STUDIES ON INTEGRATED MANAGEMENT OF CHICKPEA WILT CAUSED BY *FUSARIUM OXYSPORUM* f. sp. *CICERI*

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Dedicated to My Beloved Father

DECLARATION

I hereby declare that the thesis entitled **"STUDIES ON INTEGRATED MANAGEMENT OF CHICKPEA WILT CAUSED BY *FUSARIUM OXYSPORUM* f. sp. *CICERI*"** embodies the results of the original research work carried out by me at the Department of Environmental Science in the School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow. The work presented in this thesis has not been submitted for the award of degree or diploma to this or any other University.

Jyoti Srivastava

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PREFACE

Grain legumes hold a significant place next to cereals in lieu of their economic and nutritional importance as human food. Legumes are particularly important to the livelihoods of poor farmers of rain fed areas as they provide food, animal feed and income. The major food legumes in the developing countries comprise of: broad bean (*Vicia faba)*, chickpea (*Cicer arietinum*), cluster bean or gaur (*Cyamopsis teragonoloba*), dry bean (*Phaseolus vulgaris*), dry pea (*Pisum sativum*), groundnut (*Arachis hypogaea*), horse gram (*Macrotyloma uniflora*), lablab bean (*Lablab purpureus*), lathyrus (*Lathyrus sativus*), lentil (*Lens culinaris*), moth bean (*Vigna aconitifolia*), mung bean (*Vigna radiata*), pigeon pea (*Cajanus cajan*), soybean (*Glycine max*) and urd bean (*Vigna mungo*). Food legumes are the most versatile of the human foods as they contain many of the nutrients required for a healthy human body. They are low cost source of protein and energy as compared to animal protein. Due to radical increase in the population in the developing countries, the demand for food legumes has doubled in the last quarter century as people look for cheaper alternatives to meet their daily protein requirements. Food legumes, with their inherent ability to fix atmospheric nitrogen, play a cardinal role in maintaining soil fertility and sustainability of production systems. Apart from contributing significant amount of nitrogen, the legumes act as barrier to disease development for cereals, provide diversified weed control options, increase organic matter and reduce leaching losses.

Food legumes fetch higher prices in comparison to cereals and hence are increasingly being grown to supplement farmer’s income, thus enhancing livelihoods. The ability of the pulses to thrive better than other crops under harsh climate and fragile ecosystems make them potential candidates for diversification of cropping

systems. Sustainable agriculture, oriented towards market economy also demands inclusion of pulses in cereal based cropping system, for boosting up overall productivity and sustainability of the systems and to enhance farmer's income. Recent advances in genetic improvement and management techniques, for legumes have enhanced the feasibility of their cultivation in non-traditional niches and cropping systems, contributing to enhanced crop diversification biodiversity and income generation.

Chickpea is amongst the principal grain legumes grown in India having a large share in global production. There is a huge gap between demand and supply of chickpea and as such India has to import chickpea from Mexico, Australia, and Canada, spending huge foreign currency every year. Among the many factors contributing to low production of chickpea, lack of an assured market also contributes significantly for low production of chickpea like other pulses. This is on account of the minimum support price (MSP) as announced by the government. The MSP is high for chickpea; it is calculated on the basis of cost of cultivation which is low for chickpea, the reason being it is largely a rainfed crop so farmers have little to invest in fertilizers, insecticide, fungicides etc. Therefore it comes into prominence that while assigning the MSP due credit should also be given to other aspects like improvement in soil fertility, soil structure and thus overall contributions to sustainable agricultural production. Along with recent approaches in managing the crop production due concern should also be given to market infrastructure like storage warehousing postharvest and processing facilities so as to smoothen the chickpea economy on one hand and sustain the protein requirement on the other hand. In changing agricultural scenario there is a dire need to revisit the chickpea research priorities, and strategies so that the country remains competitive in the world market. To achieve this, the only

option is to increase the production per unit area per unit time as the scope of area expansion under chickpea is very limited. Chickpea, like other legumes is prone to many biotic and abiotic stress, of this *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is of primary concern. As such the present study was undertaken, to explore natural and ecofriendly measures in combating the disease.

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ABBREVIATIONS

FAO	Food and Agricultural Organization
FAOSTAT	Food & Agriculture organization of the United Nations Statistical D. Base
BOD	Biological oxygen demand
ANOVA	Analysis of Variance
DAS	Days after sowing
WHC	Water holding capacity
rpm	Rotations per minute
v/v	Volume in Volume
sp.gr.	Specific gravity
PCM	Phase Contrast Microscope
ppm	Parts Per Million
mm	Millimeter
µl	Micro liter
ml	Milliliter
SD	Standard deviation
CMC	Carboxy Methyl Cellulase
CZB	Czapek's Dox Broth
U.V.	Ultraviolet light
PG	Poly galacturonase
w/v	Weight in Volume
PME	Poly Methyl Esterase
C_x	Cellulase enzyme
PMG	Poly Methyl Galactouronase
PTE	Pectin Transeliminases
SE	Standard error

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Introduction

Chickpea opined vernacularly as chana botanically, as *Cicer arietinum* L. is amongst the third largest produced crop plant in the world. The phenomenal rise in the production of chickpea worldwide can be assigned to its myriad uses; it's high protein content, palatability and its essential role in animal and human diet. It's pivotal role in improving soil fertility, particularly in dry areas has assigned it a special significance in the development of sustainable agriculture of the arid and semiarid tropical regions across the globe.

1.1 Origin

Cicer arietinum L. (2n=16, n=8) which is largely a rainfed crop known by the common names, chickpea (UK), Garbanzo bean (Latin America), Bengal gram (Indian), Hommes, Hamaz (Arab world), Shimbra (Ethiopia) and Nohud and Loblebi (Turkey) is a self-pollinated rabi crop, upto 1% cross pollinated (Smithson *et al.,* 1985; Singh, 1987). It is the principal grain legume in India and is extremely popular in the food habit. In Mesopotamia and Palestine it is not sown in a wild state, but is found as an escape. The genus *Cicer* has 39 species. Besides *Cicer arietinum* L. i.e. the chickpea, 07 annual wild or weedy species and 31 perennial ones exists. Vavilov (1929) mentioned the Central Asia, Hindustan, near Eastern, Mediterranean center as primary source of origin and the Ethiopian center as secondary. These diverse centers formed the core area inhabited by the wild *Cicer* species. The crop was familiar to the ancient Greeks, Hebrews and Egyptians. It has been introduced in recent times to tropical Africa, Central and South America, South East Asia and Australia (Van Der Maessen, 1987).

The Chickpea moved from its ecological optimum foothills with high light intensity, long days, moderately high temperatures, well drained soils to the plains of India and has adapted quite well here. Anthropogenic distribution has been proven

well by its presence as cultivated chickpea in Hacilar, Turkey dated 5450 (B.C.). Sanskrit name chanaka, as well as carbonized names point to the presence of chickpea in India only about 250 years ago.

Van Der Maessen (1987) believed that the species originated in the Southern Caucasus and Northern Persia. However, Ladizinsky (1975) reported South East Turkey as the center of origin of chickpea based on the presence of closely related annual species, *C. reticulatum* Ladizinsky and *C. echinospermum* Davis. Morphologically as well as the seed protein profile of these two species resemble widely to the cultivated species *C. arietinum*. Wild *C. reticulatum* is interfertile with the cultivated pulse and morphologically closely resembles cultivated *C. arietinum*. Hence, it is regarded as the wild progenitor of chickpea (Ladizinsky, 1975).

Botanical and archaeological evidence show that chickpea were domesticated in the Middle East and Ethiopia since antiquity. Brought to the new world, it is now in Mexico, Argentina, Chile, Peru, U.S and Australia. Wild species are most abundant in Turkey, Iran, Afghanistan and Central Asia (Duke, 1981).

1.2 The Plant

Chickpea belongs to family Fabaceae order Rosales according to Bentham and Hooker's classification. The scientific name *Cicer arietinum* has been derived from the Roman word Cicer, owing to the resemblance of the seed to the head of a ram and the word arietinum derives its name from 'aries' meaning 'ram'. The plant is a much branched erect or spreading annual, 25-50 cm tall. The leaves are imparipinnate with 9 to 15 pairs of ovate, elliptic or obovate leaflets with serrate margin. All parts of the plant are clothed with glandular hairs, the secretions of which are rich in oxalic and malic acids that impart a sour taste to the leaves and fruits. The flowers, varying in colour from white to pink, are usually borne singly. The pods are small cup upto 3×2

cm but inflated and contain one or two seeds which are angular with a prominent beak and small hilum at the anterior end. The seed coat may be wrinkled, smooth or rough, ranging in colour from white, red, brown, to nearly black. The cotyledons are thick and yellowish. The stamina column is diadelphous (9+1) and the ovary is sessile, inflated, and glandular-pubescent, seed colour cream, yellow, brown, black or green rounded to angular, seed coat smooth or wrinkled tuberculate, laterally compressed with a median groove around two third of the seed, anterior beaked; germination crypto-cotylar (Duke, 1981; Cubero, 1987; Van der Maessen, 1987).

1.3 The crop-Chickpea

Chickpea are grown in India as a post monsoon winter season (rabi) crop that requires cool, dry weather for their optimum growth. They are susceptible to frost, but are highly drought tolerant because of their deep root system. It is a good crop and gives better yields under irrigated conditions in low rainfall areas also. Excessive rains after sowing or at flowering stage adversely affect the crop yield. The advent of early summer in tropics reduces the yield. Silty clay loams or deep loams without the presence of soluble salts were found as the best soils for chickpea growth (Moolani and Chandra, 1970). Such soils retain up to 200 mm moisture in the soil profile up to a depth of 1 m (Saxena, 1987). The maximum nutrient availability from the soil is at a pH range of 5.7 to 7.2 (Mahler *et al*., 1988).

Chickpea requires good soil aeration. Therefore, heavy soils require care in seedbed preparation. In such soils a rough seedbed is useful as it is not prone to surface compaction due to winter rains which may hinder seedling emergence (Kay, 1979). Chickpea is highly sensitive to salinity and sodicity in the soil (Chandra, 1980). Salinity has an adverse effect on dry-matter production and uptake of phosphorus, zinc, and iron (Dravid and Goswami, 1987). Increase in salinity (chloride

or sulfate) leads to a decrease in nodule weight, leghemoglobin content number and weight of pods per plant and biological yield. Salinity also restricts the outward movement of fixed nitrogen from the nodules, retards the translocation of nitrogen to the seeds and increases its accumulation in the leaves and pod walls (Ram *et al*., 1989).

Seed is either broadcasted or planted in rows. Two types of chickpeas are cultivated: small seeded (desi type) with generally brown to bright yellow testa colour and large seeded (Kabuli type) with salmon white testa colour.

Small seeded types are the characteristic of the Indian subcontinent and large seeded types of the Mediterranean and Western hemisphere regions. White or yellow coloured large seeds command the highest price in Indian market. The crop is most popular in the Northern Plains of India including Uttar Pradesh, Madhya Pradesh, Rajasthan, Haryana, Punjab, Bihar, West Bengal and parts of Maharashtra because of the conducive ecological condition for good growth. Chickpea production is also reported in peninsular India, in the states of Karnataka and Andhra Pradesh. The growing period and productivity of the crop declines in south of Central India. This is attributable partly due to shortness of the winter season. A similar trend is discernible, in the west east direction and in the gangetic plains. Notwithstanding its distribution throughout the country, five states *viz.,* Madhya Pradesh, Rajasthan, Uttar Pradesh, Maharashtra and Andhra Pradesh together contribute 86 percent of the chickpea production.

1.4 Economic importance

Chickpea seeds are a major source of human food and animal feed because of their high content of lysine-rich protein (Jukanti *et al.*, 2012). It has highest nutritional composition of dry edible grains containing vitamins, carbohydrates, proteins and

minerals (Table 1). It does not contain any antinutritional factor. It has considerable amount of fat contents ranging between 3.8-10.2 percent in different cultivars. Chickpea seed is valued for its high nutritive value, with 25.3 to 28.9% protein content (Muehlbauer and Rajesh, 2008; Hulse, 1991). In addition to source of proteins it has carbohydrate 38-59%, fiber 3%, oil 4.8-5.5%, ash 3%, calcium 0.2%, and phosphorus 0.3%. Its protein and carbohydrate digestibility varies from 76 to 78% and from 57 to 60% (Hulse, 1991; Huisman and Venderpoel, 1994). It is used as medicine for bronchitis, catarrh, aphrodisiac, cutamenia, cholera, constipation, diarrhea, dyspepsia, flatulence, snakebite, sunstroke and warts. The blood cholesterol level is decreased by the acids in it. Its seeds are considered antibilious (Duke, 1981). Rastogi (1993) compiled the reported chemical constituents present in chickpea seedlings as Isoliquiritigenin, Isoliquiritigenin-4glucoside, 3,4,7-trihydroxyflavone, daidzein, pratersein, p-coumaric acid, garbenzol and biochanin-7-glucoside.

Table 1 **Chickpea seeds, nutritional value**

S. no.	Ingredients	Value per 100 g (3.5 oz)
1	Energy	686 kJ (164 kcal)
2	Carbohydrates	27.42 g
3	Sugars	4.8 g
4	Dietary fiber	7.6 g
5	Fat	2.59 g
6	Saturated	0.269 g
7	Monounsaturated	0.583 g
8	Polyunsaturated	1.156 g
9	Protein	8.86 g
10	Water	60.21 g
11	Vitamin A equiv.	1 µg (0%)
12	Thiamine (Vit. B1)	0.116 mg (9%)
13	Riboflavin (Vit. B2)	0.063 mg (4%)

14	Niacin (Vit. B3)	0.526 mg (4%
15	Pantothenic acid (B5)	0.286 mg (6%)
16	Vitamin B6	0.139 mg (11%)
17	Folate (Vit. B9)	172 μg (43%)
18	Vitamin B12	0 μg (0%)
19	Vitamin C	1.3 mg (2%)
20	Vitamin E	0.35 mg (2%)
21	Vitamin K	4 μg (4%)
22	Calcium	49 mg (5%)
23	Iron	2.89 mg (23%)
24	Magnesium	48 mg (13%)
25	Phosphorus	168 mg (24%)
26	Potassium	291 mg (6%)
27	Sodium	7 mg (0%)
28	Zinc	1.53 mg (15%)

Percentages are relative to US recommendations for adults, Source: USDA Nutrient database

1.5 Area and production

India is the principal chickpea-producing country with 73.3% of the world acreage and 67.4% of the production. Pakistan ranks second with 7.3% of world acreage and 5.7% of production followed by Australia (4.2% acreage, 6.2% of production), Iran (4.1% acreage, 2.3% of production) and Turkey (0.3% acreage, 0.37% of production) (FAOSTAT, 2014). In much of the world, chickpea is cultivated in semi-arid environments and on soils of poor agricultural quality which combined with yield losses caused by biotic and abiotic constraints, mainly drought, have given rise to average yields of 0.9-1.8 t/ha across these areas of cultivation which is considerably below the theoretical potential (FAOSTAT, 2014). In India Madhya Pradesh is the leading producer followed by Rajasthan. The major chickpea producing states in India are listed in Table 2.

Table 2 Area production and yield of gram during 2013-14 and 2012-13 in major producing states

Area: Million Hectare
Production: Million Tonnes
Yield: kg/hectare

		2013-14#					2012-13				
S. no.	**State**	**Area**	**(%) to All India**	**Production**	**(%) to All India**	**Yield**	**Area**	**(%) to All India**	**Production**	**(%) to All India**	**Yield**
1	Madhya Pradesh	3.48	34.06	3.82	38.63	1096	3.13	36.71	3.81	43.16	1219
2	Rajasthan	1.92	18.82	1.64	16.59	852	1.25	14.70	1.28	14.46	1020
3	Maharashtra	1.82	17.8	1.62	16.42	891	1.12	13.14	0.85	9.67	763
4	Andhra Pradesh	0.59	5.73	0.85	8.59	1449	0.68	7.99	0.76	8.63	1119
5	Karnataka	0.92	8.95	0.57	5.76	622	0.97	11.37	0.62	7.05	643
6	U.P	0.58	5.64	0.48	4.81	824	0.60	7.09	0.68	7.65	1119
7	All India	10.22	100	9.88	100	967	8.52	100	8.83	100	1036

Source: Directorate of Economics Statistics, Department of Agriculture & Co-operation
Fourth Advance estimate

1.6 Diseases of Chickpea

There are many abiotic and biotic factors responsible for the heavy yield losses in chickpea. Of the many biotic stresses limiting its production in dry regions diseases are the main culprits. A large number of diverse fungal species attack the crop. So far 47 pathogens have been noticed to cause some or the other disease on the chickpea crop (Table 3).

Table 3 Diseases of chickpea (*Cicer arietinum* L.)

S. No	Pathogen	Disease	References
1	*Acrophialophora fusipora* (Saksena) Samson	Wilt	Nene *et al*., 1989,1996
2	*Alternaria alternata* (Fr.) Keiss.	Blight of leaves, flowers, pods	Aulakh, 1969; Gaur and Singh 1990; Shukla *et al.,* 1976; Nene *et al.,* 1989,1996
3	*Alternaria brassicicola* (Schwein.) Wilt	Wilt; leaf spot, leaf and stem blight	Nene *et al.,* 1996
4	*Alternaria tenuissima* (Nees. ex. Fr.)Wilt	Leaf spot, leaf and stem blight	Narayan, 1993; Nene *et al.,* 1996
5	*Ascochyta rabiei* (Pass.)	Leaf blight	Nene *et al.,* 1996; Luthra *et al.,* 1935.
6	*Aspergillus flavus* Link: Fr.	Seedrot, seedling rot	Nene *et al.,* 1996
7	*Botrytis cinerea* Pers ex. Fr.	Lesions on leaf and stem blight	Joshi *et al*., 1969; Nene *et al.,* 1996.
8	*Colletotrichum capsici* (Snyd.) Butl. and Bisby	Anthracnose, Stem blight	Nene *et al.,* 1996
9	*Colletotrichum dematium* (Pers. ex Fr.) Grev.	Stem blight	Mishra *et al*., 1975; Nene *et al.,* 1996.
10	*Fusarium equiseti* (Corda) Sacc.	Damping off, Collar rot	Nene *et al.,* 1996
11	*Fusarium lateritium* Nees f. sp. *ciceri* DC.	Roots	Jain *et al.,* 1960
12	*Fusarium lini* Bolly	Wilt	Mukerji, 1986
13	*Fusarium oxysporum* f. sp. *ciceri* (Padw.) Matuo. and Sato.	Wilt	Nene *et al*., 1996
14	*Fusarium orthoceras* f. sp. *ciceri* Padw.	Wilt	Mishra, 1955
16	*F. Solani* (Mart) Sacc.	Wilt	Bhargava *et al.,* 1981; Grewal

			et al., 1974; Nene *et al.*, 1989, 1996.
17	*Fusarium* sp.	Wilt	Uppal *et al.*,1935a
18	*Leveillula taurica* var. *macrospora* Uppal *et al.*, anamorph; *Oidiopsis taurica* (Lev.) Salm	Powdery mildew	Khune *et al.*, 1979; Nene *et al.*, 1989, 1996.
19	*Macrophomina phaseolina* (Tassi)Goid. {=*Rhizoctonia bataticola* (Taub) Butl.}	Foot rot, charcoal rot	*Anonymous* 1950; Nene *et al.,* 1989, 1996.
20	*Mycosphaerella rabiei* Kova. {anamorph-*Phyllosticta rabiei.* (Pass) Trot = *Ascochyta rabiei* (Pass) Lab.}	Blight	Aujla, 1964; Nene *et al.,* 1989,1996; Sattar, 1934
21	*Mystrosporium* sp.		Mitra, 1935; Nene *et al.,* 1989, 1996.
22	*Neocosmospora vasinfecta* E. F. Smith	Wilt	Haware *et al.*, 1976; Nene *et al.*, 1989, 1996.
23.	*Oidium* sp.	Powdery mildew	Patel *et al.*,1949
24.	*Operculella padwicikii* Khesw.	Root rot, dead stems	Gupta,1974; Nene *et al.*, 1989
25.	*Ozonium texanum* var. *parasiticum* Thirum	Wilt	Mishra,1955; Nene *et al.*, 1989, 1996
26.	*Pellicularia filamentosa* (Pat) Rogers.	Stem rot	Uppal *et al.,* 1935a
27.	*P. rolfsii* (Sacc.) west. (anamorph-*Sclerotium rolfsii* Sacc.)	Wilt, root and stem rot	Rajendra *et al.*, 1967.
28.	*Phacidiopycnis padwickii* (Khes.) B. Sutton :{= *Opercullela padwickii* Khes.}		Nene *et al.*, 1996
29.	*Phoma medicaginis* Malbr. and Roun	Blight	Nene *et al.*, 1989, 1996
30.	*Phoma rabiei* (Pass) Khune	Pods and leaves	Khune *et al.,* 1980
31	*Phytopthora megasperma* (Dresch)	Foot blight, oospores in foot region	Nene *et al.,* 1989, 1996; Suryanarayana *et al.*, 1968.
32.	*Pleospora infectoria* Fuckel	Common	Nene *et al.,*1989
33	*Poronia indica* S. Ahmad	Saprobe	Ahmad, 1946.
34	*Pythium ultimum* Trov	Damping off	Nene *et al.,* 1989, 1996
35	*Rhizoctonia bataticola*	Common	Nene *et al.,* 1989

	(Taub) Butl.		
36	*R. solani* Kuhn {=*Thanetophorus cucumeris* (Frank) Donk}	Root rot and wilt	Nene *et al.,* 1989, 1996; Siradhna *et al.,* 1982
37	*Sclerotinia Sclerotiorum* (Lib.) De Bary	Stem rot	Nene *et al*., 1989, 1996.
38	*Sclerotium rolfsii* Sacc. {teleomorph *Athelia rolfsii* (Curzi) Tu and Kimbrough}	Roots	Jain *et al.,* 1960; Nene *et al.,* 1989, 1996
39.	*Scopulariopsis brevicaulis* (Sacc.) Bain	Leaf and stem blight	Nene *et al*., 1996
40.	*Stemphylium sarcinaeforme* (Cav.) wilt	Blight, leaves stems , pods	Das *et al*., 1961; Prasad *et al.,* 1969
41	*Trichoderma harzianum* Rifai	Common, foot rot	Nene *et al*., 1989, 1996; Kaiser *et al.,* 1975.
42	*Trichothecium roseum* Link	Hyperparasite on rust *Uromyces.* spp.	Ahmad, 1970
43	*Uromyces ciceris arietini* (Grogn) Jacz. and Boyes	Leaf rust	Bahadur *et al.,* 1970; Roy 1949
44	*Uromyces ciceris arietini* (Grogn.) Jacz.	Rust	Bahadur *et al*., 1967,1970; Nene *et al*.,1989,1996
45	*Uromyces striatus* Schr.	Rust	Nene *et al*., 1996
46	*Uromyces viciae-fabae* (Pers.) Schr.	Rust	Nene *et al*., 1996
47	*Verticillium alboatrum* (*K*lebahn)	Wilt	Nene *et al*., 1996

The perusal of reports on the disease (Table 3) indicates that the low yield of chickpea is due to its susceptibility to several diseases. *Fusarium* wilt has become a major factor limiting chickpea production causing annual loss of 60-70 percent in yield (Mandivia *et al.,* 2002). Hence this problem of concern i.e. wilt of chickpea is taken up for the present work.

1.7 Objectives

The major Objectives of the present study are:

1. Survey and collection of diseased chickpea plants from different farmers field in Kanpur.
2. Isolation, purification and pathogenicity test of wilt causing pathogen.
3. Isolation and identification of *Trichoderma* and *Aspergillus* species from the rhizosphere of Chickpea.
4. Studies on morphological and cultural characters of the pathogen and its identification.
5. Study of the symptoms caused by *Fusarium oxysporum* f. sp. *ciceri.*
6. Histopathological studies of the pathogen.
7. Comparison of the production of certain enzymes by the pathogen.
8. Extraction of essential oils from different medicinal plants and their efficacy against the *F. oxy.* f. sp. *ciceri.*
9. Effects of various botanicals (*viz*., seeds powders, cakes, and essential oils) against the pathogen.
10. Effects of various fungitoxicants on the wilt pathogen.

Review of Literature

Chickpea is a major pulse crop grown in India and the wilt is relatively more serious than other diseases. First mention of wilt in India dates back to early part of this century (Butler, 1918). Later records reveal that Pearl (1923) noticed wilt disease of chickpea in Central Provinces and Barar, India during the crop season 1921-22. The disease was considered due to an apparently undescribed *Fusarium*. To control the disease, the use of resistant varieties was promoted as black chickpea was found more resistant than usual Burmese type. Mcrae (1924) continued to observe the wilt of chickpea, conducted a few experiments and failed to establish the casual relationship of various strains of *Fusarium* isolated from diseased plants of chickpea and inferred that relatively high soil temperature was responsible for wilt in the fields. Prasad and Padwick (1939) made 300 isolates from wilted plants of chickpea from Karnal and Delhi, India collected in 1937 and 1938. They grouped them in 13 groups according to certain major characteristics and stated that Chickpea plants might harbor a wide range of *Fusarium* types causing two distinct diseases- seed rot and wilt. The single spore cultures were morphologically alike and belonged to sub section *Orthocera.* Padwick (1941) made exhaustive studies and stated (i) *Fusarium orthocera* var. *ciceri* as cause of chickpea wilt, (ii) this fungus was able to survive well in roots and stems, even in apparently healthy appearing plants growing among diseased ones, (iii) farmyard manure hastened disappearance of the fungus, (iv) a species of *Trichoderma* and *Aspergillus* highly antagonistic to *F. orthocera* var. *ciceri* in Petri dishes was ineffective to control the disease in the field, (v) among 56 cultivars tested against wilt, Imperial Pusa types 9, 28 and 52 were highly susceptible, IP 28 was intermediate and IP 22, 63, 69 and 83 were resistant and (vi) cultivar IP 78 was not affected in 1939 but had 32 percent infection in the season.

The association of chickpea wilt with *F. orthoceras* var. *ciceri* as pathogen was reported repeatedly by several workers (Malik and Khan, 1943; Motiramani, 1947; Benloch, 1949; Mehta *et al.,* 1950). Raheja and Das (1949) analyzed the influence of cultural treatments on the incidence of wilt as (i) early wilt occurred within a fortnight after germination and it was of lower intensity, (ii) well grown plants were found to be more susceptible, (iii) interspacing of rows made no difference in the incidence of wilt and (iv) the wilting made no difference in the rate or quantity of flower production.

Manucheri and Mesri (1966) identified *Fusarium lateritium* var. (f. sp.) *ciceri* causing serious wilting in chickpea crops in Iran and reported yellowing from top to bottom in the diseased plants, the xylem of such plants turned black. Mosahebi (1968) again recorded the same observation in Iran and assessed, chickpea wilt as the most serious disease. He opined that wilting was caused by *F. lateritium* f. sp. *ciceri* and wounds made by insect larvae or nematodes facilitated the entry of the pathogen either directly or indirectly.

Echandi (1970) from Peru made repeated isolations from more than 250 wilted chickpea plants which invariably yielded *Fusarium oxysporum,* but not the *Fusarium lateritium.* Nema and Khare (1973) made a detailed report of wilt of Bengal gram in Madhya Pradesh, India. It stated that the wilt disease occurred at two phases of growth either at the early seedling stage or at the flowering stage. They observed damage upto 61 percent at the former phase and 43 percent at later stage. All kinds of Bengal gram *viz.* Kabuli, deshi, pink, green and black, irrespective of the seed size or plant type were found susceptible to this disease. The wilt was also observed associated with *Fusarium oxysporum* var. *ciceri, Rhizoctonia bataticola, Operculella padwickii, Sclerotium rolfsii* and *Sclerotinia sclerotiorum*; but in the above two

prominent stages of growth, wilting was mainly due to one of the three fungi *viz.* *Fusarium oxysporum* var. *ciceri, R. batatiticola* or *S. rolfsii. Fusarium* was found in vascular tissues of invaded plants**.** All the findings firmly related to *Fusarium oxysporum* f. sp. *ciceri* as the main causal organism of wilt in chickpea.

2.1(a) Nomenclature of the fungus causing wilt in chickpea

The observations of wilting in chickpea were commenced in 1915, but the cause of wilt remained a point of controversy, though it was considered to be a certain species belonging to genus *Fusarium* (Pearl, 1923). Prasad and Padwick (1939) called it as *Fusarium orthoceras* var. *ciceri.* This was the time when workers on *Fusarium* were busy in grouping morphological and cultural variations in sections or subsections or species or varieties. Raillo (1935) in Russia studied morphological characters useful in taxonomy and concluded, (i) the form of the apical cell was the guiding character in species determination, (ii) the incurvature of conidia, length of apical cell, number of septa and width of conidia were the characters used in separating subspecies and varities and (iii) cultural characters such as pigment, presence of sclerotia and mode of spore formation were useful in separating forms only. She also studied variability in *Fusarium* by initiating cultures from single conidium and found that the form of the apical cell and incurvature of conidia remained constant in cultures developed from single conidium; the number of septa was constant in separate isolates within a single conidial culture: the length and width of conidia varied considerably in separate isolates within a single conidial cultures; the number of sclerotia varied greatly in separate isolates within a single conidial cultures ; and the mode of spore formation (pionnotes, pseudo-pionnotes and sporodochia) varied in separate isolates within a single conidial cultures. Wollenweber and Reinking’s 65 species of *Fusarium* were grouped into 55 species by Raillo (1950).

Snyder and Hansen‘s system (1940 and 1945) was based primarily on morphology of macroconidia. Their work was an outcome of extensive single conidial analysis of cultures of *Fusarium* species under identical conditions of substrate and other environmental conditions. Their work illustrated the importance of cultural variation in taxonomy. Snyder and Hansen (1940 and 1945) reduced the 16 sections of Wollenweber and Reinking (1935) into 9 species where species *oxysporum* was represented by section *Elegans* and *Orthocera* was the subsection among three sub sections *viz., orthocera, constrictum* and *oxysporum* in section *Elegans*. The pathogen causing chickpea wilt in California was identified as *Fusarium lateritium* Nees. Emend. Snyd. and Hans. f. *ciceri* (Padw). Erwin (1958) and Westerlund *et al.* (1974) identified the chickpea wilt fungus from the region adjacent to the above one as *Fusarium oxysporum* Schlect. f. sp. *ciceri* (Padw.) Matuo and Sato. Armstrong and Armstrong (1981) considered these two pathogens names to be synonyms. Henceforth, *Fusarium orthocera* var. *ciceri* became *Fusarium oxysporum* f. sp. *ciceri* (Matuo and Sato, 1962; Chattopadhyaya and Sen Gupta, 1967; and Booth, 1971). Since then *Fusarium oxysporum* Schlechtend f. sp. *ciceri* (Padwick) Matuo and Sato is universally and uniformly recognized as the causal organism of wilt of chickpea. However, in the present thesis the name of the causal organism of chickpea wilt is referred as *Fusarium oxysporum* f. sp. *ciceri* (Padw.) Snyder and Hansen.

2.1(b) Pathogenic variability of the pathogen

Chauhan (1962) made three groups on the basis of mycelium and colony types from 22 isolates of *Fusarium orthoceras var. ciceri*. It was inferred that there was a paramount correlation between virulence and the toxicity of the *Fusarium* toxin and that the isolates were differentiated into five groups on the basis of percentage mortality and filtrate toxicity in pot inoculations. Chauhan (1962) addressed these as

physiological variations. However, the subdivision of *formae speciales* on the basis of virulence to a particular set of differential host cultivars, that vary in disease resistance were called races as Haware and Nene (1982) presented evidence of the existence of at least four races of *Fusarium oxysporum* f. sp. *ciceri* in India. Phillips (1988) reported a distinct race 6 from San Luis Obispo. In all the above reports the race classification was based upon the resistance / susceptible reaction, to the ten chickpea lines *viz.*, Annegeri, BG 212, C104, Chaffa, CPS1, JG62, JG74, JG315, K850 and L550 against the wilt pathogen *F. oxy.* f. sp. *ciceri*. Rahman *et al.,* (1988) reported race 0, 1, 2, 3, 4 and 6 and two new pathotypes from India.

Kunwar *et al.* (1989) worked on the histopathology of JG 62 and JG 74 which were susceptible and resistant to Race 1 and 4 respectively. They found that JG 62 (susceptible) wilted with both the races within 4 weeks and the histopathological details were (i) inter and intra cellular hyphae in the pith, xylem and cortex, (ii) the epidermis was disintegrated, (iii) hypertrophy of cortical and pith cells occurred and (iv) a mucilage like substance was found present whereas with JG 74, 5.2 and 14.7 percent plants wilted with race 1 and 4 respectively. The wilted plants revealed scanty intercellular mycelium in the pith, xylem and cortex without any trace of hypertrophied cells. Paul *et al.,* (2001) using the susceptible variety JG 62 selected pathogenic isolates of wilt fungus and recorded 25 isolates as fast growing, 17 as slow growing and 9 were medium growing types. On the basis of virulence on variety JG 62 none of the seedlings survived after a week of inoculation with 27 isolates (highly virulent) whereas 15 isolates proved to be moderately virulent which caused death of 66.7 percent seedlings and 9 isolates were classified as least virulent. Paulkar and Raut (2004) grouped 4 isolates of *F. oxy.* f. sp. *ciceri* as separate ones on the basis of morphological and cultural characters. A susceptible reaction was exhibited by FOC-4

on JG 62, while FOC-3 showed resistant reaction against ICCV-2, ICCV-20 and Warangal. The isolates produced different sizes of colonies due to variation in radial mycelial growth. Potato dextrose agar supported maximum growth while poor growth was observed in Kirchoff's agar medium.

2.1(c) Survey and disease incidence

During spring of 2005 to 2009 Benfreha *et al.* (2014) conducted survey at three agro-climatic zones of north-western Algeria through seven sites on wilt of chickpea. The presence of the disease was found in all the 50 fields. All the 50 fields, selected for sampling were spaced 3 to 5 km apart and were visited between March and late June. Plants showing symptoms of yellowing or wilting were collected from each of the site. The infected plants were then kept in paper envelopes, air-dried at room and stored at 18°C until further usage. Isolations were then made from the wilted plants of chickpea. It was summarized that *Fusarium* wilt was present in all the plots surveyed in the north-western Algeria. Symptoms were present at all physiological stages with varying degrees from one field to another and from one season to another. These symptoms were similar to those reported in Tunisia, Morocco, Spain, California and India (Westerlund *et al*., 1974; Nene and Reddy, 1987; Halila and Strange, 1996; El-Aoufir, 2001).

Gosh *et al.* (2013) conducted a survey between December and March 2010-2011 in the four major chickpea growing state (Andhra Pradesh, Karnataka, Madhya Pradesh and Chhattisgarh) in central and southern parts of India. The districts in each region were selected randomly. A total of 400 fields covering 17 districts were surveyed. The number of fields visited per district ranged from 20 to 30 and a distance of 15 - 20 km was allowed between sites, but the distance was greater where chickpea fields were far apart, resulting in a smaller number of sites visited in such districts.

Dry root rot and collar rot diseases were found at all the sites and incidence ranged from 8.9% - 10.3% and 7.1% - 10.5% respectively, irrespective of cultivar type and locations. Incidence of wilt and black root rot disease ranged from 9.7% - 13.8% and 6.6% - 7.4% respectively.

Singh and Dhaiya (1973) recorded the losses from 10 to 100 percent in practically all the chickpea growing states. They emphasized need for locating reliable resistant donor parent, as in field condition a cultivar free from wilt became susceptible in later years. Grewal *et al*. (1974) on the basis of monthly isolations from wilted plants throughout the crop season summarized that *F. solani* was obtained by 24.7 percent, *F. oxysporum* f. sp. *ciceri* by 15.5 percent, *Rhizoctonia solani* by 8.5 percent, *Rhizoctonia bataticola* by 8.3 percent, *Operculella padwickii* by 6.2 percent and *F. monoliforme* by 1.6 percent wilted plants of chickpea collected from 12 villages of Delhi. Haryana and IARI, New Delhi H.A.U Hisar and P.A.U. Ludhiana, Punjab, India.

Gurha and Trivedi (2008) in a survey in Karnataka state reported *Fusarium oxysporum* f. sp. *ciceri* incited wilt upto 70 percent where moisture was good. Several other investigations also reported cent per cent loss in the yield under specific conditions for the disease (Haware and Nene, 1980; Navas-Cortés *et al*., 2000; Anjaiah, 2003).

2.2 Isolation, Purification and pathogenicity test

2.2.1 Isolation of the pathogen

In a study conducted by Fisher *et al*. (1982) Komada's medium (Komada, 1975) was used to isolate a highly virulent strain of *Fusarium oxysporum* f. sp. *ciceri* from diseased chickpea plant while it's confirmation was made on carnation leaf agar medium.

Benfreha *et al.* (2014) made isolations from stem, collar, root segments and rhizosphere areas of symptomless plants to determine the occurrence of vascular infections. The samples pieces of individual plants were cut into 5 to 10 mm-long segments, surface sterilized (0.2% NaOCl for 2 min), plated on PDA: 250 g potato, 20 g of agar and 20 g of dextrose per liter of distilled water), and incubated at 22°C and a 12 h photoperiod for 5 to 7 days was then given (Erskine *et al*., 1990; Landa *et al.*, 2001). The analysis of the isolated microflora quantitatively and qualitatively varied according to the treated parts of infected plants. It was inferred that the isolated fungal species were associated with the complex of *Fusarium* wilt. The presence of *F. oxysporum* was always dominant and was isolated from all parts of the plant with an average of 43.26% and followed by *Fusarium solani* with 31.61%. The remaining species colonized different parts of the plant: *F. culmorum* (stem, collar and root); *F. equiseti* (collar), *Sclerotinia* spp. (root and rhizosphere), *Rhizoctonia solani* (collar and rhizosphere) found to cause collar rot and root rot in chickpea.

Patel *et al.* (2001) carefully studied certain cultural characters of *F. oxy.* f. sp. *ciceri* isolates collected from Annigeri, Bijapur, Bidar and Dharwad (Karnataka), in India, like the type of colony, pigmentation, sporulation and conidia and observed similarity among three isolates. In an another report by Paulkar *et al*. (2004) the 4 isolates of *F. oxy* f. sp. *ciceri,* isolated from Amaravati (FOC-1), Akola (FOC-2), Buldhana (FOC-3) and Nagpur (FOC-4), were found to be morphologically different. Variable pigmentations among the different isolates of *F. oxy.* f. sp. *ciceri* were reported by Honnareddy and Dubey (2007). They reported that the isolates showed pigmentation which varied from normal white to violet, greenish violet, brown, reddish violet, yellowish pink and dark green. Singh *et al*. (2010) in an *in vitro s*tudy collected certain isolates of *F. oxy.* f. sp. *ciceri* from Anand and Arnej (Gujarat), and

assessed the variability and the virulence among the different isolates of *F. oxy.* f. sp. *ciceri* causing vascular wilt in chickpea and reported that there was no significant difference in morphology and virulence among the three isolates.

2.2.2 Purification of the pathogen

Barnet and Hunter (1972) purified *Fusarium oxysporum* f. sp. *ciceri* using single spore isolation method and maintained them on PDA slants throughout the investigation by periodical transfer. Sumitra (2006) reported that *F. oxy.* f. sp. *ciceri* was sub cultured on PDA slants and allowed to grow at 27 ± 1ºC for ten days and such slants were preserved in a refrigerator at 5ºC and revived once in 30 days. Ahmad (2010) obtained pure culture of *F. oxy.* f. sp. *ciceri* on Czapek dox agar medium and multiplied it on Waksman's agar medium (Glucose 10 g; Peptone 5 g; Potassium dihydrogen phosphate 1 g, Magnesium sulphate 0.5 g, Distilled water 1000 ml). Hend *et al*. (2012) isolated certain dominant rhizospheric fungi were from tomato plants severely affected by wilt disease, caused by *F. oxysporum* f. sp. *lycopersici*, using soil plate method.

2.2.3 Pathogenicity test

Echandi (1970) for the first time successfully proved the Koch's tests. He inoculated chickpea plants with monoconidial isolates of *Fusarium oxysporum* f. sp. *ciceri*, and observed that the symptoms developed were identical to those manifested in fields, and the pathogens were easily reisolated from inoculated plants. His other findings were that wounding of roots increased susceptibility of plants; thirty days old plants were more susceptible to wilt than 10 to 20 days old plants and wilt was more severe in light sandy soil than on heavy clay soil.

Westerlund *et al.* (1974) collected diseased plants from central coastal area of California and reported *F. oxy.* f. sp. *ciceri* in 6 percent wilted plants and *F. solani* in

47 percent and stated that *F. oxy.* f. sp. *ciceri* required a wound for efficient infection whereas *F. solani* f. sp. *pisi* did not. Kunwar *et al.* (1989) observed that in cultivars JG 62, 50.7 percent of plants inoculated with race 1 and 98.4 percent inoculated with race 4 of *F. oxy.* f. sp. *ciceri,* wilted within 44 weeks.

Nikam *et al.* (2007) used sick soil inoculation technique to confirm the pathogenicity of the pathogen in earthen pots under greenhouse condition by using susceptible cultivar JG 62. The culture of *F. oxy.* f. sp. *ciceri* was multiplied on sand maize flour medium (1:1) and sterilized in autoclave at 15 lbs for 30 min. The flasks were then inoculated aseptically with pure culture of *F. oxy.* f. sp. *ciceri* and incubated at room temperature for 15 days. The prepared inoculum (after 15 days) was taken and mixed with sterilized sand plus soil mixture (1:1) at 100 g inoculum per kg soil. The above prepared potting mixture (sand + soil + inoculum) was then filled in disinfected earthen pots. The pots were watered slightly and incubated for four days. Before sowing the seeds of, JG 62 Uninoculated pot served as control. Wilting was observed after 25 days of inoculation. The plants showed typical wilting symptoms characterized by chlorosis and drooping of leaves and finally death of the plant. Artificially inoculated plants showed identical wilting symptoms as observed by the naturally infected chickpea plants in the field.

Barhate and Dake (2006) collected chickpea wilted plants from 5 tehsils of Ahmadnagar districts of Maharastra. Six isolates of *F. oxy.* f. sp. *ciceri* from wilted plants were found pathogenic to cultivar JG 62. These were also tested on the ten well known differentials. The reactions confirmed the presence of Race 1 in all the tehsils of Ahmad Nagar, though the isolates were morphologically and culturally different to each other.

Trivedi and Gurha (2007) demonstrated existence of Race 1, 2, 3, and 5 in six blocks of Jhansi district of Bundelkhand region, Uttar Pradesh, India. They also recorded the presence of a new pathotype (isolate FOC 6) as this isolate was found highly virulent on all the differentials with either susceptible or highly susceptible reactions. They reported the presence of race 1, 3, and 5 from Uttar Pradesh. Nikam *et al.* (2011) confirmed pathogenicity of the *F. oxy.* f. sp. *ciceri* by sick soil inoculation technique in earthen pots under greenhouse conditions by using susceptible cultivar JG 62.

2.2.4 Nutritional requirement of the pathogen

Pandey *et al.* (2001) made attempts to study effects of synthetic and non-synthetic culture media and observed that (i) maximum growth of *F. oxysporum* f. sp. *ciceri* was obtained on Richards medium followed by Nash and Snyder, Czapek's and Potato dextrose medium whereas least growth was achieved on modified Asthana and Hawkers medium and Glucose Asparagine medium, (ii) sporulation was excellent on Richards mediums followed by potato dextrose medium and Asthana Hawkers medium, (iii) microspores were formed on Joeff's medium and macrospores on glucose asparagine medium, (iv) chlamydospore were formed fairly on oatmeal medium, Nash and Snyder, potato dextrose and Czapek's medium and (v) the media which promoted best mycelial growth exhibited good results in Chlamydospore formation.

Dikkar *et al.* (2003) made an attempt to investigate about the nutritional requirements of *F. oxy.* f. sp. *ciceri* using 12 different solid and liquid media. Among liquid media, maximum dry mycelial weight was recorded on Richard's medium. Among solid media the fungus grew well on Richards agar followed by PDA and CZA. Growth on different carbon sources and nitrogen sources showed variations in

utilizations as maltose, mannitol, D glucose and sucrose supported maximum dry mycelial weight as well as sporulation. Lactose was poorly utilized, which was the finding contradictory to the results of Kushwaha (1971). Dikkar *et al*. (2003) noted KNO3 as the best N- source followed by NaNO3 in supporting mycelial growth and sporulation.

Farooq *et al.* (2005) investigated eight media for the growth of *F. oxy.* f. sp. *ciceri* and found Czapek's dox agar and chickpea meal agar media as the best. They reported glucose as the best source of carbon whereas peptone was the best source of nitrogen. Nikam *et al*. (2007) studied the growth characters and sporulation ability of the isolated *F. oxy.* f. sp. *ciceri* on different agar culture media. The media used were potato dextrose agar, host leaf extract agar, Sobouraud's agar, Richard's agar and oat meal agar medium and found that the maximum growth was obtained on host leaf's extract medium of susceptible cultivar JG-62 (90 mm) followed by Richard's agar (86 mm) and potato dextrose agar (83 mm). All the three media proved to be significantly superior in favoring the growth of *F. oxy.* f. sp. *ciceri* than the rest of the media tested. The least growth was observed on oat meal agar (71mm). Hence, in the present study, for the multiplication and maintenance of *F. oxy.* f. sp. *ciceri*, PDA medium was used.

2.3 Isolation and characterization of certain antagonistic fungi from rhizospheric soil

A variety of methods have been evaluated in isolating antagonist harbouring the rhizospheric soil. Arumugam *et al.* (2013) isolated antagonists from the soil collected from the rice field by serial dilution technique. They used nutrient agar medium for the isolation of *P. fluorescens*. One ml of soil suspension from dilutions (10^{-5} and 10^{-6}) was aseptically added to sterile Petri plates containing ten ml of sterile medium and incubated at 28±2ºC for 48 h. After incubation, well separated individual colonies were picked up and transferred to nutrient agar media slants and the pure cultures so

obtained were stored in a refrigerator for further use. In the same manner the fungus *Trichoderma viride* was also isolated using the dilutions (10^{-3} and 10^{-4}), which was aseptically added to sterile Petri plates containing twenty ml of PDA medium and were then incubated at 37ºC for 3 days.

Hend *et al.* (2012) isolated antagonistic fungi *viz. A. niger, P. citrinum, T. viride* and *T. harzianum* from soil samples of various farm fields of Riyadh region, Saudi Arabia. These fungi were isolated by soil plate methods, as described by Dhingra and Sinclair (1995) using PDA medium. *Trichoderma* spp. was isolated on selective media of Elad and Chet (1983) and were maintained on PDA slants, stored at 4°C till further use. Based on microscopic studies, the pathogen was identified as *F. oxysporum* f. sp. *phaseoli* on the basis of presence, shape and size of macro- and micro-conidia (Leslie and Summerel, 2006). On the basis of cultural characters and microscopic observations, fungi isolated from various fields were identified as, *Aspergillus flavus, Aspergillus niger, Chetomium* sp. *Cladosporium cladosporioides, F. equiseti, Penicillium citrinum, Penicillium* spp., *Trichoderma harzianum,* and *Trichoderma viride.*

Elad and Chet (1983) isolated *Trichoderma* spp. collected from the rhizosphere soils of vanilla from different locations of Thrissur and Ernakulam districts of Kerala (India) by using selective media. Dominant rhizospheric fungi of the tomato plants were isolated from plants severely affected by wilt disease caused by *F. oxysporum* f. sp. *lycopersici* by Dhingra and Sinclair (1995) using potato dextrose agar medium. Jayalakshmi *et al.* (2003) isolated *Trichoderma harzianum* from rhizosphere of healthy pigeon pea plants. Two hundred and forty-two actinomycetes strains were isolated by Cao Li Xiang *et al*. (2004) from the leaves and

roots of healthy and wilting banana plants. Majority of the reported microorganism were either *Streptomycetes* or *Streptomyces griseorubiginosus*-like strains.

Merkuz and Getachew (2012) collected a total of 101 soil samples randomly from different major chickpea growing districts in Northwestern Ethiopia as indicated (Merkuz, 2011c). From each field, soil samples were collected from relatively healthy looking chickpea plant from about 10 cm depth. Samples were collected in plastic bags, dried in the laboratory and grounded with mortar and pestle and then kept in the refrigerator at 4ºC until needed. From each sample, 10 g of soil was added to 90 ml of distilled sterilized water and vigorously shaken using a shaker for 20-30 minutes. From this, fivefold serial dilution was made by pipetting 10 ml into additional dilution water. From the final dilution (10^{-5}), aliquots of 1 ml each were spread on 9 cm diameter plates, containing 20 ml of PDA and to reduce bacterial contaminants antibiotic (50 mg/l streptomycin) were added and incubated at 25ºC for 7-10 days. *Trichoderma* colonies which developed on PDA, were identified based on visual and microscopic observations according to Rifai (1969); Samuel *et al*. (2002) and some isolates were identified at CAB-International Plant Clinic and reference cultures maintained at Debre Zeit Agricultural Research Centre. Isolates were kept in test tubes containing autoclaved sand, wheat bran and chickpea bran mixed with molasses (10 g sand, 20 g wheat bran + 20 g chickpea bran + 5 ml molasses) at 40ºC (Elad *et al*., 1980).

2.4 Symptoms of fusarium wilt on chickpea

Fusarium wilt of chickpea has been ascribed as seed-borne and seeds harvested from wilted plants when mixed with healthy seeds had the potential to carry the wilt fungus to new areas and establish the disease in the soil to economic threshold levels within three seasons (Pande *et al*., 2007). The disease has been reported to occur at seedling

and flowering stage of plant growth. The symptoms which are discernible are drooping of petioles and rachis, yellowing and drying of leaves from base to upward, browning of vascular bundles, improper branching, withering of plants and finally death of plants (Westerlund *et al*., 1974; Prasad and Padwick, 1939). The pathogen makes it entry into xylem vessel and invades the whole of the vascular system, which ultimately induces the symptoms of yellowing and wilting. In the absence of host plant the pathogen can survive up to six years (Haware, 1993). Chauhan (1962) reported the initial symptoms of the disease due to pathogen infection to be vein clearing of leaves and decrease in the chloroplast and starch formation in mesophyll cells. Whereas, Erwin (1957) characterized chickpea wilt by yellowing of leaves and necrosis of the xylem. Leaves of the wilted plants turned grayish green, then became dull yellow and wilted. The xylem and pith become darkened and discoloured. Moreover, internal discoloration of pith and xylem are fairly visible if the stem and root of the wilted plants are split vertically (Saxena and Singh, 1987). The disease resulted in reduced plant population, reduced spear size and sub-optimal yield (Ravikumar *et al.*, 2007).

2.5 Histopathological study of the pathogen

Chickpea wilt pathogen affects the xylem vessels of plants during the crucial phase of pathogenesis. Its effect on the host is both local and systemic, but because of the involvement of more than one tissue at the same time and possible dilution of the factors in the xylem fluid, a rigorous delineation of the sequences of events during pathogenesis becomes somewhat difficult. The pathogen gains entry into the xylem vessel. The vascular fungi are facultative parasites, they obviously find the xylem environment relatively free of severe host reactions Ahmad (2010).

Diseased plant roots when split opened showed discoloration of internal tissues. The symptoms of chickpea wilt observed were similar to those recorded earlier by Narasimhan (1929); Westerlund *et al*. (1974); Haware *et al*. (1986b) and Frisullo *et al*. (1989).

2.6 Studies on morphological and cultural characters of the pathogen

In *F. oxysporum* there is a considerable variation in cultural characteristics. Chauhan (1962) found variation among 22 isolates with respect to their mycelium type, colony colour, toxin production and pathogenicity. Isolates also vary in their physiology and virulence. Kistler (1997) and Tantaoui *et al*. (1996) described that the *Fusarium* wilt fungus is an asexual species. Isolates are more similar genetically within a *forma specialis* and are assumed to have a monophyletic origin. The *Fusarium* species contains *formae specialis,* group with morphologically identical forms. (Snyder and Hansen, 1954). Several *formae specialis* have been described by Messiaen and Cassini (1981).

Gupta *et al.* (1986) Saxena and Singh (1987) reported that *Fusarium oxysporum.* f. sp. *ciceri* is septate, profusely branched growing on potato sucrose/dextrose agar at 25ºC initially white turning light buff or deep brown later, fluffy or submerged. The growth becomes felted or wrinkled in old cultures. Various types of pigmentation (yellow, brown, crimson) can be observed in culture. Macro-conidia are borne on the conidiophores, which are thin walled, 3 to 5 septate, with both ends pointed and measuring 3.5-4.5 x 25–65 µm. In old cultures chlamydospores are formed, which are rough or smooth walled, intercalary or terminal and may be formed singly, in chains or pairs.

Couteaudier and Alabouvette (1990) revealed that the macroconidia are thin walled usually with three or four septa, straight to slightly curved, slender, a foot-

shaped basal cell and curved apical cell. They are generally produced from phialides on conidiophores by basipetal division. The microconidia are ellipsoidal and either have no septum or a single one. Both are formed from phialides in false heads by basipetal division. They are important in secondary infection. The chlamydospores are globose and have thick walls and are formed from either hyphae or by the modification of hyphal cells. These are important as endurance organs in soils where they serve as inocula in primary infection. The teleomorph or sexual reproductive stage, of *Fusarium oxysporum* is unknown (Leslie and Summerell, 2006).

Cultural characteristics of *F. oxy.* f. sp. *ciceri* were studied on different agar media by Nikam *et al.* (2007) who observed that host leaf extract agar (90 mm) followed by Richard's agar (86 mm) and Potato dextrose agar (83 mm) produced significantly good growth and profuse sporulation and used PDA medium for the maintenance and multiplication of the *F. oxy.* f. sp. *ciceri*. However, Chauhan (1962); Prasad and Patel (1964) and Kewate (1986) reported host leaf extract agar and Richard's agar medium as best for the ramification and sporulation of *F. oxy.* f. sp. *ciceri.*

Murumkor and Chavan (1985) observed that the growth of the fungus appears cottony white, wrinkled in old cultures at 25°C on chickpea meal agar. The fungal hypha appeared septate and profusely branched. On simple, short conidiophores micro-conidia were produced. Micro and macroconidia were straight to curved or oval to cylindrical in shape measuring 2.5-3.5 x 5-11 µm.

2.6.1 Effect of abiotic factors on wilting

Root diseases in germinal and wilt incited by *Fusarium oxysporum* f. sp. *ciceri* in particular has long been found influenced by soil moisture, temperature and types of soil *etc.* Padwick (1942) observed a well-marked correlation between wilt and sub-

soil dryness while recording wilt disease incidence in Delhi and Karnal. He also noted that late sowing reduced the incidence of wilt. Padwick and Bhagwagar (1943) conducted an experiment during 1938-1942 in which chickpea was sown at weekly interval from 23 September to 28 October. The incidence of wilt decreased with corresponding increase in grain yield, when sowing was delayed upto at least mid-October. The percentage of wilt shrank from 11.5 percent in the 30th September sowing to 1.8 percent in that of 14th October. They observed abrupt wilting when soil moisture was 24 percent as all the seedlings died within 15 days while at 16.7 and 18.4 percent moisture level, time required for wilting was longer. Raheja and Das (1957) conducted experiments to find out effect of cultural treatments on the incidence of wilt of chickpea. The experiments involved the parameters like, (i) date of sowing, (ii) spacing between rows of the crop and (iii) depth of seeding between. The results showed early wilt occurrences in about 10 to 15 days after sowing. Late wilt was observed after14th March (podding stage). The treatments did not affect early wilt but late wilt was significantly higher when deep sowing (5 inches) was practiced.

Chauhan (1962, a) on the basis of experiments in sand cultures inferred that alkaline reaction of the soil was favourable to growth of the plants, seed yield and seed number. Wilt disease intensity increased with lowering of pH 9.2. Thus checking the disease without the crops growth and yield was a possible by altering the soil pH According to Chauhan (1962b), wilt was most severe on sandy soil and least on clay loam soil.

In another study Chauhan (1963) reported a contradictory finding that 83.33 percent mortality of chickpea plants due to wilt took place at 25 percent soil moisture (on oven dry weight basis) and only 13.33 percent mortality at 10 percent soil moisture. Grewal (1969) reported *Fusarium* spp. as erratically pathogenic and the wilt

was probably due to an interaction between high temperature and moisture stress. Chandra and Tomar (1973) surveyed Haryana state, India opined that the factors for wilt disease were numerous like temperature, moisture, level, variety, crop rotation, soil type, time and method of sowing *etc*. Kaiser and Sen Gupta (1975) in an innovative experiment demonstrated that growth of *F. oxy.* f. sp. *ciceri* was more towards chickpea roots than towards roots of non-host plants. Kotasthane *et al.* (1976) again observed more severe wilt in light soil than on heavy soil. *F. oxy.* f. sp. *ciceri* also found producing toxins.

Chauhan (1961) obtained maximum toxin production in Richard's medium at 28-31°C. Boiled and non-treated filtrates, both caused wilting of chickpea in 36 h. Addition of 25µl/ml Ambramycin and 30 ppm Cholromycetin delayed the time for toxin induced wilt time from 36 h in non-treated to upto132h. The survival of inoculum of wilt *F. oxy*. f. sp. *ciceri* through chlamydospore was noticed by Sharma and Gupta (1983). They observed that the chlamydospore of the pathogen remained viable throughout the high temperature of summer months during non-cropping period in naturally infected roots of chickpea at soil depths of 5, 10 and 15cm. The fungus did not survive in roots placed on the soil surface. According to Navas-Cortes *et al*. (2000) winter sowing or planting early spring pushes the epidemic significantly, slowed the development level and reduces the final amount of disease. Mehmood *et al*. (2013) also reported that rain fall and soil variables (temperature and moisture) had a significant positive effect on *Fusarium* wilt disease.

2.6.2 Role of crop debris in chickpea wilt

The emphasis on sanitation was endorsed by the findings of Erwin and Snyder (1958). They stated that the disease spread by pieces of infected root or stem and the surface of seed infected during threshing. Haware and Nene (1996) confirmed the fungus

Fusarium oxysporum f. sp. *ciceri* from all parts of infected plant including seed. They studied the mode of survival of the fungus in crop residues during 1978-84. They collected the wilted plants in March-1978, buried one lot in the soil and brought the other lot in laboratory. The fungus could not survive in the leaflets stored in lab or soil for more than two months but it could survive in terminal branches for six months in the lab and nine months in the soil for at least 72 months. The pathogen within stem and root portions of crop residues could survive over 200 days in soil which was kept continuously wet.

Stevenson *et al*. (1997) showed presence of hyphae within root xylem. In severe cases of wilt the stem xylem vessels were invaded by the fungus up to five internodes above the point of seed attachments. They suggested that wilt symptoms were directly caused by occlusion of vascular tissue by hyphae. The infection induced the increased production of phytoalexin *viz.,* medicarpin and maakianin particularly in the roots of resistant cultivars.

Satyaprasad and Ramarao (1983) observed that (i) the root exudates from susceptible chickpea cultivars stimulated the mycelial growth and conidial as well as chlamydospore germination of wilt pathogen while the root exudate from resistant cultivars inhibited them, (ii) more amino acids were detected in susceptible chickpea cultivars JG 62 than in the resistant CPS-1and (iii) the presence of aspartic acid, asparagine and histidine were detected in root exudates of JG 62 while cysteine and galactose were consistently present in those of CPS-1.

Shinde and Deshmukh (1989) confirmed experimentally the differential response of seedling root exudates of resistant, tolerant and susceptible cultivars of chickpea. They showed that the germination of conidia of the wilt pathogen *F. oxy.* f. sp. *ciceri* in the root exudates from seedling roots of resistant cultivars JG 74 and JG

315 was less than 9% percent from tolerant cultivars, it was around 62 % from susceptible cultivars (JG62 and Sel 436) was more than 82 percent while in sterile water (control) the conidial germination was 80.62%. Moreover, less colony growth occurred on PDA media containing exudates from resistant cultivars whereas susceptible plants had a stimulatory effect. Singh *et al*. (1984) demonstrated that extracts of soil amended with crop residues of mature and immature wheat, pea, chickpea and lentil inhibited growth of *F. oxy.* f. sp. *ciceri* and *Rhizoctonia bataticola*. Cicerin and arietin were detected in the seeds of chickpea, Ye *et al*. (2002). Both possessed antifungal properties against *Fusarium oxysporum, Botrytis cinerea* and *Mycosphaerella arachidicola.* Mandivia *et al.* (2002) analysed the total phenol contents in root exudates from 10 days old seedling. A significant inverse relationship was found between wilt susceptibility and total phenol content in root exudates of chickpea seedlings.

Padghan and Baviskar (2009) studied root exudates of soybean sunflower, sorghum groundnut, urid and mungbean against chickpea effect were recorded in sorghum root extract medium 28.00 mm and 54.34 percent respectively which was at par with groundnut root medium 30.00 mm and 51.08 percent compared to control 96.33 mm Chaudhary *et al.* (2009) noticed that non polar fraction of sorghum root exudates was effective against *Alternaria alternata, Coanophora cucrbitarum, F. oxy.* f. sp. *ciceri, F. udum, Rhizoctonia bataticola, Sclerotinia sclerotiorum* and *Sclerotium rolfsii*.

2.7 Studies on cell wall degrading enzymes

The cell walls of living plants carry out diverse functions, one of which is the exclusion of pathogens. When plant cells die, their walls provide a source of nutrients for many organisms including potentially pathogenic microorganisms. The complex

chemical composition and physical structure of plant cell walls make them difficult to penetrate and degrade. Various polymers, including cellulose and protein, are embedded in a matrix of highly branched polysaccharides (McNeil *et al*., 1984; Selvendran and O'Neill, 1987). One of the simplest of these polysaccharides, pectin, is a major component of the middle lamella and acts as intercellular cement (Talmadge *et al*., 1973). De Bary (1886) postulated an involvement of cell wall degrading enzymes in plant disease more than 100 years ago.

Plant pathogenic fungi produce an array of extracellular degradative enzyme that may be important in pathogenesis (Walton *et al.,* 1990). The penetration of pathogen into parenchymatous tissues is facilitated by the breakdown of the internal cell wall, which consists of cellulose, pectin and hemicellulose. The degradation of each of these substances is brought about by the action of one or more set of enzymes secreted by the fungal pathogen. (Agrios, 2005). The disease causing organisms enter the host tissue through mechanical pressure exerted by the growing germ tube or dissolving the host cell wall through secretion of toxins or enzymes. The enzymes produced by pathogens affect chemistry of cell wall which is accompanied by cell wall degradation (Albersheim and Jones, 1969). The composition of pectin is different in different plant species and dependent on the age and maturity of the plant parts (Niture, 2008). Several phyto-pathogenic fungi secrete pectin degrading enzymes that depolymerize pectin, a major component of cell wall and middle lamellae during plant pathogenesis. Pectinases are the first enzymes secreted by fungal pathogens when they attack plant cell walls (Collmer and Keen, 1986; Idnurm and Howlett, 2001). These enzymes are essential for fungal pathogens that do not have specialized penetration structures as well as for necrotrophic pathogens during the late stages of the invasion process (De Lorenzo *et al*., 1997).

Polygalacturonase and other pectinolytic enzyme have been isolated from a wide variety of bacterial and fungal plant pathogens (Collmer and Keen 1986; Cooper 1984). Implications of pectinolytic enzymes also include the release of oligosaccharide that can serve as elicitors or suppressors of plant defense response (Davis *et al.,* 1984). On the other hand, pectinolytic enzymes of fungal origin attract the most attention since they offer tremendous potential to the industry. Some of their applications are in retting of flax and vegetable fibres, de-pectinisation and clarification of fruit juices, extraction of oils from vegetables and citrus peels, manufacturing of paper and pulp and pretreatment of pectic waste water (Moyo *et al*., 2003; Saito *et al*., 2004; Jayani *et al*., 2005).

Cellulose degrading enzymes or cellulases have been shown to be produced by several phyto-pathogenic fungi. In living plant tissues cellulolytic enzymes play a major role in softening and disintegration of cell wall material. It has been found that they help in penetration and spread of the pathogen in the host and cause collapse and disintegration of cellular structure, thereby aiding the pathogen in the production of disease. A study of the polygalactouranse activity in different Iranian isolate of *Fusarium oxysporum* revealed that the enzyme activity was higher in the highly virulent than in weakly virulent isolates (Zamani *et al.,* 2000). While another study focusing on the production of the pectic and cellulolytic enzymes by three soil borne fungal pathogen of chickpea *viz*., *F. oxy.* f. sp. *ciceri*, *Sclerotium rolfsii*, and *Macrophomina phaseolina* found that the enzyme production was highest in *S. rolfsii* as compared to the other two fungus (Chattopadhyay, 1999).

Rajeswari (2014) conducted experiments on the effect of culture filtrates of *T. viride* (1%), *T. harzianum* (1.5%), and *P. fluorescens* (2%) on the *in vitro* inhibition of Pectinolytic enzymes of *Fusarium oxysporum*. The activity of poly methyl esterase

(PME), endo polymethyl galacturonase (PMG), and exo polymethyl galacturonase (PMG), Pectin transeliminases (PTE) produced by *Fusarium oxysporum* was higher, when compared to control. Maximum inhibition of above pectinolytic enzymes (PME, endo, exoPMG, endo and exo PTE) was shown by *T. viride* treatment followed by *T. harzianum* and *P. fluorescens.*

2.8 Disease management

2.8.1 Through bioagents

Management of plant disease through biological control has been considered as a viable alternative method as against the use of chemical pesticide and cultural practices (Cook, 1983; Agrios, 2005). It is used mainly for the seed borne inoculum and the effect is short lived. Highly resistant varieties are neither available nor can be effective against different races of the pathogen prevalent in the country (Meki, *et al*., 2009; Merkuz *et al*., 2011c; Merkuz, 2012). Biological control using microbes is becoming a critically needed component of plant disease management, particularly in reducing root diseases (Nautiyal, 2000; Meki *et al.*, 2009). The use of biocontrol agents is gaining momentum as it is environment friendly and also compatible with other models of agriculture: organic, biological and integrated pest/pathogen management (Monte and Llobell, 2003). Biological control of soil borne plant pathogen is a potential alternative to the use of chemical pesticides which have been proven harmful to the environment (Hend *et al.,* 2012). Different mode of action of bio control active micro-organism in controlling fungal plant disease include hyperparasitism, predation, antibiosis, cross protection, competition for site and nutrient and induced resistance production of siderophores and so on (Sumeet and Mukerji, 2000; Heydari and Pesaarakli, 2010).

During competition, biocontrol agents compete for nutrient and space with pathogens and are rapid colonizer and proliferate at a rate, out numbering the pathogen (Chincholkar *et al.*, 2000; Sumeet and Mukerji, 2000). Antibiosis is an important mechanism used by biocontrol agents to suppress diseases by produce volatile and non-volatile antibiotics which disrupt the cell contents of pathogenic microorganisms before coming in contact with the biocontrol agent (Dennis and Webster, 1971; Yong *et al.*, 1985). Lysis is by secretion of cell wall degrading enzymes, such as chitinases and ß-1, 3-glucanases as suppression of plant diseases (Wu *et al.*, 1986). According to Loper (1988), siderophore producing microbes act as biocontrol agents by limiting the amount of iron available to potential plant pathogens. Mycoparasitism occurs when one fungus exists in intimate association with another, from which it derives some or all of its nutrients while conferring no benefits in return (Lewis *et al.*, 1991). The mechanism of mycoparasitism involves different kinds of interactions like coiling of hyphae around the pathogen, penetration and production of haustoria (Nigam *et al.*, 1997).

Padwick in (1941) reported bio control of wilt pathogen *Fusarium oxysporum* f. sp. *ciceri* in the laboratory by a species of *Trichoderma* which was highly antagonistic to the pathogen in Petri dish. But it proved useless in soil and *Aspergillus* which retarded infection was subsequently proved in-effective. Kaiser and Sengupta (1975) isolated a pathogenic strain of *Trichoderma harzianum* causing foot rot of chickpea. On artificial inoculations the fungus produced typical foot rot symptoms in chickpea seedlings within 6-8 days. Singh *et al.* (2003) determined the efficacy of *T. harzianum, T. hamatum, T. viride, Gliocladium virens, P. fluorescens* and *Bacillus subtilis* against wilt pathogen *F. oxy.* f. sp. *ciceri. T. harzianum* recorded the highest control of the pathogen both *in vitro* and under field conditions. The efficacy of *T.*

harzianum against chickpea wilt was confirmed by several workers again (Singh *et al*., 2007, Barhate and Dake, 2006). Baviskar and Padghan, (2008) Nikam *et al.* (2007) proved in pot cultures studies that *T. viride* was the most effective in reducing chickpea wilt incidence. In Ethiopia, *Trichoderma viride* has been found to reduce the radial growth of *F. solani* and mortality of faba bean due to root rot caused by this pathogen (Tesfaye, 1999). Dwivedi and Enespa (2014) found that at 50 and 75% concentration *T. longibrachiatum* completely inhibited the growth of *F. oxysporum* f. sp. *lycopersici* and *F. solani.*

2.8.2 Through botanicals

Vast fields in developing countries are blessed with abundant plants with fungicidal potential with easy formulations and application attracting lower capital investment than synthetic fungicides (Anjorin and Salako, 2009). Plant extract have been tested against *Fusarium oxysporum* for their inhibitory effect and their control efficacy under greenhouse conditions (Bower and Locke, 2004; Sahayaraj *et al*., 2006 and Amadi *et al*., 2010). Phytofungicides could be prepared or formulated from the leaves, seeds, stem bark or roots of plants and could be applied in the form of extract, powders and cakes or as plant exudates (Owino and Wando, 1992). Further, these are safe and effective in view of being systemic in their action and lack residual effect, easily biodegradable and exhibit stimulating effect on the plant metabolism. Antimicrobial properties of numerous plant extracts, polar and non-polar fractions, their pure compounds, and essential oils have been investigated by many researchers against different strains of *Fusarium* (Gomez-Rodriguez *et al.,* 2003; Irum, 2007, Riaz *et al.*, 2008, Hassannein *et al*., 2008; Ghorbany *et al*., 2010) Ark and Thompson (1959) showed that garlic extracts contain a potent fungicide. They were able to effectively protect peaches against brown rot (*Monilinia fructicola*) with deodorized

garlic extract preparations. Mandhare and Suryawanshi (2008) took six plants extract viz. *Allium sativum, Azadirachta indica, Eucalyptus* species, *Nerium indicum, Ocimum sanctum* and *Zingiber officinale* inhibited 55.5 and 22.2 percent respectively. Other three plant species were proved ineffective in inhibiting the growth of the fungus.

In vitro screening of twenty five plant extracts belonging to various families were tested against wilt causing fungi *Fusarium oxysporum* by poisoned Food Technique. Extract of *Chenopodium ambrosioides* was the most effective of all the botanicals and showed excellent antimicrobial activity by complete inhibition of mycelial growth of the test fungi (Minz *et al.,* 2014). Methanol extracts of *A. sativum* bulbs could possibly be used for controlling this soil borne fungus (Sahayaraj *et al.,* 2006). Dwivedi and Enespa (2012) evaluated the antifungal activity of extracts of *Tinospora cordifolia* (leaves), *Moringa oleifera* (bark) and *Trachyspermum ammi* (seeds) at three concentrations *viz.*, 25, 50, 75% (v/v) *in vitro* by poisoned food technique against *F. oxy.* f. sp. *lycopersici* and *Fusarium solani*. At 75% concentration of the extract there was complete inhibition of *F. oxy.* f. sp. *lycopersici* by *M. oleifera* and of *Fusarium solani by T. cordifolia.*

Pyrethrin is used as grain protectant against insects. It is obtained from the seeds or flower of *Chrysanthemum cineraria folium* which is grown in Africa, Ecuador, and Kenya. It has a short residual activity and degrades rapidlyon exposure to air moisture or sunlight and hence, may require frequent applications. Pyrethrin can be used until harvest, as there is no waiting interval required between initial application and harvest of food crops (Casida and Quistad 1995). The mode of action of pyrethrin drug by which it kills insects is by disrupting the Na (sodium) and K (potassium) ion-exchange in insect nerves and disrupting the normal transmission of nerve impulses.

Soil amendments' strike the minds of agriculture scientist and farmers to combat the root disease. Padwick (1941) reported that farmyard manures hastened the disappearance of the wilt fungus Chauhan (1963) amended the soil by mixing oilcakes, he observed significant reduction in incidence of wilt incited by *F. oxysporum* f. sp. *ciceri* in soils treated with groundnut, sesame or mustard oilcakes. The last gave the best result (8.33 percent mortality; non treated 63.33%). Nikam *et al.* (2007) found that combined application of *T. viride*, ground nut cake and neem cake in soil were effective in controlling chickpea wilt caused by *F. oxy.* f. sp. *ciceri.*

2.8.3 Through essential oils

Volatile compounds from plants, especially essential oils, have antimicrobial, fungicidal and insecticidal activities (Wilson *et al.,* 1997). The effectiveness of plant essential oils as soil fumigants to manage bacterial wilt (caused by *Ralstonia solanacearum)* in tomato has been studied (Pradhanang *et al*., 2003) some essential oils have significant efficacy against *R. solanacearum* (Momol *et al*., 1999) and against several soil-borne fungi of tomato (Momol *et al.*, 2000). Formulations of pepper extract, synthetic cinnamon oil, clove oil, cassia extract, neem oil, and mustard oil, , were assayed against *Phytophthora nicotianae* (Bowers and Locke, 2004). Essential oils from pepper, mustard, cassia tree and clove suppressed disease development caused by *F. oxysporum* f. sp *melonis* on muskmelon and reduced the population density of *F. oxysporum* f. sp *chrysanthemum* in greenhouse experiments (Bowers and Locke, 2000).

Singh *et al.* (1980) found that essential oils from *Cymbopogon martinii*, *C. oliveri*, and *Trachyspermum ammi* exhibited strong antifungal activity against *Helminthosporium oryzae.* In northern Cameroon, the essential oils of plants *Xylopia aethiopica, Vepris heterophylla* and *Luppia rugosa* are used for protection of stored

grains from attack of stored grain insect pests (Ngamo *et al*., 2007). The essential oil vapours distilled from anise, cumin, eucalyptus, oregano and rosemary were also reported as fumigants and caused 100% mortality of the eggs of *Tribolium confusum* and *Ephestia kuehniella* (Tunc *et al.,* 2000). Ocimum oils, extracts, and their bioactive compounds have been reported to have insecticidal activities against various insect species (Ofori *et al.,* 1998; Keita *et al.,* 2001).

2.8.4 Through fungitoxicants

One of the most common ways to control plant diseases is through the use of chemicals. Chemicals are effective against the pathogen because they either inhibit germination, growth or multiplication of the pathogen. A true understanding of the mechanism of all the chemicals employed against the pathogen is yet lacking but it has been found that the chemicals are effective either as protectants, at the point of entry of the pathogen or they act systemically through the plant. Chemicals act by inhibiting the ability of the pathogen to synthesize certain cell wall components; damaging the cell membrane of the pathogen; by forming complexes and thus inactivating certain coenzymes, and enzymes. Systemic fungicides and antibiotics are absorbed and translocated internally. They inhibit ergosterol biosynthesis and some often interfere with respiratory mechanism. (Agrios, 2005)

Manucheri and Mesri (1966) suggested seed treatments with mercury compounds to eradicate wilt disease menace. Verma (1976) treated seeds with bavistin or carboxin at the rate of 0.25% which protected plants in potted soil infested with *F. oxysporum* f. sp. *ciceri. Sclerotium rolfsii* or *Rhizoctonia bataticola.* These fungicides, found to absorb and translocate in seedlings, persisted up to 12 days. They were also found to protect seedlings in field for 30 days or more in the field where

root rot and wilt of chickpea by the above pathogens were known to be epiphytotic each year.

Haware *et al.* (1978) found the wilt fungus in the hilum of chickpea seeds. It was eradicated by seed treatment with Benlate T (benomyl+Thiram). Dikkar *et al*. (2001) assayed some systemic (carbendazim, difanoconazole thiophanate–methyl, propiconazole, hexaconazole and ridomil (metalaxyly) and non-systemic (thiram, ziram, mancozeb, captan and cholrothalonil) fungicides. He reported that among all the tested fungicides carbendazim showed cent per cent inhibition of mycelial growth at the lowest concentration. Singh and Jha (2003) experimentally demonstrated that thiram and Bavistin (carbendazim) among the seven fungicides tested reduced the incidence of wilt when used as seed treatments and soil drenching. Both the fungicides increased the grain yield under field conditions. Singh *et al.* (2009) in an integrated management trial found Bavistin 2g/kg seed, Thiram 2.5g/kg seed, bavistin (1g/kg seed)+thiram (1.25g/kg seed) and fungi nill 4.0g/kg seed were superior in increasing seed germination and reducing plant mortality. Seed treatment with bavistin +thiram and Bavistin alone was most effective in increasing seed germination and reducing plant mortality under field conditions. They also recorded that *Trichoderma viride* and thiram gave similar results. Both fungicides decreased the disease incidence and increased the grain yield under field conditions.

Srivastava *et al*. (2011) reported that the radial colony growth of *Fusarium oxysporum* f. sp. *psidii* associated with rhizospheric soil of guava was completely inhibited by carbendazim 50 per cent WP at high concentration like 100, 1000 and 10,000 ppm.

In order to protect the farmer's economy use of chemical fungicides, though a non-ecofriendly, pollution creating device, sometimes become necessary. Since the

pathogen is a facultative saprophyte and can survive in soil for upto six years in the absence of susceptible host (Haware *et al*., 1978 and 1986), use of resistant varieties was looked up as an economical and practical solution but development of susceptibility in resistant cultivars along with constant evolution of variability in the pathogen has been reported (Nikam *et al*., 2007).

2.8.5 Integrated management

Integrated management is looked upon as an efficient viable option in controlling the plant diseases as they curb the risk of developing resistance by the pathogen to the fungitoxicants and also promote plant health and sustainable agriculture. It encompasses various strategies like minimum use of chemicals for checking the pathogen population, encouragement of beneficial bioagents, to reduce pathogen inoculum, modification of cultural practices and use of resistant varieties (Bendre and Barhate, 1998).

In an investigation carried by Steinberg *et al.* (2004) he found that use of spent mushroom compost along with cattle manure significantly reduced the disease incidence of both *Fusarium* wilt (*F. oxysporum*) and dry root rot (*Rhizoctonia solani*) in chickpea. Landa (2005) reported that the use o *B. subtilis* GB03 and *P. fluorescens* RG 26, when applied either alone or each in combination with non-pathogenic *F. oxysporum* Fo 90105, proved as excellent treatment in suppressing *Fusarium* wilt of chickpea. Similarly Chand and Singh (2005) reported plant extracts, of eucalyptus (*Eucalyptus globulus*), jatropha (*Jatropha multifida*), neem (*Azadirachta indica*), garlic (*Allium sativum)* were able in significantly reducing the wilt incidence in gram, except for *Calotropis procera.*

A combination of neem cake + carbendazim + *T. harzianum* was found significantly effective in controlling the disease *Fusarium* yellows caused by

Fusarium oxysporum f. sp. *gladioli* (Sharma *et al*., 2005). Coriander wilt caused by *Fusarium oxysporum* f. sp. *coriandrii* was successfully controlled by the application of two bioagents *viz.*, *Trichoderma harzianum, Pseudomonas fluorescens* and two fungicides, carbendazim (0.1%) and mancozeb (0.25%) at 45 DAS (Singh, 2009)

Constant evolution of the pathogen and development of resistance to various chemicals has created a stir to search for such integrated management practices which can substantially control the wilt disease besides helping in making it more environments friendly. Hence the present study was undertaken to visualize the problem afresh and put forth in the following pages.

Materials and Methods

3.1 Survey and collection of plants

To obtain information on the occurrence and incidence of the wilt disease of chickpea, periodical and planned collection of the wilted plants were carried out throughout the crop season for the years (2011-2012 and 2012-2013) in nearby villages of Unnao (Magarwara, Singrossi, Sikandarpur) and Kanpur (Mandhana, Rooma, Singhpur) districts of U.P.

IIPR (Indian Institute of Pulse Research) farm fields were also visited in addition to the above locations farm. The wilted plants samples were collected from each by randomly locating 1 sq. meter quadrate in five replicates from different fields. Thus the maximum wilted plants were collected, put to observation, isolations and further studies.

3.1.1 Disease Incidence

Disease incidence was calculated in percent following the procedure as suggested by Chester (1950).

$$\text{Percent disease incidence} = \frac{\text{Total number of diseased plants}}{\text{Total number of plants in the field}} \times 100$$

3.2 Isolation of the causal organism

The root of each plant sample was washed thoroughly to remove the sticking soil remnants. Then the root of the plant was cut into three small pieces with the help of sterilized scalpel. These root pieces were surface sterilized in 0.01 % mercuric chloride ($HgCl_2$) solution for one minute. Such sterilized root pieces were washed in running sterilized distilled water, so as to remove the traces of mercuric chloride on the surface of these root pieces. Excess moisture was removed, by putting these pieces in between two folds of sterilized blotting papers, under aseptic condition in the inoculation chamber.

Four to five bits of root sections were plated at an equal distance from

each other and Petri dishes were incubated for 5 days at 25±25°C in a BOD incubator. The Petri dishes were observed daily for the growth of the causal fungus. As soon as the growth of the causal fungus was observed it was transferred to PDA slants. The culture tubes with these fungal cultures were examined microscopically and the tubes having the cultures of *Fusarium oxysporum* f. sp. *ciceri* were sub cultured.

3.2.1 Single spore culture

Pure culture of the fungus was obtained by adopting dilution method (Keitt, 1915). Four sterilized culture tubes containing 10ml sterilized distilled water in each was taken. Small piece of PDA along-with the pathogen culture was transferred to the first culture tube, and shaken vigorously till a homogenous spore suspension was obtained. Then 1ml of the spore suspension from the first test tube was transferred into second test tube containing 9ml of sterilized water. This process was repeated 4 more times, so that the fourth test tube contained a much diluted homogenous spore suspension. Thus homogenous spore suspension was obtained in a dilution of 1/10, 1/100, 1/1000, 1/10000, 1/10000 in the culture tubes. They were examined by dipping the inoculation loop in them. The single loop was placed on a glass slide and examined for single spore under a microscope. The single spore containing dilution was then used to transfer a single spore to freshly prepared PDA on it.

3.2.2 Identification of *Fusarium oxysporum* f. sp. *ciceri*

Monoconidial cultures of the different isolates of *Fusarium* found pathogenic on chickpea were identified following Booth (1971). The slides from each culture were prepared in lactophenol and cotton blue stain. These slides were examined under the microscope. For size comparison (length and width) 100 conidia for each septation group were measured with the help of ocular micrometer and the averages were

worked out. On the basis of the morphological and cultural characters observed, the *Fusarium* was identified following the taxonomic system of Booth (1971).

3.3 Isolation and identification of antagonistic fungi

Isolations of the antagonistic fungi were done by using serial dilution technique. The rhizosphere soil from roots of healthy plants was taken onto a Petridishes. One gram of such soil was mixed in 10 ml of sterilized water and shaken for 20 to 30 min on a magnetic stirrer. Serial dilutions of the above soil suspension was made (10^{-2} to 10^{-6}) with sterile distilled water in each test tubes (9ml per tube). Aliquot of (0.5ml) was spread onto PDA plates (20 ml) in triplicate and incubated at 28°C for 7 days. As soon as the growth of the antagonistic fungus was observed it was transferred to PDA slants. The culture tubes with these fungal cultures were examined microscopically and the tubes having the cultures of the antagonist were sub cultured. The fungal colonies were purified and identified by available literatures.

The isolated bioagents were identified on the basis of their colour, morphological characters as well as sporulating structure and conidia under Phase Contrast Microscopy (PCM). The antagonistic fungi *i.e. Trichoderma* species (*T. harzianum* and *T. virid*e) were purified by hyphal tip technique (Gilman, 1957) and identified on the basis of morphological and cultural characteristics as per the key given by Barnett and Hunter (1972), Bissctt (1991) and Rifai (1969); and *Aspergillus spp.* (*A. niger,)* by Gilman (1957). These were used for *in vitro* and *in vivo* evaluation against *and F. oxysporum* f. sp. *ciceri.*

3.4 Pathogenicity test

3.4.1 Multiplication of the fungal inoculum

For pathogenicity test, each of the isolate was multiplied on soil maize meal medium, (Miller, 1946), comprising 190 gm of field soil sieved though 2mm sieve, 10 gm of finely grounded maize meal and 70 ml of distilled water. The sieved soil and maize meal were thoroughly mixed and 200 gm of this mixture was taken in each 500ml flask. Seventy ml of sterilized distilled water was then added to wet the soil maize meal mixture. These flasks were sterilized in an autoclave at 15 lbs. per square inch pressure for 20 min for two consecutive days. Later these flasks were inoculated with fungal mycelium growing on PDA and incubated at 25℃ in BOD incubator for 20 days for the growth of the fungus. For each *Fusarium* isolate separate flasks was maintained for multiplication of the fungus. (The same procedure was employed for multiplying antagonistic fungus inoculum).

3.4.2 Preparation of pots infested with *Fusarium oxysporum* f. sp. *ciceri*

For testing the pathogenicity of chickpea cultivars against various *Fusarium* isolates and for assessing the effectiveness of different biocontrol agents and fungitoxicants against the pathogen the plastic pots of 15 cm size were used. For all the pot experiment the normal field soil was used. This soil was dried under the sun passed through 2 mm sieve sterilized by autoclaving for 20 minute at 15 lbs. pressure/sq. inch for two consecutive days. The plastic pots of 15 cm size were washed and dipped in 5 per cent Lysol solution and rinsed thoroughly with sterilized water before use. These pots were filled upto 10 cm height with a mixture of sterilized soil maize meal (190:10) and 5% (w/w) wilt fungus inoculums and multiplied on soil maize meal medium. The contents were gently tapped down and were covered on the top with sterilized soil upto 2 cm height and were given light irrigation with sterilized distilled

water. These pots were covered with butter paper on the top to avoid aerial contamination and were incubated for 7 days at room temperature (18ºC-25ºC) before sowing. For each *Fusarium* isolate separate pot was prepared.

3.4.3 Pathogenicity Test

The pathogenicity test of the fungus isolates recovered from the affected root were made on healthy plants of the host separately in order to establish pathogenic nature of all the isolates according to Koch's postulates (Koch, 1876).

To test the pathogenicity of all the isolates separately, the infested pots of different isolates were sown with susceptible variety JG62 @ 10 seed per pot. The seeds to be tested for wilt were surface sterilized in 1:1000 mercuric chloride solutions for 2 minutes followed by washing in running sterilized distilled water. These were sown on the top layer of the pots, these pots were then placed on wooden benches closed on all the side with double layer of muslin cloth. When the plants showed wilting they were pulled out from the pots carefully and soil adhering to the roots washed off in running water. Reisolations were made from the roots as well as from the basal portions of the stem of the wilted plants. The typical symptoms were recorded. Reisolates were examined and found to be the same pathogen that has been taken to infest the pots. Thus Koch's postulates were proved and pathogenicity test were carried out successfully. The mortality percentage of seedlings of JG 62 a known susceptible genotype was recorded for each isolate to work out the pathogenic nature, as per the scale of Trivedi and Gurha (2007) which is as follows:

Highly pathogenic - Above 80% mortality

Moderately pathogenic - 50-79.9% mortality

Weakly pathogenic - Less than 50% mortality

3.5 Study of symptoms caused by different *Fusarium* isolates

The disease symptoms under natural conditions of infection were carefully studied and recorded. The symptoms in pot culture were studied from its initial stage to complete wilting for different fusarium isolates separately.

3.6 Histopathological studies

Histopathological studies were carried out by cutting sections of roots and stems of freshly wilted chickpea plants collected from pots. These sections were stained in modified Amann's lactophenol (Maneval, 1936) cotton blue stain; comprising phenol (pure crystals) 20 gm, lactic acid 20 gm, glycerin 40 gm, 1% aqueous solution of cotton blue 10ml and distilled water 10 ml. Later these sections were placed in lactophenol without dye to remove excess stain. These sections were examined under the microscope for the presence of fungal mycelium, spores and its course in the plant tissues and for other effects.

3.7 Cultural study of the pathogen

Cultural studies paved the way to find the out comparative growth rate of different isolates of pathogen, and the variations in the detailed morphology of the isolates.

3.7(a) Preparation of medium

One semisynthetic medium (PDA) was taken for the study of radial growth of the pathogen. The different ingredients of the medium were dissolved in distilled water separately before mixing together and the medium was sterilized in autoclave at 15 lbs. pressure per square inch for twenty minutes.

Potato dextrose agar medium

1	Pealed potato	200gm
2	Dextrose	20gm
3	Agar agar	20gm
4	Distilled water	1000ml.

3.7(b) Radial growth studies of isolates of *Fusarium oxysporum* f. sp. *ciceri*

For the study of radial growth of isolates, 20 ml sterilized PDA medium was poured into sterilized petridishes and when the medium solidified, equal discs of 5mm diameter of the fungal growth cut by cork borer, were placed in the center of each petridish. After inoculation the petridishes were incubated for 10 days at 22±1ºC. Each treatment was replicated three times. Radial growth of the pathogen was observed in millimeter in two directions at right angles to each other after every 24 hours; there after average was calculated. Different cultural characters were recorded for all the pathogenic isolates of *Fusarium*.

3.8 Study of abiotic factors affecting wilt

Knowledge of optimum soil conditions, temperature and availability of inoculums for wilting is absolutely necessary to formulate effective disease management strategies. To gain an insight as to the effect of these abiotic factors on wilting, all the isolates of *Fusarium oxysporum* f. sp. *ciceri* were multiplied separately on soil maize meal medium (Miller, 1946) and then mixed in equal quantities for preparing the pots as described earlier.

3.8.1 Effect of soil composition

To find out the effect of soil moisture and aeration on wilt incidence, sand in varying proportions *viz.,* 0, 5, 15, 25, 35 percent was mixed with normal field soil (sandy loamy) of Kanpur. Keen and Raczowski box method (1921) as described by Piper (1944) was used to measure density, specific gravity, maximum water holding capacity and percent pore space of soil and soil sand mixtures.

The method consists of Brass cylinder box (dimeter 5 cm and height 1.6 cm) with perforated base. The air dried soil or soil sand mixture were crushed and sieved through 2mm sieve. A filter paper was fitted upon the perforated base of the box and

weighed. The box was packed with the soil sample by adding small quantities at a time and tapping the box after each addition of soil to ensure even packing upto nearly the top. The surplus overtopping soil was sliced off with a sharp blade. The box was placed for 24 hours in a small tray containing 1cm deep water. The box, from the tray was then removed and weighed after wiping the excess water. Consequent to keeping the box in water for 24 hours the soil at the top bulged. This was sliced off and weighed in a porcelain dish. The box was again weighed with the residual wet soil sample. The soil in the box and the dish containing expanded soil was dried in an oven at 105ºC for 6hrs. Both the box and the dish were again weighed and the set of readings, thus recorded were used for calculating various factors.

Calculation

Weight of box +filter paper = 'a'gm

Weight of box + air dry soil = 'b' gm

Weight of box + wet saturated soil = 'c' gm

Weight of box + wet residual soil *i.e.*

after removal of wet expanded soil = 'd' gm

Weight box + residual wet soil

after drying at 105ºC = 'e' gm

Weight of empty porcelain dish = 'f' gm

Weight of dish + wet expanded soil = 'g' gm

Weight of dish + expanded soil

after drying at 105ºC = 'h' gm

Internal volume of the box = 'v' ml

1. Apparent density = (b -a) /v

2. Absolute specific gravity = (e-a)/ v-(d-e)

3. Maximum water holding capacity = (c-a)-(b-a)/ (b-a) ×100

4. Percent pore space = (d-e)/v×100

5. Volume expansion of 100gm soil = (g-h) + (h-f/sp. gr.) /v×100

3.8.2 Effect of inoculum level

To determine the effect of inoculum level on wilting the plastic pots (100 mm) were filled with (i) sieved soil with (25%) sand w/w, (5%) maize meal and (5%) wilt inoculum (w/w) upto 90 mm; (ii) sieved soil with (25%) sand (w/w), (5%) maize meal and (5%) wilt inoculum (w/w) upto 65 mm. Over it a layer of 30 mm sieved soil and (25 %) sand was filled. (iii) sieved soil with (25%) sand (w/w), (5%) maize meal and 5% wilt inoculum w/w upto 35 mm. A layer of 65 mm sieved soil and (25 %) sand (w/w) was put on the infested soil. In all the cases soil, sand and maize meal used were sterilized. In all the sets, the seeds were sown and a thin layer of soil sand mixture was placed over it.

3.8.3 Effect of temperature

The temperature effect was recorded by keeping the pots in especially made spore proof cages. Maximum and Minimum temperature were regularly recorded during December- January (2012-13) the range of temperature was 8-17ºC and during February- March (2013) 21-28ºC. In each experiment the seeds were sown after surface sterilization as described earlier. Ten seeds were sown in each pot with 5 replications. Seeds of single plant progenies of highly susceptible varieties JG 62, Kanpur local and Ujjain Local were used in the experiments.

3.8.4 Role of different crop debris in multiplication of wilt pathogen *Fusarium oxysporum* f. sp. *ciceri*

Role of different crop debris in the multiplication of wilt pathogen was ascertained by collecting the debris of pigeon pea, chickpea, wheat, urid, linseed and mustard from the farmer’s field. Healthy plant debris (200 gm) free from infections was washed

under running tap water and soaked in distilled water for 24 hrs. The soaked debris were boiled in the same water and filtered. Along with these debris potato dextrose broth medium was also prepared to compare the results. The potato dextrose broth medium was prepared by the method described earlier excluding agar. The Erlenmeyer flask containing the respective medium were sterilized and inoculated as per the method mentioned above.

3.9 Comparative studies on the production of certain enzymes produced by *Fusarium oxysporum* f. sp. *ciceri* (isolate 1)

In order to study the production of cellulolytic and pectinolytic enzymes the pathogen was grown in 100 ml of Czapek's broth amended with Carboxy Methyl Cellulose (CMC) and Sodium polypectate as the carbon source respectively. The Czapek's broth was then inoculated with 8 mm of mycelial disc from 10 day old culture of the pathogen and was kept in incubation for 7, 14 and 21 days at 28±1ºC. The fungal cultures were then filtered twice through Whatman filter paper no. 42 and centrifuged at 2000 rpm for 20 min and the clear supernatant was taken as the enzyme source.

Cellulase activity was assayed according to the viscometric method (Muse *et al.,* 1972). One percent CMC was prepared in 0.1M sodium acetate-acetic acid buffer pH 5.2 (as substrate).The reaction mixture consisting of 4 ml of CMC, 1 ml of buffer solution and 2 ml of enzyme solution (culture filtrate) were taken and mixed thoroughly. The mixture was immediately put into the Ostwald-Fenske viscometer (150 size with minimum efflux time of 15-20 sec for double distilled water) maintained at 37ºC. The efflux time was recorded at an interval of 30 minutes for two hours (*viz.,* 30 min, 60 min, 90 min and 120 min).

The reduction in viscosity was expressed as percentage loss in viscosity over water (control) and was calculated by the following formula:

Percentage loss in viscosity $= \frac{To-T1}{To-Tw} \times 100$

Where T_0 = Flow time of reaction mixture at zero hours

T_1 = Flow time of reaction mixture at particular interval

T_w = Flow time of distilled water

The same procedure was repeated to assay Polygalacturonase (PG) enzyme except that one percent sodium polypectate was used as substrate prepared in 0.1M sodium acetate-acetic acid buffer pH 5.2.

3.10 Disease management

3.10.1(a) Efficacy of antagonist *in vitro*

The antagonists *T. harzianum, T. viride* and *A. nige*r were inoculated according to dual culture technique of Johnson and Curl (1972) and Tu (1980) on PDA Petri dishes and the inhibition as well as interaction pattern was recorded.

(a) *Interaction behavior of Antagonist with the pathogen*:

Interaction behaviour was studied by inoculating them in different possible combinations on PDA Petridishes and the interaction pattern was recorded according to the key of Johnson and Curl (1972):

A Mutual intermingling of the two organisms

B Mutual Inhibition on contact

C Mutual inhibition at a distance

D Inhibition on contact, the antagonist continues to grow, at unchanged or reduced rate though the colony of the inhibited organisms

E Inhibition at a distance, the antagonist continues to grow resulting in a clear zone at an unchanged or reduced rate

Data Analysis: All values were expressed as mean ± SD, n = 3 and the results on the effect of different bioagents on the colony growth of the pathogen were analysed by analysis of variance (ANOVA with replication), P≤ 0.5 was considered statistically significant.

3.10.1(b) Efficacy of antagonists *in vivo*

The antagonists *T. harzianum T. viride* and *A. niger* were mixed in different percentages at 2, 4, 6, 8, % (w/w) infested soil of sterilized soil sand mixture in 15 cm plastic pots. The seeds (10 @ seeds per pot) of susceptible varieties JG 62, Kanpur local and Ujjain local were surface sterilized and sown as per the methods described earlier and their effects were recorded.

3.10.2 Toxicity of certain botanicals against the wilt pathogen

The potencies of different management trials in the subsequent experiments were assayed against Isolate 1.

3.10.2(a) Plant extracts

Leaves of eleven medicinal plants species *viz. Acacia nilotica, Aegle marmelos, Azadirachta indica, Callistemon lanceolatus, Cassia fistula, Polyalthia suberosa, Pongamia pinnata, Syzgium cumini, Tamarindus indicus, Terminalia arjuna* and *Zizyphus jujuba* were collected and washed with sterile water. It was then crushed with equal amount of water (w/w). Centrifuged at 5000 rpm for 15 minutes. The filtrate was then taken as 100% stock solution. For the preparation of alcoholic extract alcohol was used instead of water. Concentration of 100, 200 and 500 ppm were prepared in PDA medium amended with leaves extract as in food poison technique (Zentymer, 1955). The medium in petriplates was then inoculated from 7 day old culture of the pathogen. Inoculated petriplates were then kept at 27ºC±1in BOD Incubator. Each experiment was then replicated thrice and percentage inhibition was

then calculated according to the formula: (dc-dt))/dc×100. Control was taken as plain PDA. Data was analysed using ANOVA with replication.

3.10.2(b) Seed powder

The seeds of black pepper (*Piper nigrum*) and cumin (*Cuminum cyminum* L) were crushed in a mortar and pestle and a 50% (w/v) stock solution was prepared in sterilized water. It was then passed through a double layered muslin cloth and finally filtered through Whatman filter paper no1. Different concentration *i.e.,* 10%, 20% and 30% (v/v) were prepared by adding 90 ml, 80 ml, and 70 ml of sterile water into the stock solution. The solution was heated up to 40-50°C for 10 minutes to avoid contamination. The extract was stored at 4˚C to avoid any further chemical alteration or contamination.

The above extracts were then added to 90 ml of Czapek's medium and allowed to solidify. The activity of all the extract was then tested against the pathogen by poisoned food technique. In control plates, sterilized distilled water was added with Czapek's-dox agar medium in place of the seed extract. Each treatment was replicated three times. After solidification, the medium in Petriplates was inoculated from 7 day old culture of the pathogen *Fusarium oxysporum* f. sp. *ciceri* in the center of each Petriplates and incubated at 25±2˚C. The colony diameter of the pathogens was then measured after 7 days of incubation. Data recorded was subjected to Analysis of variance (ANOVA) and the values were expressed as mean±SE of three replications.

3.10.2(c) Oil cakes

Stock solution of mustard (*Brassica juncea*), til (*Sesamum indicum*) and neem (*Azadirachta indica*) oilcakes were prepared by taking twenty grams of each oilcake and soaking it for two hours in 100 ml of distilled sterilized water. A double layered

muslin cloth with Whatman No. 1 filter paper was used for the filtration of the extract. This served as stock solution. It was kept under UV light for ten minutes to further avoid any sort of contamination. Different concentrations of the extract *viz*. (10%), (20%) and (30%) were prepared by adding 90 ml, 80 ml, 70 ml quantity of sterile water into the stock solution. The stock solution was heated up to 50°C for 10 min. The activity of the three oilcake extract was tested against the pathogen by poisoned food technique (Zentymer, 1955). The 10, 20 and 30% conc. of oilcake extracts were mixed separately in 90 ml sterilized Czapek's-dox agar medium and poured into Petriplates. Plain water in place of oilcake extract served as control plate. Each treatment was replicated three times. After solidification of the medium, 5 mm. diameter plugs from 7 days old colony of *Fusarium oxysporum* f. sp. *ciceri* was inoculated in the center of each Petriplates and incubated at 25±2˚C. The colony diameter of the pathogen was measured after 7 days of incubation. Data were analysed using analysis of variance for three replications and values were expressed as mean ±SE.

3.10.3 Potency of certain essential oils against FOC

Leaves of certain plants *viz., Ocimum sanctum* (Lamiaceae) *Cymbopogon jwarancusa* L (Poaceae) *Eucalyptus citriodora* (Myrtaceae), *Muraya koenigii* (Rutaceae), *Tagetus erecta* (Asteraceae), *Cymbopogon martini* (Poaceae) *Oroxylum indicum* (Bignonaceae) and seed powder of *Daucus carota* (Apiaceae) and *Cucurbita pepo* (Cucurbitaceae) were put to hydro distillation using Clevenger apparatus (Clevenger 1928). Essential oil so obtained was used to prepare 1000, 500, and 250 ppm (µg/ml concentration in PDA medium). Each treatment was replicated three times. After solidification of the medium. The medium in petriplates was then inoculated from 7 day old culture of the pathogen *Fusarium oxysporum* f. sp. *ciceri* in the center of each

petriplates and incubated at 25±2˚C. The colony diameter of the pathogens was then measured after 7 days of incubation. The results were subjected to ANOVA followed by Dunnett's multiple range for replicate values.

3.10.4 Effect of some fungitoxicant on the mycelial growth of FOC

Five fungitoxicant were evaluated for their efficacy against the pathogen both *in vitro* and *in vivo*. Captan, Bavistin (Carbendazim), Diniconazole (FOB), Mancozeb (Indofil M-45), and Zineb (Indofil Z-78) were tested *in vitro* using food poison technique. The percentage inhibition in radial mycelia growth of the pathogen was calculated after 7days by impregnating the PDA plates with the fungitoxicant, replicated thrice.

The above fungitoxicants were also evaluated for their efficacy to control the wilt disease in infested pots. The pots were filled with 10% pathogen (w/w) in sieved soil and sand mixture sterilized at 15 lb. pressure per square inch for 20 min. Seeds of highly susceptible variety JG 62 were surface sterilized in 0.01% mercuric chloride solution for 2 min and then washed in running distilled water. Seed dressing with fungitoxicant *viz.* carbendazim, captan, diniconazole, mancozeb and zineb before sowing were done at the dose of 0.3%, 0.1%, 0.03%, 0.3%, 0.2% respectively. Seeds dressed with fungitoxicants were sown at 10 seeds per pot replicated five times. Control pots comprised of surface sterilized seeds without any dressing. Percentage mortality was observed in each case.

3.10.5 Integrated management

Two seed extract *viz.*, *Piper nigrum* and *Cuminum cyminum* were taken along with carbendazim to assess their efficacy in controlling the pathogen both *in vitro* and *in vivo* at different concentrations.

3.10.5(a) ***In vitro assay***

To determine the integrated effect of botanicals and fungitoxicant an *in vitro* test was undertaken followed by pot experiment. The experiment included the following treatments. T1: *Cuminum cyminum* extract (10%) + Carbendazim (0.3%); T2: *Cuminum cyminum* extract (20%) + Carbendazim (0.3%); T3: *Cuminum cyminum* extract (30%) + Carbendazim (0.3%); T4: *Piper nigrum* extract (30%) + Carbendazim (0.1%); T5: *Piper nigrum* extract (30%) + Carbendazim (0.2%); T6: *Piper nigrum* extract (30%) + Carbendazim (0.3%).

The above mentioned treatments set were prepared as mentioned earlier in the text. (i) All the treatment set *viz.*, T1, T2, T3, T4, T5, and T6 were assayed *in vitro* using PDA agar medium and colony growth was measured after 7days.

3.10.5(b) ***In vivo*** **assessment under pot conditions**

The above treatment set *viz.,* T1, T2, T3, T4, T5, T6 were assayed under pot conditions. Infested pots were prepared as described earlier. The pots were filled with 10% pathogen (w/w). Seeds of susceptible variety JG 62 were surface sterilized with 0.01% mercuric chloride solution and were then dressed with the mentioned concentrations of the fungitoxicant in the treatment sets as described earlier. The pots were sprayed with the botanicals as when required and were observed for 25 days. Each treatment was replicated five times with seven seeds per pot.

Results

4.1 Survey and collection of diseased plants

Disease prevalence was ascertained across six villages *viz.,* Magarwara, Singrossi Sikandarpur, Mandhana, Rooma, Singhpur and the fields of Indian Institute of Pulses Research (IIPR) Kanpur by repeated surveys for two consecutive years (2011-2012 and 2012-2013).

The method of recording the data of healthy and wilted plants and the determination of percentage disease incidence was described in Chapter-3 and the results are presented in Table 4, Fig.1 and Plate (1 and 2).

Table 4. Survey of wilt disease of Chickpea (*Cicer arietinum* L.) during two crop seasons in six villages located around two districts of U.P.

S. No	Name of the villages	Name of the districts	Average percentage of disease incidence (2011-2012)	Average percentage of disease incidence (2012-013)
1.	Magarwara	Unnao	40.01±0.44	35.70±0.15
2.	Singrossi		35.79±0.73	30.75±0.09
3.	Sikandarpur		25.63±0.38	38.64±0.06
4.	Mandhana	Kanpur	29.56±0.47	38.54±0.16
5.	Rooma		33.56±0.54	36.03±0.26
6.	Singhpur		31.36±0.49	33.54±0.26
7.	Fields of IIPR		35.14±0.36	30.90±0.20

Values shown are the percent mean ± SD of five fields for each village
Average temperature 2011-12 max temp 30ºC min temp 3.3ºC.
Average temperature 2012-13 max temp 29.3ºC min temp 5.3ºC.

The perusal of data reveals that in the two crop seasons (2011-2013) the disease incidence varied from (25.63 %) to (40.01%) across the above mentioned locations. In 2011-2012 crop season Sikandarpur had the lowest (25.63%), while Mandhana had the highest disease severity (40.01%), while Singrossi recorded (35.79%), Mandhana (29.56%), Rooma (33.56%), Singhpur (31.36%) disease incidence. In 2012-2013 Singrossi had the lowest (30.75%) and Sikandarpur recorded the highest (38.64 %) disease incidence, while Magarwara, Mandhana, Rooma and Singhpur recorded 35.70%, 38.54%, 36.03%, 33.54% and 30.90% wilt incidence respectively.

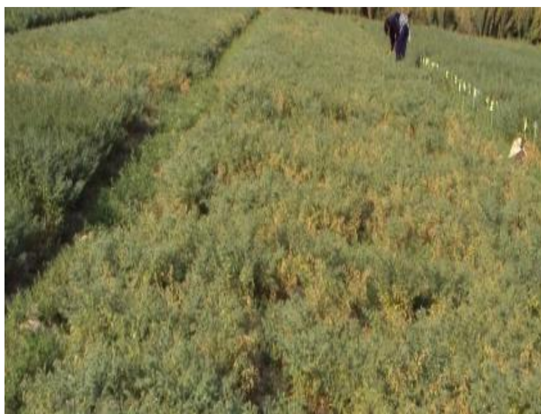

Plate 1 Wilt disease of chickpea in the IIPR farm field

Plate 2 Wilt disease in field (Mandhana)

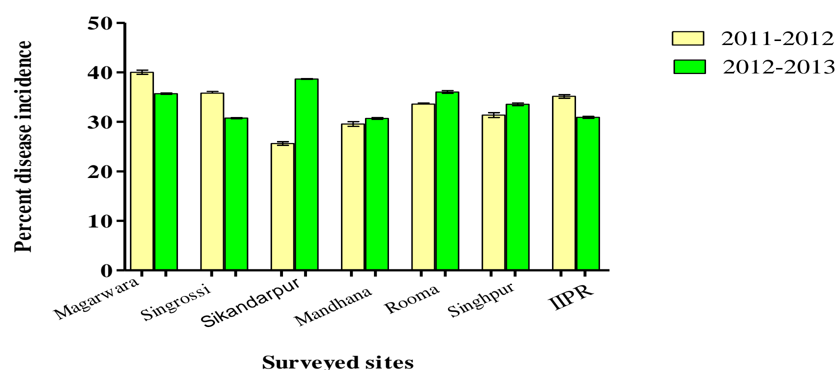

Fig 1 Incidence of *Fusarium* wilt in 6 villages across two Districts (Unnao, Kanpur) during 2011-2012 and 2012-2013

It was noted that temperature also has direct bearing on wilt disease however there was no correlation between wilting percentage and the crop season or the location. The survey also depicted that wilt disease is a cause of concern because the wilt affected plants yield neither straw nor seed and the percentage of loss is high on three fronts; (a) loss of seeds sown (b) loss of productive space of field (c) the net loss in straw and seed production.

4.1.1 Symptomatology of the disease as observed in the fields

On the basis of seeds sown by farmers two types of crops were observed (i) Broadcast crop and (ii) the crop sown in rows. At the first glimpse, the wilted plants occurred in small isolated patches with somewhat circular outline in the former while in the later type of crop design the wilt disease appeared progressing along the lines (Plate 1 and 2).

(a) After one month of sowing the seedlings in the fields showed various symptoms. *Fusarium* infestation was characterized by drooping of apices and loss of turgidity, flaccidity including flaccidity of individual leaves followed by a dull green discoloration. When uprooted the root region remained healthy and showed no discoloration.

(b) However, symptoms were more discernible 6 to 8 weeks after sowing when the plants were in the vegetative phase. Drooping of apices was prominent. The leaves became chlorotic, turned yellow in most of the cases then straw coloured or light brown.

(c) The affected plants sometimes showed retarded growth and ultimately the death. The stems when splitted vertically downwards, black discoloration of internal tissues was observed. Most often, at the time of flowering and poding stage , the symptoms of above typical wilt were seen in one or two branches of individual plants where the roots of such plants yielded *Fusarium* cultures (Plate 3 and 4).

Plate 3 Wilting symptoms in Chickpea at podding stage showing normal looking pods without seeds

Plate 4 Wilting symptoms in Chickpea at early, mid and late stages of wilt (right to left)

4.2 Isolation, Purification and Identification of *Fusarium oxysporum* f. sp. *ciceri*

Infected plants were collected from six villages *viz*., Magarwara, Singrossi, Sikandarpur, Mandhana, Rooma and Singhpur and also from farms of Indian Institute of pulses research IIPR, (Kalyanpur) Kanpur. Isolations were made on potato dextrose agar medium and culture of each isolate was purified by single spore culture as per the methodology described in chapter 3. These pathogenic cultures were studied morphologically, culturally and then identified with the help of standard text (Booth 1971).

4.2(a) Description of the pathogen *Fusarium oxysporum* f. sp. *ciceri* (Padw.) Snyder and Hansen

Hyphae abundant, septate, branched, forming a mycelium which developed a thin stroma on the substratum; Stroma coloured and of various shades. Aerial mycelium mostly abundant, white occasionally pink or lilac pionnotes lacking, conidia formed on free conidiophores, hyaline, mostly one celled.

Microconidia often agglutinated into false heads, one celled straight, ellipsoid, oval or slightly curved. Macroconidia with thin delicate walls and indistinct septation

with highly vacuolated contents, nearly straight or slightly curved. Chlamydospores were terminal and intercalary sometime in chains, globose to pear shaped, smooth, usually one celled and numerous.

4.2(b) Identification

Microconidia abundant, ellipsoid to ovoid or slightly curved but not pear shaped. Macroconidia thin walled, chlamydospores terminal, and intercalary on short lateral branches in mycelium. These characters belong to section *Elegans*. Sporodochia lacking, Macroconidia not constricted nor these possessed typical foot cell. These were straight or slightly curved, not more than 4µm broad. These characters are of species *oxysporum.* By the abundance, shape and size of microconidia, macroconidia and chlamydospores the fungus was identified as *Fusarium oxysporum* f. sp. *ciceri* (Padw.)

4.3.1 Measurement of morphological character

Isolate 1

Colour of substrate-Light yellow

Structure	Septation	Measurements	Frequency
Microconidia	0	3.75-9.0µm x 2.5-3.1µm	Frequent
Macroconidia	1	4.5-12.0 µm x 2.5-3.6 µm	Rare
	2	5.0-9.8 µm x 2.4-3.5 µm	Rare
	3	7.4-10.4 µm x 1.7-3.24 µm	Most Frequent
	4	10.5-12.0µm x 2.5-3.0 µm	Frequent
	5	22.5-26.5 µm x 2.5-3.5 µm	Very rare
Chlamydospores	-	4.4-11.1µm diameter	Frequent
Mycelium width	-	1.5-5µm	

Isolate 2

Colour of substrate- Pale

Structure	Septation	Measurements	Frequency
Microconidia	0	3.4-7.1 µm x 1.25 -2.1 µm	Frequent
Macroconidia	1	4.9-9.4 µm x 1.75-2.4 µm	Frequent
	2	5.4-8.6 µm x 2.0-3.4 µm	Frequent
	3	6.4-11.4 µm x 2.5-3.75 µm	Most Frequent
	4	7.0-17.0µm x 2.4-3.23 µm	Rare
	5	12.5-17.5 µm x 2.5-3.1 µm	Very rare
Chlamydospores	-	6.0-10.5µm diameter	Frequent
Mycelium width	-	1.5-5µm	

Isolate 3
Colour of substrate- Violet

Structure	Septation	Measurements	Frequency
Microconidia	0	4.5-4.9 µm x 1.84 -3.23 µm	Frequent
Macroconidia	1	6.5-10 µm x 1.25-3.75 µm	Frequent
	2	7.0-12.4 µm x 2.1-3.24 µm	Most Frequent
	3	8.5-17.5 µm x 2.5-3.75 µm	Most Frequent
	4	15.4-20.5µm x 2.5-3.1 µm	Rare
Chlamydospores	-	7.5-12.0µm diameter	Frequent
Mycelium width	-	2.0-2.25µm	

Isolate 4
Colour of substrate-Olive Buff

Structure	Septation	Measurements	Frequency
Microconidia	0	3.9-9 µm x 2.0 -3.4 µm	Frequent
Macroconidia	1	8.3-12.1 µm x 1.5-2.3 µm	Most Frequent
	2	9.4-10.3 µm x 2.0-3.75 µm	Frequent
	3	9.5-11.5 µm x 2.5-3.0 µm	Frequent
Chlamydospores	-	6-10.5µm diameter	Frequent
Mycelium width	-	1.5-3.25µm	

Isolate 5

Colour of substrate- Dirty white

Structure	Septation	Measurements	Frequency
Microconidia	0	4-9 µm x 1.48-2.49µm	Frequent
Macroconidia	1	8.5-9.75 µm x 1.5-2.5 µm	Frequent
	2	10-14.9µm x 1.25-2.25 µm	Most Frequent
	3	10.5-13.5µm x2.75-3.1 µm	Frequent
	4	15-24µm x2.5-3.1 µm	Rare
Chlamydospores	-	4.5-11.5µm diameter	Frequent
Mycelium width	-	2.5-5µm	

Isolate 6

Colour of substrate- Purple

Structure	Septation	Measurements	Frequency
Microconidia	0	5.5-7.4 µm x 3.0-3.25 µm	Frequent
Macroconidia	1	10-11.7 µm x 3.0-3.25µm	Frequent
	2	11.2-13.0 µm x 3-5.0 µm	Frequent
	3	12.5-13.5µm x 3.5-4.5 µm	Most Frequent
	4	13-14.8µm x 3.5-5.0 µm	Rare
	5	14.5-20 µm x 3.5-5.25 µm	Very rare
Chlamydospores	-	5-8.5µm diameter	Frequent
Mycelium width	-	1.5-2.75µm	

Isolate 7

Colour of substrate- Hyssop Violet

Structure	Septation	Measurements	Frequency
Microconidia	0	3.4-7.1 µm x 1.25 x2. µm	Frequent
Macroconidia	1	8.5-20 µm x 2.5-5.5 µm	Frequent
	2	10.5-15.5 µm x 2.5-5.5 µm	Rare
	3	12.0-25.0 µm x 2.5-5.0µm	Frequent
	4	25.0-45.0 µm x 3.0-4.5 µm	Very rare
Chlamydospores	-	5-10µm diameter	Very rare
Mycelium width	-	1.75-2.75µm	

The isolate (1, 4, 5 and 7) possessed bigger microconidia in the range of 3.75-9.00µm whereas other isolates have smaller ones in the range of 3.4-7.4µm. Chlamydospores were most frequent in isolate of 5, 4, 2, 1 while the other isolate showed lesser frequency (Plate 5,6 and 7).

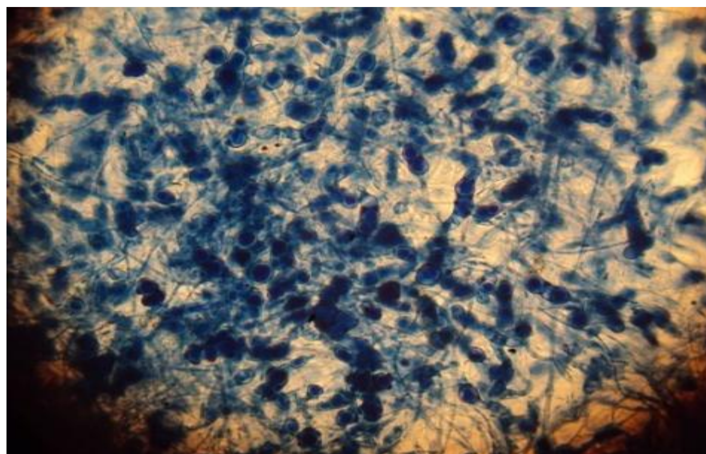

Plate 5 Isolate 1 Chlamydospore

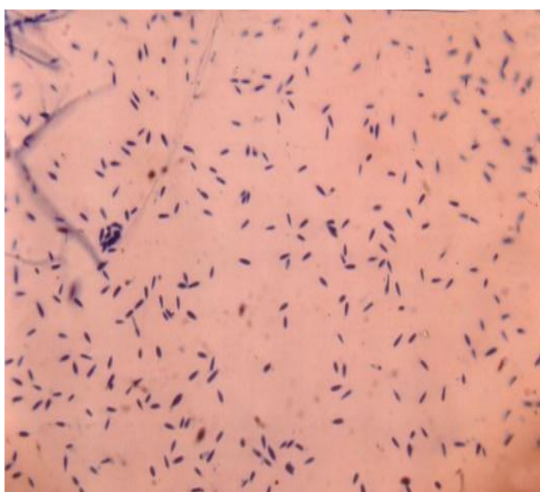

Plate 6 Isolate 3 Microconidia

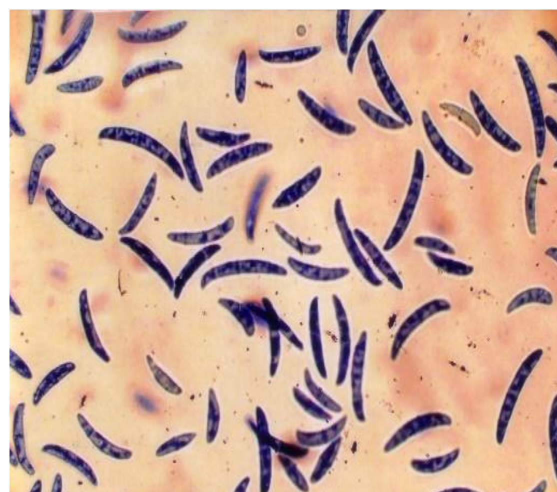

Plate 7 Isolate 6 Macroconidia

4.3.2 Radial growth rate and area enhancement of isolates of *Fusarium oxysporum* f. sp. *ciceri* on potato dextrose agar medium

The Petri dishes of potato dextrose agar medium were inoculated by Garett's disc method in the center with each isolate separately and incubated in B.O.D incubator at 25±1ºC according to methods described in chapter-3. The data are presented in Table 5, Fig 2 and Plate (8 and 9). The perusal of data indicated that isolate 1, 3 and 7 advanced at a better pace while isolate 2, 4, 5, 6 were slow in growth.

The rate of growth and advancement of colonies of other isolates were not different significantly. The colonies of all the isolates upto 48 hours were creeping at a slow rate then the isolate advanced at a better speed upto 120 hours. The resultant

diameter after 120 hrs of incubation in descending order was isolate 1, 3, 7, 6, 4, 2, 5. If grouped on the basis of growth as fast, medium and slow where the growth was above 7 cm in between. It was inferred that there existed no correlation between the morphological characters and the radial growth of the isolates.

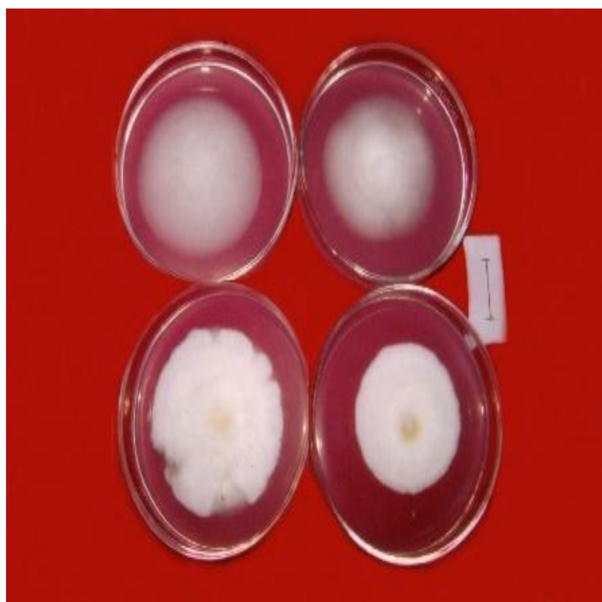

Plate 8 Radial growth of *Fusarium* isolates 1, 2, 3, 4 (from left to right on PDA)

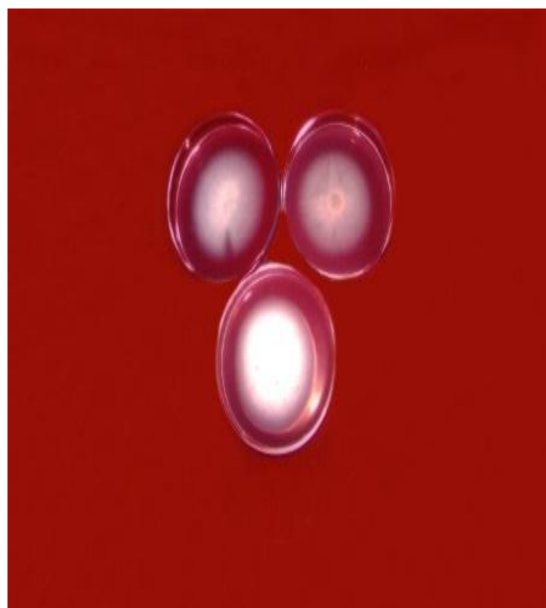

Plate 9: Radial growth of *Fusarium* isolates 5, 6, 7 (from left to right on PDA)

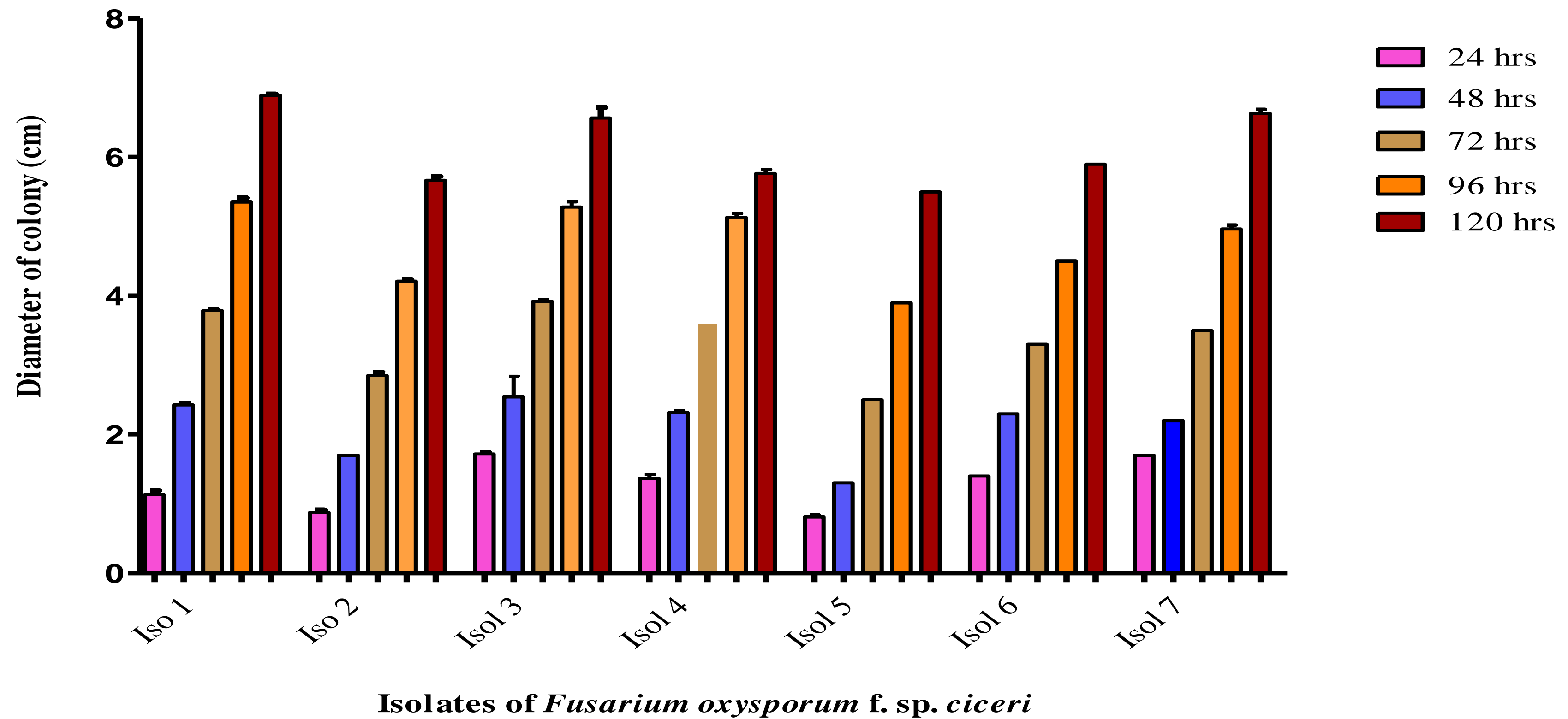

Fig 2 Radial growth of isolates of FOC on PDA after 120hrs

Table 5 Radial Growth (diameter in cm) and area (in sq. cm) of Isolates of *Fusarium oxysporum* f. sp. *ciceri* on potato dextrose agar medium

Isolate no	Radial Growth (in cm) and area of colony in sq. cm.														
	24 hrs			48 hrs			72hrs			96hrs			120 hrs		
	Dia (cm)	Area in sq cm	Growth rate	Dia (cm)	Area in sq cm	Growth rate	Dia (cm)	Area in sq cm	Growth rate	Dia (cm)	Area in sq cm	Growth rate	Dia (cm)	Area in sq cm	Growth rate
1	1.13±0.05	1.01±0.10	-	2.42±0.02	4.62±0.09	1.29±0.06	3.790.01	11.28±0.05	1.36±0.03	5.35±0.06	22.51±0.51	1.56±0.06	6.89±0.02	37.33±0.22	1.54±0.05
2	0.88±0.01	0.60±0.02	-	1.66±0.05	2.48±0.20	0.78±0.05	2.85±0.04	6.39±0.21	1.18±0.09	4.21±0.01	13.94±0.10	1.36±0.03	5.67±0.05	25.23±0.51	1.45±0.02
3	1.72±0.02	2.32±0.05	-	2.67±0.06	5.63±0.28	0.95±0.06	3.92±0.02	12.09±0.12	1.24±0.08	5.28±0.07	21.93±0.60	1.36±0.05	6.56±0.15	33.89±1.57	1.28±0.16
4	1.36±0.05	1.46±0.12	-	2.31±0.02	4.21±0.10	0.95±0.08	3.56±0.05	9.99±0.32	1.25±0.08	5.13±0.05	20.70±0.46	1.57±0.11	5.76±0.05	26.13±0.52	0.63±0.11
5	0.81±0.02	0.52±0.02	-	1.26±0.05	1.26±0.11	0.45±0.08	2.47±0.05	4.78±0.22	1.2±0.1	3.86±0.05	11.74±0.34	1.4±2.72	5.46±0.05	23.48±0.49	1.6±2.7
6	1.36±0.057	1.4±0.12	-	2.26±0.05	4.03±0.20	0.9±0.1	3.26±0.05	8.3±0.29	1±0.1	4.5±0.05	16.14±0.41	1.26±0.05	5.86±0.05	27.04±0.53	1.3±0.11
7	1.6 ±0.05	2.18±0.14	-	2.16±0.05	3.69±0.19	0.5 ±1.36	3.46±0.05	9.4±0.31	1.3±0±00	4.96±0.05	19.38±0.44	1.53±1.4	6.63±0.05	34.57±0.60	1.6±0.11

Values shows are the mean ± SD of three replicates

4.4 Pathogenicity test

The pathogenicity test was conducted on the known highly susceptible variety JG62. Identification of pathogenic cultures was confirmed by comparing the characters of cultures maintained at IIPR Kanpur and Plant pathology lab, Department of Botany, D.A-V College Kanpur. The monoconidial isolates of *F. oxysporum* f. sp. *ciceri* were classified in seven groups on the basis of discernible characters such as colour of mycelium, colour of substratum, radial growth, sporulation and frequency of various types of spore on a single medium *i.e.* Potato dextrose agar.

The seven isolates differing in cultural and morphological variety were tested against known variety of JG 62 as suggested by Haware and Nene (1982) and Phillips (1988). The isolates were multiplied on soil maize meal medium and wilt infested pots were prepared as per the method described in chapter-3. Surface sterilized seeds of JG 62 @ five seeds per pot and ten pots were used for each isolate. The reactions were recorded for fifty seeds sown in wilt infested pots for each isolate. The pathogenic nature of isolate were presented as per the scale of Trivedi and Gurha (2007) based on the mortality percentage of chickpea seedlings on universal susceptible variety JG 62.

Highly pathogenic = above 80% mortality,

Moderately pathogenic = 50-79 %

Weakly pathogenic ≤ 50% mortality.

The results presented in Table 6 indicates isolate 3 as highly pathogenic and isolate 7 as weakly pathogenic while the rest were moderately pathogenic.

Table 6 Percentage mortality of Chickpea in *Fusarium* infested pots @ five seeds per pot

S. no.	*Fusarium oxysporum* f. sp. *ciceri*	Total no of seed sown in ten pots	Seedlings emerged	No. of plants wilted	Percentage mortality of seedlings
1	ISO-1	50	47	31	65.95
2	ISO-2	50	45	26	57.77
3	ISO-3	50	47	41	87.23
4	ISO-4	50	48	32	66.66
5	ISO-5	50	49	37	75.51
6	ISO-6	50	48	35	72.91
7	ISO-7	50	48	22	45.83

4.4(a) Symptoms caused by FOC on chickpea plants under *in vivo* condition

The seeds of susceptible chickpea variety JG 62 were surface sterilized and sown in wilt infested pots as per the methods described in Chapter 3. The drooping of seedlings started from upper part of the plants. Later the petioles and rachis along with leaflets drooped resulting in collapse of entire plant. The leaves become chlorotic, turned yellow then straw coloured or light brown. In the infected plants there was no rotting, drying or discoloration of roots. The affected plants suffered from retarded growth and ultimately the seedling died. The disease in pot cultures generally appeared after 20 days of sowing. The collapsed seedlings when uprooted, usually exhibited uneven shrinking of the stem both above and below the collar region. When the stems of the infected seedlings were splitted vertically from the collar region downwards, brown black discoloration of internal tissues was conserved. The seedling when dried at an early age, only browning of the internal tissues was observed.

4.4(b) Nature of wilt

The infected plants from infested pots were put to isolations from root, collar, stem leaves and apices. The root and collar region yielded the culture of *Fusarium oxysporum* f. sp. *ciceri* in all the isolations. However, the isolations recovered the pathogen from the stem and apices were frequent to the level of 80 percent. Leaves yielded the cultures of the wilt pathogen rarely. If the wilted plants were set aside for 4-5days then the leaves also yielded *F. oxysporum* f. sp. *ciceri* upto 20 percent indicating travelling mycelium in conducting tissues even after the death of the plants. These observations confirms the systemic nature of the disease.

4.5 Histopathology of chickpea plants infected with FOC

The longitudinal sections of roots infected with *Fusarium oxysporum* f. sp. *ciceri* showed presence of fungal mycelium in the tracheids and vessels of xylem tissue. In a few cases chlamydospores were found present but tyloses were rare. The clogging of vessels by fungal mycelium and tannin in the tracheids was frequent but no macro or microconidia were observed in the sections (Plate 10 and 11)

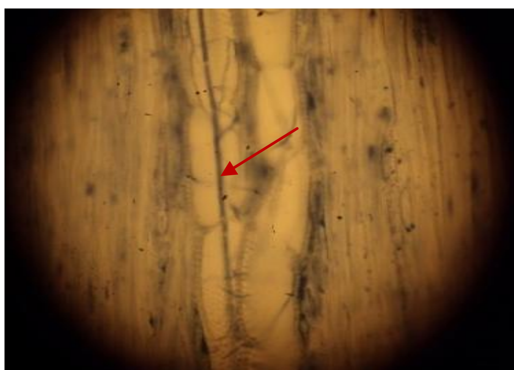

Plate 10

Plate 11

Plate (10, 11) LS of chickpea root infected by *Fusarium oxysporum* f. sp. *ciceri* showing fungal mycelium in the vessels and tracheids of xylem tissues.

4.6. Abiotic factors influencing the disease

Knowledge of optimum soil condition, temperature and availability of pathogenic inoculum for cent per cent wilting of susceptible plants is a prerequisite in order to have a greater insight about the various abiotic factors affecting the wilt disease and correspondingly devise better management practices. Hence all the isolates of *Fusarium oxysporum* f. sp. *ciceri* were multiplied separately on soil maize meal medium (Miller, 1946). Then all the isolates of *Fusarium oxysporum* f. sp. *ciceri* were mixed in equal quantities (w/w) for preparing the pots as described earlier in Chapter 3. The results are presented subsequently.

4.6(a) Effect of soil composition on wilting

Addition of sand in normal sandy loam soil of Kanpur in different proportions changed the composition of soil which resulted in differences in the time lag of wilting. Pure soil sand and mixtures of this soil with sand to the levels of 5, 15, 25, and 35 per cent were taken. Addition of sand in higher proportion shortened the time required to wilt. However, 25 and 35 per cent sand mixture seemed equally conducive for the wilt disease incited by *Fusarium oxysporum* f. sp. *ciceri* therefore, in further experiment, (25%) sand with the same soil was taken as standard test medium. The changes in various soil factors were calculated. The apparent density as well as absolute specific gravity increased with addition of sand in soil whereas water holding capacity per cent pore space and volume of expansion of 100 gm soil showed gradual decrease. Of these factors (31.4±1%) water holding capacity and (43.5%) pore space was found to be favorable for causing wilt (Table 6).

Table 7 Effect of soil composition on days to cent per cent wilting of plants with levels of equal inoculum of all mixtures of all isolates of *Fusarium oxysporum* f. sp. *ciceri*

S. no	Composition of pot soil	Apparent density	Absolute specific gravity	Water holding capacity	Per cent pore space	Volume expansion of 100gm soil	Days taken to per cent wilting
1.	Pure soil (sieved) 100%	1.24	2.08	36.3	45	2.66	40
2.	Pure soil + sand (sieved) 5%	1.3	2.15	35.0	44.7	2.4	31
3.	Pure soil + sand (sieved) 15%	1.36	2.26	33.1	44.1	2.0	27
4.	Pure soil + sand (sieved) 25%	1.42	2.33	31.4	43.5	1.7	20
5.	Pure soil + sand (sieved) 35%	1.47	2.4	28.9	43	1.52	19

4.6(b) Effect of depth of inoculum on wilting

The placement of inoculum at different depths in the pots indicated that:

(i) The availability of inoculum just after seed germination was highly injurious to seedlings causing wilt within twenty days of sowing.

(ii) The delayed contact of roots with the inoculum, wilt symptoms was considerably delayed.

(iii) Inoculum at the base of pot (35 mm depth) and at the middle (65 mm depth) has less significant difference on time lag to create wilt symptoms (Table 8, Fig 3). The data portends that immediate contact of inoculum to germinating seeds was the best suited for quick pathological screening.

Table 8 Time (in days) taken for wilting in chickpea variety (JG 62) in relation to placement of equal level of inoculum (mixture of all the isolates) of FOC at different depths

S. no.	Depth of inoculum in 100mm size pot	Time taken for cent per cent wilting of JG 62 seeds (DAS) by *F. oxysporum* f. sp. *ciceri*
1.	Entire pot filled with inoculum i.e. inoculum upto surface of the pot	18.3
2.	90 mm from base of the pot	30
3.	65 mm from base of the pot	43
4.	35 mm from base of the pot	47.66

Values shown are the average of three replications

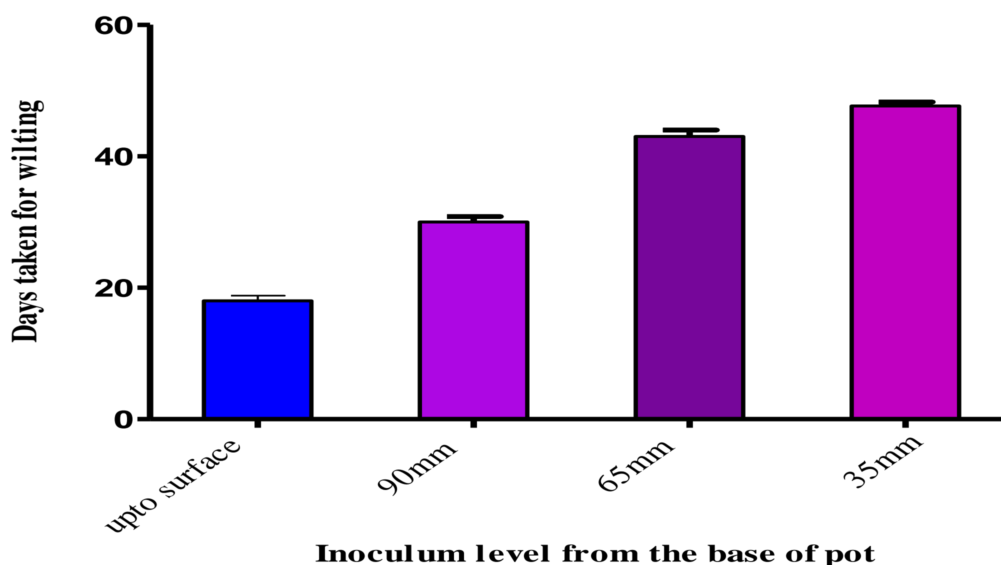

Fig 3 Time in days taken for cent per cent wilting in chickpea variety JG 62 in relation to placement of equal level of inoculum (mixtures of all isolates) of FOC

4.6(c) Effect of temperature on wilting

The temperature ranges of 18-17ºC and 21-28ºC were found to influence the wilt phenomenon significantly. Low temperature delayed the wilt development. However, about twenty days from the date of emergence of seedling were significant to wilt the plants at higher ranges of temperature. Within the varieties tested *viz.,* JG 62, Kanpur

local (desi) and Ujjain local no significant difference was observed in time taken to wilting, Table 9.

Table 9 Time (in days) taken for wilting in chickpea due to equal levels of inoculum of *Fusarium oxysporum* f. sp. *ciceri* at different ranges of temperature

S. no.	Varieties	Time taken for wilting (in days)									
		Temperature ranges 8-17ºC					Temperature range 21-28 ºC				
		Seeds sown per pot	Total seeds in 5 pots	Seedlings emerged	No of wilted plants	Days taken to wilting	Seeds sown per pot	Total seeds in 5 pots	Seedlings emerged	No of wilted plants	Days taken to wilting
1.	JG 62	10	50	48	46	29	10	50	47	45	19
2.	Kanpur local	10	50	45	44	30	10	50	48	48	19
3.	Ujjain local	10	50	48	48	27	10	50	49	47	20

4.6(d) Role of different crop debris in multiplication of primary inoculum of *F. oxysporum* f. sp. *ciceri*

Generally the wilt pathogen of chickpea survives in the soil and crop debris. The nutrients available in the crop debris are sufficient enough for multiplication of pathogen. To find out the precise reaction of these debris in growth and sporulation of FOC, broth media were prepared and the pathogen was multiplied as per the method mentioned in chapter-3. PDA broth was also prepared and inoculated at the same time to compare the result of debris broth medium with it. The results have been presented in Table 10.

The results indicated that urid straw was the best suited for the growth as it yielded maximum dry weight (1060 mg) as compared to linseed straw which produced minimum mycelium (359 mg). If arranged in ascending order of dry mycelium weight produced by the different debris straw it would be linseed<pigeonpea<chickpea<wheat straw<and urid straw. The biomass produced by Potato dextrose broth was next to urid straw debris as it was 940 mg. The production of micro and macroconidia was maximum in PDA broth medium with per ml count of

$8.5x10^3$ and $5.6x10^3$ respectively though chickpea, urid and wheat straw yielded $1.5x10^2$, $1.6x10^2$ and $1.4x10^2$ macroconidia per ml respectively but microconidia were minimum in chickpea and wheat straw with the per ml count of $5x10^2$ and $9x10^2$ only. The pH in all the broth medium shifted to alkalinity after the growth of the fungus *Fusarium oxysporum* f. sp. *ciceri*.

Table 10 Growth and sporulation of FOC (iso1) on different Crop Debris broth and its comparison with potato dextrose broth

S. No	Name of media	Dry wt. of mycelium (mg)	pH of medium		Microconidia Count/ml	Macroconidia Count/ml
			Initial	Final		
1	PDB	940	6.0	6.5	$8.5x10^3$	$5.6 x10^3$
2	Wheat straw	648	6.4	9	$9 x10^2$	$1.4 x10^2$
3	Linseed	359	6.2	9	$5x10^3$	$9.6 x10^2$
4	Urid	1040	6.5	9	$6.6x10^3$	$1.6 x10^2$
5	Chickpea	580	6.1	8	$5 x10^2$	$1.5 x10^2$
6	Pigeon pea	500	6.5	8.5	$1.2 x10^2$	$5.2 x10^2$

4.7 Production of cellulolytic and pectinolytic enzymes by *Fusarium oxysporum* f. sp. *ciceri*

It was observed that *Fusarium oxysporum* f. sp. *ciceri* (iso1) produced cellulolytic and pectinolytic throughout the incubation period of 7, 14 and 21 days. Maximum activity of enzymes was observed on the 14th day of incubation. Polygalacturonase (PG) registered 74.46% loss in viscosity at 90 min interval on 14th day while Cellulase recorded 79.09% viscosity loss at the same time interval.

The perusal of data in the given Table 10 clearly suggest that enzyme activity continued to increase with the age of the culture recording 25, 31.75, 46.01 and 54.79 percent loss in viscosity by the Polygalacturonase (PG) enzyme and 48.09, 60.62, 65.35 and 70.2 percent by Cellulase after 30, 60, 90 and 120 min time intervals on the

7th day respectively. On the 14th day of incubation the enzymatic activity of the PG enzyme at 30, 60, 90, and 120 min was 57.61, 63.67, 74.76, 68.69 percent while cellulase recorded 59.11, 66.55, 79.09, 69.10 percent loss at the same time interval. On 21st day of the incubation, the loss in viscosity by PG after 30 min was 48.35% at 60 min, 58.24%, at 90 min, 61.54% and at 120 min 65.94%; and with cellulase it was 40.15%, 42.38%, 56.50% and 54.28% respectively. It is very clearly evident that after 14 days the enzymatic activity declined. Both the enzymes showed maximum production after an interval of 90 minutes. The results also states that the pathogen *Fusarium oxysporum* f. sp. *ciceri* produced more cellulase (Cx) enzyme as compared to PG enzyme after same days of incubation and time intervals.

Table 11 Assay of Cellulase and PG enzyme production by FOC iso1 (by viscometric method)

Percent Viscosity loss							
S. No.	**Enzyme**	**Incubation in days**	**Time interval in minutes**				
			00	**30**	**60**	**90**	**120**
1.	**PG**	**7**	00	25	31.75	46.01	54.79
		14	00	57.61	63.67	74.76	68.69
		21	00	48.35	58.24	61.54	65.94
2.	**Cellulase**	**7**	00	48.09	60.62	65.35	70.20
		14	00	59.11	66.55	79.09	69.10
		21	00	40.15	42.38	56.50	54.28

4.8 Disease Management

4.8(a) Efficacy of antagonist *in vitro*

Among the ecofriendly methods of control the antagonistic fungi play an important role to keep the pathogenic fungi *Fusarium* at bay, thus protecting the crop plants. Therefore, experiments were designed to find suitable control of wilt through antagonists *viz., Trichoderma harzianum, T. viride* and *Aspergillus niger in vitro* and in pot cultures following the methods described in chapter 3. The efficacy of antagonist fungi *viz., T. harzianum, T. viride* and *A. niger* were worked out according

to dual culture technique of Johnson and Curl (1972) and Tu (1980). In the dual culture the growth of the two fungi *i.e.,* the isolates of *Fusarium oxysporum* f. sp. *ciceri* (isolate 1 to 7) and the species of *Trichoderma* or *Aspergillus* was observed in terms of their interaction and the per cent inhibition of isolate of *F. oxysporum* f. sp. *ciceri* using the formula of Kaushik and Arora (2002):

Percent inhibition = {(C-T)/C} x 100

Where C and T are the growth in mm in control and treatments respectively as described in chapter 3 “Materials and Methods”. The perusal of data in Table 12, Plates (12-17) and Fig 4 revealed that the growth of all the isolate 1 to 7 of *Fusarium oxysporum* f. sp. *ciceri* was inhibited considerably by the antagonistic fungi *viz.*, *T. harzianum, T. viride* and *A. niger*. The interaction pattern between the isolates of wilt pathogen and *T. harzianum* was either of D or E type. It was D type (with isolate 1, 2, 3, 5, 7) meaning growth of antagonist *i.e., T. harzianum* continuing after coming in contact with *F. oxysporum* f. sp. *ciceri*. Whereas with the other isolates (4, 6) it was E type, *i.e.* inhibition at a distance and the antagonist continued its growth resulting in a clear zone either at unchanged or reduced rate. The inhibition % ranged from 20.11(isolate 7) to 65.78 (isolate 6) while it was 36.15, 56.64, 61.76, 52.39, 55.65 percent, with isolate 1, 2, 3, 4, and 5 respectively.

The percentage reduction in the colony growth of FOC isolates with *T. viride* ranged from 36.16 (isolate 4) to 63.30 (isolate 5). It recorded an inhibition per cent of 56.26, 59.76, 53.40, 58.79 and 48.09 with isolate 1, 2, 3, 6 and 7 respectively while the interaction pattern was of C, D, and E type *i.e.* (i) mutual inhibition at a distance (ii) the antagonist continues to grow after coming in contact with other organism (iii) inhibition at a distance and antagonist continued to grow resulting in a clear zone at

an unchanged or reduced rate respectively. It was E type with isolate 2 and D type with isolate 1, 3, 4, 7 while C type with isolate 6 and 5.

The interaction pattern between the wilt pathogen *F. oxy.* f. sp. *ciceri* isolates and *A. niger* revealed minimum inhibition of the former if compared with species of *T. harzianum* and *T. viride.* The interaction pattern was of B, C and D type. Isolates 2, 3, 6, 7 showed B type of interaction where mutual inhibition of fungal colonies took place when they came in contact with each other. They exhibited 23.33, 17.95, 25.55 and 14.16 percent inhibition respectively while isolate 1 and 5 exhibited C type of interaction pattern and percentage inhibition was 47.22% and 40.28% respectively. Isolate 4 exhibited D type of interaction with 21.29 % inhibition. Isolate 3 was the least controlled by *A. niger* whereas for isolate 1, radial colony growth was checked upto 47.22 percent. So there was no correlation in the type of interaction and the percent inhibition of the pathogen. It was inferred if either the antagonist or the pathogen were in close vicinity of root system the other one will not reach it, so if the wilt pathogen was on the rhizoplane of chickpea it would infect the susceptible variety irrespective of the presence of antagonistic species, that was the possible reason surmised for the ineffectiveness of antagonistic species (like *Trichoderma* or others) in certain cases.

Table 12 Inhibition of radial growth of different isolates of *Fusarium oxysporum* f. sp. *ciceri* due to *T. harzianum, T. viride, A. niger* and their respective interaction pattern according to Johnson and Curl 1972

S. No.	Isolate /Antagonist	Radial growth of isolates on 7th Day in mm(in presence of the antagonist)	Percent inhibition	Type of interaction
1	Isolate 1(Control)	57.16±0.62	0.00	
	Isolate 1+*T.harzianum*	37.16±1.31	36.15±0.87	D
	Isolate 1+*T.viride*	25.0±0.81	56.26±1.74	D
	Isolate 1+*A. niger*	30.16±0.84	47.22±1.82	C
2	Isolate 2(Control)	57.83±0.62	0.00	
	Isolate 2+*T.harzianum*	25.07±0.49	56.64±1.03	D
	Isolate 2+*T.viride*	23.27±0.60	59.76±1.27	E
	Isolate 2+*A. niger*	44.33±0.57	23.33±1.21	B
3	Isolate 3(Control)	61.26±0.91	0.00	
	Isolate 3+*T.harzianum*	23.42±0.46	61.76±0.91	D
	Isolate 3+*T.viride*	28.55±0.48	53.40±0.96	D
	Isolate 3+*A. niger*	50.26±0.52	17.95±1.04	B
4	Isolate 4(Control)	49.0±0.81	0.00	
	Isolate 4+*T.harzianum*	22.62±0.55	52.39±1.37	E
	Isolate 4+*T.viride*	31.25±0.65	36.16±1.64	D
	Isolate 4+*A. niger*	38.56±0.49	21.29±1.23	D
5	Isolate 5(Control)	59.5±1.08	0.00	
	Isolate 5+*T.harzianum*	26.38±0.46	55.65±0.90	D
	Isolate 5+*T.viride*	21.83±0.62	63.30±1.28	C
	Isolate 5+*A. niger*	35.53±0.55	40.28±1.13	C
6	Isolate 6(Control)	54.48±0.40	0.00	
	Isolate 6+*T.harzianum*	18.64±0.26	65.78±0.61	E
	Isolate 6+*T.viride*	22.45±0.72	58.79±1.63	C
	Isolate 6+*A. niger*	40.55±0.60	25.55±1.37	B
7	Isolate 7(Control)	64.5±0.41	0.00	
	Isolate 7+*T.harzianum*	51.52±0.46	20.11±0.87	D
	Isolate 7+*T.viride*	34.48±0.61	48.09±1.17	D
	Isolate 7+*A. niger*	55.36±0.76	14.16±1.44	B

Values shown are the mean ± SD of three replicates, significant at ($p<0.05$)

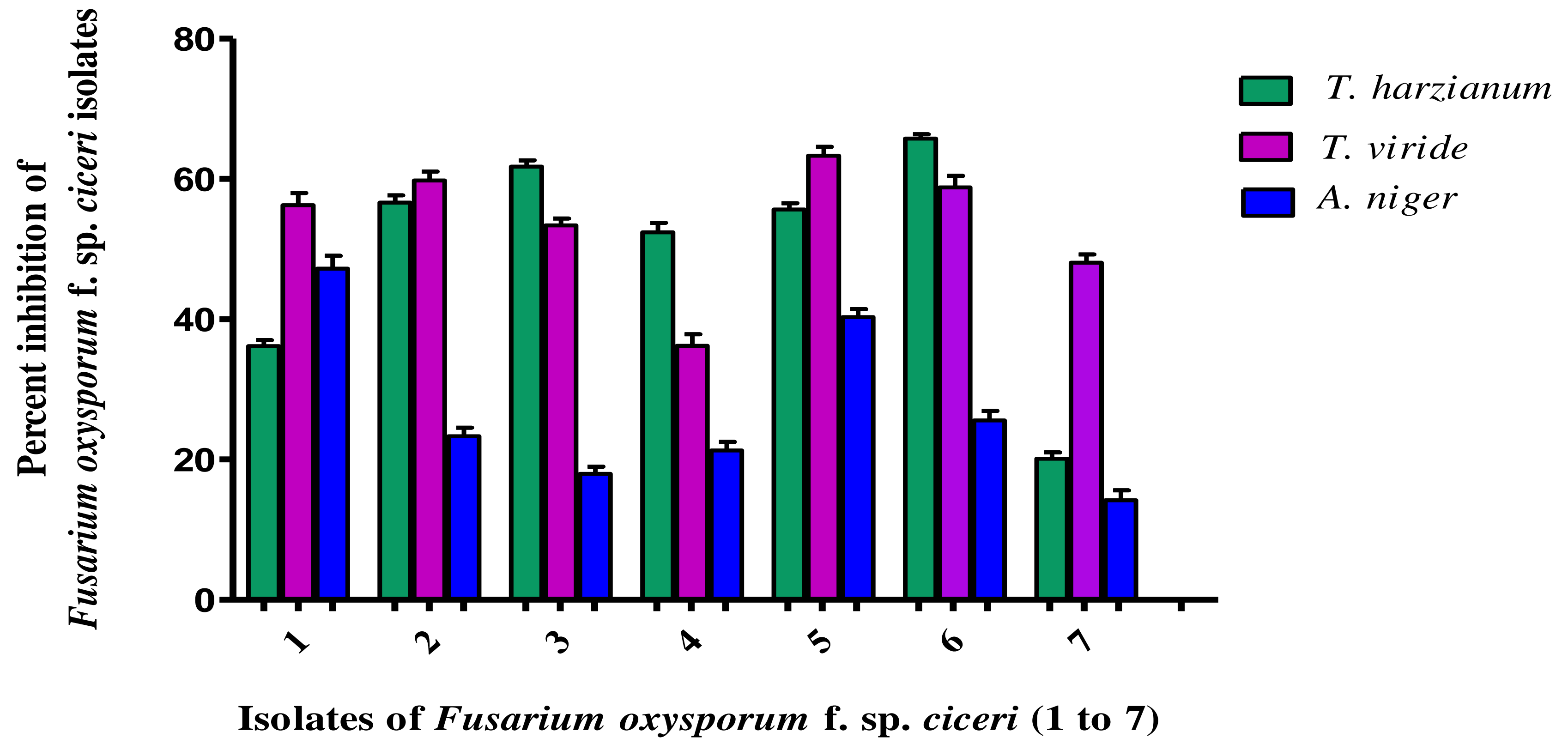

Fig 4 Percent inhibition of FOC isolates by *Trichoderma* spp. and *A. niger* on 7th day of inoculation

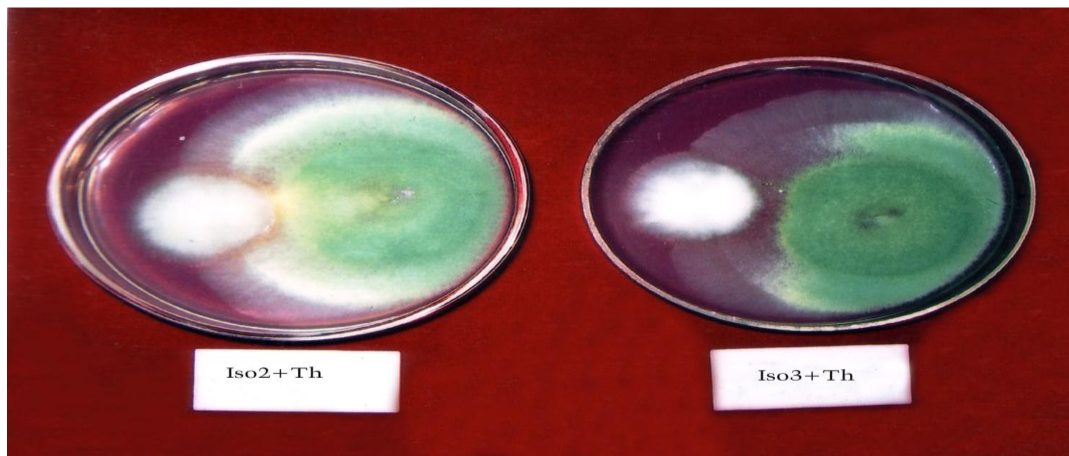

Plate 12

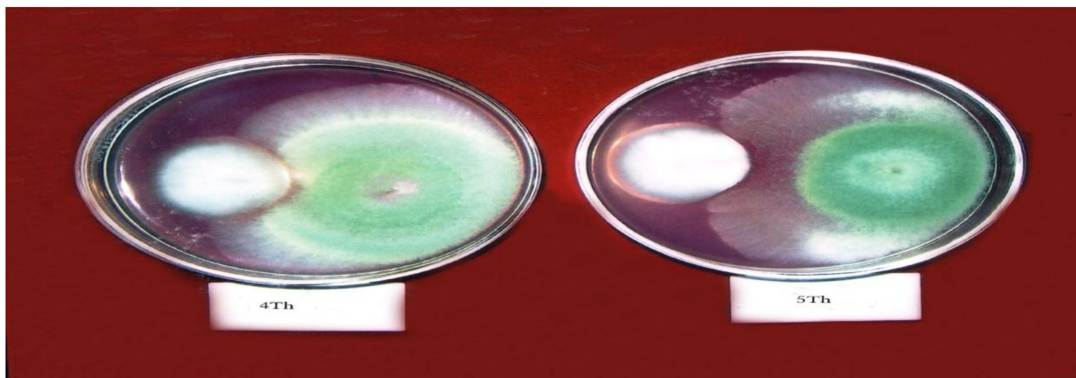

Plate 13

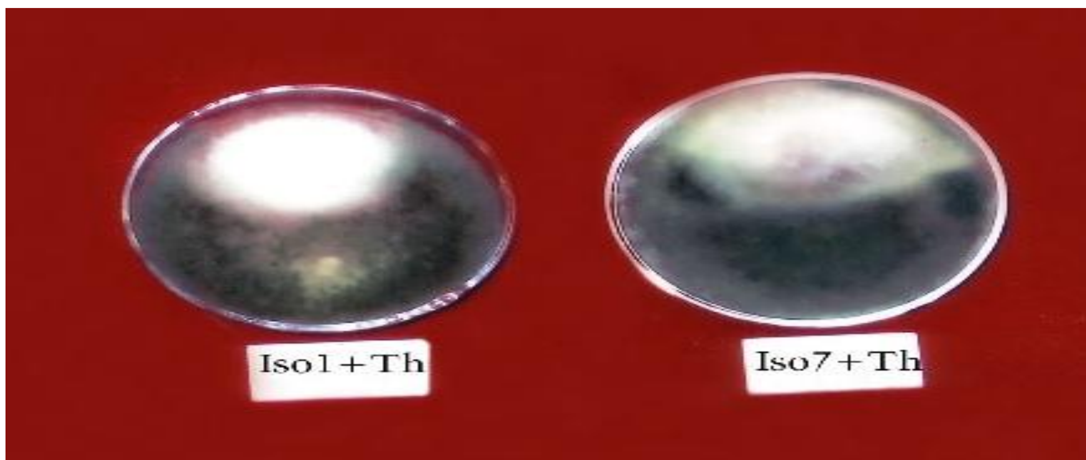

Plate 14

(Cont…….)

Plate 15

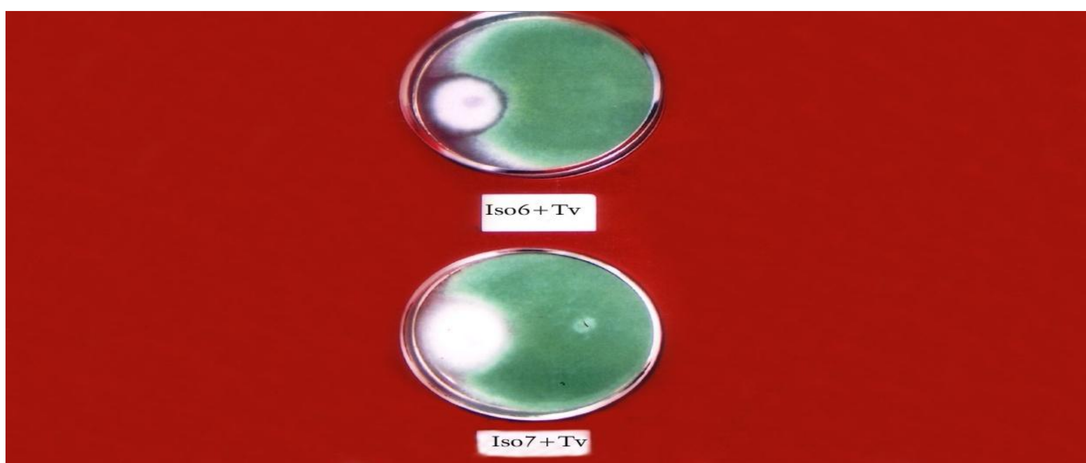

Plate 16

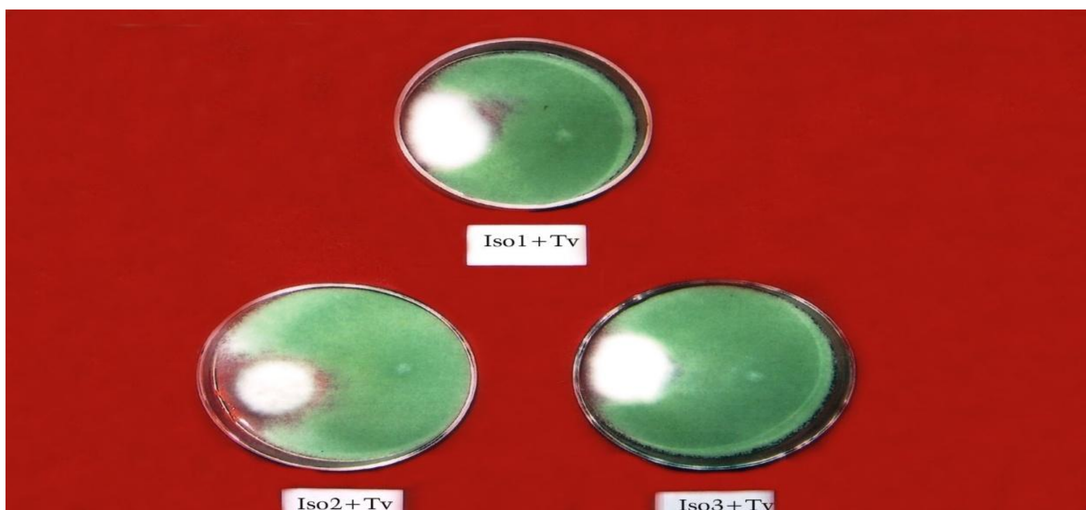

Plate 17

Plate (12-15) Interaction of *Fusarium* isolates with *T. harzianum* and Plate (16-17) Interaction of *Fusarium* isolates with *T. viride*

4.8(b) Efficacy of antagonists *in vivo*

The effectiveness of antagonists *viz., T. harzianum T. viride* and *A. niger* in different concentrations were tested against three highly susceptible varieties JG 62, Kanpur Local and Ujjain Local in pot cultures as described in chapter 3.The results were presented in Table 13.

The data indicates that all the three antagonist *viz.*, *T. harzianum, T. viride*, and *A. niger* were effective at all levels of inoculum percentage against the three susceptible varieties JG 62, Kanpur local, and Ujjain local in pot cultures as described in chapter 3. *T. harzianum* at 2, 4, 6, 8 percent controlled wilt as its incidence was reduced to 44, 18.6, 6.52, and 0.00 in JG 62. Varieties, Kanpur and Local and Ujjain Local responded to the antagonist more quickly as there were no incidence of wilt at 6 percent level of application of the antagonist. The same varieties with the same sequence of concentrations of *T. viride* were tested in infested pots. The results revealed 48.92, 38.29, 17.39 and 0.00 per cent reduction in wilt incidence at 2, 4, 6, 8 per cent of antagonist respectively.

A. niger at same inoculum percentage checked the wilt incidence by 45.8, 19.1, 16.3, 6.25 per cent respectively. The efficacy of *A. niger* was lesser as it required 10.00 per cent inoculum of *A. niger* (w/w) to check the wilt incidence completely. Though *T. harzianum* was the best antagonist but *T. viride* or a combination of the above described bioagents could be used to effectively check the wilt incidence.

Table 13 Effect of different concentration of *Trichoderma* spp. and *A. niger* on incidence of wilt of chickpea caused by the mixture of equal amount (w/w) of all the 7 isolates of *F. oxysporum* f. sp. *ciceri* in pot cultures

S. No	Different concentrations of the antagonists with (5%) mixed inoculum of *F. oxysporum* f. sp. *ciceri*		No. of seeds sown @5 seeds per pot	No. of seedlings emerged	No. of plants wilted	Percent incidence of wilt
1	*T. harzianum* 2%	JG62	50	50	20	44
		Kanpur Local	50	46	17	36.9
		Ujjain Local	50	45	15	33.3
2	*T. harzianum* 4%	JG62	50	48	9	18.6
		Kanpur Local	50	47	16	34.04
		Ujjain Local	50	49	13	26.5
3	*T. harzianum* 6%	JG62	50	46	3	6.52
		Kanpur Local	50	49	0	0
		Ujjain Local	50	48	0	0
4	*T. harzianum* 8%	JG62	50	50	0	0
		Kanpur Local	50	47	0	0
		Ujjain Local	50	48	0	0
5	Control	JG62	50	50	50	100
		Kanpur Local	50	48	48	100
		Ujjain Local	50	49	49	100
6	*T. viride* 2%	JG62	50	49	24	48.97
		Kanpur Local	50	47	21	44.68
		Ujjain Local	50	48	19	39.58
7	*T. viride* 4%	JG62	50	47	18	38.29
		Kanpur Local	50	48	16	33.33
		Ujjain Local	50	49	13	26.5
9	*T. viride* 8%	JG62	50	50	0	0
		Kanpur Local	50	49	0	0
		Ujjain Local	50	49	0	0
10	Control	JG62	50	49	49	100
		Kanpur Local	50	48	48	100
		Ujjain Local	50	46	46	100

11	*A. niger* 2%	JG62	50	48	22	45.8
		Kanpur Local	50	45	16	35.5
		Ujjain Local	50	47	18	38.29
12	*A. niger* 4%	JG62	50	47	9	19.1
		Kanpur Local	50	48	14	29.1
		Ujjain Local	50	49	16	32.6
13	*A. niger* 6%	JG62	50	49	8	16.3
		Kanpur Local	50	48	11	22.9
		Ujjain Local	50	45	10	22.2
14	*A. niger* 8%	JG62	50	48	3	6.25
		Kanpur Local	50	47	2	4.25
		Ujjain Local	50	46	1	2.17
15	*A. niger* 10%	JG62	50	48	0	0
		Kanpur Local	50	47	0	0
		Ujjain Local	50	46	0	0
16	Control	JG62	50	49	49	100
		Kanpur Local	50	46	46	100
		Ujjain Local	50	48	48	100

4.9 Control through Botanicals (cold water and alcoholic extract)

Cold water and alcoholic extracts were prepared separately from 11 angiospermic plants species including herbs, shrubs and trees. The data are presented in Table (14,15) Fig 5. From the perusal of data it could be easily envisaged that:

(i) In all the treatments efficacy of alcoholic extracts in inhibiting the pathogen was more as compared to water extracts,

(ii) as the percentage of concentration of the phytoextracts was increased the radial colony growth of the pathogen decreased, that was somewhat in proportionate manner.

(iii) There were three plant species whose extract exclusively inhibited the growth of *F. oxyrsporum* f. sp. *ciceri* these are *Aegle marmelos Azadirachta indica jus.*and *Callistemon lanceolatus.*

(iv) *Syzygium cumini* was the least effective among the plant varieties tested

(v) The efficacy of *Pongamia pinnata* in inhibiting the growth of *Fusarium oxysporum* f. sp. *ciceri* was a new record.

4.9(a) Alcoholic extracts

The data in Table 15, Fig 5 portends that at 500 ppm *Callistemon lanceolatus* checked the radial colony growth of the pathogen by 97.55 per cent, *Azadirachta indica* (96.08 %), *Aegle marmelos* (94.62 %), Acacia *nilotica* (80.30 %), *Zizyphus jujuba* (70.62 %), *Polyalthia suberosa* (58.89 %), *Tamarindus indicus* (61.68 %), *Pongamia pinnata* (62.31%), *Terminalia arjuna* (58.79 %), *Cassia fistula* (47.4 %) and *Polyalthia suberosa* by 58.89 % percent (significant at $P<0.05$).

At 200 ppm there were four plant species which checked the growth of the pathogen more than 40 percent, they were *Callistemon lanceolatus* (58.49%), *Azadirachta indica* (60.43 %), *Aegle marmelos* (58.49 %) and *Acacia nilotica* (46.47 %). Alcoholic extracts of *Callistemon lanceolatus* showed 50.69 percent reduction.

At 100 ppm. *Azadirachta indica* checked growth by 47.16 %, *Aegle marmelos* (44.98 %), *Acacia nilotica* (31.62 %), *Zizyphus jujuba* (30.88 %), *Polyalthia suberosa* (34.15 %), *Tamarindus indicus* (28.54 %), *Pongamia pinnata* (24.17 %), *Terminalia arjuna* (24.87 %) and *Cassia fistula* (21.64 %).

4.9(b) Water extracts

It was observed from the data in Table 14, Fig 5 that water extracts were able to check the growth of the fungus significantly at higher concentration (500 ppm). *Callistemon lanceolatus* checked the growth of the pathogen by 92.20 per cent. *Azadirachta indica* (79.54%), *Aegle marmelos* (83.25%), *Acacia nilotica* (70.20%). *Zizyphus jujuba* (57.26%), *Polyalthia suberosa* (40.89%), *Tamarindus indicus* (45.51%), *Pongamia pinnata* (39.35%) and *Terminalia arjuna* (37.91%) percent (significant at $P<0.05$).

However, at lower concentrations the reduction in the colony growth was not very effective.

At 100 ppm concentration water extract of *Syzygium cumini* showed 11.94 percent reduction in the radial colony growth while *Callistemon lanceolatus* showed 40.36 per cent reduction followed by *Azadirachta indica* (37.46%), *Aegle marmelos* (35.69%), *Acacia nilotica* (28.31%), *Zizyphus jujuba* (24.68%), *Polyalthia suberosa* (22.76%), *Tamarindus indicus* (21.62%), *Pongamia pinnata* (18.78%), *Terminalia arjuna* (18.48%) and *Cassia fistula* (15.58%) (significant at $P < 0.05$).

Table 14 Efficacy of water extracts of certain plants against *Fusarium oxysporum* f. sp. *ciceri* (iso1) at different concentrations

S. no	Name of plants	Percent reduction in the colony growth of the pathogen at different concentration of water extract					
		100ppm		200ppm		500ppm	
		Radial growth (mm)	Percentage Inhibition	Radial growth (mm)	Percentage Inhibition	Radial growth (mm)	Percentage Inhibition
1	*Acacia nilotica L*	48.6±1.21	28.31±1.79	42.76±1.36	37.78±1.98	20.63±0.55	70.20±0.79
2	*Aegle marmelos L*	43.6±1.15	35.69±1.70	35.66±1.17	48.11±1.70	11.6±0.52	83.25±0.74
3	*Azadirachta indica*	42.4±1.24	37.46±1.84	31.06±1.00	54.80±1.46	14.16±1.05	79.54±1.53
4	*Callistemon lanceolatus*	40.43±0.75	40.36±1.11	29.06±0.90	57.71±1.31	5.4±0.52	92.20±0.76
5	*Cassia fistula L.*	57.2±1.20	15.58±1.77	53.06±1.00	22.80±1.46	45.0±1.00	35.02±1.44
6	*Polyalthia Suberosa*	52.36±0.55	22.76±0.81	48.1±0.9	30.02±1.30	40.93±0.90	40.89±1.30
7	*Pongamia pinnata*	55.06±0.90	18.78±1.33	49.06±1.10	28.61±1.60	42.00±1.00	39.35±1.44
8	*Syzygium cumini L.*	59.7±0.60	11.94±0.89	56.1±1.01	18.38±1.47	49.0±1.00	29.25±1.44
9	*Tamarindus Indica*	53.13±1.02	21.62±1.51	50.1±1.01	27.11±1.47	37.73±0.64	45.51±0.92
10	*Terminalia arjuna*	55.2±0.64	18.48±0.94	51.76±0.68	24.69±0.99	43.00±1.00	37.91±1.44
11	*Zizyphus jujuba*	51.06±1.00	24.68±1.48	44.06±1.10	35.89±1.60	29.6±1.21	57.26±1.75
	Control	67.8±1.31		68.74±1.09		69.26±0.64	

Values shown are the mean ± SD of three replicates, significant at ($p<0.05$)

Table 15 Efficacy of alcoholic extracts of certain plants against *Fusarium oxysporum* f. sp. *ciceri* (iso1) at different concentrations

S. No.	Name of plant	Alcoholic Extract					
		100ppm		200ppm		500ppm	
		Radial growth (mm)	Percent Inhibition	Radial growth (mm)	Percent Inhibition	Radial growth (mm)	Percent Inhibition
1	*Acacia nilotica*	45.9±0.65	31.62±0.97	36.66±0.57	46.47±0.84	13.43±1.40	80.30±2.05
2	*Aegle marmelos*	36.9±0.70	44.98±1.04	28.43±0.75	58.49±1.09	3.66±0.57	94.62±0.84
3	*Azadirachta indica*	35.16±1.15	47.61±1.72	27.1±0.85	60.43±1.24	2.67±0.57	96.08±0.84
4	*Callistemon lanceolatus*	33.1±1.15	50.69±1.71	25.26±1.10	63.11±1.60	1.66±0.6	97.55±0.84
5	*Cassia fistula*	52.6±0.61	21.64±0.90	50.06±1.10	26.91±1.61	35.83±1.27	47.45±1.86
6	*Polyalthia Suberosa*	44.2±0.8	34.15±1.19	45.06±0.90	34.20±1.31	28.03±1.00	58.89±1.47
7	*Pongamia pinnata*	50.9±1.15	24.17±1.71	43.06±1.10	37.12±1.60	25.7±0.51	62.31±0.76
8	*Syzygium cumini*	56.13±1.02	16.38±1.52	53.03±0.95	22.57±1.38	43.03±0.95	36.90±1.39
9	*Tamarindus Indica*	47.96±1.26	28.54±1.88	42.1±0.85	38.54±1.24	26.13±1.02	61.68±1.50
10	*Terminalia arjuna*	50.4±1.25	24.87±1.86	46.13±1.10	32.65±1.61	28.1±1.01	58.79±1.48
11	*Zizyphus jujuba*	46.4±0.52	30.88±0.78	41.13±0.80	39.95±1.17	20.03±1.05	70.62±1.54
	Control	67.13±1.80		68.5±1.32		68.2±0.9	

Values shown are the mean ± SD of three replicates, significant at ($p<0.05$)

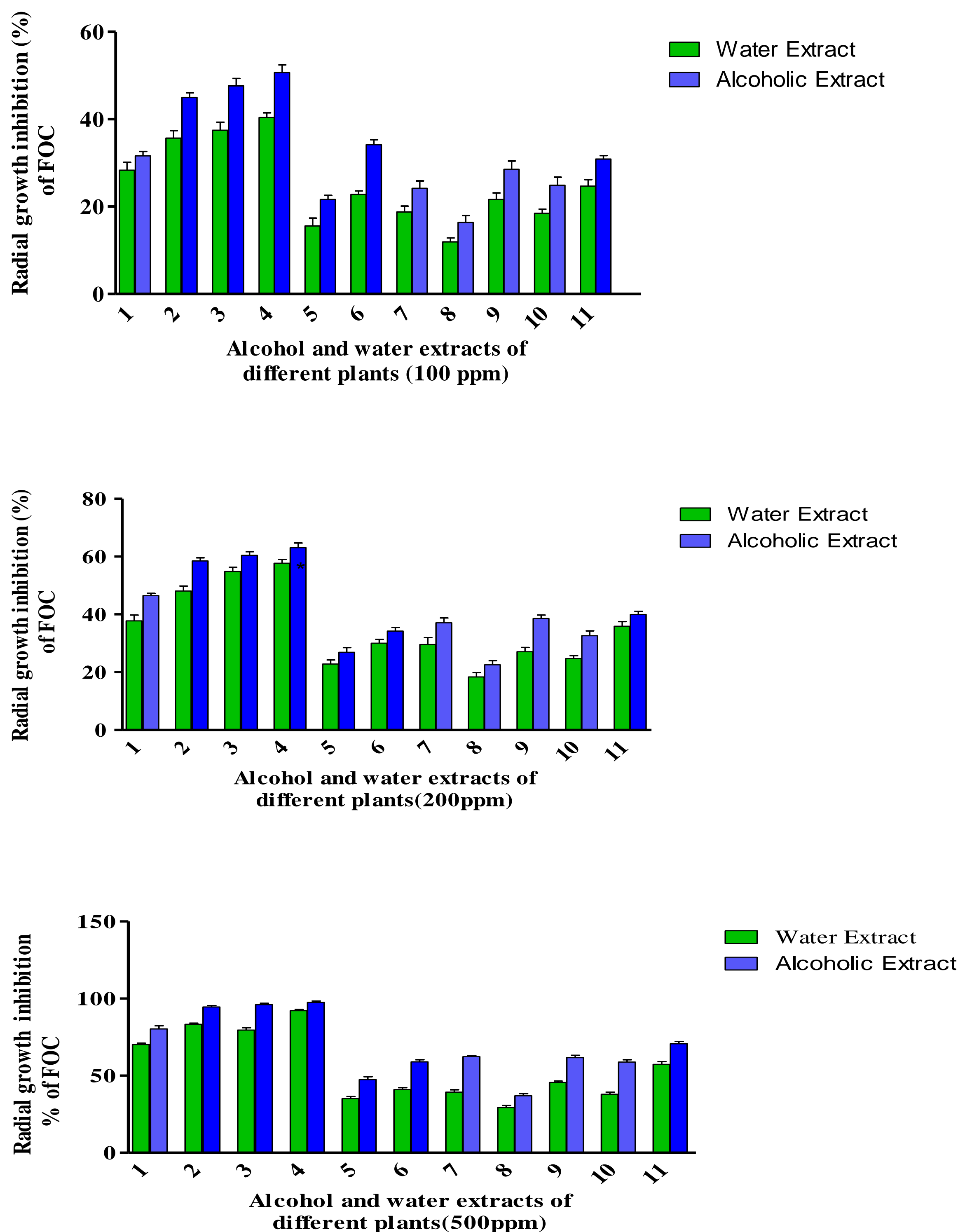

Fig 5 Radial growth inhibition of FOC (iso1) by plant extracts at 100, 200, 500 ppm concentration. (1. *Acacia nilotica* 2. *Aegle marmelos* 3. *Azadirachta indica* 4. *Callistemon lanceolatus* 5. *Cassia fistula* 6. *Polyalthia suberosa* 7. *Pongamia pinnata* 8. *Syzygium cumini* 9. *Tamarindus indicus* 10. *Terminalia arjuna* 11. *Zizyphus jujuba*)

4.9(c) Seed powders

Two seed powders were selected *viz.* black pepper (*Piper nigrum*) and cumin (*Cuminum cyminum*) for evaluation of their efficacy against the wilt pathogen at 10 20 and 30 percent. The extracts were prepared as described in chapter 3. It was observed that the extract of *Piper nigrum* at 30 percent was effective upto 90.53% in controlling the growth of the pathogen (significant at $P<0.05$). *Cuminum cyminum* extract showed significant reduction in controlling the growth, at 30 percent concentration it caused 74.38% inhibition of the pathogen. *Piper nigrum* extracts showed better efficacy in controlling the radial colony growth of the pathogen and inhibited the growth by 81.28% and 69.23% at 20 and 10 per cent concentration respectively, while *C. cyminum* showed 69.70% and 65.71% reduction in the colony growth respectively at the same concentrations (Table 16, Fig 6 Plate 18, 19).

Table 16 Potency of certain seed powders against *Fusarium oxysporum* f. sp. *ciceri* (iso1) at different concentrations on the 7th day of inoculation

S. No.		Seed Powder Extract				
		Cuminum Cyminum		*Piper nigrum*		Control
	Concentration (%)	Radial growth (cm)	Percent Inhibition	Radial growth (cm)	Percent Inhibition	Radial growth (cm)
1.	10	2.28±0.60	65.71±1.32	1.6±0.07	75.97±1.30	6.66±0.08
2.	20	2.05±0.02	69.70±0.3	1.27±0.072	81.28±1.06	6.76±0.03
3.	30	1.71±0.03	74.38±0.32	0.63±0.03	90.53±0.53	6.7±0.05

Values shown are the mean ±SE of three replications, significant at ($p<0.05$)

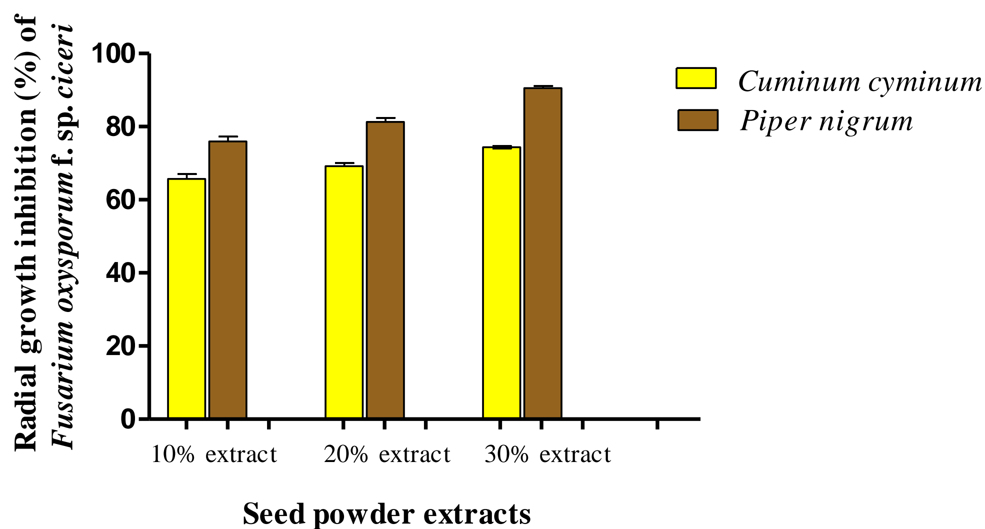

Fig 6 Radial growth inhibition of FOC (iso1) by *P. nigrum* and *C. cyminum* at different concentration on 7th day of inoculation

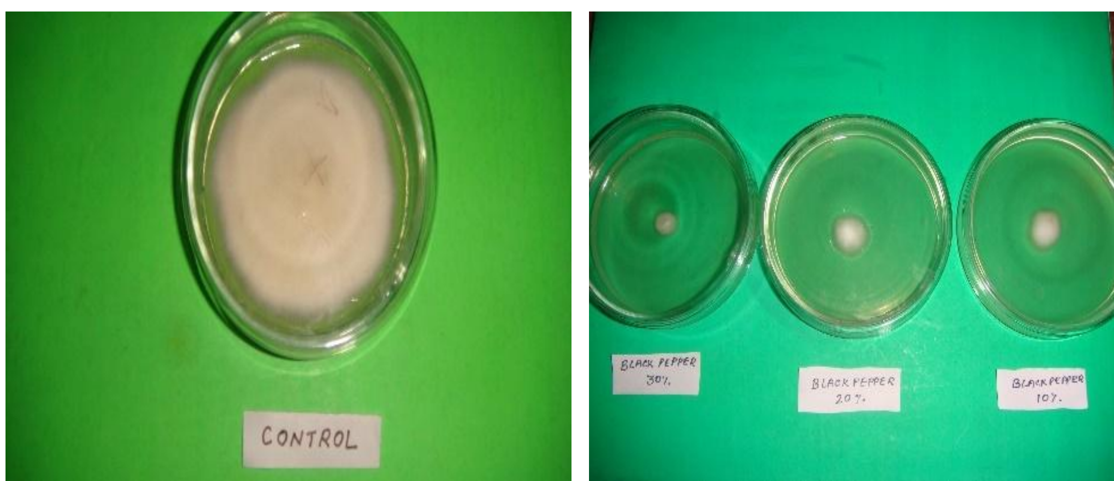

Plate 18 Black pepper (*P. nigrum*) efficacy against FOC (iso1) showing growth of the pathogen on 7th day of inoculation

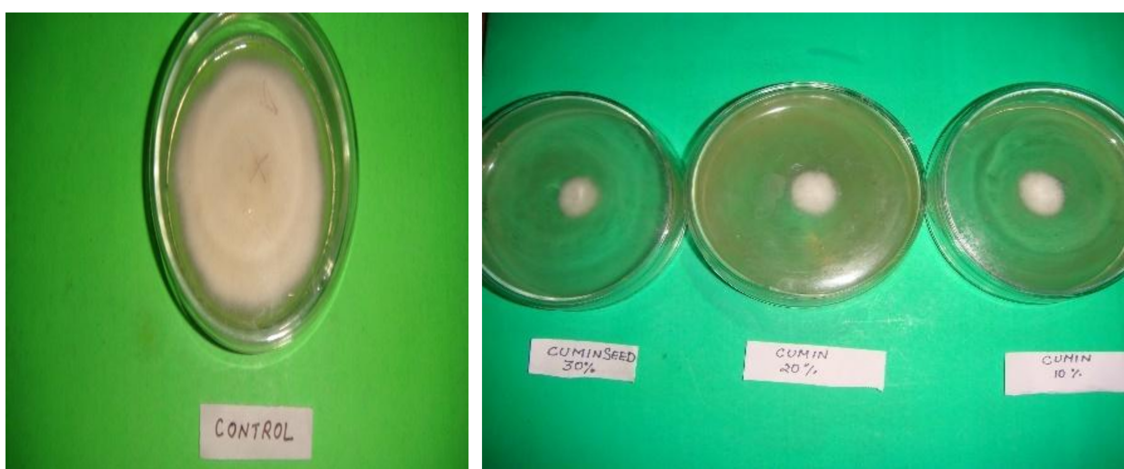

Plate 19 Cumin (*C. cyminum*) efficacy against FOC (iso1) showing growth of the pathogen on 7th day of inoculation

4.9d Efficacy of oil cakes

The oil cakes were assessed against the pathogen as they are most easily available to the farmers. It was observed that all the oil cakes showed significant reduction in the growth of the pathogen. Mustard oilcake at 30 percent concentration showed maximum efficacy and was successful in checking the growth the growth by 80.25% while neem oilcake showed 68.74% reduction in the growth followed by til oilcake which showed 66.75% reduction at the same concentration (significant at $P<0.05$). It was observed that even at low concentration the oilcakes were fairly effective in controlling the growth. At 20 percent concentration mustard oilcake showed 69.59% neem oilcake 62.55% and til oilcake 50.24% reduction in the growth. The mustard, neem and til oilcakes at 10 percent concentration showed 64.21%, 50.75% and 44.91% inhibition in the radial colony growth of the pathogen respectively (Fig 7, Table 17, Plate 20). It was envisaged that oilcakes are fairly effective in controlling the pathogen and can be readily employed in managing the disease.

Table 17 Efficacy of oil cakes against *Fusarium oxysporum* f. sp. *ciceri* at different concentrations on the 7th day of inoculation

		Oil Cakes						
		Mustard		Til		Neem		Control
S. No.	Concentration (%)	Radial growth (cm)	Percent Inhibition	Radial growth (cm)	Percent Inhibition	Radial growth (cm)	Concentration (%)	Radial growth (cm)
1	10	2.35±0.05	64.21±0.72	3.61±0.01	44.91±0.54	3.23±0.03	50.75±0.76	6.56±0.03
2	20	2.01±0.04	69.59±0.67	3.3±0.02	50.24±0.66	2.48±0.01	62.55±0.55	6.63±0.06
3	30	1.31±0.07	80.25±1.01	2.21±0.03	66.75±0.33	2.08±0.01	68.74±0.35	6.66±0.03

Values shown are the mean ±SE of three replications, significant at ($P<0.05$)

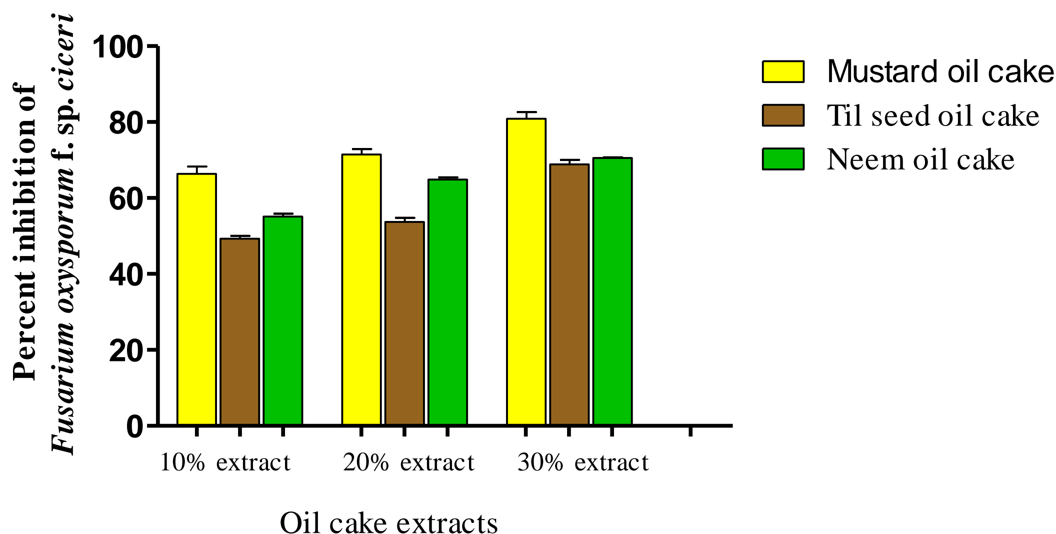

Fig 7 Percent inhibition of Mustard, Til and Neem oil cakes against FOC (iso1) at different concentration on 7th day of inoculation

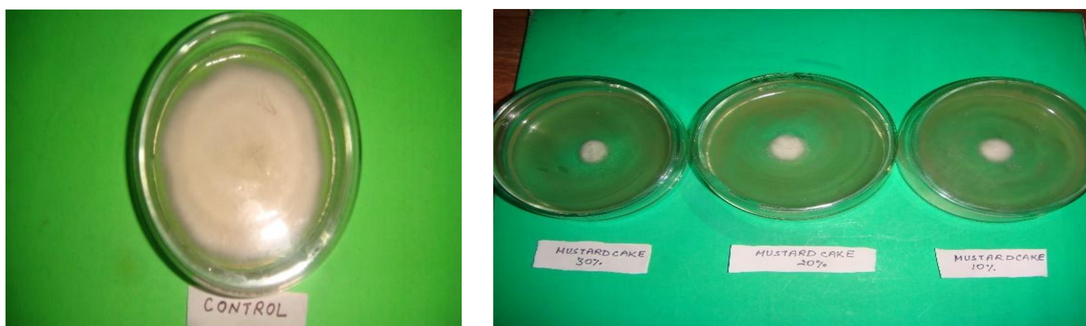

Plate 20 Mustard oil cakes efficacy against FOC (iso1) showing growth of the pathogen at different concentration

4.9(e) Through essential oils

Assessment of certain essential oil extracts were explored to find better alternatives for managing the pathogen. The perusal of data in the Table 18, Fig 8, 9 and10 clearly brings to light that a majority of the essential oils were successful in reducing significantly the growth of *Fusarium oxysporum* f. sp. *ciceri.*

Ocimum sanctum was the most effective among the tested oils inhibiting the growth by 77.32% followed by *Cymbopogon jwarancusa* 73.62% at 1000 ppm. The inhibition of the pathogen by *Cucurbita pepo* (pumpkin seed oil, 51.99%) and by *Daucus carota* (carrot seed oil, 54.17%) and Eucalyptus oil (67.97%) were new records at the same concentration, while *Tagetes erecta* showed (67.48%), *Cymbopogon martinii* (54.17%), *Oroxylum indicum* (50.95%) and Murraya *koenigii*

(34.71%) reduction in the growth .

At 500 ppm the reduction in the growth by *Cucurbita pepo, Cymbopogon jwarancusa, Cymbopogon martini, Daucus carota, Eucalyptus citriodora, Murraya koenigii, Oroxylum indicum, Ocimum sanctum* and *Tagetes erecta* were characterized by 47.04, 62.24, 44.36, 51.71, 46.07, 24.50, 42.17, 67.17, 52.45% respectively (significant at P<0.5).

At 100ppm five essential oils *viz., Cucurbita pepo (*40.86%), *Cymbopogon jwarancusa* (47.78%), *Daucus carota* (47.00%) *Ocimum sanctum* (62.29%) and *Tagetes erecta* (41.33%) controlled the growth above 40 percent while the rest were below it. The following key points were reflected:

(i) Higher concentration of essential oil checked the pathogen growth to a large extent.

(ii) Pumpkin (*Cucurbita pepo*) and carrot seed oils (*Daucus carota*) were fairly effective against the pathogen at all the concentrations.

(iv) *Ocimum sanctum* and *Cymbopogon jwarancusa* showed maximum inhibition against the pathogen while *Murraya koenigii* was the least effective among all

Table 18 Percent inhibition of FOC (iso 1) by some essential oils at 250, 500 and 1000ppm concentrations

S. No.	Name of the Plant/ Plant part used	Percent inhibition of colony growth of *Fusarium oxysporum* f. sp. *ciceri* at different Concentration (µg/ml)					
		250(µg/ml)		500(µg/ml)		1000(µg/ml)	
		Radial growth (cm)	Percent inhibition	Radial growth (cm)	Percent inhibition	Radial growth (cm)	Percent inhibition
1	*Cucurbita pepo* (seed)	4.0±0.11	40.86±1.78	3.6±0.05	47.04±1.13	3.25±0.10	51.99±1.08
2	*Cymbopogon jwarancusa* (leaves)	3.53±0.08	47.78±0.73	2.56±0.03	62.24±0.78	1.78±0.06	73.62±1.23
3	*Cymbopogon martini* (leaves)	4.1±0.08	39.38±1.45	3.78±0.03	44.36±0.25	3.1±0.03	54.17±0.62
4	*Daucus carota* (seed)	3.58±0.03	47.00±1.15	3.28±0.03	51.71±0.24	3.0±0.02	55.65±0.61
5	*Eucalyptus citriodora* (leaves)	4.15±0.05	38.61±1.77	3.66±0.01	46.07±0.51	2.16±0.09	67.97±1.29
6	*Murraya koenigii* (leaves)	5.26±0.04	22.10±1.80	5.13±0.04	24.50±0.12	4.41±0.04	34.71±0.99
7	*Oroxylum indicum* (leaves)	4.26±0.06	36.90±1.41	3.93±0.11	42.17±1.32	3.31±0.10	50.95±1.90
8	*Ocimum sanctum* (leaves)	2.5±0.00	62.29±0.66	2.23±0.08	67.17±0.98	1.53±0.04	77.32±0.85
9	*Tagetes erecta* (leaves, flowers)	3.96±0.04	41.33±1.44	3.23±0.07	52.45±0.89	2.20±0.03	67.48±0.53
	Control	6.76±0.12		6.8±0.05		6.76±0.06	

Values shown are the mean ±SE of three replications.

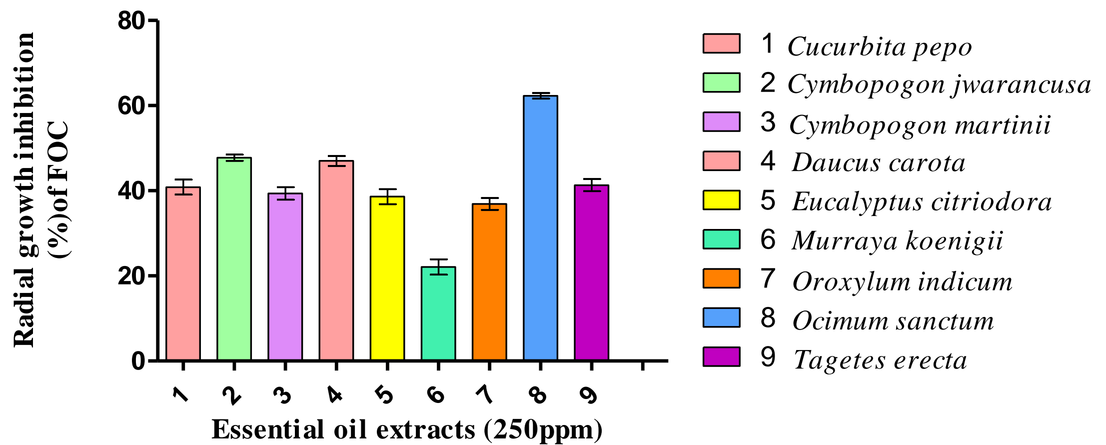

Fig 8 Percentage inhibition of *Fusarium oxysporum* f. sp. *ciceri* (iso 1) by Essential oils at 250 ppm

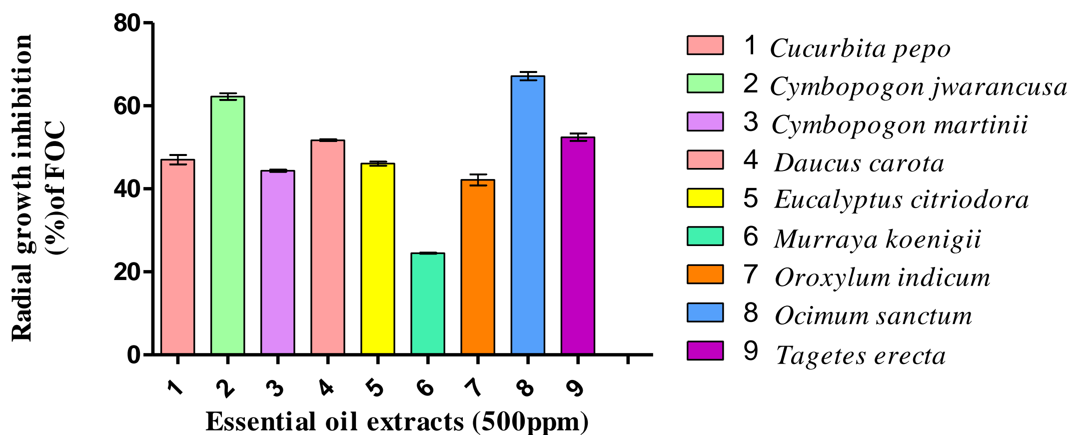

Fig 9 Percentage inhibition of *Fusarium oxysporum* f. sp. *ciceri* (iso 1) by Essential oils at 500 ppm

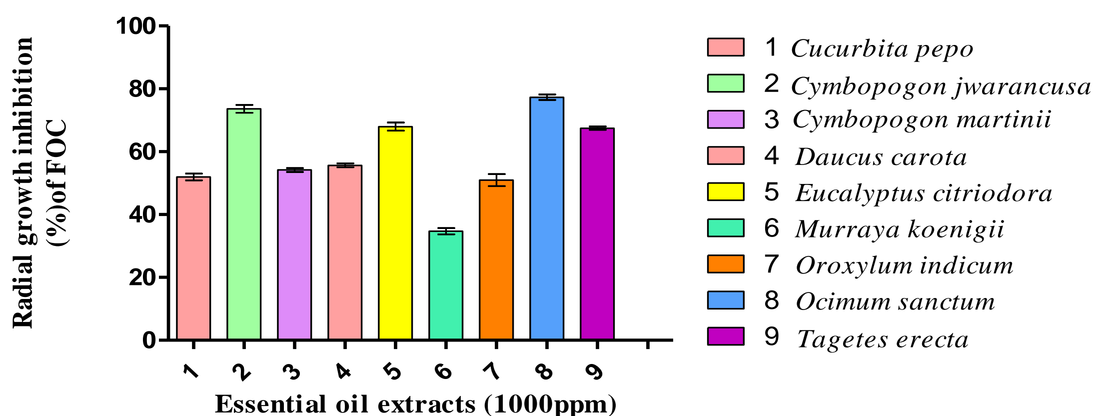

Fig 10 Percentage inhibition of *Fusarium oxysporum* f. sp. *ciceri* (iso 1) by essential oils at 1000 ppm

4.10 Effect of different fungitoxicant on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* and their efficacy in pot cultures

Five fungitoxicants were assayed for their efficacy both *in vitro* and *in vivo*. The perusal of data in Table 19, Fig11 revealed that all the five fungitoxicant tested *in vitro* significantly checked the mycelial growth of the wilt fungus *Fusarium oxysporum* f. sp. *ciceri*. Carbendazim (bavistin) proved most effective as was evident from the table 19 that the growth was controlled upto 92.64%, while Captan, diniconazole (FOB), mancozeb (Indofil M -45) and zineb (Indofil Z -78) were significant in checking the growth (P<0.5) by 81.31%, 79.41%, 64.70 and 67.64% respectively.

Table 19 *In vitro* assessment of certain fungitoxicant against FOC (iso 1)

S.no	Fungicide	Conc. in Percentage (%)	Avrg. diam in(cm)	Inhibition over control
1	Carbendazim(bavistin)	0.3	0.5±0.05	92.64±0.84
2	Captan	0.1	1.23±0.08	81.31±1.33
3	Diniconazole	0.03	1.4±0.05	79.41±0.84
4	Mancozeb(IndofilM-45)	0.3	2.4±0.1	64.70±1.47
5	Zineb(IndofilZ-78)	0.2	2.2±0.11	67.64±1.69
	Control		6.8±0.0	00.00

Values are the mean of three replicates ±SE

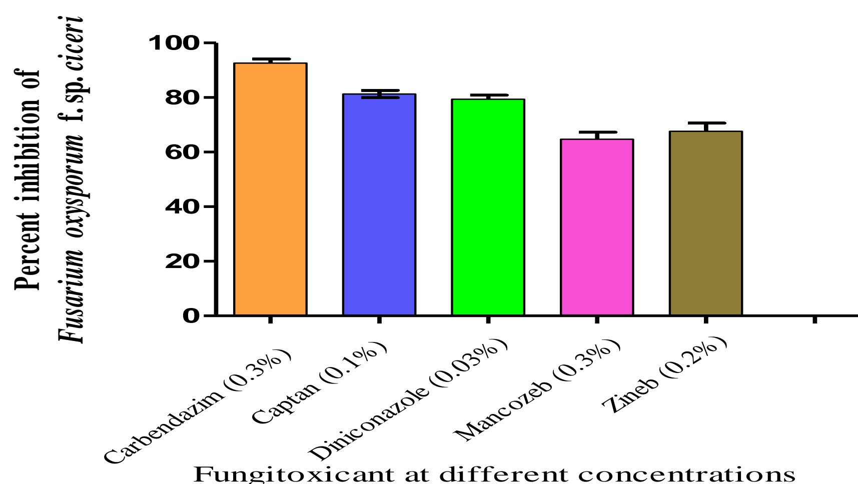

Fig 11 Percent inhibition of radial colony growth of FOC (iso 1) by different fungitoxicants

In the pot experiments all the fungitoxicants significantly reduced the wilt disease as depicted in Table 20. Carbendazim (bavistin) again proved the best fungitoxicant showing only 4% mortality captan was the next better fungitoxicant showing 8% mortality followed by diniconazole (FOB) 14.2, Mancozeb (Indofil M-45) 16.6 and Zineb (Indofil Z -78) 12.7% mortality. Thus carbendazim proved better fungitoxicant followed by Captan.

Table 20 Effect of fungitoxicant on plant mortality on variety JG62 under infested pot condition against FOC (iso 1)

S. no	Fungicide	Conc. in Percent age (%)	Seeds sown	No. of plants Emerged	No of plants wilted	Percentage Mortality
1	Carbendazim (Bavistin)	0.3	50	50	2	4
2	Captan	0.1	50	50	4	8
3	Diniconazole (FOB)	0.03	50	49	7	14.2
4	Mancozeb (Indofil M-45)	0.3	50	48	8	16.6
5	Zineb (Indofil Z-78)	0.2	50	47	6	12.7
	Control		50	49	49	100

4.11 Assay of integrated management of chickpea wilt using botanicals and fungitoxicants

The integrated management trial using botanicals and fungitoxicant was investigated both *in vitro* and under pot conditions. The experiment included the following treatments. T1: *Cuminum cyminum* extract (10%) + Carbendazim (0.3%); T2: *Cuminum cyminum* extract (20%) + Carbendazim (0.3%); T3: *Cuminum cyminum* extract (30%) + Carbendazim (0.3%); T4: *Piper nigrum* extract (30%) + Carbendazim (0.1%); T5: *Piper nigrum* extract (30%) + Carbendazim (0.2%); T6: *Piper nigrum* extract (30%) + Carbendazim (0.3%).

The above mentioned treatment set was prepared as mentioned earlier in the text. (i) All the treatment set *viz.,* (T1, T2, T3, T4, T5, andT6) were assayed *in vitro* using PDA agar medium and colony growth was measured after 7days.

4.11(a) *In vitro* assay

Integration of fungitoxicant with the botanicals gave tremendous result in controlling the colony growth of the pathogen. It was observed that all the treatment set were effective in controlling the growth of the pathogen cent per cent as depicted in Table 21 except for T1, comprising of *Cuminum cyminum* 10% and carbendazim 0.3% which showed an inhibition of 87.72%. The result were reinstated by the reduction in the dry mycelial weight of all the pathogen as T1, T2, T3, T4, T5, and T6 recorded 0.39, 0.30, 0.13, 0.28, 0.05 and 0.02 gm respectively as compared to control (without any treatment) which was observed to be 0.48 gm after 7 days of incubation.

Table 21 *In vitro* assay of integrated management trial using botanical and fungitoxicant

S. No.	Treatment set	Radial growth in (cm)	Percentage inhibition	Mycelial weight(gm)	
				Fresh	Dry
1	T1	0.83±0.05	87.72	2.68±0.01	0.39±0.00
2	T2	00	100	1.93±0.11	0.30±0.01
3	T3	00	100	1.03±0.06	0.13±.05
4	T4	00	100	1.15±0.05	0.22±0.01
5	T5	00	100	0.73±0.06	0.05±0.01
6	T6	00	100	0.37±0.06	0.02±0.0
	Control	6.76±0.12		3.2±0.05	0.43±0.03

Values shown are the mean ±SD of three replicates

4.11(b) *In vivo* assay under pot conditions

Seed dressing with the fungitoxicant and regular sprays of the botanicals was found to be effective in controlling the wilt menace drastically. The data in Table 22 clearly suggest that all the treatment set were very effective in controlling the disease except for the first treatment incorporating cumin extract (10%) and carbendazim (0.3%) which showed a mortality percentage of 3.03, while all the rest treatment showed cent per cent control of the disease.

The findings clearly highlight that integrated management of the wilt disease through botanicals and fungitoxicant was more superior as compared to the other management trials when done solely in controlling the pathogen both *in vitro* and under *in vivo* conditions.

Table 22 Assay of integrated management under pot conditions

S.no	Conc. in Percentage (%)	Seeds sown	No. of plants Emerged	No of plants wilted	Percentage Mortality
1	T1	35	33	1	3.03
2	T2	35	34	0	0
3	T3	35	35	0	0
4	T4	35	34	0	0
5	T5	35	35	0	0
6	T6	35	35	0	0
	Control	35	34	34	100

Plate 21 Control of wilt disease through integrated approach (Seed powder + fungitoxicant)

Discussion

Indian agriculture continues to be largely rainfed with nearly 60 % of the cultivated area not having any access to irrigation. Even if the country's full irrigation potential is realized, half of its cultivated area will continue to be to be under rainfed farming (Venketeswaralu and Rao, 2011). Rainfed agriculture is largely practiced in arid, semiarid and sub humid regions in the country, where the annual precipitation is lower than the evapotranspiration demand. Under this scenario the significance of chickpea reaches its peak as it is predominantly a rainfed crop and is one of the very important protein rich crops from human as well as animal diet point of view. Moreover, among pulses it occupies first rank for area (10.2 mha), production 9.88m tones (fourth advance estimate, Agricultural Statistics at a glance 2014). Among all the factors responsible for low productivity, wilt caused by *Fusarium oxysporum* f. sp. *ciceri* accounts for the substantial losses ranging from 60-70 percent (Mandivia *et al.,* 2002; Gurha and Trivedi, 2008). Wilt is such a disease that eliminates the crop from the fields and the enthusiasm of growers. Though its first mention was made by Butler in 1918, but Prasad and Padwick (1939), by their exhaustive studies confirmed (i) *Fusarium orthoceras* var. *ciceri* as cause of wilt, (ii) the fungus was able to survive in roots and stems, (iii) farmyard manure hastened disappearance of the fungus and (iv) a species of *Trichoderma* and *Aspergillus* were antagonistic *in vitro* but were unable to control the disease in fields. Later on several workers confirmed the association of chickpea wilt with *Fusarium orthoceras var. ciceri* as pathogen (Malik and Khan, 1943; Motiramani, 1947; Benlloch 1949; Mehta *et al.,* 1950).

By the elimination of 7 sections out of 16 sections of Wollenweber and Reinking (1935) by Snyder and Hansen (1940 and 1945) where species *oxysporum* was represented by section *Elegans* in which *orthocera* was the pathogen causing the

wilt of chickpea, it was renamed as *Fusarium oxysporum* Schlect f. sp. *ciceri* (Padw.) Matuo and Sato.

5.1 Survey and collection of plant samples

The assessment of wilt disease of chickpea was clarified by the results of periodic surveys conducted in two consecutive crop season, 2011-2012 and 2012-2013 in 6 villages belonging to Unnao and Kanpur district of Uttar Pradesh along with the fields of Indian Institute of Pulses research (IIPR), Kalyanpur and Kanpur. The results depicted that the percentage of wilted plants varied from 25.6 to 40.01% in the two crop season. It was inferred that (i) *Fusarium oxysporum* f. sp. *ciceri* was the main causal agent of the disease, (ii) wilting of chickpea plant was not specific to location or direction or situation of the farmer's field and (iii) there was no correlation between wilting percentage and crop seasons surveyed. This disease also emphasized losses on three fronts (i) loss of seeds sown (ii) loss of production space (iii) net loss of straw and seed. Our findings are similar to Abera *et al.* (2011) who reported that in two cropping season 2006-2007, and 2007-2008, in five randomly selected farmers' fields mean incidences of wilt recorded in each district were Gondar Zuria, 34.16% and 34.11%; Dembia, 37.90% and 35.36%; LiboKemkem, 34.74% and 28.81%; Fogera, 34.74% and 28.81%; Dejen, 34.74% and 28.81% and Enemay, 33.34% and 37.64% indicating that fusarium wilt was highly distributed in all the surveyed chickpea-growing areas of northwestern part of Ethiopia.

Padwick (1941) noticed complete destruction of variety IP 28 though cultivar IP 78 was not affected in 1939 but had 32 percent infection in the next crop. Benloch (1949) found chickpea plants largely destroyed by *Fusarium*. Mosahebi (1968) noted that wounds made by nematode and insect larvae were responsible for wilting. He observed yellowing of leaves and discoloration of vascular elements as

main symptoms. Nema and Khare (1973) recorded 61 percent wilting at seedling stage and 43 percent at flowering in a detailed survey report of Madhya Pradesh. Singh and Dahiya (1973) recorded the losses from 10 to cent per cent in practically all the chickpea growing states in India. Kunwar *et al.* (1989) inoculated variety JG 62 with Race 1 and Race 4 separately and observed 50.7% and 98.4% wilting within four weeks.

Thus from the reports, and the present survey data on wilted plants, the lower percentage of wilt in the villages surveyed would have been due to (i) uneven distribution of inoculum in the soil, (ii) chances of escape from the pathogen attack and (iii) variability in the pathogen. But the percentage of losses remained on the higher side from its first detection in the fields (Butler, 1918 and Pearl, 1923)

5.2 Symptomatology of the disease

The symptoms of wilt in the field commenced generally after one month of sowing. The seedlings began losing turgidity and the plants tended to lie down with chlorotic leaves and sometimes with stunting of stems. When uprooted the roots appeared healthy with almost no discoloration. When the plants were in vegetative phase, wilting symptom was pronounced by drooping of apices, yellowing of leaves followed by drying and death of the entire plant. The yellowing of leaves in most cases was followed by browning. The roots of such plants showed no rotting, drying or discoloration, though splitted vertically revealed black or dark brown discoloration of internal tissues. In cases of late wilt, that was at the time of flowering and podding stage the above typical wilt symptoms were observed in one or two branches of the healthy plants.

Narasimhan (1929) also noticed similar symptoms like drooping of leaves followed by typical wilting and necrosis of tissues in the collar and roots. The affected

plants easily broke away at the collar region. But in some cases he recovered a species of *Fusarium* and in others a *Rhizoctonia*. Dastur (1935) mentioned the symptoms in detail as drooping of tender apical parts with slight loss of colour, followed by chlorosis and leaves hanging down limply. The later were not easily shed but with time the wilted plants may turn brown and continue to stand thus in the field for a long time. The root system showed no external signs of rot. Such roots from wilted plants yielded a species of *Fusarium*. Erwin (1958) characterized wilting by leaf yellowing and xylem necrosis in California. Chauhan (1962) observed (i) acropetal vein clearing of leaves possibly indicating path of toxin movement, (ii) the number of chloroplast and starch formation in mesophyll cells decreased, (iii) leaves turned yellow and then drooped and (iv)younger plants were more susceptible. Sinha (1973) noticed that the seedling of chickpea if attacked die and dry out soon. But upto the month of January and early February plants remained healthy and usually in the third week of February the disease spread with typical wilting symptoms. Thus drooping of apices, yellowing of leaves, chlorosis followed by browning, apparently symptomless roots with no rotting or browning of internal tissues were the similar symptoms as observed in the present studies.

Moreover, the symptoms caused by *Fusarium oxysporum* f. sp. *ciceri* in susceptible chickpea variety JG 62 in wilt infested pot cultures showed drooping of seedling from upper part of the plant, followed by loss in turgidity of petioles and leaflets resulting in collapse of entire plant. The leaves became chlorotic, later turned straw colored or light brown. These wilted seedling when uprooted depicted uneven shrinking on both sides of collar region and dark brown to black discoloration in the radial longitudinal section. Such infected seedlings when put to reisolations from root, collar, stem, and apices yielded the same pathogen *F. oxysporum* f. sp. *ciceri* identical

to the original one. However, leaves yielded the culture rarely. If these wilted plants were set aside for 4-5 days then such leaves were able to produce the wilt fungal cultures in upto 20% cases. From the studies it was confirmed that the mycelium travelled through the conducting tissues even after the death of the plants *ipso-facto* confirming the systemic nature of the disease.

5.3 Histopathology of chickpea plants infected with *Fusarium oxysporum* f. sp. *ciceri*

The Histopathology was studied by observing radial and tangential longitudinal sections of stem and root of wilted plants. It revealed travelling mycelium in vessels and tracheids. Chlamydospores were observed frequently in xylem tissues of stem and roots whereas no macro or macroconidia were observed. The tyloses were of rare occurrence in the roots and in stems, they were altogether absent. In a few cases browning of vessel and tracheids was noticed. In certain cases mycelium was observed in cortical region also. Kunwar *et al.* (1989) studied histopathology in susceptible variety JG 62 infected with race 1 and race 4. They observed inter and intracellular hyphae in pith, xylem and cortex with epidermal cells disintegrated. Hypertrophy of cortical and pith cells occurred due to mucilage like substance. Stevenson *et al.* (1997) also observed occlusion of vascular tissues by mycelial growth of the pathogen. In more serious cases they noted invasion of stem xylem vessels upto five internodes above the point of seed attachment. In artificially inoculated plants that showed signs of wilting had hyphae within the root xylem.

5.4 Culture medium of the pathogen

Pandey *et al.,* 2001; Dikkar *et al*., 2003 presented effect of different culture media on growth and sporulation of FOC. The media used comprised of 6 synthetic and 6 semi synthetic media. Among solid media the fungus grew well on Richards' agar followed by PDA and CZA. Among the liquid media investigated maximum dry mycelial

weight was recorded on Richards' medium. Maltose mannitol, D glucose and sucrose supported maximum dry mycelial weight as well as sporulation. Lactose was poorly utilized. Among nitrogen sources tested KNO3 was the best followed by NaNO3 for supporting the mycelial growth and sporulation. Paulkar and Raut (2004) found potato dextrose agar medium for maximum growth while Kirchoff's agar was poorest to support fungal growth among the 6 media tested *viz.* Ashby's medium, Asthana and Hawker medium, Czapek's medium Kirchhoff's medium potato dextrose agar medium and Richard's agar medium. Farooq *et al.* (2005) found Czapek's dox agar and chickpea meal agar media as best among the eight media tested. They reported glucose as the best source of carbon and peptone as the best source of nitrogen. Growth of the fungus was maximum at 30ºC and most suitable pH was 6.0-7.0. The growth reduced drastically below 15ºC and above 35ºC.

On the basis of most of the above findings Trivedi and Gurha (2007) used potato dextrose agar medium for comparing the growth of different isolates of chickpea wilt fungus. Similarly, in the present studies PDA was used for isolations and for further experimentations wherever necessary.

Out of 420 isolations made from the wilted plants collected from 6 different villages and isolations made from IIPR, Kanpur 7 groups of isolates were sorted out according to their morphological and cultural and discriminative characters. These isolates were assigned the numbers 1 to 7 sequentially. From the data it was inferred that the cultural variants of *F. oxysporum* f. sp. *ciceri* were not affected by the direction and location or other edaphic factors of the field soil.

5.5 Pathogenicity test

The pathogenic potential of different isolates was determined on the percent wilt incidence on the highly susceptible variety JG 62. Using the parameter of Trivedi and

Gurha (2007) considering seedling mortality above 80 percent as highly pathogenic, seedling mortality 50-79% as moderately pathogenic and seedling mortality less than 50 percent as weakly pathogenic. The results depicted isolate 3 as highly pathogenic and the rest as moderately pathogenic. Thus there existed no correlation between morphological and cultural characters of isolate as well as their pathogenic behavior. Our results comply with the various other findings. Paul *et al.* (2001) in an investigation recorded 25 isolates of *Fusarium oxysporum* f. sp. *ciceri* as fast growing, 17 as slow growing and 9 were reported as medium growing types. On the basis of virulence on variety JG 62 none of the seedling survived after a week of inoculation with 27 isolates, whereas 15 isolates proved to be moderately virulent (mortality 66.6%) and 9 were classified as least virulent.

The symptoms produced during test of pathogenicity were exactly identical to those described earlier by Westerlund *et al.*, 1974; Cabrera *et al.,* 1985. Reisolations studies revealed the presence of the same fungus identical to the original one obtained from naturally wilted plants. The morphological and cultural characteristics of the *F. oxysporum* f. sp. *ciceri* obtained were similar to those reported earlier (Gupta *et al.,* 1986). Characteristic wilt symptoms such as drooping of leaflets and yellowing of the leaves starting from apical part, progressing downward and final wilting of the whole plant were observed. Demirci *et al.* (1999) isolated *F. oxy.* f. sp. *ciceri* from both plants and seeds and its pathogenicity was tested and proved individually. Nikam *et al.* (2011) also confirmed the pathogenicity of the *F. oxy.* f. sp. *ciceri* using sick soil inoculation technique in earthen pots on susceptible cultivar JG 62.

5.6 Studies on cultural and morphological characters of the pathogen

Based on the radial growth and colour of the substratum the isolate were grouped into seven isolates. Radial growth of different isolates was studied on PDA medium. On

the basis of radial growth as fast, medium and slow where the growth was above 7.0 cm, in between 6.0-6.9 cm and below 6 cm respectively in 120 hrs. Isolate 1, 3 and 7 had medium pace of growth and the rests showed slow growth.

Isolate 4,5,7 showed bigger macroconidia in the range of 3.75-9 μm whereas others had smaller ones in the range of 3.25-7.5 μm Chlamydospore were most frequent in isolate 2,4 while others showed lesser frequency. Three septate macroconidia were most frequent in occurrence in most of the isolate.

Results are in agreement with Paulkar and Raut (2004) who grouped 4 isolates of *F. oxysporum* f. sp. *ciceri* as separate ones on the basis of morphological and cultural characters. The isolates produced different sizes of colonies due to variation in the radial fungal growth. Mushrif and Khulbe (2010) demonstrated biochemical variability among three isolates of *Fusarium oxysporum* f. sp. *ciceri*. These findings also support that there is no correlation between the cultural morphological and pathogenic behavior of different isolate of *F. oxy.* f. sp. *ciceri*. Gupta *et al.* (1987) reported similar observations in disease plants in which leaves turn yellow and straw colored and the conidiophores were 3 to 5 septate, thin walled, pointed both ends, fused and measuring 3.5 to 4.5 x 25 to 65 μm. Macroconidia were fewer than micro conidia, borne singly or branched in old culture. They were rough or smooth walled, intercalary or terminal and may be formed singly, in chains or pairs.

5.7 Assessment of abiotic factors affecting wilt

(i) Soil factors

Knowledge of Climate, soil conditions and availability of pathogenic inoculum for cent percent wilting of susceptible plants has been the prerequisite for studies of wilt disease. Hence, the normal soil of Kanpur was mixed with sand and the physical soil factors such as water holding capacity (WHC), percent pore space (PPS), absolute

specific gravity, volume expansion of 100 gm soil were altered and calculated along with their effects on wilting. It was observed that 31±1% WHC and 43.4 percent pore space was most conducive for wilting it was possible by adding 25 % sand to the normal sieved soil. The other soil factors like volume expansion of soil and absolute specific gravity were having no correlation with wilt development.

The results were in agreement with the findings of Padwick (1942) where he observed a well-marked correlation between wilt and subsoil dryness. Sattar *et al.* (1953) reported 24% soil moisture was conducive for wilting in chickpea. Kotasthane *et al.* (1976) observed more severe wilt in light soil than on heavy soil. Similar results were presented by Sugha *et al.,* 1994; Andrabi *et al.,* 2011 who reported moisture and temperature, as key environmental factors that influence the development of the pathogen.

(ii) Depth of inoculum

The depth of inoculum in the pots proved significantly effective in altering the time lag of wilting. The immediate contact of inoculum to germinating seeds was the best suited for quick pathological screening as the plants wilted in 31 days at the temperature range of 21 ºand 28ºC. Raheja and Das (1957) also noticed that late wilt was significantly higher when deep sowing (5 inches) was practiced.

(iii) Temperature

Between the two temperature range occurring during the crop season that were 8-17ºC and 21-28ºC, the later was most favorable for wilting as it required 21-28, the days for developing wilt symptoms depending on the varieties. Sattar *et al.* (1953) in the pot experiments precisely noticed that soil temperature 25ºC lowered the wilt incidence. Kushwaha (1971) found optimum temperature for growth of wilt fungus to be 25ºC.

Bhatti and Kraft (1992) tested a large seeded kabuli chickpea (Burpee 5043) and small seeded desi chickpea (JG 62) at various temperature ranging from 10 to 30°C with a range of inoculum densities of pathogen. Wilt severity did not increase with increased inoculum levels of 104 or 105 microconidia and macroconidia/ml. However wilt symptoms were more severe at high temperature (25 and 30° C) than those at lower temperature (10, 15 and 20° C). The symptoms of chickpea wilt disease increased by decreasing soil moisture.

(iv) Role of different crop debris in multiplication of primary inoculum of wilt pathogen (FOC)

The chickpea wilt pathogen survives in the soil and crop debris generally. Therefore the growth and sporulation of FOC was assessed on Potato dextrose broth, wheat straw, linseed, Urid straw chickpea and pigeon pea broth media. It was found that urid straw was the best suited for the growth as it yielded maximum dry mycelium (1060) as compared to linseed straw which produced minimum biomass (360 mg). The biomass produced by Potato dextrose broth was next to urid debris straw (940 mg). If arranged in ascending order of dry mycelium weight it would be linseed, pigeon pea, chickpea, wheat straw and urid straw. The macroconidia produced per ml were minimum in chickpea and wheat straw, whereas it was maximum in potato dextrose broth. The pH in all the broth shifted to alkalinity.

It was notable that there existed no correlation between the biomass production and micro and macroconidia count per ml. By the results it was inferred that the crop debris play an important role in perpetuation and multiplication of the wilt inoculum.

Chauhan (1963) observed significant reduction in wilt incidence by mixing oilcakes of groundnut, sesame and mustard, the last gave the best result (8.33% mortality compared with control's 63.33%. Singh *et al.* (1981) controlled *Rhizoctonia*

bataticola root rot of chickpea by amending the soil with wheat or maize or sorghum straw. Nene (1996) recorded maximum wilting of chickpea in sorghum chickpea, maize chickpea, and wheat chickpea, intercropping using highly susceptible cultivar JG 62 over three crop seasons.

5.8 Production of cellulolytic and pectinolytic enzyme by *Fusarium oxysporum* f. sp. *ciceri*

The plant cell wall is a heterogenous structure of polymers which circumvents the cell containing cytoplasm (Karr and Albersheim, 1970). Exocellular enzymes like pectinolytic, hemicellulolytic, proteolytic and cellulolytic enzymes have been ascribed for the degradation of cell wall. These enzymes have been credited with the capacity of attacking each of the major polymeric component of the cell wall (Wheeler, 1975) and plant pathogens have been reported to secrete a range of such enzymes (Riou, 1991).

The maximum enzymatic activity by PG and Cellulase was shown on 14th day after 90 min of incubation registering 74.76 and 79.09 % reduction in viscosity. However, on 21st day, after 120 min of incubation they registered 70.20 and 54.28% reduction in viscosity respectively. The results indicate that FOC produces cellulolytic and pectinolytic enzymes and that the production of Cellulase was higher than PG.

Our results are in confirmation with the reports of Mehta *et al.,* 1974 who reported that maximum production of PG and C_x enzyme took place in between 4 to 12 days in culture filtrates of *A. solani* and *Alternaria tenuis*. The gradual loss in production of the enzymes PG and Cellulase (C_x) during long incubation period could be attributed to slow inactivation of PG and C_x by the appearance of oxidized phenols in the semi ripe tomato fruit medium (Chaurasia *et al.,* 2014) similar observation were also reported by Balasubramanian and Srivastava (1973). Dwivedi and Enespa (2015) in an investigation reported that the activity of cellulolytic

enzymes by *F. oxysporum* f. sp. *lycopersici* was more in 14th day old culture and the enzyme activity subsequently decreased with increase of culture age.

Sadik *et al*. (1983) have illustrated that a large number of pathogens have the ability to produce more cellulolytic than pectolytic enzymes. Production of cell wall degrading enzymes by pathogens facilitates the successful invasion of the host tissue (Gothoskar *et al.,* 1955; Singh and Hussain, 1968; Singh, 1984 and Tamuli *et al.* 2008).

In general, these enzyme activities are much higher in compatible host-pathogen interaction than in incompatible interaction involving resistant plants. The plant cell wall may be protected from above' fungal enzymes if the activity of the same is inhibited by biomolecules released by the host during pathogenesis.

Disease management

5.9 Control of wilt disease through bioagents

Fusarium oxysporum f. sp. *ciceri* (FOC) and *Trichoderma* spp. share the same ecological niche and in the present investigation the interaction of different isolates of FOC with the antagonist *Trichoderma harzianum, Trichoderma viride* and *Aspergillus niger* were worked out and the interaction behavior was recorded as per the terminology of Johnson and Curl (1972).

A- Mutual intermingling of the two organisms

B- Mutual inhibition on contact

C- Mutual inhibition at a distance

D-Inhibition on contact, the antagonist continues to grow at unchanged or reduced rate through the colony of the inhibited organism

E-Inhibition at a distance the antagonist continues to grow resulting in clear zone at unchanged or reduced rate

It was notable that 'A' type of interaction was not found anywhere with either of the three antagonist tested against the pathogen. It indicated that all the antagonist were effective in checking the growth of the pathogen. 'D' and 'E' type of interaction were peculiar in the fact that the former exhibited no clear zone at the point of interaction while the latter formed a clear zone of inhibition in between the two organisms. The observations led to conclude that (i) wherever the antagonist and the above pathogen was making a colony the antagonist would neither intermingle nor parasitize the pathogen, though hampered the growth of the pathogen in most of the cases and (ii) either of the pathogen isolate or the antagonist was juxtaposed to root zone the counteracting would never come nearer to it. So if the pathogen was present on the rhizoplane of susceptible chickpea plant then the antagonist will be unable to save the same plant from fusarial attack.

Hence the application of the antagonist should be in the soil where the seeds were to be sown or the seeds should be dressed by the antagonist before sowing so that the nascent roots emerging should come in contact with antagonist first. The efficacy of the antagonist was also tested using three highly susceptible varieties JG 62 Kanpur local and Ujjain Local in pot cultures. The observations were that *T. harzianum* successfully controlled the wilt attack on JG 62 cultivar as the wilt incidence was reduced to 44, 34, 6.52 and 00 % by the application of 2, 4, 6, 8 percent (w/w) of the antagonist. Thus both the species of *Trichoderma* were able to control the wilt at 8 percent. Our results comply with Boureghda and Bouznad (2009) who also reported high degree of chickpea wilt control by *T. harzianum, T. atroviride* and *T. longibrachiatum.* The results bear semblance with various investigations stating the use of *T. harzianum* and *T. viride* as biocontrol agents. Species of *Trichoderma* when added to the soil or applied as seed treatments have been found to grow readily

along with the developing root system of the treated plant (Howell *et al.*, 2000; Harman, 2006).

Different *Trichoderma* species have been extensively tested as biocontrol agents against wide range of plant pathogens and several of them have been found potent against many soil-borne plant pathogenic fungi (Calvet *et al.,* 1990; De *et al.,* 1996; Reddy *et al.,* 2000; Meki *et al.,* 2009;). Dubey *et al.*(2011) characterized secondary metabolite *viz.* 6-noxylene alcohol, massollactone, methyl cyclopentane , methyl cyclohexane , N –methyl pyrollidone, dermadin , ketotriol, koningin-A, 3-methyl heptadecanol, 2 methyl heptadecanol, palmitic acid, 3-(2-hydroxypropyl)-4-(hexa2-4- dineyly)-2-2(5H)-furanone and 3- (propenone)-4-(hexa -2-4- dineyl)-2-2(5H)-furanone in all the 13 metabolites produced by *T. harzianum* and *T. viride* effective against *F. oxy.* f. sp. *ciceri* by GC-MS/MS technique. Temesgen (2002) indicated *T. hamatum* and *T. koningii* controlled soil borne infection of 30 day-old seedlings by *R. solani* and *Fusarium* spp. and increased the grain yield of faba bean.

However *Aspergillus niger* showed a maximum of 47.22% reduction in colony diameter of FOC but under pot conditions was 100 percent successful in controlling the wilt disease at 10 (%) w/w inoculum. The inhibitory effects of *Aspergillus* spp. against several plant pathogens have been reported (Geetha *et al.*, 2005, Gachomo and Kotchoni, 2008). The positive response of bean plants on the addition of *A. niger* have been reported due to the fungistatic activity or the plant growth promoting activities in soil. (Whipps and Mc Quilken, 1993; Bashar and Rai, 1994; Singh *et al.*, 2002). Hend *et al*. (2012) stated that Under pot conditions, maximum control of the wilt disease was observed with *T. harzianum* (44.4%) whereas *A. niger* reduced wilt to (35.6%), as compared to FOL inoculated plant, reduce wilt incidence and boosted plant growth significantly.

5.10 Effect of plant extracts, seed powders and oilcakes against the pathogen

In recent years, the need to develop fungal disease control measures using photochemical as alternative to synthetic chemicals has become a priority of scientists worldwide (Reddy *et al*., 2007). Antifungal action of plant extracts has great potential as they are easy to prepare and apply.

Alcoholic and water extracts of 11 plants were prepared and assayed at 100, 200 and 500 ppm against FOC namely *Acacia nilotica, Aegle marmelos, Azadirachta indica, Callistemon lanceolatus, Cassia fistula, Polyalthia suberosa, Pongamia pinnata, Syzygium cumini, Tamarindus indicus, Terminalia arjuna,* and *Zizyphus jujuba*

It was observed that maximum inhibition in the colony growth was observed with alcoholic extract as compared to water extract. That extracts were more efficient at higher percentage of concentration. Three plant species *viz. Callistemon lanceolatus, Azadirachta indica* and *Aegle marmelos* controlled the growth of FOC more than 90% with C*allistemon lanceolatus* being successful to the extent of (97.55%) at 500 ppm. The successful inhibition in growth of FOC by *Pongamia pinnata* (62.31%) in the present study is a new record. *Syzygium cumini* was the least effective among the tested plant varieties.

Similar studies were conducted by Satish *et al.* (2007) who studied aqueous extracts of fifty-two plants from different families for their antifungal potential against eight important species of *Aspergillus*. Among 52, twelve extracts recorded significant antifungal activity against one or the other *Aspergillus* species tested. Similarly, Pundir and Jain (2010) analysed the potencies of 22 plant extracts against food associated fungi and found that clove and ginger are more efficient than other plant extracts.

Two seed powders were assayed for their efficacy against the pathogen at 10, 20 and 30% concentration. At 30 % concentration *Piper nigrum* checked the growth to 90.53% while *Cuminum cyminum* followed closely behind with 74.38%. The results highlight that the seed powders showed significant reduction in the growth even at low concentration of percentage. Both of them at 10% concentrations showed greater than 65% inhibition in the radial colony growth of the pathogen. Results are in conformity with Feng hu *et al.* (2013) who observed that essential oil of *C. cyminum* at 100 mg/ml completely inhibit the mycelial growth of *Sclerotinia sclerotiorum.* Among three oilcakes which were assayed against the pathogen mustard oilcake was the most efficient causing 80.25% reduction in the growth of the pathogen followed by neem (68.75%) and til oilcake (66.75%). It was interesting to note that even at 10 percent concentration the oilcakes were fairly effective and showed inhibition greater than 50% excluding til oilcake which showed 44.91% reduction in the growth of *F. oxysporum* f. sp. *ciceri*. Our results are in accordance with the findings of Nikam *et al.* (2007) who depicted that combined application of *T. viride,* ground nut cake, and neem cake in soil gave better control against chickpea wilt caused by *F. oxy.* f. sp. *ciceri*.

Phytofungicides have been reported to be harmless to beneficial organisms such as pollinating insects, earthworms and to humans (Rotimi and Moens, 2003). Khalid *et al*. (2002) stated that their toxic effect is normally of an ephemeral nature disappearing within14-21days. Antimicrobial action of plant extracts have great possibilities as they are easy to formulate and apply. Furthermore, these are safe and effective in view of being systemic in their action and lack residual effect, they are easily biodegradable and exhibit stimulating effect on plant metabolism. Several authors have confirmed the antifungal properties of several plant parts and

phytochemicals (Giridhar and Reddy, 1996; Benharref and Jana, 2006; Satish, 2007; Dwivedi and Enespa 2012). The active constituents which are considered responsible for the antifungal properties of various phyto-chemicals are generally low molecular weight phenolics (hydroxybenzoic acid, flavanoids, hydroxycinamic acid, acetophenone, stilbenes and lignans) as well as oligo or polymeric forms such as hydrolysable and condensed tannins and lignins (Close and McArthur 2002; Okwu, 2004).

5.10 (d) Essential oils

Several studies are going on to explore the potential of essential oils as antifungal agents (Ko *et al.,* 2003; Oxenham *et al.,* 2005). In this study, 9 essential oils have been studied against FOC at 250, 500 and 1000 ppm. The fungus is plant pathogenic and is responsible for disease at field levels. So considering the importance of essential oils as ecofriendly agents, they were studied against the fungi. It is interesting to note that the essential oils of *Ocimum sanctum* and *Cymbopogon jwarancusa* tested, exhibited high antifungal activity against FOC at 1000 ppm. An interesting finding was that seed powder of *Cucurbita pepo* and *Daucus carota* also showed significant reduction in the colony growth of the *Fusarium oxysporum* f. sp. *ciceri* demonstrating 54.16 and 58.33 percent inhibition. However, *Muraya koenigii* was the least effective.

Recently the use of fungicidal chemicals has fallen into disfavour because of their detrimental effects on non target organisms and environment pollution (Evelyn *et al.,* 2004). If natural plant products can reduce population of soil-borne plant pathogens and control disease development, then these plant extracts have potential as environmentally safe alternatives and as components in integrated pest management programs (Bowers and Locke, 2004). Moreover, the essential oils as such may be

exploited for their fungitoxic potency because of the synergistic activity of their different compounds (Tripathi *et al.,* 2004; Sharma and Tripathi, 2006).

Application of essential oils or their main components as a biorational alternative to conventional fumigants in field conditions will require further experimentation (Pradhanang *et al.,* 2003). Attempts have been made to explore the mode of action of essential oils against few fungi, where hyphal wall synthesis was found to be affected by the oils (Zambonelli *et al.,* 1996; Sharma and Tripathi, 2006). The information was found in the literature concerning mode of action of essential oils on/in the fungal cell in order to promote fungistatic or fungicide effect. In general, inhibitory action of natural products on moulds involves cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intercellular and extracellular enzymes (El-Mougy, 2009).

5.11 Efficacy of fungitoxicant against the *Fusarium oxysporum* f. sp. *ciceri*

The efficacy of fungitoxicant *viz.* Carbendazim (Bavistin), Captan, Diniconazole (FOB), Mancozeb (Indofil M-45), Zineb (Indofil Z-78) were assessed on the mycelial growth both *in vitro* and *in vivo.* Carbendazim was found to be the most effective fungicide both *in vitro* and *in vivo* as it inhibited the mycelial growth upto 94% and plant mortality upto 2% respectively. The results are in fair agreement with Dikkar *et al*. (2001) who found carbendazim to be the most effective. Singh and Jha (2003) demonstrated thiram and bavistin to reduce the incidence of wilt when used as seed treatment and soil drenching. Shovan *et al.* (2008) assayed five fungitoxicants namely Tilt-250 EC, Vitavax-200 75% WP, Rovral 50 WP, Dithane M-45 80 % WP and Cupravit 50% WP at 100, 200 and 400 ppm for their efficacy against the radial colony growth and mycelial dry weight of *F. oxysporum* and observed that there was complete inhibition of radial growth at all the selected concentration.

5.12 Management of *Fusarium oxysporum* f. sp. *ciceri* by using botanicals and fungitoxicants

To determine the integrated effect of botanicals and fungitoxicant an *in vitro* test was undertaken followed by pot experiment. The experiment included the following treatments. T1: *Cuminum cyminum* extract (10%) + Carbendazim (0.3%); T2: *Cuminum cyminum* extract (20%) + Carbendazim (0.3%); T3: *Cuminum cyminum* extract (30%) + Carbendazim (0.3%); T4: *Piper nigrum* extract (30%) + Carbendazim (0.1%); T5: *Piper nigrum* extract (30%) + Carbendazim (0.2%); T6 : *Piper nigrum* extract (30%) + Carbendazim (0.3%).

The above mentioned treatments set were prepared as mentioned earlier in the text. (i). All the treatment set *viz.,* (T1, T2, T3, T4, T5, and T6 were assayed *in vitro* using PDA medium, colony growth was measured after 7 days. All the treatment sets were able to significantly check the growth of the wilt pathogen both *in vitro* and under pot conditions. All the treatment sets showed 100 per cent reduction in radial colony growth except for treatment T1 which inhibited the pathogen to 87.74%. The results were reinstated by the reduction in the dry mycelial weight of the pathogen in the treatment sets T1, T2, T3, T4, T5, and T6 as they recorded 0.39, 0.30, 0.13, 0.28, 0.05, 0.02, gm respectively as compared to control (without any treatment) which was observed as 0.48 gm after 7 days of incubation.

Our result are in conformity with Hossain *et al*. (2013) who performed integration of soil treatment with *T. harzianum* isolate T-75 and *A. indica* leaf extract and seed treatment with Provax-200, The treatments appeared to be significantly superior in reducing *Fusarium* wilt and in improving seed yield of chickpea compared to any single or dual application of them in the field. The results of the study exhibit the importance of integrating selective microbial antagonist, botanical extract and

fungicide to achieve appropriate management of *Fusarium* wilt and increase of seed yield in chickpea.

Similar findings were reported by Sultana and Ghaffar (2010) and Nikam *et al.* (2007) who reported that the soil borne diseases of crops incited by species of *Fusarium* are cost-effective to be managed through integration of microbial antagonist, fungi toxicants or organic amendment. Different mechanisms have been suggested as being responsible for their combined or single effect on yield improvement and fungal inhibition. Raju (2005) reported that the lowest disease (pigeon pea wilt) incidence (6.6%), and the highest number of nodules per plant (23.3), fresh weight per plant (6.3 g), and dry weight per plant (2.2 g) were obtained with *T. viride* + carbendazim.

Kapoor *et al.* (2006) found that amendment with bioagent Tricoguard at 2.5 kg or 62 kg FYM ha^{-1} + *Lantana camara* (10 t ha^{-1}) + spray with carbendazim at pre flowering stage was most conducive in managing the root rot-wilt complex disease in pea.

Summary and Recommendations

It is evidenced by various studies that chickpea wilt remains a major biotic constraint limiting the production of the crop. Prudent management of the disease calls for an amalgamation of various cultural practices and the exploration of new avenues which help in the sustainable development of agriculture and are also environment friendly. The present study was taken up to explore disease management through a combination of various approaches, such that it caters to the need of the farmers as well as the environment.

1. Periodic surveys during 2011-12 and 2012-13 cropping season across the six villages of *viz.* Mandhana, Rooma, Singhpur (Kanpur) and Magarwara, Singrossi, Sikandarpur (Unnao) and fields of IIPR Kanpur demonstrated that:

(a) (i) Wilt is a major disease affecting the chickpea crops, (ii) *Fusarium oxysporum,* f. sp. *ciceri* is largely associated with it, (iii) the losses encompassed ranged from (25.63-40.01%), (iv)There was no correlation between area, crop season, and the percentage of wilted plants. It was also noticed that the loss encumbered is on three fronts (i) loss of seeds sown, (ii) loss of productive space, (iii) the net loss of straw and seed productivity.

(b) Two basic patterns were observed on the basis of seeds sown by the farmers (i) broadcast crop and (ii) crop sown in rows. At a glance, the wilted plants occurred in small patches with somewhat circular outline in the former, while in the latter type the wilt disease appeared progressing along the lines.

The isolation, purification of cultures, subsequently proving of Koch's postulate confirmed the wilt causing fungus being *Fusarium oxysporum* f. sp. *ciceri* (Padw.) Snyder and Hansen.

2. Creation of symptoms artificially in pot cultures and the histopathological studies revealed drooping of seedlings from apices followed by loss in turgidity of leaflets

and petioles resulting in collapse of the entire plant. The leaves become chlorotic, turned yellow then straw colored or light brown. Most often the affected plant showed stunting. The collapsed seedlings when uprooted exhibited uneven shrinking of stem on both the sides of collar region, when splitted vertically downwards black discoloration of internal tissues was observed. In the fields when the wilt disease occurred at the flowering and podding stage, the above typical symptoms were noticed in one or two branches of individual plants. The pods were devoid of any seeds. The roots of such plants on isolation yielded the cultures of *F. oxysporum* f. sp. *ciceri*.

3. The histopathology of such affected plants depicted the presence of fungal mycelium in tracheids and vessels of xylem tissues in longitudinal sections of roots. In a few cases chlamydospores were found present but tyloses were very rare. The clogging of vessels by fungal mycelium in the tracheids was frequent but no macro or microconidia were observed.

4. (a) The isolates of *F. oxysporum* f. sp. *ciceri* after purification were grouped according to their discernible cultural and morphological characters into seven groups. The isolates if grouped according to their radial growth on PDA then isolate1, 3, 7 were medium growing and the rest were slow growing where the criteria for growth was fast growing if colony growth was greater than 70 mm, medium growing if growth was between 60-69 mm and slow growing, below 60 mm in 7 days.

(b) The observation on their morphological characters were (i) microconidia most frequent in isolate 4 and 5 and in others frequent (ii) macroconidia one septate most frequent in isolate 4 and in isolate 2, 3, 5, 6 frequent, rare in isolate 1 and very rare in isolate 7 and (iii) three septate macroconidia were frequent in all the isolates.

5. If reckoned on the basis of pathogenic behavior then isolate 7 was weakly pathogenic isolate 3 was highly pathogenic while the rest of the isolates were moderately pathogenic.

6. (a) It was observed that 31.4±1 percent water holding capacity and 43.5 percent pore space of the soil was the most conducive for wilting that was possible by adding 25% sand to the normal sieved soil (sandy loam) of Kanpur. The other soil factors like volume expansion of soil and absolute specific gravity were having no correlation to wilt development.

(b) The depth of inoculum in the pots proved significantly effective in altering the time lag of wilting. The immediate contact of inoculum to germinating seeds was the best suited for quick pathological screening as the plant wilted in 31 days at the temperature range of 21-28°C.

7. Production of cellulolytic and pectinolytic enzymes was observed throughout 7, 14 and 21 days of incubation. It was noticed that (i) there was more production of cellulolytic enzyme than pectinolytic enzyme (ii) maximum enzymatic activity was observed on the 14 day of incubation with cellulose registering 79.09% per cent loss in activity after 90 min while PG recorded 74.76% at the same time interval and day (iii) both the cellulolytic, Cellulase and pectinolytic Polygalacturonase (PG) enzymes showed a visible decrease in their activity on 21st day of inoculation..

8. Interaction of different isolates of FOC with the bioagents *T. harzianum T. viride* and *A. niger* was studied *in vitro* and *in vivo* separately and the interaction behaviour was recorded as per the terminology of Johnson and Curl (1972).

A Mutual intermingling of the two organisms.

B Mutual Inhibition on contact.

C Mutual inhibition at a distance.

D Inhibition on contact, the antagonist continues to grow, at unchanged or reduced rate though the colony of the inhibited organisms.

E Inhibition at a distance, the antagonist continues to grow resulting in a clear zone at an unchanged or reduced rate.

The dual culture of either of any antagonist with the 7 isolate inhibited the growth of the pathogen effectively to the level of 14.16-65.78 %. (a) The interaction pattern of *T. harzianum* with isolate 1, 2, 3, 5 and 7 was of D type whereas the isolate 4, 6 showed E type of interaction pattern *T. viride* revealed C type of interaction with the isolate 5, 6. D type of interaction with isolate 1, 3, 4 and 7 and E type with isolate 2. In case of *A. niger,* B type of interaction was shown by isolate 2, 3, 6 and 7. C type of interaction was depicted by isolate 1and 5. D type of interaction was seen with isolate 4 only while E type of interaction was not observed. The observation led to infer that (i) wherever the antagonist and the above pathogen was making a colony, the antagonist would neither intermingle nor parasitize the pathogen, though hampered the growth of the pathogen in most of the cases (ii) either of the pathogen isolates or the bioagents was juxtaposed to root zone the counter acting would never come nearer to it so if the pathogen was present on the rhizoplane of susceptible chickpea plant then the antagonist will be unable to save the same plant from fusarial attack.

(b) Hence the application of the antagonist should be in soil where the seeds were to be sown or the seeds should be dressed by the antagonist before sowing. So that the emerging nascent roots should come in contact with antagonist first.

(c) The efficacy of all the three bioagents were tested using 3 highly susceptible varities JG 62, Kanpur local and Ujjain local in pot cultures. *T. harzianum* was able to check the wilt incidence by 44, 18.6, 6.5, 00 percent while *T. viride* at same level of inoculums was able to control the incidence by 48.97, 38.29, 17.39, 00 percent of JG

62. However, 8 percent *T. viride* (w/w) proved cent per cent effective against wilt disease in all the varieties tested. The wilt incidence after the application of 2, 4, 6, 8, 10, per cent antagonist *A. niger* (w/w) was 45.8, 19.1, 16.3 and 6.25, 00 sequentially. Thus both the species of *Trichoderma* used were able to control wilt disease when applied at 8% concentration (w/w).Whereas the antagonistic *A. niger* checked the wilt incidence completely when applied at 10 per cent concentration (w/w).

9. The nutrients available in the crop debris and soil were sufficient enough for multiplication of pathogen. Therefore, the growth and sporulation of *F. oxysporum* f. sp. *ciceri* (isolate 1) was assessed on mustard, pigeon pea, chickpea, linseed, urid straw wheat straw and potato dextrose broth media. It was found that urid straw, wheat straw was the best suited for the growth as it yielded maximum dry weight mycelium (1060 mg) as compared to linseed straw which produced minimum biomass (360mg).

The microconidia produced per ml were minimum in chickpea and wheat straw, whereas it was maximum in potato dextrose broth followed by urid straw. Linseed straw produced maximum macroconidia ($9.6x10^2$). The pH in all the broth medium was shifted to alkalinity (pH 6-9). By the results it was inferred that the crop debris plays an important role in perpetuation and multiplication of the wilt inoculum. So, sanitation of the fields after harvesting in Rabi and Khariff crop season may help to control the wilt disease.

10. Control of wilt disease through botanicals (*viz.* plant extracts, seed powders and oilcakes) were assayed during the course of investigation.

(a) Plant extracts

Control of wilt disease by applying cold water and alcoholic extracts of 11 species of medicinal plants were studied by assaying them using food poison technique *in vitro.*

It was inferred that (i) in all the cases the efficacy of alcoholic extracts in inhibiting the pathogen was more as compared to water extracts and (ii) as the percentage of concentration of phytoextracts increased, percentage inhibition also increased that was somewhat in a proportionate manner.

There were 3 plant species whose extract inhibited the growth of FOC to the extent of more than 90%, at 500 ppm, these notable plant species were *Aegle marmelos, Azadirachta indica* and *Callistemon lanceolatus*. There was only 1 plant species *Syzygium cumini* whose water extract checked the growth below 30%, while all the other water extracts inhibited the growth above 30 % at the same concentration mentioned above.

(b) Among the seed powders tested both *Piper nigrum* and *Cuminum cyminum* showed excellent result in checking the radial colony growth of the pathogen at all concentrations. Both of them at 30 % concentration controlled the growth to 90.53 and 74.38% respectively.

(c) Among the three oilcakes tested *viz.* mustard, til and neem at three concentrations (10, 20, 30 %) all of them significantly checked the growth of the pathogen. At higher concentrations all the oilcakes showed superior result in controlling the growth of the pathogen. The most notable among them was mustard oil cake showing 80.25% inhibition followed by neem 68.74 and til 66.75%.

11. 9 Essential oils were extracted and assayed at 250, 500 and 1000 ppm. 4 plant species inhibited the pathogen greater than 60% and these were *Tagetes erecta, Eucalyptus citriodora, Ocimum sanctum* and *Cymbopogon jwarancusa* at 1000 ppm. Inhibition of the pathogen by C*ucurbita pepo* and *Daucus carota* were new findings in the present investigation.

12. The efficacy of five fungitoxicants *viz.* (Bavistin) carbendazim, Captan,

diniconazole (FOB), mancozeb (Indofil M- 45) and Zineb (Indofil Z-78) were evaluated on the mycelial growth of FOC. They were tested in infested pot using highly susceptible variety JG 62. Carbendazim was found to be the most efficacious both *in vivo* and *in vitro* as it inhibited the mycelial growth by 92.64% and reduced the plant mortality to 4%. The next was captan that resulted in 81.31% inhibition of colony of FOC and checked the wilt in infested pots upto 92%.

13. Integrated management trial using botanicals and fungitoxicant in six treatment sets were assayed *viz.* T1: *Cuminum cyminum* extract (10%) + Carbendazim (0.3%); T2: *Cuminum cyminum* extract (20%) + Carbendazim (0.3%); T3: *Cuminum cyminum* extract (30%) + Carbendazim (0.3%); T4: *Piper nigrum* extract (30%) + Carbendazim (0.1%); T5: *Piper nigrum* extract (30%) + Carbendazim (0.2%); T6: *Piper nigrum* extract (30%) + Carbendazim (0.3%). All the treatments set were extremely effective in controlling the pathogen both *in vitro* and under infested pot conditions. All the treatment were cent per cent effective except for treatment set1 comprising of *Cuminum cyminum* (10%) + carbendazim(0.3%) which showed an inhibition of 87.72% and mortality % of 3.03.

The present study brings to take off level (i) with clear identity of the pathogen involved in wilting of chickpea (ii) existing variability in *Fusarium oxysporum* f. sp. *ciceri*. (iii) cellulolytic and pectinolytic enzyme secretions by the pathogen (iv) efficient disease management with botanicals, bioagents essential oils and fungitoxicant. (v) sanitation as an important exercise proved experimentally, (vi) integrated approach of managing the disease as superior to the above management practices when done singly.

RECOMMENDATIONS

The findings of the present study reveal that use of integrated management trials either singly, like use of alcoholic extracts of plants *viz. Callistemon lanceolatus, Azadirachta indica, Aegle marmelos*; oilcakes, like mustard, neem and bioagents like *Trichoderma harzianum, Trichoderma viride*. *Aspergillus niger;* or in combination like using botanicals and fungitoxicants *viz. Piper nigrum* and *Cuminum cyminum* extracts along with carbendazim were potent in checking the growth of the pathogen and can be looked upon as viable options to control the wilt menace effectively in fields.

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Appendix

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EFFICACY OF SOME FUNGAL ANTAGONIST AGAINST CHICKPEA WILT PATHOGEN *FUSARIUM OXYSPORUM* f. sp. *ciceri*

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Abstract: *Chickpea is a well-known rainfed crop of high value. Wilt caused by Fusarium oxysporum f.sp. ciceri (FOC) is the major seed, soil borne disease which results in excessive damage to the crop. The present study was aimed to determine the potentiality of locally isolated bioagents (Trichoderma Harzianum, Trichoderma viride and Aspergillus niger) against seven isolates of Fusarium oxysporum f.sp.ciceri causing chickpea wilt. Under in vitro conditions all the tested antagonist species inhibited the radial growth of the pathogen. Among all the bioagents the inhibition of the pathogen was least with A. niger and maximum with T. harzianum. Under pot experiments all the treatments were able to significantly control the wilt incidence. The bioagents at different concentrations viz. (2%, 4%, 6%, 8% w/w) were tested against the susceptible variety viz. JG62. T. harzianum and T. viride at 8% and A. niger at 10% concentration (w/w), inhibited the wilt incidence upto 100%.*

Keywords: *Bioagents, Chickpea, Wilt.*

Introduction

Chickpea is amongst the predominant grain legume crop grown in India. It's pivotal role in maintaining soil fertility particularly in dry areas has assigned it a special significance in the development of sustainable agriculture of the arid and semi-arid tropical regions. Amongst the major biotic constraints limiting its yield in the Indian subcontinent and the Mediterranean Basin[1], *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceri* holds a cardinal place causing annual losses ranging from to 10% to 100% under conditions favourable for the disease[2- 3].

The disease is primarily managed by resistance breeding programme. The high incidence of pathogenic variability and mutability limits the effectiveness of any naturally selected resistance against the pathogenesis[4]. Disease management with fungicides is uneconomical because of the soil and seed borne nature of the pathogen besides it being hazardous to the environment[5]. Fungicides not only contribute to ground water pollution but also cause loss of non-target beneficial flora and evolving of fungicidal resistance variant of the pathogen[6].

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Management of plant disease through biological control has been considered as a viable alternative method as against the use of chemical pesticide and cultural practices [7-8]. Different mode of action of bio control active micro-organism in controlling fungal plant disease include hyper-parasitism, predation, antibiosis, cross protection, competition for site and nutrient and induced resistance.[9]

Aspergillus species are well known for producing various kinds of active compounds including antifungal and antibacterial[10-11]. Effectiveness of Aspergillus species against tomato wilt, brinjal wilt and foot rot of black pepper causing pathogens , *Fusarium oxysporum* f. sp. *lycopersici* *Fusarium solani* and *Phytopthora capsici* respectively have been reported.[12-13] *Trichoderma* species have become popular biological agents to protect crop against plant pathogen all over the world[14] . They can parasitize fungal pathogen and produce antibiotics [15]. *Trichoderma* species were found as effective biological inducers of plant's own defence mechanism in coconut [16], cucumber [17], and tomato [18-20]

The present study was undertaken to estimate the effectiveness of *T. harzianum, T. viride* and *A. niger* species against chickpea wilt pathogen *Fusarium oxysporum* f. sp. *Ciceri.*

Material and methods

A. Sample Collection

All the 7 isolates of *Fusarium oxysporum* f. sp. *ciceri* (FOC) used in the present study were isolated from roots of wilt infected chickpea plants collected from across six farm fields of Kanpur and Unnao district. The antagonists *viz T. harzianum T. viride* and *A. niger* were isolated from the rhizosphere soil of healthy chickpea plants.

B. Isolation Purification and maintenance of the pathogen

The root of each collected plant sample was washed thoroughly in running tap water and then surface sterilized in 0.01% mercuric chloride solution for one minute. The sterilized root pieces were then kept in potato dextrose agar (PDA) medium, incubated for 5 days at 25ºC. As soon as the growth of causal fungus was obtained it was transferred to PDA slants.

Pure cultures of the pathogenic fungus was obtained by adopting dilution method of Keitt[21]. Small piece of PDA culture along with the pathogen was transferred to a tube containing 10 ml sterile distilled water, shaken vigorously till a homogenous suspension was obtained, 10 fold serial dilution was made up to 10^{-4}. Single loop containing a single spore was then transferred to PDA plate. The colonies so obtained were then transferred to PDA slants and kept at 4ºC for further use.

C. Isolation of the Bioagents

Isolation of the bioagents *viz.* (*T. harzianum, T viride* and *A. niger*) was done from the rhizosphere soil of healthy chickpea plants by serial dilution method of V. N Pathak [22].One ml of soil suspension from dilution of 10^{-5} and 10^{-6} was aseptically added to sterile plate containing 15-20 ml of PDA . After incubation individual colonies were picked up with sterile loop and transferred to PDA slants and kept at 4ºC for further use.

D. Identification of the Fungal strains (Pathogen and Bioagents)

Based on the microscopic studies all the 7 isolates of pathogenic *F. oxysporum* f. sp. *ciceri* were identified on the basis of size and

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shape of the micro and macroconidia [23]. All the bioagents isolated *viz T. harzianum, T. viride* and *A. niger* were identified on the basis of morphological and cultural characteristics [24-27].

E. In-vitro Evaluation of antagonistic behaviour of fungal antagonist against of isolates *F. oxysporum* f. sp ciceri

All the bioagents were inoculated according to dual culture technique of Johnson and Curl [28] on PDA petridishes and the inhibition of the radial growth of the test pathogen in treated and control plates were recorded after one week of incubation. Percent inhibition of mycelial growth of the pathogen was calculated using formula [29]:

$$I(\%) = (C-T)/C*100$$

Where I = percent inhibition, C= colony diameter in in control, and T= Colony diameter in treatment

F. Study of the interaction pattern of the pathogen and antagonist *in -vitro*

The interaction pattern among the pathogen and bioagents was studied according to the key of Johnson and Curl [28] where:
A is mutual intermingling of the two organism
B is mutual inhibition on contact
C is mutual inhibition at a distance
D is Inhibition on contact, the antagonist continues to grow, at an unchanged or reduced rate through the colony of the inhibited organism
E is inhibition at a distance, the antagonist continues to grow resulting in a clear zone at an unchanged or reduced rate.

G. Efficacy of the antagonist in pot experiments

Multiplication of the inoculum of the7 test fungal isolates as well as the bioagents (*T. harzianum T. viride* and *A. niger*) were done as per the method of Miller[30], comprising of 190 gm of field soil sieved through 2 mm sieving mesh , 10gm of finely grounded maize meal and 70 ml of distilled water. The 200 gm of this soil maize meal medium was sterilized in 500 ml of Erlenmeyer flask. Later these flask were inoculated with the test fungal isolates and the antagonist and incubated at 25 ±2ºC for 20 days to obtain the respective inoculums.

H. Preparation of pots infested with F. oxysporum f. sp. ciceri.

For all the pot experiments 15 cm pots were taken, surface sterilized in 5 percent Lysol and then rinsed thoroughly. The pots were then filled with sterilized soil maize meal medium (190:10) and 5 % (w/w) wilt fungus inoculum multiplied on soil maize meal medium. The antagonist *T. harzianum, T. viride* and *A. niger* were mixed at different concentrations *viz.*2, 4, 6, 8 and 10% (w/w) in infested soil sand mixture in 15 cm plastic pots. The seeds @ 5 seeds per pot of susceptible variety JG62, were surface sterilized and sown for a total of 10 pots. The pots were lightly irrigated as and when required.

I. Statistical analysis

All values were expressed as mean ± SD, n = 3 and the results on the percent reduction of colony growth of the FOC isolates *in-vitro* were analysed by analysis of variance (ANOVA). $P \leq 0.05$ was considered statistically significant. Statistical evaluation was carried out using SAS system and the mean values were compared using the Least Significant Difference (LSD) at $P<0.05$.

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3. Results

The perusal of data in Table 1 reveals that the growth of all the isolates of Fusarium oxysporum f. sp. ciceri was inhibited considerably by the bioagents, *T. harzianum, T. viride* and *A. niger (*P<0.05)

A. Inhibition of colony growth of FOC isolates by the bioagents and their respective interaction pattern.

The interaction pattern between the isolates of wilt pathogen and *T. harzianum* was either of D or E type. It was D type (with isolate 1, 2, 3, 5 and7) meaning growth of antagonist *i.e T. harzianum* continuing after coming in contact with *F. oxysporum* f. sp. *ciceri*. Whereas with the other isolates it was E type, meaning inhibition at a distance and the antagonist continued its growth resulting in a clear zone either at an unchanged or reduced rate. The inhibition was significant (P<0.05) ranged from 20.11% (isolate7) to 65.78% (isolate 6) while it was 36.15, 56.64, 61.76, 52.39, 55.65, with isolate 1, 2, 3, 4, and 5 respectively.

The percentage reduction in the colony growth of the FOC isolates with *T. viride* was significant(P<0.05) ranging from 36.16% (isolate 4) to 63.30 %(isolate 5). It recorded an inhibition of 56.26, 59.76, 53.40, 58.79, 48.09 % with isolate 1, 2, 3, 6 and 7 respectively while the interaction pattern was of C, D, and E type meaning (i) mutual inhibition at a distance (ii) the antagonist continues to grow after coming in contact with other organism (iii) inhibition at a distance and antagonist continued to grow resulting in a clear zone at an unchanged or reduced rate respectively. It was E type with isolate 2 and D type with isolate 1, 3, 4 and 7 while C type with isolate 6 and 5.

The interaction pattern between the wilt pathogen *F. oxysporum* f. sp. *ciceri* isolates and *A. niger* revealed minimum inhibition of the former if compared with species of *T. harzianum* and *T. viride.* The interaction pattern was of B, C and D type. Isolates 2, 3, 6, 7 showed B type of interaction where mutual inhibition of fungal colonies took place when they came in contact with each other. They exhibited 23.33, 17.95, 25.55, and 14.16 % inhibition respectively while isolate 1 and 5 exhibited C type of interaction pattern and inhibition was 47.22 and 40.28 % respectively. Isolate 4 exhibited D type of interaction with 21.29 % inhibition.

B. Efficacy of antagonist under pot conditions

The results in Table 2 clearly indicates that all the three bioagents viz. *T. harzianum T. viride* and *A. niger* were effective at all levels of inoculum percentage against the susceptible variety JG62 chickpea seeds. *T. harzianum* at 2. 4. 6 and 8% controlled the wilt as its incidence was reduced to 45.8, 13.1, 16.3, 6.25 and 0%.

T. viride when tested in infested pot with the same sequence of concentrations revealed 48.97, 38.29, 17.39 and 0 % wilting of seeds (JG62). *A. niger* was tested in the same manner. The results showed 45.8, 19.1, 16.3, 6.25 and 0% wilting at 2, 4, 6, 8 and10 % inoculum respectively. It can be inferred that efficacy of *A. niger* was lesser when compared to the other two bioagents, as it required 10% inoculum of *A. niger* (w/w) to check the wilt incidence completely.

Though *T. harzianum* was the best antagonist but *T. viride* and *A. niger* or a combination of the above species may be utilized to check the wilt menace effectively.

4. Discussion

In recent years growing concern against the use of chemical pesticides has forced the

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scientific community to look for various alternative measures to manage plant diseases. The use of biocontrol agents is gaining momentum as it is environment friendly and also compatible with other models of agriculture: organic, biological and integrated pest/pathogen management [31]. Biological control of soil borne plant pathogen is a potential alternative to the use of chemical pesticides which have been proven harmful to the environment [32]. The fungal antagonist may compete for ecological niche by consuming available nutrients and by secreting a spectrum of biochemical. These biochemical may include cell wall degrading enzymes, siderophores, chelating iron, and a wide variety of volatile and non-volatile antibiotics [33] .

The present study was undertaken to assess the effectiveness of *T. harzianum T. viride* and *A. niger* against the chickpea wilt pathogen *F. oxysporum* f.sp. *ciceri*. The *in-vitro* assay revealed that all the three bioagents rapidly colonized the medium and were effective in checking the radial growth of the pathogen. *T. harzianum* was the most effective and was able to reduce the pathogen growth (isolate6) upto 65.78%, while *T. viride* followed closely behind with 63.30% inhibition (isolate 5).

The results are in agreement with various investigations stating the use of *T. harzianum* and *T. viride* as biocontrol agents. *Trichoderma* species when added to the soil or applied as seed treatments have been found to grow readily along with the developing root system of the treated plant. [34-35].

However *A. niger* showed a maximum of 47.22 % reduction in colony diameter of FOC whereas under pot conditions it was 100 percent successful in controlling the wilt at 10% inoculum. The inhibitory effects of *Aspergillus spp*. against several plant pathogens have been reported [36-37] .The positive response of bean plants on the addition of *A. niger* have been reported due to the fungistatic activity or the plant growth promoting activities in soil.[38-39],[29] Alwathnani et al.[32] found that under pot conditions, *T. harzianum* and *A. niger* boosted plant growth significantly and reduced the wilt incidence to (44.4%) and (35.6%), respectively as compared to FOL inoculated plant. The antagonistic potential of the bioagents has been attributed to fungistatic effect [40] or might be due to the secretion of antibiotics by the fungi or other inhibitory substances produced by the antagonists. [41-43]

5. Conclusion

The present study indicates the success of the bioagents *T. harzianum T. viride* and *A. niger* against FOC. *T. harzianum* was found to be most effective under *in vitro* and pot experiments followed by *T. viride* and *A. niger* to control chickpea wilt. The above bioagents could be used as eco-friendly cost effective alternative for the biological control which may help to obtain higher yield and promote sustainable agriculture.

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TABLES

Table 1: Inhibition of radial growth of different isolates of *F. oxysporum f. sp.ciceri* due to *T. harzianum T. viride* and *A. niger* and their respective interaction pattern

S. No	Isolate /Antagonist	Radial growth of isolates on 7th Day in mm(in presence of the antagonist)	Percent inhibition	Type of interaction
1	Isolate 1(Control)	57.16±0.62	**0.00**	
	Isolate 1+*T.harzianum*	37.16±1.31	36.15±0.87	D
	Isolate 1+*T.viride*	25.0±0.81	56.26±1.74	D
	Isolate 1+*A.niger*	30.16±0.84	47.22±1.82	C
2	Isolate 2(Control)	57.83±0.62	**0.00**	
	Isolate 2+*T.harzianum*	25.07±0.49	56.64±1.03	D
	Isolate 2+*T.viride*	23.27±0.60	59.76±1.27	E
	Isolate 2+*A. niger*	44.33±0.57	23.33±1.21	B
3	Isolate 3(Control)	61.26±0.91	**0.00**	
	Isolate 3+*T.harzianum*	23.42±0.46	61.76±0.91	D
	Isolate 3+*T.viride*	28.55±0.48	53.40±0.96	D
	Isolate 3+ *A. niger*	50.26±0.52	17.95±1.04	B
4	Isolate 4(Control)	49.0±0.81	**0.00**	
	Isolate 4+*T.harzianum*	22.62±0.55	52.39±1.37	E
	Isolate 4+*T.viride*	31.25±0.65	36.16±1.64	D
	Isolate 4+*A niger*	38.56±0.49	21.29±1.23	D
5	Isolate 5(Control)	59.5±1.08	**0.00**	
	Isolate 5+*T.harzianum*	26.38±0.46	55.65±0.90	D
	Isolate 5+*T.viride*	21.83±0.62	63.30±1.28	C
	Isolate 5+*A. niger*	35.53±0.55	40.28±1.13	C
6	Isolate 6(Control)	54.48±0.40	**0.00**	
	Isolate 6+*T.harzianum*	18.64±0.26	65.78±0.61	E
	Isolate 6+*T.viride*	22.45±0.72	58.79±1.63	C
	Isolate 6+*A. niger*	40.55±0.60	25.55±1.37	B
7	Isolate 7(Control)	64.5±0.41	**0.00**	
	Isolate 7+*T.harzianum*	51.52±0.46	20.11±0.87	D
	Isolate 7+*T.viride*	34.48±0.61	48.09±1.17	D
	Isolate 7+*A. niger*	55.36±0.76	14.16±1.44	B

Values shown are the mean ± SD of 3 replicates, significant at $p \leq 0.05$

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Table 2: Effect of different concentrations of Trichoderma spp. and *A. niger* on incidence of wilt of chickpea caused by *Fusarium oxysporum* f. sp. Ciceri (isolate 3) at 10 %(w/w) inoculum in pot cultures

S.no	**Different concentrations of the antagonists with 10 % mixed inoculum of *F. oxysporum* f.sp.*ciceri***			**No. of seeds sown @5seeds per pot**	**No. of seedlings emerged**	**No. of plants wilted**	**Percent incidence of wilt**
1	*T. harzianum*	2%	JG62	50	50	20	44
2		4%	JG62	50	48	9	18.6
3		6%	JG62	50	49	3	6.52
4		8%	JG62	50	50	0	0
5	Control		JG62	50	50	50	100
6	*T. viride*	2%	JG62	50	49	24	48.97
7		4%	JG62	50	47	18	38.29
8		6%	JG62	50	46	8	17.39
9		8%	JG62	50	50	0	0
10	Control		JG62	50	49	49	100
11	*A. niger*	2%	JG62	50	48	22	45.8
12		4%	JG62	50	47	9	19.1
13		6%	JG62	50	49	8	16.3
14		8%	JG62	50	48	3	6.25
15		10%	JG62	50	48	0	0
16	Control		JG62	50	49	49	100

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FIGURE

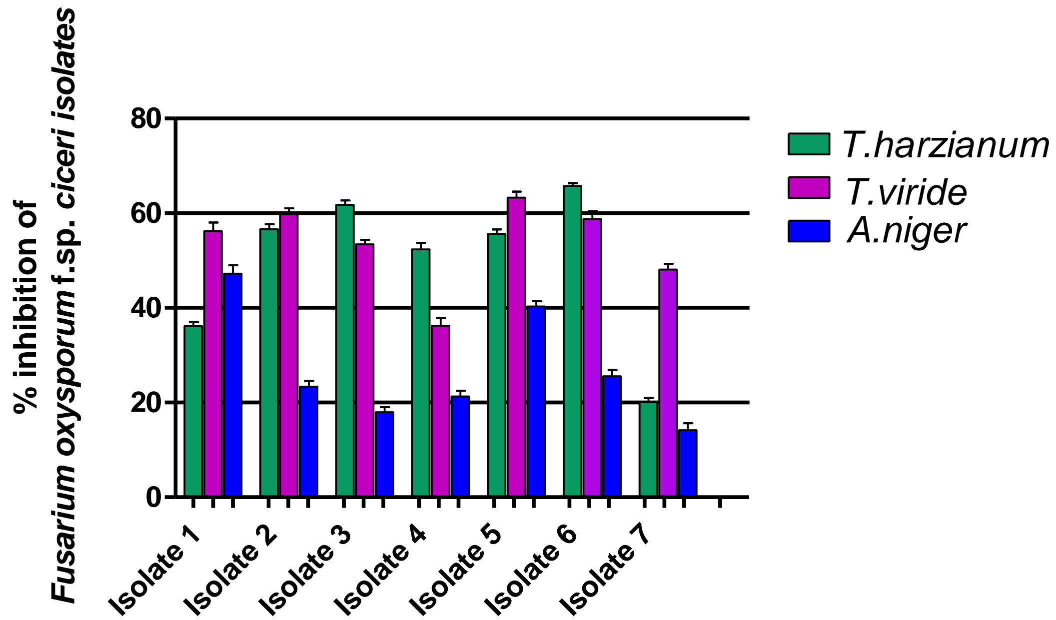

Figure1: Percent inhibition of *F. oxysporum f.sp. ciceri* isolates with the bioagents: All tested bioagents showed significant reduction in the colony growth of FOC isolates. T. harzianum was the most effective among the tested bioagents.

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Assessment of antifungal potentials of some plant extracts against chickpea wilt

Jyoti Srivastava, SK Dwivedi

Abstract
Chickpea enjoys a major share in the pulse cultivation across the globe because of its multifarious uses and its pivotal utility in human diet. Fusarium wilt caused by *Fusarium oxysporum f.sp. Ciceri* is amongst one of the major constraints limiting its yield. The present study addresses the efficacy of four plant extracts in controlling the growth *Tamarindus* of wilt pathogen. For the present study aqueous and alcoholic extracts of *Callistemon lanceolatus, indicus, Terminalia Arjuna* and *Zizyphus jujuba* were prepared at three different concentrations *viz.* 100, 200 and 500ppm concentrations. Alcoholic extracts of all the tested plants were superior as compared to their water counterparts at all concentrations. At 500ppm alcoholic extract of *Callistemon lanceolatus* was found to be the most effective among the tested plant varieties inhibiting the growth to (97.55%) followed by *Zizyphus jujuba* (70.62%), *Tamarindus indicus* (61.68%) and *Terminalia arjuna* (58.89%).

Keywords: Chickpea, wilt, plant extracts.

1. Introduction

Grain legumes play a pivotal role in improving the livelihood, nutritional security of farmers and populations in less developed countries as well as in sustainable agriculture of the arid and semi-arid regions worldwide. Chickpea (*Cicer arietinum* L. diploid, 2n=16) is considered to be one of the founder crops of modern agriculture [1]. It is an important source of human food and animal feed and a significant factor in improving soil fertility.

Of the many biotic stresses limiting its production in the Mediterranean basin and Indian subcontinent Fusarium wilt caused by *Fusarium Oxysporum* f. sp. *ciceri* is the most notable [2]. Heavy annual losses are encountered due to Fusarium wilt epidemic which may reach 100% under conditions favorable for disease [3-4]. Conventional approach of disease management through chemical pesticides is under public scrutiny due to the potential harmful effects on environment, their undesirable effects on non-target organism and possible carcinogenicity [5]. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market [6]. Other practices like crop rotation soil solarization, pathogen free seed have been employed but with limited success.

Hence persistent effort to seek better alternative is inevitable. An interesting vista is the use of natural plant products in controlling the fungal disease due to their being ecofriendly [7] and positive role in sustainable agriculture. Phytofungicides could be prepared or formulated from the leaves, seeds, stem bark or roots of plants and could be applied in the form of extract, powders and cakes or as plant exudates [8].Vast fields in developing countries are blessed with abundant plants with fungicidal potential with preparation and application attracting lower capital investment than synthetic fungicides [9]. Plant extract have been tested against *Fusarium oxysporum* for their inhibitory effect and their control efficacy under greenhouse conditions [10-12] .The present study was undertaken to evaluate the potential of some local plant leaves extracts in reducing the population of *Fusarium oxysporum* f.sp. *ciceri*.

2. Material Method

2.1 Isolation and identification of the test fungal strain

The pathogen *Fusarium oxysporum* f. sp. *ciceri* (FOC) used in the present study was isolated from roots of wilt infected chickpea plants collected from the farm fields of Kanpur and

Unnao district. Roots from diseased plant specimens were sliced into 2 to 3 bits and placed in mercuric chloride (0.01%) solution for 3 minutes and rinsed with sterile distilled water. The pieces were then picked using a pair of sterile forceps, blotted dry and placed in Potato Dextrose Agar (PDA) plates and incubated for 7 days at room temperature (27 ± 2 °C). The isolated fungi was identified on the basis of colour, morphological characters as well as sporulating structure and conidia under microscope and confirmed by available literature [13].

2.2 Source of plant material

Callistemon lanceolatus, Tamarindus indicus, Terminalia arjuna and *Zizyphus jujuba* were collected from nearby areas of Kanpur and Unnao district. Fresh plant material was collected in resealable plastic bags.

2.3 Determination of mycelial inhibition by poisoned food technique

In order to study the effectiveness of some local trees as botanical toxicant , leaves of 4 medicinal plants species viz. *Callistemon lanceolatus, Tamarindus indicus, Terminalia arjuna* and *Zizyphus jujuba* were collected and washed with sterile water. The leaves were air dried and then grinded with the help of pestle and mortar. It was then crushed with equal amount of water (w/w).Centrifuged at 5000rpm for 15 minutes. The filtrate was then taken as 100% stock solution. For the preparation of alcoholic extract alcohol was used instead of water. The extracts were poured in screw cap bottles to avoid contamination. Concentrations of 100, 200 and 500ppm were prepared in PDA medium amended with leaves extract as in food poison technique [14]. The medium in petriplates was then inoculated from 7 day old culture of the pathogen. The Petri dishes containing media devoid of the extracts with same amount of distilled water served as control. Inoculated petriplates were kept at 27 ºC±1in BOD Incubator. Each experiment was then replicated thrice and percentage inhibition was then calculated according to the equation:

$$\% (I) = (dc-dt))/dc\times 100$$

Where I is percentage inhibition, dc is growth of the pathogen in control plate and dt is growth of the pathogen in treatment set plate.

2.4 Statistical analysis

All values were expressed as mean ± SD, n= 3 and the results on the percent reduction of colony growth of the FOC isolates *in vitro* were analyzed by analysis of variance (ANOVA). $P\leq 0.05$ was considered statistically significant.

3. Result

Plant extract obtained from four medicinal plants were evaluated for their potential fungitoxicity. We compared percent inhibition of *Fusarium oxysporum* f .sp. *ciceri* using alcoholic and water extract of the above mentioned four plants at different concentrations as presented in Table 1. One way ANOVA, (followed by Dunnett's multiple comparison test) Test was used to make all comparisons. The assay was performed in triplicate and SD has been shown by error bars. At 100ppm, 200ppm and 500ppm concentrations of different plant extracts, percent inhibition of *Fusarium oxysporum* f .sp. *ciceri* were significant (P<0.001). It was observed that increasing the concentration of the plant extract both of water and of alcoholic extract caused an increase in the percent inhibition of the radial growth of the pathogen *Fusarium oxysporum* f.sp. *ciceri* (FOC). However, it was noticeable that *Callistemon lanceolatus* and *Zizyphus jujuba* proved to be more promising fungitoxicant. At 500ppm alcohol extract of *Callistemon lanceolatus* significantly controlled the growth to (97.55%) (P≤ 0.001) followed by *Zizyphus jujube (*70.62%) *Tamarindus indicus* (61.68%) and *Terminalia arjuna* (58.79%) while aqueous extract of *Callistemon lanceolatus* at 500ppm controlled the growth upto (92.20%), *Zizyphus jujuba* (57.26%), *Tamarindus indicus* (45.51%). *Terminalia arjuna* was the least effective among all the tested plant varieties and showed only (37.91%) reduction with water extract in the colony growth of the pathogen.

Table 1: Percent inhibition in the radial growth of *Fusarium oxysporum* f. sp. *ciceri* at different concentrations of water and alcohol extracts:

S. No	Name of plants	Concentration in ppm	Water extract		Alcohol extract	
			Radial growth in mm	Percent Inhibition	Radial growth in mm	Percent inhibition
1	*Callistemon lanceolatus*	100	40.43±0.75	40.36±1.11	33.1±1.15	50.69±1.71
		200	29.06±0.90	57.71±1.31	25.26±1.10	63.11±1.60
		500	5.4±0.52	92.20±0.76	1.66±0.6	97.55±0.84
2	*Tamarindus indicus*	100	53.13±1.02	21.62±1.51	47.96±1.26	28.54±1.88
		200	50.1±1.01	27.11±1.47	42.1±0.85	38.54±1.24
		500	37.73±0.64	45.51±0.92	26.13±1.02	61.68±1.50
3	*Terminalia arjuna*	100	55.2±0.64	18.48±0.94	50.4±1.25	24.87±1.86
		200	51.76±0.68	24.69±0.99	46.13±1.10	32.65±1.61
		500	43.00±1.00	37.91±1.44	28.1±1.01	58.79±1.48
4	*Zizyphus jujube*	100	51.06±1.00	24.68±1.48	46.4±0.52	30.88±0.78
		200	44.06±1.10	35.89±1.60	41.13±0.80	39.95±1.17
		500	29.6±1.21	57.26±1.75	20.03±1.05	70.62±1.54
	Control	100	67.8±1.31		Control	67.13±1.80
		200	68.74±1.09			68.5±1.32
		500	69.26±0.64			68.2±0.9

Values shown are the mean ± SD of 3 replicates, significant at $p\leq 0.05$

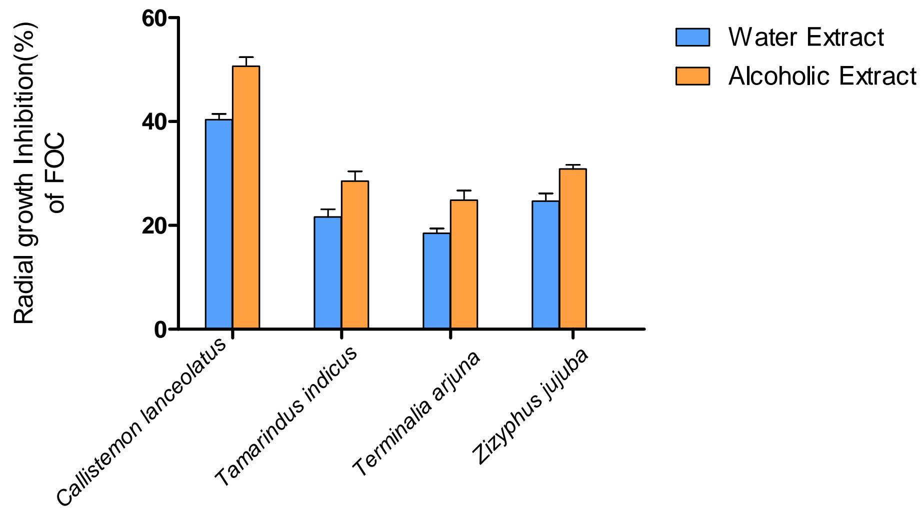

Fig 1: Radial colony growth inhibition (%) of FOC at 100ppm

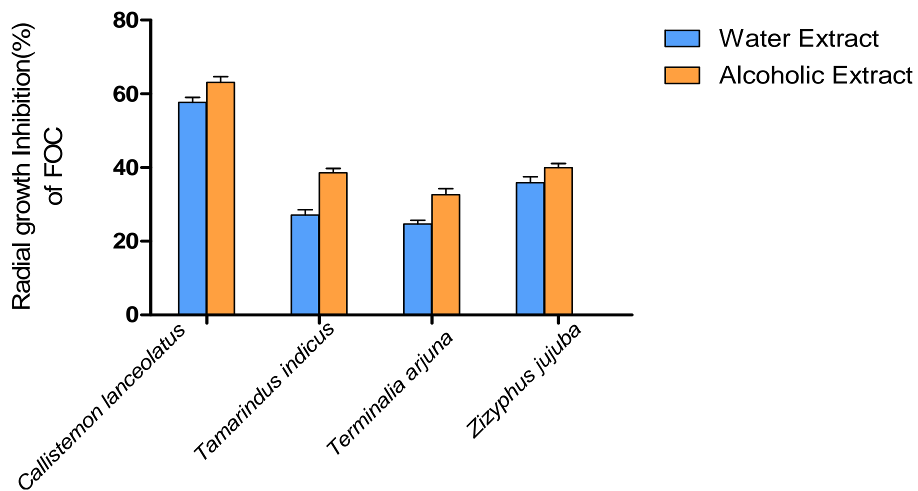

Fig 2: Radial colony growth inhibition (%) of FOC at 200ppm

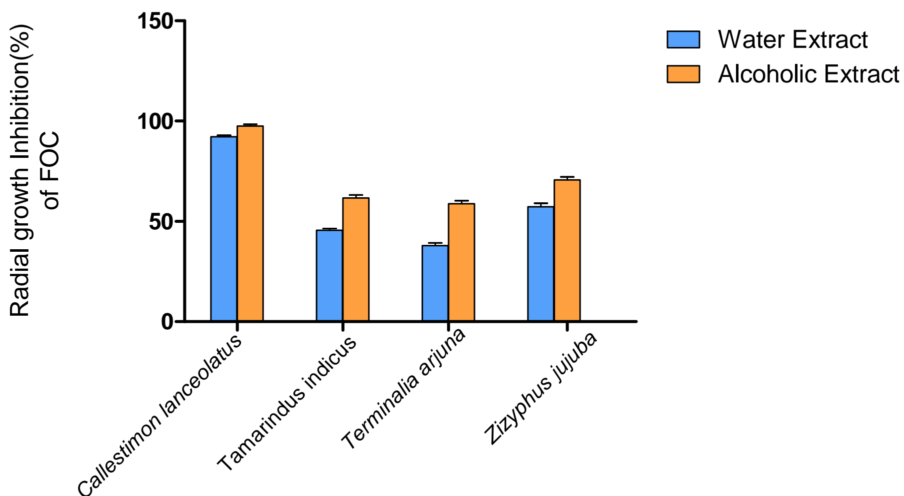

Fig 3: Radial growth inhibition (%) of FOC at 500ppm

4. Discussion

In the recent years, the need to develop fungal disease control measures using phytochemicals as alternative to synthetic chemicals has become a priority of scientists worldwide [15]. Ark and Thompson [16] showed that garlic extracts contain a potent fungicide. They were able to effectively protect peaches against brown rot (*Monilinia fructicola*) with deodorized garlic extract preparations. Singh *et al.* [17] found that essential oils from *Cymbopogon martinii*, *C. oliveri*, and *Trachyspermum ammi* exhibited strong antifungal activity against *Helminthosporium oryzae.* Plant fungicides have been reported to be safe to beneficial organisms such as pollinating insects, earthworms and to humans [18]. Khalid *et al.* [19] reported that their toxic effect is normally of an ephemeral nature disappearing within 14-21days. Antifungal action of plant extracts has great potential as they are easy to prepare and apply. Several authors have confirmed the antifungal properties of several plant parts and phytochemicals [20-22]. The active constituents which are considered responsible for the antifungal properties of various phyto-chemicals are generally low molecular weight phenolics (hydroxybenzoic acid, flavanoids, hydroxycinnamic acid, acetophenone, stilbenes and lignans) as well as oligo or polymeric forms such as hydrolysable and condensed tannins and lignins [23-24]. Antimicrobial properties of numerous plant extracts, polar and non-polar fractions, their pure compounds, and essential oils have been investigated by many researchers against different strains of *Fusarium* [25-29].

5. Conclusion

A large number of earlier workers have reported anti-fungal properties of several plant species. Anjorin [32] reported that combination of two or more plant extracts proved more effective and could reduce the risk of resistance developing by the target fungi. The present study indicates that plant extracts of *Callistemon lanceolatus*, *Tamarindus indicus, Terminalia arjuna* and *Zizyphus jujuba* can serve as a cheap, easily available, cost effective, and a holistic option of managing the wilt of chickpea.

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