded from these results that a concentration dependent relationship of heavy metals (Cr/Ni) was observed with reniform nematode and root-rot

fungus. The heavy metals in the soil can enhance the reduction in plant growth and yield of chickpea in presence of *R. reniformis* / *F. solani.*

The amount of heavy metals viz. Cr and Ni in chickpea was estimated on dry weight basis by Atomic Absorption Spectrophotometery (AAS). It was interesting to note that Cr was accumulated by plants in lesser amount than Ni and the amount of heavy metals was more in roots than in shoots. The concentration of the heavy metal accumulation was more in inoculated plants than the uninoculated plants. Moreover, the heavy metals were accumulated in greater amounts by plants inoculated with *F. solani* than the plants inoculated with *R. reniformis.* It was further noticed that the accumulation of Cr and Ni in plants increased with an increase in their concentration. It can be concluded from the results that the higher accumulation of Cr / Ni was recorded in plants infected with the test pathogens (*R. reniformis* / *F. solani*) and treated with heavy metals (Cr / Ni) as compared to those plants uninfected but treated with heavy metals (Cr / Ni).The higher accumulation of Cr / Ni in the plants infected with either *R. reniformis* of *F. solani* might be responsible for greater reduction in plant growth and others parameters of chickpea.

The studies were conducted on the effect of Cr and Ni on the life cycle of *R. reniformis* in chickpea. Results revealed that the penetration, development and multiplication of reniform nematode, *R. reniformis* were inhibited and delayed by the presence of heavy metals viz. chromium and nickel as compared to control. The penetration of nematodes in control started on first day after inoculation which was however delayed by one day in plants grown in pots treated with either Cr or Ni. Females with slight swelling were first observed on 5^{th} day in control whereas such females were recorded on 8^{th} and 7^{th} day in plants treated with Cr and Ni, respectively. The fully swollen females, females with matrix and females with eggmasses were first recorded on 10^{th}, 14^{th} and 16^{th} day of inoculation in the control plants, respectively. The corresponding

stages of development of reniform nematode were first recorded on 12th, 18th and 20th day, and on 11th, 16th and 18th day of inoculation in plants treated with Cr and Ni, respectively. The average number of eggs per eggmass was significantly reduced in both Cr (48) and Ni (52) treated plants as compared to control (69). The eggs took 4 days to hatch into second stage juveniles in control, while in Cr and Ni treated soil, eggs hatched in 5 and 4 days respectively. The second stage juveniles were recorded on 20th day in control as against 25th day in Ni and 22nd day in Cr treated soil, respectively. The third stage of female and male juveniles were recorded on 23rd day in control, but, these stages of development were recorded on 29th and 25th day of inoculation in Cr and Ni treated soil, respectively. Similarly, fourth stage of female and male juveniles were recorded on 25th day in control, while in Cr and Ni treated soil these stages were recorded on 33rd and 28th day after inoculation, respectively. The immature females and adult males were recorded on 28th, 39th and 33rd day, respectively in the corresponding treatments. In this way the life cycle of *R. reniformis* on chickpea was delayed by 11 days and 5 days in the presence of Cr and Ni respectively as compared to control. The number of immature females and adult males were significantly reduced in plants treated with either Cr or Ni as compared to control. The total population of the nematode was also reduced in Cr and Ni treated pots as compared to control on the day of recovery of immature females. The female and male ratio (female: male) was 1.17:1.00 in control as against 1.00:1.30 and 1.00:1.21 in Cr and Ni treated soil, respectively.

The effect of Cr and Ni on the efficacy of oil-cakes viz. mustard, neem, castor, mahua, linseed and sesame, fungal biocontrol agents (*Paecilomyces lilacinus* and *Trichoderma harzianum*) and Bavistin in the management of *R. reniformis* / *F. solani* infecting chickpea was studied. The results clearly revealed that plant growth and yield of chickpea was significantly reduced in

the pots individually treated with *R. reniformis*, *F. solani*, Cr and Ni. Moreover, these parameters of chickpea plants was synergistically reduced in the pots treated with the test pathogen (*R. reniformis* / *F. solani*) in combination with the heavy metal (Cr / Ni) except the plants grown in Cr-treated soil and inoculated with *F. solani* , in which the reduction in plant growth was not synergistic. It was found that the growth and yield of chickpea was significantly improved in presence of neem cake, mustard cake, *T. harzianum*, mahua cake, castor cake and sesame cake as compared to untreated-uninoculated plants. However, the application of linseed cake, *P. lilacinus* and Bavistin did not show any significant improvement in the plant growth and yield as against untreated-uninoculated plants. The best protection of chickpea plants against the *R. reniformis* was recorded by the application of *P. lilacinus* followed by Bavistin, neem cake, mustard cake, castor cake, *T. harzianum* and mahua cake. These treatments significantly reduced the reproduction factor of reniform nematode which consequently increased plant growth and yield of chickpea as compared to untreated-inoculated plants. Similarly, the best protection of chickpea plants against *F. solani* was recorded by the application of Bavistin, followed by *T. harzianum*, neem cake, linseed cake, mustard cake, castor cake and mahua cake. These treatments also significantly reduced the disease index of *F. solani* and increased the plant growth and yield of chickpea. The application of neem cake, mustard cake, castor cake, *T. harzianum* and mahua cake also reduced the damage caused by heavy metals (Cr / Ni). The application of neem cake, mustard cake, castor cake, mahua cake, *T. harzianum*, *P. lilacinus* and Bavistin were found to be effective in managing the damage caused by combined effect of heavy metal (Cr / Ni) and *R. reniformis*. Similarly, the application of *T. harzianum*, neem cake, mustard cake, castor cake, mahua cake, linseed cake and Bavistin were also found to be

effective in managing the damage caused by combined effect of heavy metal (Cr / Ni) and *F. solani*.

Different chickpea varieties viz. Annegiri-1, Avarodhi, CSJD, JG-74, Gauraw, Gaut, Gulab, K-850, KGD-1168, KUSCR-2, KWR-108, Pant-186, Phule-G 8602, Phule G 92028, Phule G 96020, Pragati, Pusa-1103, Pusa-120, Pusa-1060, Radhey , Sadabahar, Vardan, Vijay, WCG-2 (Surya) and XVSCR-2 responded differently to the *R. reniformis*, *F. solani* and heavy metals viz. Cr and Ni. There was an adverse effect of the test pathogens and heavy metals on the growth of chickpea varieties, irrespective the level of its resistance against pathogen or heavy metal.

Out of 25 chickpea varieties, 8 were highly susceptible (Annegiri-1, KUSCR-2, Pant-186, Pragati, Pusa-1103, Pusa-120, Radhey and Vardan), 8 susceptible (Avarodhi, Gaut, Gulab, K-850, Phule G 96020, Pusa-1060, Vijay and XVSCR-2), 5 tolerant (CSJD, JG-74, Phule G 92028, Sadabahar and WCG-2 (Surya)), 3 moderately resistant (Gauraw, KGD-1168 and KWR-108) and one resistant (Phule G 8602) against the reniform nematode, *R. reniformis*, and 7 were highly susceptible (Annegiri-1, KUSCR-2, Pusa-1103, Radhey, Vardan, Vijay and XVSCR-2), 12 susceptible (Avarodhi, CSJD, Gauraw, Gaut, Gulab, K-850, Pant-186 , Phule G 92028, Phule G 96020, Pragati, Pusa-120 and Pusa-1060), 3 tolerant (JG-74, KGD-1168 and Sadabahar), 1 moderately resistant (WCG-2 (Surya)) and 2 resistant (KWR-108 and Phule- G 8602) against the root- rot fungus, *F. solani*. Similarly, out of 25 chickpea varieties, 15 were susceptible (Annegiri-1, CSJD, JG-74, Gauraw , KGD-1168, KUSCR-2, KWR-108, Phule G 92028, Phule G 96020, Pragati, Pusa-120 , Sadabahar , Vardan, Vijay, and XVSCR-2), 7 tolerant (Avarodhi, Gaut, Gulab, K-850, Pusa-1103, Pusa-1060 and WCG-2 (Surya)), two moderately resistant (Pant-186 and Radhey) and 1 resistant (Phule G 8602) to heavy metal, chromium, and 2 were highly susceptible (Pragati and XVSCR-2), 19 susceptible

(Annegiri-1, Avarodhi, CSJD, JG-74, Gaut, Gulab, K-850, KGD-1168, KUSCR-2, Phule G 92028, Phule G 96020, Pusa-1103, Pusa-120, Pusa-1060, Radhey , Sadabahar, Vardan, Vijay and WCG-2 (Surya)), 3 tolerant (Gauraw, KWR-108 and Pant-186) and one resistant (Phule G 8602) against the nickel.

It was interesting to note that the chickpea variety, Phule-G 8602 showed resistance against both the pathogens and heavy metals. Therefore, this variety was once again tested for its resistance to check whether the resistance persisted if the plants grown in the soil treated with either Cr or Ni even in the presence of either *R. reniformis* or *F. solani*. The results indicated that the variety Phule-G 8602 showed the resistance towards *R. reniformis, F. solani* and both the heavy metals (Cr and Ni) even when the same chickpea variety was grown in soil infested with the test pathogen (*R. reniformis/F. solani*) and contaminated with these heavy metals. Therefore, this variety may be recommended to farmers to grow in the fields infested with reniform nematode and root-rot fungus and contaminated with Cr and Ni after making field trials.

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