CHAPTER-I
INTRODUCTION
Air, water and soil are the most important components of the environment on which survival of living being is dependent. The rapid growth of population and industrialization has been responsible for air, water and soil pollution. This has ultimately threatened the existence of plants and animals and endangered the survival of human race. A major threat to our civilization comes from the use of polluted water. Pollution of water and soil mostly results from discharges of domestic sewage containing organic and inorganic substances and industrial effluents discharge such as distilleries, sugar factories, tanneries, paper making factories, textiles, printing, dyeing, coal mines and plant based on chemical technology, radio-nucleosides and thermal effluent.

Water is the most important natural resource and is used for drinking, bathing, irrigation, navigation and industrial purposes. The water hygiene, sanitation and drainage have been topmost priorities ever since the beginning of human civilization. The ancient, religious and mythological scriptures and archeological document have plenty of evidence to support the existence of consciousness for
water hygiene and drainage. The first documented concern for the refuse arising from industrial process to be kept out of stream, to be rendered harmless before reaching stream or to be utilized or got rid of otherwise discharge into running water, is from the statement in paper of appointment of the British Royal Commission in 1865 (Wilson and Calvert, 1913).

The released water from domestic and industrial sources after used is known as waste water which contain large amount of organic and inorganic substances. Waste water is generally classified as domestic, industrial or agricultural according to its sources. Waste water originated from industrial sources is known as industrial effluent. In an excellent review on the environmental biotechnology (Mudrack et al., 1987) designated the mixture of industrial effluent and community effluent as 'mix effluent'.

The discharge of untreated industrial effluent is one of the most significant factors for pollution of aquatic ecosystem and irrigating lands. The effluent contain acids, alkalies, heavy metal, toxic inorganic, organic compound in dissolved and suspended forms. Agro-soil receiving these industrial effluents by irrigation or other means were adversely affected. The irrigation of agro-ecosystem by
effluents, may affected the soil pH, salinity, soil ionic balance and availability of essential mineral to plants and many more physico-chemical properties of soil which are vital for plant growth.

The cottage industries of woolen carpets, rug and durries have grown very rapidly in various part of the world. These industries utilize chemical and water in large quantity. The untreated waste water is let out as effluent to aquatic and terrestrial system around the industrial complex. The agro-ecosystem, waste lands and aquatic system in the vicinity of woolen industries of eastern Uttar Pradesh have been receiving the significant amount of Na, Ca, Cr(VI) Cl, OH, SO₄, NH₄, CO₂, CH₃COO, dye and solid at extremes of pH separately by the irrigation, with untreated enriched effluent from scouring, dyeing and processing of woolen carpet (Mishra and Ambasht, 1988). Tripathi (1975) has reported the physico-chemical properties of effluent of a chemical and fertilizer factory at Sahupuri near Varanasi.

Textile effluents constitute a major part of the total industrial effluents of U. P. Ajmal and Khan (1985) analysed the effluent of the Modi Textile Factory Ltd. Modinagar U. P. (MTE) and studied its effect in various concentration on certain physico-chemical properties of soil and germination and growth of kidney bean
Phaseolus aureus and lady's finger Abelmoschus esculentus. The effluent was found to be rich in various type of solids, BOD, COD, Cl⁻, SO₄²⁻, Na⁺, K⁺, Ca²⁺ and Mg²⁺, deficient in dissolved oxygen and highly alkaline is nature. At all the concentration tested, there was an increase in the water soluble salts, electrical conductivity, cation exchange capacity, pH, NH₃ – N, phosphorus, organic matter and NH₄ OAc-extractable Na⁺, K⁺, Ca²⁺, and Mg²⁺ of the soil. The greatest changes were recorded with 100% effluent, the most marked being in the organic matter of soil, followed by NH₃ – N, K⁺, Na⁺, P⁺, Ca²⁺ and Mg²⁺. The other effluent concentration also changed soil composition accordingly.

In India 106.6 million hectares of land is under crop cultivation out of which only 37 million hectare is being irrigated. A major drawback of Indian agriculture is that 66% of the cultivated land is still dependent on rain water. Thus due to inadequate irrigation facilities farmer used disposed sewage and industrial effluent for irrigation purposes. Inhabitant farmer of the effluent vicinity irrigate their vegetable, crops, cereal, pulses etc. with untreated effluents. Organic matter and other nutrients present in sewage and effluent changed the physico-chemical properties of soil. The physical and
chemical characteristic of the soil influence the existence of soil microorganism (Griffin, 1972 and Garrett, 1981).

Soil moisture governs indirectly the activity and population dynamics of the microorganism in soil (Griffin, 1969 and 1972). Soil water is one of the most important factor influencing the growth and survival of soil borne pathogens. In plant disease control, the knowledge of soil water plays an important role with regard to microbial growth, antagonism, host exudation and other factor, which affect pathogen in soil (Rovira, 1965; Cook and Flentje, 1967; Griffin, 1969 and Cook and Papendick, 1970).

The application of sewage and industrial effluent to land has also been practiced during recent years as an alternative means of treatment and disposal. This supplies not only water but also the manorial ingredient and plant nutrients. Many workers have monitored the effect of sewage and sewage sludge on the chemical properties and heavy metal content of the soil, the germination of seeds, growth and development of plants (Heukelekien, 1953; Dunlop et al., 1961; Ko and Duckstein, 1972; Hohla et al., 1978; Wollan et al., 1978; Feigin et al., 1979; Saavedra et al., 1984; Rupp and Saurcrbrey, 1987; Oran and Demalach, 1987 and Asano and
Pettrygrone, 1987). Other workers have studied the effect of various industrial effluents on the chemical composition of soil and the germination of seeds and growth of different crop plants (Ghosh, 1966; Dolar et al., 1972; Rajannan and Oblisamy, 1979; Ajmal and Khan, 1983, 1984a; Srivastava and Sahai, 1987; Kadioglu and Algur, 1990; Tripathi et al., 1990; Elcey and Tiwari, 1991; Dulari, 1996 and Singh, 2003).

Soil not only provided support to the plants but also water and nutrients. Sewage and industrial effluent of brewery, dairy, distillery, pharmaceutical, sugar, textiles and woolen carpet dyeing factories contain high amount of organic matter and other nutrients especially nitrogen, phosphorus and potassium. Therefore sewage and industrial effluent of various factories can be utilized and applied in agricultural field as a source of fertilizer after proper dilution (Day, 1973; Degodyuk, 1983; Linden et al., 1981; Bossart and Barth, 1985; Ajmal and Khan, 1983, 1984a, 1985; Srivastava and Sahai, 1987; Kadioglu and Algur, 1990; Patel and Kumar, 1991 and Tiwari et al., 1993). Several workers have reported the accumulation of heavy metal in soil as a result of sewage irrigation (Haines, 1984; Grey et al., 1986; Elliot and Linn, 1987; Meshref et al., 1989; Shrivastava et al., 1989; Smith et al., 1990; Singh, 1992 and Kumari, 1996).
**Effect of Industrial Effluent on Micro-flora and Plant Diseases**

Industrial effluents released by the various industries, enter into river, wasteland or used to irrigate adjacent agricultural field by farmers. Presence of various inorganic and organic matters in the effluent is well known. The pollutants have direct or indirect effects on microbial community also and the plant susceptibility to microbial pathogen may be altered to influence disease development. Thus the plant disease caused by microorganisms may be either enhanced or suppressed depending upon the nature of disease, host, concentration and the diversity of pollutants. It is becoming evident that interaction between pollutant, plant and microorganisms including the pathogen can affect pathogenesis (Rai and Upadhyay, 1988). Soils polluted with different pollutants in a range of concentration are known to exert diverse effect on qualitative nature of mycoflora.

Downing (1971) observed toxic effluent to inhibit microbial population in activated sludge treatment plants. Curds (1973) reported that change in sewage substrates results in variation in treated effluents. Leach *et al.*, (1978) also noted suppressed microbial activity by additions of Penicillin and Streptomycin in biological
waste treatment plants. Chandra (1990) observed the inhibition of fungal growth in nutrient medium amended with different concentrations of sewage and sludge. The fungi *Fusarium oxysporum* f. sp. *lini, Curvularia lunata, Trichoderma harzianum, Aspergillus luchuensis, Cladosporium cladosporoides, Penicillium citrinum, P. granulatum* and *A. niger* were inhibited.

In the area of industrial waste water treatment, low microbial species diversity was found. Lester *et al.*, (1979) and Mousen and Davis (1984) observed that accumulation of microbial population to toxicant were accompanied by reduction in species diversity. Raw distillery water was very toxic to the soil microorganisms (Juwarkar and Dutta, 1990). The growth rate of *Rhizobium* and *Azotobacter* was also reduces due to application of raw waste water. Allen *et al.*, (1953) opined that the total count of bacteria (*Escherichia coli* and *Streptococcus faecalis*) probably constitutes a better index of pollution in water than examination of either of the microorganism alone. Vasiliev and Vavilin (1982) observed that higher concentration of pollutants results in poor performance of the treatment process. Fayes *et al.*, (1988) reported the effect of some industrial waste, on bacteriological characteristic of soil and water due to sewage treatment.
In the application of industrial effluent to form one of the crucial factors to be considered is the possibility of contamination of soil and ground water. The effects of adding wastes to soil on potentially pathogenic microorganisms such as *Nocardia asteroides* (Beaman *et al.*, 1976 and Orchard, 1979) that are usually present in relatively low numbers (Orchard and Goodfellow, 1974 and Orchard *et al.*, 1977) were of great importance.

Application of sewage sludge compost as soil amendment may be proved useful to decrease the activity of certain important disease (Levis *et al.*, 1981). The percent incidence of wilting was recorded more in control field in comparison to sewage irrigated field (Chandra, 1990). The per cent incidence of wilting of linseed and gram were recorded more in control field in comparison to sewage irrigated field (Singh, 1992).

**The Rhizosphere Micro-Flora**

Rhizosphere refers to the zone of soil in which microbial activity is affected by the root of any plant species which may release organic substrates. The rhizosphere is the narrow soil zone, surrounding living plant roots, which contains root exudats, sloughed off root remains and large population of microorganisms of various
nutritional groupings (Baker and Cook, 1974). This zone has been considered to be of special importance because a root pathogen essentially traverses through this zone before infecting the roots therefore, the complex biology of rhizosphere has drawn a considerable attention of microbiologists. Garret (1956) mentioned that comparatively little attention was paid on the rhizosphere of diseased plants which has ecological significance in the disease development and pathogens population dynamics. The rhizosphere micro-flora influences the inhibition or stimulation of soil-borne plant pathogens (Rovira and Campbell, 1975). It is considered important, as the rhizosphere microbes may control the growth and spread of pathogens by antibiosis (Bowen, 1978 and Neuman, 1978).

The microbial population in and around root includes bacteria, actinomycetes, fungi, yeast and protozoa. Many investigators have shown that the fungi are dominant contributors to soil biomass. Vancura and Kunc (1977) have demonstrated that bacteria can dominate the rhizosphere by selectively inhibiting bacteria or fungi with antibiotics (Anderson and Domsch, 1978). The rhizosphere population could be regarded as a stable community around a particular plant species in a specific soil or alternatively as an unstable succession of population. Microorganisms may also produce plant
growth regulators (Brown, 1974 and Lynch, 1976). Although it is not known that they can produce these in sufficient quantity to affect the plant. The rhizosphere micro-flora may be particularly critical in effecting the breakdown of organic matter and basic minerals and in antibiotics produced by bacteria, actinomycetes and fungi showed toxic effect on many plant parasitic fungi (Gupta and Tandon, 1977). The rhizosphere is an interesting habitat for the study of microbial activity because of its relevance to crop production. The changing nature of rhizosphere effect is usually correlated with the nature and amount of root exudates. It has been reported that the root exudates have distinct selective action on rhizosphere microorganisms which result in stimulation of certain groups and suppression of the others (Lockhead, 1940 and Yoshida and Sakai, 1962). The work has been done on different microbiological aspects of the rhizosphere which has been reviewed by several workers (Starkey, 1929; Rovira and Davey, 1974; Katzenelson et al., 1948; Clark, 1949; Garrett, 1956; Buxton, 1957; Saksena, 1969; Garrett, 1970; Brown, 1975; Coleman, 1976; Hick and Chan, 1989 and Upaohay and Rai, 1992).

It is now well established fact that normally the micro-flora in non rhizosphere are less in number than in rhizosphere. Several worker have reported that variation are found in rhizosphere

The population of rhizosphere microorganism is greater at lower moisture level than higher ones whereas it is reverse in case of non rhizosphere soil (Griffin, 1963). The biology of pathogenic fungi is adversely affected due to increase in gross microbial population.

The rhizosphere micro-flora were changed by altering the environmental factors (Clark, 1948; Rovira, 1959 and Rouatt et al., 1963). Pollutants induce biochemical and physiological changes in the host plant and alter the physical and chemical environmental of the host surface which may favour infestation by the pathogen. Darbyshire and Greaves (1973) reported that the magnitude of the rhizosphere response in term of microbial number is markedly influenced by biological as well as chemical factors. Webley et al., (1952) suggested that different species of plant may develop root region flora with different characteristics. Thom and Humfeld (1932) observed higher number of microorganism in soil in proximity to the root of certain plants than in soil away from the roots.

Soil amendment with various organic substances have been done by various workers to study their effects on
microorganisms. Inhibition of growth of *Rhizoctonia solani* in chitin amended soil was reported by Sneh *et al.*, (1971). Sanikidze (1969) reported the enhancement of fungal development in soil on addition of ammonium sulphate, ammonium nitrate and calcium cyanamide. Mercury tolerant strains of *Aspergillus niger* from urambo soil (Gibson, 1953) and *Trichoderma* spp resistant to thiram (Richardson, 1954) reflect the diversity of the effect of fungicides on soil fungi. Rai and Tiwari (1977) reported the effect of soil fumigation with formation on soil mycoflora and found a decrease in number of species in treated soil samples which was due to fungicidal or fungistatic action of fumigant on propagules of the fungal species. Bourret *et al.*, (1965) observed the inhibition of chlamydospore formation in *Fusarium oxysporum* f. sp. *phaseoli* in high carbon dioxide pressure in the environment. Beraha and Powell (1953) reported the inhibition of *Monilia fruticola* by using some biphenyl derivatives. Rai and Tiwari (1975) studied the effect of a toxic chemical (HgCl₂) on soil mycoflora and reported that it was toxic to soil mycoflora for about a month but later the toxicity was reduced. A few species of *Aspergillus* and *Penicillium* showed tolerant capacity to mercuric chloride but the degree of their tolerance was based upon the concentration of chemical.
The effect of inorganic pollutants on soil microbial activity was studied by Wilke and Anne (1988). The purpose of this study was to develop a short term laboratory method to predict long term effect of inorganic pollutants on soil microbial activity. Kodama et al., (1988) studied the microbial degradation of disinfectants and treatment by activated sludge, the concentration of chlorhexidine CH in hospital or domestic waste water before or after pretreatment was determined in addition to microbiological and physico-chemical investigation. Some aspect of manganese availability and aluminium toxicity in the rhizosphere of plant is discussed by Uren (1989). Chandra (1990) reported that number of fungi g\(^{-1}\) dry soil was higher in rhizosphere in unpolluted garden soil as compared to the polluted soil. Marcos et al., (1995) studied the effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth promoting rhizobacteria.

**About the Plant, Its Wilt Disease and the Pathogen**

Tomato (*Lycopersicon esculentum* Mill.) is worlds third largest vegetable crop after potato and sweet potato. Tomato plant is a small annual or short lived perennial herbs and belong to the family
Only this species is cultivated throughout the world for its edible fruits. In India, the cultivation of tomatoes on commercial scale began towards the close of 18th century and is now cultivated both on commercial scale as well as in kitchen garden, thus providing jobs for thousands of landless people and small farmers especially in the proximity of the cities.

Tomatoes are good source of vitamin C and A and play an important role in human nutrition, especially due to its antiscorbic property. The area having under tomato cultivation in India is about 83,000 ha having total production of 7.90 lakh tones (Average 957 kg/ha [FAO, 1988]).

Tomatoes are affected by a number of biotic agents like fungi, bacteria, viruses, mycoplasma like organisms and nematodes, affecting almost all the parts of the plant such as root, leaves, stem and fruits. Among the fungal diseases vascular wilt *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen is most serious and poses a major constraint in successful cultivation of tomato especially in warm regions of the world including India. Generally farmers and small growers raise 2 – 3 crops of tomato in a year in the same field and due to the repeated cultivation of the same
or allied vegetable in the same soil year after year soil borne pathogens continue to perpetuate and affect adversely the new crop if proper care is not taken to combat the development of soil borne pathogens especially the tomato wilt Fusaria. The *Fusarium* wilt of tomato is cosmopolitan in occurrence and usually affects tomato in nursery as pre and post emergence seed and seedling rot and later causes wilting in seeded and transplanted crop. Because of its persistence in soil, infected fields become virtually useless for tomato cultivation for many years.

Tomato suffer annually from serious losses both in nursery and in field (transplanted crop) mainly due to *Fusarium* spp. of all the fusaria, *F. oxysporum* f sp. *lycopersici* predominate the wilt scenario (Kapoor, 1988). Nematode injury to plant further increase the chance of infection by pathogen. The importance of the disease could be judged from losses caused by it in different parts of world. Smith (1899) reported parasitic *Fusarium* which in some places put an end to growing of tomato in Florida. Orton (1903) reported losses of $500,00 in Florida. Ho Huang and Sun (1982) reported that losses were 26.8 to 35.3 percent in Taiwan. The accurate losses in India are not known since systematic plant disease survey on national scale had not
been carried out. However, according to some reports the damage to
tomato seedlings due to *Fusarium* spp. alone is about 15% (Singh and
Srivastava, 1953), whereas Misra (1954) reported that at the Netarhat
plateau of Chhotanagpur, Bihar, it has become impossible to grow
tomato because of the severity of *Fusarium* wilt of tomato and on an
average annual loss due to this disease was about 10 – 20% which
may go up to 80 percent in severe case. Recently a survey of those
states viz. Delhi, Maharashtra and Tamil Nadu was carried out by the
Indian Agricultural Research Institute, New Delhi (Kapoor, 1988)
which revealed that on an average 8 – 18 percent of the crop was
affected at different location and may result in the total loss (100%
wilting) of some of the cultivar at some places. In addition, the disease
was also found prevalent in peninsular part of India. Assam, Uttar
Pradesh, etc. The losses would be quite high as disease caused great
fluctuation in yields.

Soil borne pathogen is in general, more difficult to
control owing to the complex soil ecosystem and this is more
particularly in case of *F. oxysporum* f. sp. *lycopersici* because of its
persistence in the soil and other factors such as light soil root knot
nematode and tomato monoculture etc. aggravating the disease further
which results in seed and seedling rot in the nursery and wilting of adult plants in the field and pots.

Saccardo first described a *Fusarium* on decaying tomato fruit received from Italy in 1886 and was named as *Fusarium oxysporum* (Schl.) sub sp. *lycopersici* Sacc. Latter Thaxter (1890) reported a Fusarium on tomato fruit and referred its to as *Fusarium lycopersici* Sacc. However, Massee (1895) first described tomato wilt as ‘sleepy disease’ of tomato in Britain and attributed it to *Fusarium*. It is evident from his description that the tomato wilt pathogen possessed two stage, the *Diplocladium* and *Fusarium* forms, produced from the same hyphae bed only but the *Fusarium* form was capable of causing infection in tomato plants. Later Bewley (1922) showed that the *Fusarium* and *Diplocladium* forms were not stages of the same fungus but belonged to different genera and each could under definite conditions produce wilt. Wallenweber and Reinking (1935) recorded a large number of *Fusarium* species [*F. bulbigenum, V. lycopersici, F. equiseti, F. scirpi, F. scirpi, V. acuminatum (F. exebescens), F. oxysporum, F. moniliformae, F. coruleum, F. semitectum, F. lateritium, and F. culmorum*] causing will or rot of tomato. Wellman (1943) described a new species of *Fusarium* (*Fusarium retusum*) causing vascular will of tomato which produces identical symptom to
that of *F. bulbigenum*, *V. lycopersici*. The tomato wilt pathogens has been named differently by different workers from time to time but now are considered as synonyms of this organism as follows.


The fungus is a facultative saprophyte and there is considerable variation in cultural characteristics. Isolates also vary in their physiology and in their virulence.

The mycelium of *F. oxysporum* f. sp. *lycopersici* is white to pink, septate branched and intracellular. Conidia are borne in mosses (sporodochia). The sporodochia are red to orange in colour. Microconidia are generally one celled or sparingly septate, it richly scattered in the aerial mycelium and measures 8 – 18 × 2.5 – 4.8 μm. The macroconidia are three to five septate. Three septate conidia measure 25 – 66 × 2.3 – 4.3 μ while five septate 32 – 68 × 2.8 – 4.5 μ.
Chlamydospore are formed in old culture. They are smooth globose, one celled terminal and intercalary very numerous.

In India Butler (1918) was the first to report the occurrence of *Fusarium* wilt at Pusa (Bihar). Subsequently *F. solani*, *F. equiseti* c. Varma (1954), *F. semitectum* (Nedumaran and Vidyasekharan, 1982) and *Sclerotium rolfsii* (Khatua et al., 1981) have also been associated with tomato wilt. Recently, Kapoor (1988) determined the nature of wilt syndromes in different agro-climate region (Delhi, Maharashtra and Tamil Nadu) of the country and reported that pathogen involved in tomato wilt syndrome are *Fusarium oxysporum*, *F. solani*, *F. semitectum*, *F. moniliformae* (new record), *F. chlamydosporum* (new record), *Rhizoctonia solani*, *R. bataticola* and *Sclerotium rolfsii* *F. oxysporum* predominated the syndrome in Delhi and Tamil Nadu, where as *F. salani* in Maharashtra and are regarded as principal pathogen. The further reported that the prevalence of these two principal pathogens has been correlated with their aggressiveness. Association of *Rhizoctonia* with the syndrome is more common in Maharashtra and Tamil Nadu than in Delhi. In addition to the wilt, the pathogen also causes pre and post emergence seedling rot and damping off of tomato.
In India, the disease was reported more than seven decades back (Butler, 1918) and since then some stray attempt have been made by various workers to control this disease. Though breeding for disease resistance is reliable and cheapest method to combat the disease, the different agro-climatic conditions and presence of variability in the tomato wilt pathogen render it more difficult. Although, resistant varieties are available and cultivated widely elsewhere (Porte and Walker, 1941; Strobel et al., 1969; Crill et al., 1971; Collingwood and Defrancas, 1979; Visser, 1980 and Pavan and Kurozawa, 1981) but so for true resistance has not been incorporated with well adopted or preferred tomato varieties. In India as most of the cultivated varieties have succumbed to the disease (Kapoor, 1988), other forms of control mainly chemical, biological etc. must be practiced. In order to mitigate the tomato wilt menace, various measures have been attempted from time to time in other countries and rarely in India. Based on the existing information, studies on some of the important and easily available seed protestants, soil fungicides, systemic fungicides and growth regulators as well as different methods of their application were carried out so as to recommend the most effective one for further field trials (Sen and

**Microbial Interaction**

Microbial interaction is an important factor for biological control of soil-borne plant pathogens (Garrett, 1965 and Baker and Cook, 1974) and has a large bearing on the establishment of an organism in the rhizosphere. Antagonism between *Fusarium udum* and root region micro-flora of pigeon pea was studied by Upadhyay and Rai (1987) with reference to colony interaction, hyphal interference, volatile and non-volatile metabolites and staling growth product. *In vitro* studies may indicate the potentialities of antagonism of a fungus in soil. *F. udum* was observed to parasitize *Rhizopus nigricans* (Rai et al., 1977), *Cunnighamella echinulata* (Upadhyay, 1979), *Curvularia lunata* (Upadhyay, 1979), *Mortierella subtilissima* (Upadhyay et al., 1981a) and *Aspergillus luchuensis* and *Syncephalastrum recemosum* (Upadhyaya et al., 1981b). The formation of chlamydospore of *Fusarium udum* was observed frequently in side the hyphae, conidiophores and the sporangiophores of the host fungi as a consequence of mycoparasitism (Rai et al., 1977 and Upadhyay et al., 1979).
The formation of chlamydospore inside the hyphae and conidiophores or sporangiophores have been attributed to non-availability of nutrients and other physiological and environmental stresses being exerted inside the host (Rai et al., 1977).

A generalized concept of biological control has been proposed by Baker and Cook (1974) that biological control is the reduction of inoculum density or disease producing activity of a pathogen or parasite in its active or dormant stage, by one or more organisms accomplished naturally or through manipulation of the environment, host or antagonists, or by mass introduction of one or more antagonists. Emphasis have been laid on mycoparasite reactions (Dennis and Webster, 1971b; Barnett and Binder, 1973; Upadhyay and Rai, 1983b; Benhamou and Chet, 1983; Mandal et al., 1996; Aggarwal et al., 1996), inhibition of an organism by the others due to volatiles (Dennis and Webster, 1971b; Hutchinson, 1971 and Skidmore and Dickinson, 1976) and non volatile metabolites (Dennis and Webster, 1971a; Skidmore and Diekinson, 1976; Kredics et al., 2005 and Szekeres et al., 2005) and the role of staling growth products in microbial interaction (Park, 1964) mainly in view of possible biological control of plant pathogens. Baker and Cook (1974)
pointed out that biological control rarely eliminates a pathogen from the site but rather reduces its number or its ability to produce diseases.

The hyphal interaction and parasitism include defined kinds of interactions such as morphologicl changes, coiling of one hypha by the other, vacuolation, granulation and hyphal bursting, penetration, production of houstoria and lysis of the hyphae. Colony interaction, parasitism and formation of resting bodies by the parasites hosts have been studied by many investigators (Skidmore and Dickinson, 1976; Arora et al., 1979; Upadhyay et al., 1981; Flad et al., 1983 and Rai and Bashar, 1989). Several other workers have reported antagonism against the soil borne plant pathogenic fungi (Hornby, 1978; Rai and Upadhyay, 1978; Arora et al., 1979; Henis, 1984; Rai and Bashar, 1989; Kokalis, 2002; Singh et al., 2003a and Singh, 2003). Gupta et al. (2006) evaluated T. viride for the control of wilt complex disease of chick pea under field condition. Joshi and Raut (2005) have observed low incidence of seedling wilt with the use of bioagent such as Trichoderma viride and T. harzianum. Trichoderma isolated proved to be effective in controlling Phytophthora dresceleri f. sp. cajani under green house conditions (Srivastava and Malt, 2008).
Actinomycetes and bacteria play an important role in the inhibition of fungal growth. An actinomycetes *M. globosa* was found to be a destructive parasite of *F. udum* (Rai and Upadhyay, 1978). Presence of growth inhibitors in the gases emanating from bacterial and actinomycetes cultures have also been reported by French (1962) and Dennis and Webster (1971b). Chung et al. (1989) reported that the antagonistic activity against *F. salani* might have occur due to the lysis of fungal cell wall by *Streptomyces* which produce chitinase. Many antagonists produce antibiotic substance which have the ability to produce enzymes, which cause the lysis of cell wall components of the pathogenic fungi. This helps the antagonists to penetrate the host hypha and grow on it as hyperparasite (Tapio and Arja, 1989). In general bacterial antagonist showed greater inhibition of the wilt pathogen than fungal antagonist *in vitro* (Kapoor and Kar, 1988). Velvis et al. (1989) studied the role of antagonism in the decline of *Rhizoctonia salani* inoculums in the soil.

Lysis and deformation of the mycelium as *Neovossia indica* by Actinomycetes isolates act-V has been shown by Amer (1995) and Aggarwal et al., (1996). Actinomycetes also showed the lysis and degradation of the mycelia of *Pythium aphanidermatum* (Dish and Yagi, 1973 and Kausakari and Ueyama, 1975).
*Coniothyrium minitants* is a potential mycoparasite against *Sclerotium* spp. Application of *C. minitants* inoculum to soil have been reported to reduced the survival of sclerotia of *Sclerotinia sclerotiorum* (Haung, 1980; Quiken *et al.*, 1995 and Cale *et al.*, 2001). Recently Elad and Freeman (2002) have reviewed the role of antagonistic fungi in the management of foliar and soil borne diseases of crops.

**Effect of Volatile Metabolite of Fungi on Mean Radial Growth of Pathogens**

Several investigators have reported production of biologically active volatile substances by various microorganisms which have been found to inhibit or stimulate the growth of fungi (Robinson and Garrett, 1969; Dennis and Webster, 1971b; Hutchinson, 1971; Singh *et al.*, 1975; Skidmore and Dickinson, 1976; Rai *et al.*, 1981; Basar, 1990; Chandra, 1990; Singh, 1992; Bjurman and Kristensson, 1992; Wilkins and Larson, 1995a and Singh, 1996). Corbon dioxide is the most common fungal volatile which affect the hyphal growth, sporulation and spore germination. Hutchinson (1971, 73); Fries (1973) and Vilanova *et al.*, (2007) gave a comprehensive review on biological activity of volatile metabolites of fungi. Production of volatile substances in soil and their involvement in
fungistasis is well documented by Pavlica et al., (1978) and Papavizas and Lumsden (1980). Robinson and Garrett (1969) collected volatiles from culture of *Fusarium oxysporum* that inhibited germination of sporangiophore of *Cunninghamella elegans*. Dennis and Webster (1971b) have demonstrated inhibitory effect of various fungi. Hutchinson and Cowan (1972) reported that gases emanated from the culture of *Trichoderma harzianum* reduced the growth of *Aspergillus niger* and *Pestalotia rhododendri*. Volatile metabolites produced by fungi include hydrogen cyanide (Ward and Thorn, 1965), thiazole, acetaldehyde (Robinson and Garrett, 1969), ethanol (Pentland, 1967) and several other toxic substances (Hutchinson, 1971 and Fries, 1973). Production of volatile inhibitor of germination and hyphal extension by *Geotrichum candidum* has been studied by Mckec and Robinins (1988).

Streptomyces produced a range of volatile substances which showed different effects on different test fungi (Gupta and Tandon, 1977). Various degrees of inhibition of growth and spore germination of *F. udum* by different microorganisms have been observed by Upadhyay and Rai (1987). The volatile metabolites of some actinomycetes on growth of some litter decomposing fungi was studied by Rai et al., (1981). The volatile metabolites of the four species of streptomyces inhibited the mycelia growth of *Acremonium*
furcatum, Aspergillus niger, Cladosporium cladosporoides and Trichoderma harzianum. Muemlndfeld and Hans (1988) reported that asperdurinn isolated from Aspergillus duricaulis show significant antifungal activity.

**Effect of Microbial Non-Volatile Metabolite on Growth of Pathogens**

Many microorganisms produce biologically active non-volatile metabolites and toxic or general staling substances (Brain, 1949; Gottleib and Shaw, 1970; Dennis and Webster, 1971a; Singh and Webster, 1973 and Skidmore, 1976). Several non-volatile antibiotics produced by Trichoderma sp. are trichodermine (Godtfredsen and Vangedal, 1965), alamethicine (Meyer and Russer, 1967) and dermidine (Pyke and Dietz, 1966 and Meyer, 1966) which are active against a range of fungi. Aspergillus terreus has been reported to secrete geodin, terricin and terric acid (Marcus, 1947). A number of cases have been reported where metalolites are secreted by microorganism which generally have toxic effects on the microfungi (Brain, 1957). These non-volatile metabolites of the microorganisms bring about morphological changes in fungi and interfere with their metabolism. Inhibition of linear growth and/or spore germination of various fungi either directly or indirectly by fungal metabolites have
been reported by a number of investigators (Bilai, 1956; Park and Robinson, 1964; Dick and Hutchinson, 1966; Robinson et al., 1968; Glen and Hutchinson, 1969; Hutchinson, 1971; Fries, 1973 and Cowan et al., 1973). The toxicity of microbial metabolites may also cause the complete check of growth; stunting and lysis of fungal hyphae. Alexopolous and Herrick (1942) also laid emphasis on inhibitory effect of some actinomycetes on various species of fungi in culture. Hora and Baker (1972) reported that actinomycetes were quite active and capable of producing volatile materials inhibitory to spore germination. Hutchinson and Cowan (1972) reported the inhibition of growth and sporulation of *Aspergillus niger* and *Pestalotia rhododendri* by the gases produce by culture of *Trichoderma harzianum*. Furgal and Helena, 1988 found that culture filtrate of the saprophytic fungi inhibit the growth of *Rhizobium leguminosarum*. Effect of gluconase and chitinase produced by *T. harzianum* detected on the walls of *Sclerotium rolfsii*. Protease and lipase activity were also detected in the medium when the antagonist attached to mycelium of *S. rolfsii* (Elad et al., 1982). Recently the role of extra cellular enzymes produces by fungi *Trichoderma* sp. has been well documented by several investigator, (e.g. Proteolytic enzyme L Kredics et al., 2005 and chitinase Hoell et al., 2005). Effect of non-
volatile metabolites of some microbes have been observed by Bashar (1990), Chandra (1990), Singh (1992) and Singh (1996). Szekeres et al. (2005) have reviewed antagonistic metabolites produced by *Trichoderma* spp. The metabolites are linear, amphipathic palypeptides, namely peptibols and peptibiotics. They also discussed the physico-chemical and biological properties of these antibiotic compounds which include the disruption of lipid membranes, antimicrobial activities and induction of plant resistance.

**Effect of Pesticides on Growth Behavior of Pathogens**

Although a varieties of non-chemical methods (e.g. biological control, breeding for disease resistance and cultural practices) are available for controlling diseases, pesticides particularly fungicides remain a necessary component of crop production programmes due to its highly visible, effective and practical technique to manage different plant disease and in some instances the principal method of control. It has been estimated by the “Pesticides Association of India” (1975) that annual losses due to pests and diseases are about 18.4 per cent of the total produce of which losses due to disease are 25 per cent and those in storage are 7 per cent.
(Krishnamurthi, 1975). Most of this crop loss is due to fungal pathogens Cramer (1967) estimated by using FAO and other statics that about 12 per cent losses in world crop yields annually are due to diseases, excluding losses during storage.

In recent years, plenty of information is available on the effect of fungicides on the control of soil-borne plant pathogens and their activities in soil (Kannaiyan and Prasad, 1983; Kotasthane et al., 1987; Ray and Das, 1987; Singh and Dwivedi, 1988; Basar, 1990; Winter et al., 1993; Singh et al., 1992 and Souliac and Leroux, 1995). Mushogho (1968) and Richardson (1954) observed the dominance of Trichoderma in thiram treated soil. One of the prime effects of treating soil with fungicides may be that, antagonists are stimulated to rapid growth and sporulation, and that the pathogen may be weakened, and therefore, secondary control of the pathogen is obtained (Munnecke, 1972). A major objection of many of the chemical control currently in use is their indiscriminate effect on organisms other than pathogen (Bashar, 1990). It may only be necessary to weaken the pathogen, rather to kill it and make it more vulnerable to antagonism of the associated microflora (Baker and Cook, 1974).
Shukla et al., (1981) and Bashar (1990) have studied the efficacy of various fungicides against *Fusarium oxysporum* f. sp. *ciceri* and reported bavistin as the best seed dresser which increased germination of seeds but at the same time reduce the incidence of wilt disease. Souliac and Leroux (1995) found that Benzimidazole and carbendazim were very effective fungicide against stem rot of rape caused by *S. sclerotiorum*. Huang et al. (1997) reported that amendment of soil with either fermented agricultural wastes (CF - 5) or allyl alcohol at 150 – 400 ppm suppressed apothecial production of *S. selerotiorum* and enhanced the colonization of sclerotia by *Trichoderma harzianum*. Smolinska and Horbowicz (1999) reported that volatile compounds mainly isothiocyanates decreased the survival of resting propagules of three pathogenic fungi viz., *F. oxysporum* f. sp. *lycopersici* var *radicis*, *Sclerotium cepivorum* and *S. sclerotiorum*. Recently Shivpuri and Gupta (2001) and Singh (2002) observed the efficacy of some fungicides against *S. sclerotiorum* and found that bavistin, topsin-M and celest has completely inhibited the growth of the pathogen at all the concentration tested.

The effect of different insecticides on soil borne plant pathogens have been studied by several workers. Insecticides may affect soil-borne pathogens in addition to insecticidal properties
Tisserat (1977). When insecticides are applied to the soil they may affect soil borne pathogens in addition to other microorganisms and ultimately plant diseases (Bollen, 1961 and Cole and Batson, 1975). Effect of insecticides on the severity of *Verticillium* wilt was studied by Leach and Frank (1982). Aguda et al. (1984) also evaluated the efficacy of insecticides against two entomogenous fungi. Siddique Aguda et al., (1987) reported the efficacy of metasystox – R against some common fungi amongst which *Rhizoctonia solani* showed greatest sensitivity against the insecticide. Efficacy of some insecticides was evaluated against *Sclerotium rolfsii* in *vitro* and was noted that B. H. C. caused maximum inhibition of growth of the pathogen (Singh and Dwivedi, 1988). Bashar (1990) observed efficacy of some insecticides against *Fusarium oxysporum* f. sp. *ciceri*.

**Effect of Heavy Metals on Micro-flora**

In recent years extensive studies have been done on changes in the spectrum of fungal flora, biomass, decomposition, soil respiration and soil activity in heavy metal polluted soil (Freedman and Hutchinson, 1980; Nordgren et al., 1983; Yamamoto et al., 1985). The concentration of the heavy metals like Cd, Cr, Cu, Ni, Pb and Zn increases in the ecosystem as a result of continuous emission
of pollutants which exert a severe impact on their functioning (Babich and Stotzky, 1974). Toxicity of heavy metals depends on the physico-chemical properties of the recipient environment (Babich and Stotzky, 1980, 1983 and Wong et al., 1980).

The increasing pH enhanced the toxicity of Cd against Aspergillus niger (Babich and Stotzky, 1977). Various heavy metals are highly toxic to plants and animal. Their potentiality as hazardous chemicals has been widely acknowledged. The seriousness of heavy metals as environmental pollutants is further accentuated by the following facts (Antonovics et al., 1971):

i. Heavy metals tend to accumulate in organisms at various trophic levels and thus their toxicity tends to increase at upper levels of the food chain.

ii. Unlike most organic substances, heavy metals cannot be degraded by biological organisms.

iii. Tolerance to heavy metals is highly specific.

Much work has been done on the effect of heavy metals particularly copper and mercury against fungi because of their fungicidal values (Ashida, 1965 and Ross, 1975). All heavy metals adversely affect the survival growth, species diversity and interaction
of microorganisms in natural environment. For instance a decrease in the number of microbial species was noted in soils collected from heavily contaminated site with cadmium, lead, zinc and copper as compared to soils obtained from non-contaminated site (Hartman, 1974). Total number of bacteria, actinomycetes and fungi were found to be reduced in most severely zinc contaminated soils as compared to control soil (Jordan and Lechavaliar, 1975).

Cadmium compounds, both inorganic and organic, are commonly used against fungal infection. Several studies have shown varying degree of tolerance of fungi to cadmium. Ashida (1965a) reported that *Penicillium glaucum* is able to tolerate cadmium. Strain of *Fusarium oxysporum* isolated from soil heavily contaminated with Cd, Cu, Pb and Zn had greater tolerance to these heavy metals than isolateds from uncontaminated soil. The toxic effect of the Cd on fungi may be exerted on several forms of fungal development on mycelia growth, fruiting body formation and spore germination. Babich and Stotzky (1978) have reported two categories of fungi on the basis of their sensitivity to cadmium. (i) Fungi capable of growth on agar amended with upto 10 ppm Cd, but inhibited by 100 ppm Cd *Botrytis cinerea, Penicillium vermiculatum, Aspergillus flavus* and *Fomes annosus* (ii) Fungi capable of growth in the presence of 100
ppm Cd e.g. *Rhizopus stolonifers*, *Trichoderma viride*, *Penicillium asperum* and *Cunnighamella echinulata*. Cifferi and Baldacci (1945) reported that sporocidal activity of Cd is less than Cu or Hg for species of *Alternaria tenuisima* and *Plasmopara viticola*. Cd acts as a growth inhibitor for Bacteria *Bacillus circus*, *E. coli* and *Agrobacterium tumefaciens*, the actinomycetes *Nocardia paraffinae* and the filamentous fungi *Aspergillus niger*, *Rhizopus stolonifer* and *Trichoderma viride*. In general, actinomycetes are more tolerant to Cd than the bacteria (Babich and Stotzky, 1983a). Bashar (1990) reported the inhibition of growth of *Fusarium oxysporum* f. sp. *ciceri* by various concentration of Cd. Singh (1992) also recorded the inhibitory effect of Cd on growth of various fungi, bacteria and actinomycetes.

Inhibitory effect of nickel was found against *Bacillus urevis*, *Pseudomonas* sp., the actinomycetes and the filamentous fungi e.g., *Trichoderma viride*, *Rhizopus stolonifers*, *Aspergillus gigantus*, *A. niger* and *Penicillium vermiculatum*. The effect of Ni was found to be more toxic to growth of *T. viride*, *R. stolinifers*, *Penicillium vermiculatum*, *Gliocladium* sp., *Aspergillus gigantus* and *A. niger*. Toxicity of Cu has been reported against *A. niger*, *P. simplicissimum*, *A. scyptalydium* sp. and *Fusarium oxysporum* (Babich and Stotzky, 1986). The toxic effect of lead has been observed against the growth
of *A. niger*, *T. viride*, *Saprolegnia* sp. and *Achlya* sp. (Babich and Stotzky, 1983b). Magnesium showed inhibitory effect on the growth of *Rhizopus stolonifers* and *Trichoderma viride* (Babich and Stotzky, 1986).

Mercury, Cu, and Cd are potent component of several fungicides to control various plant diseases due to their toxic effects against several pathogenic fungi. Bashar (1990) reported that mercury, Cu, and Cd inhibited growth of *Fusarium oxysporum* f. sp. *ciceri*. Singh (1992) also observed that Cd, Cr, Pb, Ni and Cr inhibited the radial growth of *Fusarium oxysporum* f. sp. *ciceri* and *Fusarium oxysporum* f. sp. *lini*. Murugesan (1990) observed that increasing concentration of the Bo, Cu, Mn and Zn individually or in combination, inhibited the radial growth and hyphal dry weight of *Rhizoctonia bataticola*.

Heavy metals such as Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn are of particular concern in industrial, agricultural and domestic wastes. When such wastes are allow to enter various ecosystems, heavy metal concentration may gradually increase to toxic levels for micro-flora and fauna (Huisingh and Huisingh, 1974; Williams and Wollum, 1981; Dubey and Dwivedi, 1988). The chloride forms of Cd,
Co, Ni and Hg have been found to inhibit mycelial growth and sclerotia formation by Sclerotium rolfsii and Rhizoctonia solani (Dwivedi et al., 1986). Copper and Hg were used to control the club root disease caused by Plasmodiophora brassicae (Preston, 1931).

A survey of literature indicate that, so far little work has been done on the effect of land application of effluent on soil microflora (pathogenic and non pathogenic), particularly with reference to the pathogen Fusarium oxysporum f. sp. lycopersici causing wilt disease of tomato in relation to their incidence and severity. The present work was therefore undertaken to study the following aspects in detail.

1. Determination of physico-chemical characteristics of the effluent, tube well water and soil samples collected from effluent irrigated and controlled fields.

2. Isolation and study of microflora (Fungi and bacteria including actinomycetes) from effluent treated and untreated (control) soil samples collected from the selected fields at monthly interval.

3. Study of incidence and severity of wilt disease of tomato from the selected fields.
4. Study on the population dynamics and pathogenicity of the pathogen in the selected fields.

5. Studies on microbial interactions between the pathogenic and non-pathogenic microflora with reference to biological control of the plant disease.


7. Effect of different pesticides on growth behavior of the test pathogen.

8. Effect of effluent on growth of some dominant microflora and the test pathogen.

9. Effect of some heavy metals on growth of some dominant microflora and the test pathogen.