FURTHER STUDIES ON SAFFLOWER (Carthamus tinctorius L.) WILT CAUSED BY Fusarium oxysporum f. sp. carthami [Klisiewicz and Houston]

BY

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B. Sc. (Agri.)

Dissertation

Submitted To The Marathwada Agricultural University

In Partial Fulfilment Of The Requirement

For The Degree of

MASTER OF SCIENCE

(Agriculture)

IN

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY

MARATHWADA AGRICULTURAL UNIVERSITY

PARBHANI [Maharashtra] INDIA

1989

DEDICATED TO THE EVER

LOVING MEMORY OF MY FATHER

LATE SHRI SHANKAR RAMCHANDRA NAGALE

CANDIDATE'S DECLARATION

I, hereby declare that the dissertation

or part thereof has not been

previously submitted by

me for a degree of

any University

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PARBHANI

DATE 11 / 8 /1989

CERTIFICATE - I

Shri Shivaji Shankar Nagale has satisfactorily prosecuted his course of research for a period of not less than Four Semesters and that the dissertation entitled "Further studies on safflower (*Carthamus tinctorius* L.) wilt caused by *Fusarium oxysporum* f.sp. *carthami* (Klisiewicz and Houston)", submitted by him is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the dissertation or part thereof has not been previously submitted by him for a degree of any University.

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CERTIFICATE - II

This is to certify that the dissertation entitled "Further studies on safflower (*Carthamus tinctorius* L.) wilt caused by *Fusarium oxysporum* f. sp. *carthami* (Klisiewicz and Houston)", submitted by Shivaji Shankar Nagale to the Marathwada Agricultural University, Parbhani in partial fulfilment of the requirements for the degree of Master of Science in the subject of Plant Pathology has been approved by the student's advisory committee after oral examination in collaboration with the external examiner.

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ACKNOWLEDGEMENT

I avail this opportunity to express my most sincere and heariest sense of gratitude and inebtedness towards Dr. S.M. Pedgaonkar, M.Sc.(Agri), Ph.D., Associate Professor, Department of Plant Pathology, Marathwada Agricultural University, Parbhani (India), on whose inspiration this piece of work was under taken. It was his constant, capable and in valuable guidance, constant encouragement, esteemed interest and constructive criticism right from the selection of problem till the completion of present investigation.

I also feel to my proud privllege to express my deep sence of gratitude to Dr. C.D. Mayee, Head, Department of Plant Pathology, M.A.U., Parbhani for his valuable guidence, keen interest, constructive suggestion and criticism on the present investigations.

My cordial thanks are also to Dr. T.B. Garud, Officer Incharge and Sorghum Pathologist, S.R.S. Parbhani and his staff for providing necessary facilities for conducting the field trial.

I also wish to express my heartly thanks to Dr. B.P. Kurundkar, Assistant Professor of Plant Pathology, M.A.U. Parbhani for helpful suggestions in conducting the experiments.

I gratefully acknowledge my sincere thanks to Dr. B.K. Dhanorkar, Associate Professor of Entomology, member of Advisory Committee for his time to time suggestions and advice.

I would like to record sincere thanks and indebtedness to Dr. V.K. Patil, Dean and Director of Instruction, Faculty of Agriculture and Dr. C.P. Ghonsikar, Associate Dean and Principal, College of Agriculture, M.A.U. Parbhani for completion of research problem.

I am also thankful to all staff members of the Department of Plant Pathology, M.A.U. Parbhani for their valuable help and encouragement.

I also thankful to my colleagues and friends, S/Shri Gaikwad V.N., Sawant K.L., Gole, M.D. Patil, M.D., Chavan S.N., Jadhav P.V., Chandak V.B., Mitkar P.L., Bansode P.T., Mahajan S.J., Thete M.R., Mehtre S.P. and Dange N.M. for their direct or indirect help for making this work enjoyable.

I am highly indebted to kith and kins for their constant encouragement and moral support, especially my mother, uncle, brothers and particularly to Ashok Biradar (ASI) for constantly providing opportunities

inspiration and financial assistance through out the studies in building up my educational career.

I am also thankful to Mehetre G.A. for his valuable cooperation in providing seed of safflower for experiment.

My sincere thanks to Md. Khaleque for typing of the manuscript and Rasheed Photo Studio for help of photographs.

Finally I wish to express thanks to those who have helped med directly or indirectly.

PARBHANI

DATE 11 / 8 /1989

(S. S. Nagale)

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INTRODUCTION

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INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the major rabi oil seed crop of Maharashtra State of India. However, India occupies the largest hectarage under safflower cultivation and ranks second in production next to Mexico in the world (Weiss, 1971).

This crop is potentially an important oil seed crop because it yields various valuable and economic products like safflower edible oil, dye, oil cake, green vegetable, fodder and hull. So the safflower proved to be a valuable crop in the deccan plains viz. Maharashtra, Karnataka and Andhra Pradesh owing its ability to grow on relatively small amounts of residual moisture in soil after post-rainy season.

The per capita availability of edible oil in India is well below recommended level due to many reasons. *Fusarium* wilt (*Fusarium oxysporum* f. sp. *carthami*) is one of the important fungal diseases of this crop which limits or reduces the yield and commercial production.

Safflower being a drought resistant, it is cultivated on all types of soils including sandy soils.

But it thrives well on relatively black and medium black soils and alluvial loams. Therefore, this crop has been adapted extensively in Maharashtra and perticularly in Marathwada region of the Maharashtra State.

Fusarium wilt is appearing every year in the Marathwada region and also increasing because of continuous use of same field for safflower cultivation (Anonymous, 1985, 1986 and 1987).

Further, recurrence of root-rot disease (Macrophomina phaseolina) is also observed in the field to a considerable extent. Appearance and association of both soil borne diseases enhanced economical losses in yields. However, high incidence and intensity of Fusarium wilt posing a new threat for successful and profitable cultivation and production in the Marathwada region. Losses due to Fusarium wilt disease has been reported to the tune of 44 per cent (Jackson, 1985).

The demand of safflower oil has been increasing in the country because safflower oil contains leolic acid which helps in reducing cholesterol level in human blood. Further, the production of safflower has failed to keep pace with it. Considering, the increasing demand of oil seeds in India, it plays vital role in national economy. The past studies did not indicate various basic factors

responsible for losses in yield and also disease development, like role of plant water status and its relation in disease development, host resistance and screening techniques, effect of planting dates in development of disease, role of association of Fusarium wilt and root-rot pathogen, seed borne nature and survival ability of the Fusarium pathogen etc.

Therefore, it is thought essential and also necessary to undertake the studies on these aspects.

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CHAPTER – II

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

2.1 General

Fusarium wilt is posing a serious problem in safflower cultivation in India and particularly some parts of the Marathwada region of Maharashtra State (Anonymous, 1985, 1986, 1987 and 1988).

Safflower is subjected to a number of diseases caused by fungi, bacteria and viruses. Among these, fungal diseases are important and economic one which limit or reduce the yields and commercial production of the crop.

Important and major fungal diseases are :

Rust	: Puccinia carthami (Huiz and Cordo).
Cercospora leaf spot	: Cercospora carthami (Sander and Ramky).
Wilt and head rot	: Sclerotinia sclerotiorum (Lio-de-Barry).
Alternaria leaf spot	: Alternaria carthami (Choudhary and Klissler).
	: Alternaria alternata (Klissler),

Wilt	: Fusarium oxysporum f. sp. carthami (Kli, and Hoston).
Dry root rot	: Rhizoctonia bataticola (Macrophomina phaseolina) (Weiss, 1983)

Safflower wilt caused by (Fusarium oxysporum f. sp. carthami) was widely distributed in Marathwada region during 1987-88 crop season and the disease incidence was varied from 5.71 per cent to 18.88 per cent in general in all the districts of the Marathwada region. Nearly 50 per cent disease incidence was recorded in some villages of Hingoli and Partur taluka of Parbhani district (Mehetre, 1988). Safflower being an important oil seed crop of this region and it occupies a special position in the crop economics. The relevent literature is therefore cited.

2.2 Historical and current status of the disease

Joshi (1924) reported safflower wilt in India for the first time caused by Sclerotinia sclerotiorum wilt and caused much damage at Pusa in 1920. He found out that all Indian varieties under cultivation were affected. The infection was upto 30 per cent.

Classen et al., (1949) observed a root-rot disease firstly in 1948 in Scottsbluff plots on dry land commercial field. The phycomycetes and the species of

Fusarium and Alterneria were commonly isolated from diseased roots. All introduced varieties from India and Africa were 50-100 per cent affected with Fusarium wilt of safflower.

Thomas (1959) reported the attack of Fusarium on safflower (root infection) in the acid soils and since then cultivation of safflower was hampered for continuous period of six years.

Klisiewicz and Houston (1962) reported that Fusarium wilt of safflower was observed during May 1962 in California. Disease incidence was ten per cent and in most fields up to 50 per cent. A characterstic symptoms of diseased plant was a unilateral yellowing of the foliage followed by wilting. Yellowing begins on lower leaves and progresses upward on one side of the plant. The affected leaf tissue gradually turns brown and dies. In this way as the disease progresses the entire plant wilts. A brown discolouration of the vascular bundles appear on one side of the root and stem of plant. The Fusarium wilt of safflower was incited by a form of Fusarium oxysporum schlecht. The fungus was isolated from the tissues of roots, stem and leaves of diseased plants.

Klisiewicz and Houston (1963) reported that the form of Fusarium oxysporum Schlecht as the causal agent of vascular wilt of safflower and it's pathogenicity was conducted on six wild species of genus Carthamus and number of test plants belonging to other genera. They found that isolates from safflower were pathogenic only on the species of Carthamus and pathogenic form there by designated as Fusarium oxysporum Schlechst f. sp. carthami.

Stout (1963) observed safflower wilt and reported the causal organism Fusarium oxysporum in large planting in Yolo country.

Zimmer et al., (1963) reported Fusarium oxysporum f. sp. carthami for the first time in commercial field in California.

Klisiewicz and Thomas (1970 a) reported that two pathogenic races of Fusarium oxysporum f. sp. cathami were identified.

Klisiewicz (1975) reported that a new third race of Fusarium oxysporum f. sp. carthami was identified among isolates collected from safflower in 1973.

Recently this disease has been reported from India to cause considerable loss of the crop as high as 25 per cent (Singh et al., 1975).

Ghosal et al., (1977) reported that Fusarium wilt (Fusarium oxysporum f. sp. carthami) infected seed of safflower when contaminated with healthy seed caused the infection to healthy ones and produced the disease.

Chakrabarti and Basuchaudhary (1978) reported the incidence of wilt of safflower caused by Fusarium oxysporum f. sp. carthami from three districts of Utter Pradesh during their survey.

Nyvall (1979) has given detail account of various diseases of safflower. He has described Fusarium wilt disease caused by Fusarium oxysporum f. sp. carthami in detail, being an important disease of safflower.

Zayed et al., (1980) studied safflower root rot disease in Egypt. Fusarium oxysporum was indentified among isolates collected from safflower in 1973.

Weiss (1983) described a range of diseases which have been recorded on safflower and also enlisted common and major diseases of safflower. He stated that Fusarium wilt caused by Fusarium oxysporum f. sp. carthami is a major and wide spread disease of safflower.

Chakrabarti and Basuchaudhary (1984) reported that Fusarium oxysporum f. sp. carthami invades the tap root of

safflower (Carthamus tinctorius L.) directly or through it's root haris. Penetration of the fungus was reported to be by mechanical means. Pathogen secreted various kinds of enzymes inside the host. This helped the organism to reach the xylem vessels and colonize.

Nirmal (1985) conducted studies on wilt disease of safflower in relation to symptomatology and identification of the causal pathogen of the disease and also screened safflower varieties for resistance against Fusarium wilt in pot culture and reported five cultivars are resistant.

Quilartan (1985) reported that the major diseases of safflower in Mexico were Rust, Stemphyllium, Alternaria, Fusarium and Phytophthora.

It is reported from the oilseeds research station, Latur that wilt percentage was Zero to 24.50 per cent in screening and diseases nursary trial (Anonymous, 1989).

2.3 Management of disease/host resistance to pathogen

2.3.1 Varietal screening for resistance

Fusarium being a soil brone disease, it is very difficult to manage by chemical means. Therefore, to find out good sources of resistant genotypes and breeding resistant varieties will be the solution to minimise

economic losses. Success in evolving breeding resistant varieties against soil borne pathogens largely depends upon the efficiency of the screening technique or method used.

Numerous workers employed in various laboratory as well as field techniques in identifying the sources of resistant genotypes. The available review on this aspect is cited.

Knowles et al.,(1968) examined several seed samples of safflower (Cathamus tinctorius L.) for Fusarium oxysporum f. sp. carthami under laboratory conditions and reported that genetic stock numbers 4 (-2) -4 (-6) were resistant to wilt pathogen.

Klisiewicz and Thomas (1970 b) screened various breeding material for reaction of Fusarium oxysporum f. sp. carthami causing wilt and observed that there are two different pathogenic races on the basis of reaction on the breeding lines. They reported that Nebraska 4051 was resistant to both pathogenic races.

Klisiewicz and Thomas (1970 c) further carried out screening work of various safflower genotypes. Again they reported the presence of third pathogenic race which A-14154, 49 and 2151 were good sources of resistance to all three races.

Klisiewicz (1975) identified four races of Fusarium oxysporum f. sp. carthami among the isolates collected from safflower genotypes during the year 1973 and tested all the four races against various cultivars and available genetic sources. He found that majority of the breeding material was either susceptible or only partially resistant to race four.

Thomas and Hill (1977) studied the effect of the plant age on reaction of eight safflower cultivars to Fusarium wilt. He compared the inoculation of plant by planting seed in infected soil and plants grown 21 days in non infected soil and then inoculated by root deep procedure. He observed four types of reactions of susceptibility and resistance on the basis of cultivar reactions. He finally concluded that inoculation of plant at both the agensies needed to evaluate cultivar resistance accurately.

Klisiewicz and Urie (1982) tested germplasm of safflower which were reported resistant to Fusarium wilt (caused by Fusarium oxysporum f. sp. carthami) in field as well as in green house test under artifical inoculation. They selected initially safflower resistant to races and then observed for field resistance and selected finally for breeding purpose.

Nirmal (1985) screened fifty two safflower varieties for resistance against the Fusarium wilt in pot culture and reported that NS-1016, HOP-22, MYT-28, BLY-211 and BLY-1080 were resistant to disease.

Kulkarni (1987) screened 12 safflower varieties for varietal reaction against Fusarium oxysporum f. sp. carthami and found that genotype BLY-1080 showed resistant reaction, while cultivars N-248, JLSF-71, HOP-116 were moderately resistant.

Pedgaonkar and Mayee (1989) screened 34 safflower genotypes in vitro by water culture technique and in vivo in sick plot. JLSF-88 and N-248 showed 20 and 40 per cent wilting respectively as against 100 per cent wilting in all other genotypes tested by water culture technique. However in field tests none of the accessions was free from wilt. However, 14 cultivars were moderately resistant and 13 were tolerant.

2.3.2 Planting date

Adjustment of cultural practices are the best remedies for reducing soil borne diseases. Planting date and time is one of the important biological functions of control measure. It is well established fact that there is a definite role in early or late planting on development of disease in many crops. Selected references are cited.

Padwick and Bhagwagar (1943) conducted studies on chickpea wilt caused by Fusarium orthoceras var. ciceri on different two sowing dates and reported that late September sowing or first fortnight sowing of October increase the incidence of gram wilt, while second fortnight of October sowing decrease the wilt percentage.

Greaney (1946) conducted field experiment over a period of four years and showed that early sowing of spring wheat considerably reduce root-rot cuased by Drechslera sativum and Fusarium spp. and increase yields.

Studies conducted by Mundkur (1946) on chickpea wilt (Fusarium orthoceras var. ciceri)concluded that crops sown in September was áll most destoryed due to Fusarium wilt, while October planting showed very less incidence of the disease.

Nicholson and Sinchlair (1973) conducted studies on three different planting dateson five cultivars of soyabean at Jabalpur and reported that early sowing produce more seedling and discoloured seeds affected by Colletotrichum dematium. However, latter planting dates or late sowing increased Macrophomina Phaseolina and Sclerotium and Fusarium root rots.

Chakrabarti and Basuchaudhary (1978) studied the incidence of safflower wilt caused by (Fusarium oxysporum f. sp. carthami) and its relationship with the age of host, soil and environmental factors and reported that, disease severity increase with the age of the crop and soil stress.

Deokar et al.,(1984) conducted trial on effect of different dates of sowing on yield of different safflower cultivar. The varieties S-4, B-263-2 A and JL-2 from 26th September to 12th October, gave higher seed yields as compared to their yields at other sowing dates. However, they have not mention about appearance of wilt disease.

Nikam and Patil (1984) reported that 1st October was the optimum sowing time for Tara, S-4, A-1 cultivars in dry land and disease incidence was low.

Ramanamurthy (1985) reported that planting of safflower crop after 1st week of November reduced yields considerable due to major pest and diseases, while sowing time in 1st week of October showed less attack of pest and diseases and yields were increased substantialy.

Mehetre (1988) carried out superimposing observation on wilt incidence of safflower in dry land experiment on

different sowing dates and observed that minimum incidence of Fusarium wilt was reduced on 13th October sowing.

Pangarkar (1989) reported that sowing of safflower in 1st week of October well reduce Fusarium wilt and root rots and other insect pests. Delay in sowing increased the major disease of safflower.

2.3.3 Role of water content in plant and soil and Fusarium wilt disease development

Fusarium wilt of safflower is stated to be commonly associated with soil water deficit or soil stress condition. However, it is not yet clear the role of plant water content in relation to disease development.

Many studies of anotomical, morphological and biochemical changes and of changes in rates of physiological process caused by lack of water have been published, (Kramer 1949). Further, he discussed in detail the effect of internal water deficits on plant in relation to plant growth, stomatal opening, photosynthetic activity, biochemical changes in cell composition and permeablity of roots. According to him, due to change or deficit in soil water, affected number of physiological processes in plant and its water content.

Harrison (1970) reported that potato plants infected with Verticillium albo-atrum showed that there were lower leaf water content than the healthy plant even though disease symptoms were not appear. Further he stated that wilting appear in disease plant when water saturation deficit of 20 per cent was attained, but in healthy plants occured at marginally lower deficits. Further he concluded that due to infection of the pathogen plants were showing internal water stress through-out the season which altimately reduce the rate of photosynthesis.

Harrison (1971) studied the transpiration in potato plants infected with verticillium spp. and reported that both stomatal and cuticular transpiration of single, detached leaves were reduced by infection. A linear correlation was obtained between water saturation deficit and transpiration rate in both diseased and healthy plants until the leaves wilted, suggesting that reductions in the stomatal rate are a consequence of the greater water deficits found in diseased plants, the differences in cuticular rates probably being due to anatomical differences between healthy and diseased leaves.

Close parallels between transpiration and water deficit indicate that in diseased plants water loss is largely determined by leaf water content. Thus wilting,

commonly seen as a symptom of infection, is not the result of excessive water loss but follows a reduction in supply of water to the leaves.

Cook (1973) discussed regarding influence of low plant and soil water potential on diseases caused by soil borne fungi and concluded that low water potential make the host plant more resistant to disease, also spread of the fungus within a plant is more rapid during periods of maximum water flow. Thus, vascular wilt are characteristically more severe in wet than in dry soils, although wilt symptoms develop most rapidly under hot, dry weather.

Water deficits affected photosynthesis in plants by causing stomatal closure, changes in chloroplast activity, reduction leaf growth and senescence of leaves (Boyer, 1976; Kozlowski, 1976; McCree and Davis, 1974).

Chohan (1979) discussed the epidemiology of soil-borne diseases of groundnut and concluded that generally all Fusarium wilt diseases manifests it in dry weather conditions.

Dodd (1980) presented the photosynthetic stress translocation balance concept of predisposition to root and stalk rots in sorghum. According to this theory, root and stalk rot predisposition begins with senescence of

root tissue because of an insufficient supply of carbohydrate for normal metabolic function. The senescing cells, apparently unable to produce normal resistance metabolites, are invaded by microorganisms that are only weakly pathogenic on vigorous cells. As more root tissues are distroyed, the ability of plant to obtaine water from the soil is reduced. The plant eventually reaches the point where transpiration rates exceed water uptake and consequently permanent wilting occurs. Several microorganisms now invade and digest the remaining stalk structure. He concluded that photosynthetic stress influences the stalk root of sorghum. Further, he stated that photosynthetic stresses are affected by water deficits, destruction on leaf tissue, light reduction and mineral deficiencies.

Pruss (1980) discussed the various aspect of epidemiology of soil-borne diseases and stated that crop factor is the most important factor in disease epidemiology and which can not be separated from the environmental factors. Further, he stated that in charcoal rot of sorghum and soybean the response of the crop stress condition is of paramount importance in epidemiology.

Papendick and Campbell (1981) stated that most of the effect of water on cellular functions are closely related to the free energy of water in cells which determines

the water availability for life processes. Water potential is the fundamental concept now widely used in the biological and soil sciences for quantifying the energy of water in plants, microorganisms, soils and other related system is not yet clear.

Duniway and Gordon (1986) discussed in detail water relation and pathogen activity in soil with special reference to Phytophthora root rots and Fusarium spp. from soil and stated that low water potential in soil and infected host tissue increase the incidence and severity of Fusarium, diseases of mature wheat plants. Further he concluded that infected plants which escape the seedling blight of wheat phase of the disease may remain infected and yet appearantly healthy. The water status of the soil is a critical factor in this case because of its effect on water status of host, he concluded that water stress predisposed infected plants to rapid development of root rot disease of wheat.

Schoenewiess (1986) presented a various aspects on water stress predisposition to disease and stated that plant water deficits affect a number of processes and influence host parasite interaction. Further he discussed in detail and stated that, if plants are able to adapt water stress, are they also able to mentain a higher level of effective

defence response under stress than plant are not able to adopt. It may be that threshold levels of water status are required for predisposition to non-aggressive fungal pathogens may be lowered in adapted plants and that imposition of mild water stress by cultural practices may be an effective means of reducting the predisposing effects of drought. Then he discussed water stress predisposition to disease and concluded specifically with regards to vascular wilt fungi and stated that vascular wilts are characteristically more severe in wet than in dry soils, although wilt symptoms develop most rapidly under hot and dry weather, further he also mentioned that the effect of water stress on host predisposition to wilt fungi appear complex.

Waller (1986) reviewed and discussed in his paper on drought, irrigation and fungal diseases of tropical crops and stated that the vascular wilt pathogen, Fusarium oxysporum, is often reported to cause less damage under dry condition and it has been hypotehsised that reduce transpiration apparent under conditions of water stress delays the spread of the pathogen through the vascular tissue. However, this situation does not always occure in the tropics. Many of the stalk and root rots associated with water stress predisposition are of apparently complex etiology. Further water relation and pathogen activity in soil is also most important in soil borne pathogens.

2.4 Some basic studies of the pathogen in disease development

2.4.1 Seed parts carrying infection of *Fusarium oxysporum* f. sp. *carthami*

Species of *Fusarium oxysporum* persist for long period in the soil as a chlamydospere and as a living hyphae in plant debries. The initial inoculum from the soil is readily available and invade the safflower plant causing *Fusarium* wilt. Thus, it is reported by many workers that *Fusarium* wilt of safflower is a seed borne in nature.

Klisiewicz (1963) reported that *Fusarium oxysporum* f. sp. *carthami* was present in the seed from infected safflower. He further investigated that hyphae of the fungus were associated with the xylem and selerenchyma tissue in the pericarp and were evident in sclerenchyma and paranchyma tissue in the seed coat. Further he also observed mycelium and spores were present on the surface of some seed.

Anilkumar and Padaganur (1978) conducted an experiment on eradication of internally seed borne *Fusarium oxysporum* f. sp. *carthami* in safflower and reported that variety 7-13-3 of safflower was heavily infected and pathogen was deeply seated (internally seed borne). The use of single

fungicide could not reduced the internal seed borne inoculum, combination of two different fungicide like Ceresan and Bavistin were able to eradicate complete inoculum of the pathogen.

Nyvall (1979) discribed various diseases of safflower crop and presented epidemology of Fusarium wilts and stated that Fusarium wilt caused by Fusarium oxysporum f. sp. carthami is a internally and externally seed borne disease. Further, he reported that mycelium persists in the endosperm or embronic region in the seed while, condia are associated as a external infection.

Chakrabarti and Basuchaudhary (1984) studied the penetration and invasion by Fusarium oxysporum f. sp. carthami in safflower by way of carrying anotomical and histopathological changes, pathway of invasion and location of the pathogen inside the host tissue and stated that the pathogen invase through root hairs secrets various enzymes reach to xylem vessels and finaly colonizes in different parts of the affected tissues.

2.4.2 Saprophytic survival and determination of inoculum density of Fusarium oxysporum f. sp. carthami from sick and non-sick soils

The fungus persists in the soil for a longer period as dormant chlamydosperes and mycelium. In soil borne

diseases such as root-rots, basal stem rots and vascular wilts has three main components refered by Tarr (1972). The most important components is the servival of the pathogen between crops or alternative host either in a saprophytic phase or as a resting propagules. Therefore, inoculum and inoculum density is most important in epidemology of soil borne diseases.

Thomas (1959) studied Fusarium infection of safflower roots on acid soil.

Garrett (1970) has shown that the success of a root disease organism very much depends on the saprophytic survival of the pathogen in soil.

Smith (1970) reviewed the significance of population of pathogen Fusarium in soil and discussed saprophytic ability of the many Fusarium oxysporum f. sp. cathami and reported that Fusarium oxysporum f. sp. carthami has a rather high degree of compitative saprophytic ability than the others Fusarium spp. in the soils.

Ghosal et al. (1977) reported that acidic soil, light textured soil, high nitrogen and warm, moist weather were favourable for disease development of Fusarium oxysporum f. sp. carthami.

Chakrabarti (1980) studied the role of survival of safflower wilt pathogen Fusarium oxysporum f. sp. carthami and found out fungus could survived in soil and plant debris for a period of one year.

Kulkarni (1987) studied the saprophytic survival nature of Fusarium oxysporum f. sp. carthami of safflower wilt in different types of soil by using soil dilution plate method and concluded that medium black soil favoured and harboured the more number of pathogen propagules in the soil after 150 days in soil when compared to rest of soils.

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********** CHAPTER - III

MATERIALS AND METHODS

CHAPTER - III

MATERIALS AND METHODS

3.1 Collection of sample

Safflower (*Carthamus tinctorius* L.) plants exhibiting typical unilateral yellowing of foliage followed by wilting were collected from the cultivator fields of the Marathwada region and Central Farm of the Marathwada Agricultural University, Parbhani.

3.2 Isolation

Fungus was isolated by usual laboratory methods by planting the affected bits on autoclaved potato dextrose agar (PDA) media after surface sterilization with 0.1 per cent mercuric chloride solution. The culture obtained was purified by single hybphal tip method. Isolated fungus was identified as *Fusarium oxysporum* f. sp. *carthami* by the Mycologist, I.A.R.I., New Delhi.

Pure culture was maintained on potato dextrose agar (PDA) and was renewed after every 15 days.

3.3 Pathogenicity

In pathogenicity test, fungus isolated from freshly naturally infected plant was multiplied on PDA and incubated at 25-28^{o}C. 15 days old inoculum was added in the

sterilised pots containing sterilised soil, and allow to grow for 4 days.

Then surface sterilised seeds of susceptible safflower variety Tara were sown in the above pots. Plants showed wilting symptoms after 15 days. After the establishment of pathogenicity reisolation was carried out from the artifically infected plants. The isolates was exactly the same as that of previous one isolated from fresh field samples. The isolated pure culture was used throughout the course of experimentation.

3.4 Management of disease/host resistance to Pathogen

3.4.1 Screening of safflower genotypes against *Fusarium* wilt by water culture technique

To find out sources of resistance in various genotypes against wilt diseases, generally sick plot or pot screening techniques are used. These techniques are laborious and time consuming. Therefore, to know the disease reaction of various cultivars and to standardise quick method for screening the safflower genotypes against *Fusarium* wilt disease, a water culture technique as suggested by Nene *et al.* (1981) for screening the chickpea wilt (*Fusarium oxysporum* f. sp. *ciceri*) was tried.

The ten days old seedlings already raised were carefully uprooted. Such a seedlings were washed in a running tap water and then rined with sterilized distilled water. One seedling was transplanted in to each test tube containing 20 ml of diluated spore suspension. Spore count per ml was about 6.5×10^5 spores/ml. Five seedlings of each line were used alongwith susceptible Tara line and also non inoculated seedling of each test line as a control.

Observations were recorded for seedling mortality (Wilting) up to 14 days. Disease intensity score was recorded as per disease rating scale suggested by Mayee and Datar (1986). Root, crown and petiole portion of the affected seedling were cut and observed for presence or absence of the pathogen. Categorisation of disease reaction is presented in the Table 1 and 2.

3.4.2.1Effect of different planting dates on incidence of safflower wilt in pots under artificial inoculation

Preparation of standard bulk inoculum

Pure culture of Fusarium oxysporum f. sp. carthami already mentained on PDA was multiplied on potato dexerose liquid medium in 250 ml Erlenmeyerflask in sufficient quantity as usual way. The cultured flask were shaken

constantly during a period of growth. 15 days old culture was used in preparation of fungus soil mixture 2 kg of non autoclaved field soil was mixed in the proportion of one flask of the inoculum. Inoculated soil was throughly mixed aspectically to obtain uniform distribution of inoculum. Such inoculum contain innumerable macro and micro conidai and hyphal growth. Soil inoculum mixture was kept for 4 days to establish the inoculum in the soil, such inoculated soil was used in filling about 30 cm diameter earthen pots. Pots were watered sufficiently so as to obtain soil moisture for sowing the seeds.

The experiment was conducted on susceptible Tara and highly susceptible HOP-123 cultivar of the safflower for each treatment or planting date about 25 surface sterilized seeds were sown in sick soil pots and non-inoculated pots. For each date of sowing treatment 5 pots were maintained along with non-inoculted control. In each pot 5 seeds were sown to have a total 25 plants. Initial sowing was done on 10th September followed by an interval of 10 days up to 30th October comprosing total six different treatments of sowing dates. Observations were recorded after 12 days, on the incidence of the disease up to 60 days and wilt per cents was recorded in each treatment.

3.4.2.2 Effect of different sowing dates on the safflower wilt under natural condition

Superimposed observations were recorded regarding incidence of safflower wilt under natural conditions in an experiment, "Effect of sowing dates on different rabi, crops" conducted in Dryland Agricultural Research Centre at M.A.U. Parbhani.

The experiment was in a split plot design with three replications and having five different sowing dates of six different crops.

Number of plant wilted was recroded in safflower crop in various dates of sowing treatments and mortality percentage was calculated. Disease intensity was calculated as for disease rating score. The data was subjected to statistical analysis.

3.4.3.1 Plant water status and fusarium wilt development

Fusarium wilt disease of safflower is most commonly associated with water deficits of soil and also plant. It was therefore desired useful to study the water status of safflower in relation to development of the disease. In a series of experiments absolute water content (AWC), relative water content (RWC) and water saturation deficits (WSD) of leaf and stem portion were measured taking into

consideration the known tolerant and highly susceptible genotypes of safflower. Measurements of AWC, RWC and WSD were carried out as per the method suggested by Slatyer (1967) and Verma (1978).

3.4.3.2 Planting, inoculation and sampling

Two safflower cultivars JLSF-88 and SSF-56 representing differential host-pathogen interactions such as; tolerant and highly susceptible types respectively were selected for the experiment. They were raised in medium size earthen pots. Each line was planted in 20 pots out of which half of the pots were inoculated with Fusarium culture by using the 'sick plot technique'. The each pot 5 plants were maintained. Plant samples were derived randomly from 30th day onward after planting, at an interval of 15 days. Uniform size samples were uprooted from inoculated and uninoculated treatments and transfered immediately to previously saturated polythene bags and subsequently to the experimental trays covered with black paper to avoid any loss of water.

Pots were watered on every 8th day to maintain uniform soil moisture. Soil samples were similarly collected from the rhizosphere of the plants at the depth of 10 to 15 cm in soil moisture boxes. The boxes were kept in trays and covered with black paper. Initial weight were obtained and

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the soil moisture boxes were oven dried at 105°C for 3 hrs or more till constant weight of the sample. Rhizosphere soil moisture was derived by the following formula.

$$\text{Rhizosphere soil moisture \% (RSM)} = \frac{\text{Fresh wt. } (W_1) - \text{Dry wt. } (W_2)}{\text{Dry wt. } (W_2)} \times 100$$

AWC, RWC and WSD were determined for the plant samples, after they were properly washed and made free of soil particles.

3.4.3.3. Estimation of absolute water content (AWC) and relative water content (RWC)

The plant sample taken from the saturated polythene bag was cut in to 20 pieces of 1 to 2 mm thickness from the basal portion with sharp blade. Care was taken to maintain uniform thickness of the stem pieces and avoiding any type of tissue damage while cutting the stem discs. The cut pieces of stem were divided into two sets, each containing 10 pieces in the previously weighed small pertidishes. The initial or fresh weight of each set was recorded immediately. Set No.1 was then transfered to preheated oven at 70°C for drying for 2-4 hrs. Temperature and time of drying was adjusted according to the need and as per the age of the stem pieces. Care was taken that samples did not over dried or contained moisture, constant oven dry

weight was obtained. Absolute (total water content in the set No.1 was found out as per the formula.

$$AWC\ \% = \frac{\text{Fresh wt.} - \text{Dry wt.}}{\text{Dry wt.}} \times 100$$

3.4.3.4 Saturation of stem pieces

A foam sheet was cut in such a way that it fitted well in a China tray. On this foam sheet a total of 80 wells of 10 mm diameter located at equidistance in a 10 x 8 verticalhorizontal rows were prepared with the help of cork-borer (Fig. 2). The holes were adjusted according to the thickness of the cut samples.

Two vertical row of wells were represented by each cultivar while one row for tolerant and another row for highly susceptible was maintained. The foam sheet was placed in a China tray and saturated with distilled water. Ten stem pieces whose initial weight was recorded earlier (Set No.2) were transferred to the holes with the help of forceps at the rate of one stem pieces per hole in a wet foam sheet, which was already saturated with distilled water completely. The foam sheet was then covered with P.V.C. sheet and pressed gently to express a small amount of water from the foam in to the holes with the discs, so as to provide liquid contact between the walls of tho hole and cut

edges of the discs/pieces. The tray containing the sample was incubated at 25°C for 3 hrs.

The saturated samples from holes of the foam sheet were taken out and placed on a four filter paper pieces for each treatment. The samples were again covered with the same number of filter papers.

The sandwitch so prepared was inverted and pressed gently for 1-2 minutes to remove excess saturated water. The samples were then weighted and transferred to preheated over at 70-100°C for 2-4 hrs till constant weight was obtained. Relative water content (RWC) and water saturation deficit (WSD) was calculated according to the formula:

$$\text{Relative water content (RWC)} = \frac{\text{Initial wt. - Oven dry wt.}}{\text{Saturated wt.-Oven dry wt.}} \times 100$$

$$\text{Water saturation deficit(WSD)} = \frac{\text{Saturated wt. - Initial wt.}}{\text{Saturated wt. - Oven dry wt.}} \times 100$$

3.5 Other basic studies of pathogen :

3.5.1 Site of infestation and parts affected in the seeds due to Fusarium oxysporum f.sp. carthami

Development of seed borne fungi in various parts were studied by the method suggested by Tynar and Mckinnon (1964). An pexeriment was conducted on JLSF-88, Tara and

S-4 cultivars of safflower using artificial inoculation and naturally infected seeds.

In an artificial inoculation 100 healthy looking seeds of said varieties were sterilized as usual procedure initially and then soaked in sterilized water for 24 hrs. Such a seeds were than inoculated with pure culture of the fungus and incubated for 48 hrs to ensure the infection to seed parts. After 48 hrs of inoculation, seeds were picked with the help of sterilize forcep, washed in running tap water initially and then again surface sterilized with 0.1 % mercury chloride solution, were three successive washing were done in distile sterile water.

About 25 seeds were randomly used and testa, integument and embryo (cotyledons + hypocotyl) were separated with the help of sterilized forcep and scalpel. Such separated and removed parts of the seeds were plated on PDA aspectically and incubated at laboratory temperature (26-28^{o}C) for 6 days. The observations were recorded regarding development of fungus on separated parts and presents on fungal flora on it.

In the same way naturally infected seeds of the same variety in same way were also socked in sterilized water for a period of 48 hrs. Such a seeds were initially surface sterilized as usual procedure and cut or separated

in to 3 componant parts viz. testa, integument and embryo. Such cut or separated portions of the seeds were also plated aspectically on PDA and incubated at laboratory temperature (26-28°C) for a period of 6 days. In this case also observations were recorded on separated parts regarding development of fungus.

3.5.2 Saprophytic survival and determination of inoculum density of Fusarium oxysporum f. sp. carthami from sick and non-sick soils

To determine the inoculum density, it was desired useful to assey the potentially pathogenic propagules developed in soil. For this purpose, soil samples were collected from the sick plot at Sorghum Research Station, Parbhani and also from the non affected area (non sick soil). Monthly soil samples were collected upto 8 months and samples were processed for testing. Using the dilution plate count method and selective medium for Fusarium spp. estimation of Fusarium oxysporum f. sp. carthami population was made. The data were expressed as a number of colonies recorded from one gram of soil sample.

3.6 To study the association of Macrophomina phaseolina and Fusarium oxysporum f. sp. carthami in disease complex

Macrophomina phaseolina is a poor competitor in the soil and exist primarly in soil as a sclerosia. Safflower

crop is also suffered from the root rot due to Macrophomina phaseolina. Fusarium wilt and root rot both diseases appeared in the field. Both pathogen also infects through root and rootlets. It was therefore, though worth while to ascertain the associative effect in development of disease complex.

Sufficient quantity of sterilized soil were artifically inoculated with the pure culture of wilt and root-rot fungi in the proportion of one flask for 2 kg soil separately as stated earlier. Mixture of two pure cultures were also made in the 1:1 ratio and then it was added to sterilized soil to have inoculum of soil mixture. Soil inoculum mixture was kept for 4 days to establish the inoculum in soil. 5 pots of each treatment were maintained. Surface sterilized seeds of variety Tara were sown. Five plants were maintained in each pot to obtain sufficient plant population. Un-inoculated control was also run at the same time. Observations were recorded regarding plant mortality in each treatment.

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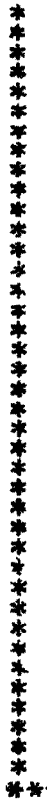
********* CHAPTER - IV



********** RESULTS

CHAPTER - IV

RESULTS

4.1 Collection of soil samples, isolation, pathogenicity, symptomatology of the *Fusarium* wilt fungus

Fusarium wilt affected plants were collected from Central Farm of the M.A.U. Parbhani. Fungus was isolated by usual laboratory method and the culture was purified by single hyphal tip method. Isolated fungus for identified as a *Fusarium oxysporum* f. sp. *carthami* by the Mycologist I.A.R.I. New Delhi. The pathogenicity was proved by making soil sick with organism. Symptoms appeared after 15 days and exhibited symptoms in similar fation as they occure in nature. Culture was maintained on PDA and renewed after 15 days interval. Such a pure culture was used throughout the investigation.

Studies were mostly conducted on host resistance and management of the disease as well as on some basic factors of the disease development. The results on various experiments are presented.

4.2 Screening of safflower genotypes against *Fusarium* wilt by water culture technique

None of the genotypes had shown resistant type of reaction out of 34 genotypes screened (Table 1 and 2).

Table 1. Screening of safflower genotypes against Fusarium wilt by water culture technique.

Sr. No.	Name of the genotypes	Wilting percentage	Disease score
1.	2.	3.	4.
1.	BSF-9-100-2	100	9
2.	BSF-9-51	100	9
3.	CTV JLSF-88	40	5
4.	S-29 A 731	100	9
5.	CTV-14	100	9
6.	MUT LSF-1	100	9
7.	708	40	5
8.	MUT A-1	100	9
9.	MUT No-168-4	100	9
10.	JL-2-2	100	9
11.	CTV-9	40	5
12.	N-887	40	5
13.	HUS-3123	100	9
14.	NRS-209-75	40	5
15.	JLSF-88	40	5
16.	Hop-123	100	9
17.	N-168-4	100	9
18.	BLY-652	40	5
19.	N-62-8	40	5
20.	JLSF-80	40	5

Contd....

Table 1. Contd...

1.	2.	3.	4.
21.	SSF-31	100	9
22.	Annegiri-1	60	7
23.	LSF-23	100	9
24.	S-4	100	9
25.	JLSF-7	100	9
26.	N-248	100	9
27.	JLSF-83	100	9
28.	NC-1585-2	80	7
29.	JLSF-98	40	5
30.	Tara	80	7
31.	HUS-3143	100	9
32.	JLSF-90	100	9
33.	SSF-56	100	9
34.	JLSF-94	60	7

1. * Based on five seedlings of each genotype.

2. The wilting percentage in distilled water (control) was nil.

Table 2. Categorisation of safflower genotypes screened against wilt by water culture technique.

Sr. No.	Particulars	Disease score	No. of genotypes
1.	Resistant	1	Nil
2.	Moderately resistant	3	Nil
3.	Tolerant	5	10
4.	Susceptible	7	4
5.	Highly susceptible	9	20

However, JLSF-88, 708, CTV-9, N-887, NRS-209-75, CTV-88, BLY-652, N-62-8, JLSF-80, JLSF-98 were observed tolerant (40 per cent wilt mortality). Cultivars Annegiri, NC-1585-2, Tara and JLSF-94 are susceptible and remaining 20 were highly susceptible. This technique seems to be suitable for preliminary screening of safflower genotypes and needs field confirmation.

4.3.1 Effect of different planting dates on incidence of safflower wilt in pots under artificial inoculation

This experiment was conducted with a view to know the optimum sowing date in relation to Fusarium wilt disease. Wilt percentage is given in Table 3. Data presented in the table revealed that wilt incidence varied

from 48 to 80 per cent in various dates of sowing. Maximum disease incidence was recorded on 20th September sowing (80 per cent) followed by 10th September sowing (72 per cent). Minimum disease incidence (wilting per centage) was recorded due to 10th October sowing (40 per cent). Sowing beyond the 10th October enhanced the disease to the extent of 64 per cent. It is evident from the above result that the planting date of safflower crop suitable between 1st week of October.

Table 3. Effect of different sowing period on the incidence of safflower wilt in pots under artificial inoculation.

Different dates of sowing	Percent plant wilted due to Fusarium wilt
10th September	72
20th September	80
30th September	60
10th October	48
20th October	56
30th October	64

* Based on 25 plants.

* There was no wilting in control pots.

4.3.2 Effect of different sowing dates on the safflower wilt under natural conditions

It was revealed from the Table 4 that the treatment difference of sowing dates were significant. The disease incidence (wilting per cent) varied from 20.00 to 37 per cent. Minimum disease incidence was recorded on 10th October sowing (20 per cent) followed by 20th October (30.66 per cent). Severity of the disease was increase after 20th October of sowing. Therefore, it is evident that the planting of safflower crop is suitable between 10th to 20th October.

Yield difference due to sowing also significant. The sowing of safflower crop on 10th October has recorded significantly highest yield (641.53 kg/ha) followed by crop sown on 20th October (627.51 kg/ha).

On the basis of wilting per cent and yield q/ha, it is clearly indicated that the sowing of safflower crop between 10th to 20th October will fatch highest yield and less incidence.

Table 4. Effect of different planting dates on the safflower wilt incidence in dry land experiment (super imposing observations).

Sr. No.	Date of sowing	Wilt (Per cent)	Yield kg/ha
1.	30th September	34.33	562.43
2.	10th October	20.00	641.53
3.	20th October	30.66	627.51
4.	30th October	35.33	461.74
5.	10th November	37.00	168.51
	S.E. ±	1.25	7.06
	C.D. at 5%	3.78	21.28

4.4 Water status and *Fusarium* wilt development

Root infecting fungi that causes visible symptoms of root disease, such as root-rot, club root and root-browning are referred as a rhizopathic fungi by Hall (1986). Root infecting fungi are most commonly associated with water deficits. It is now well established fact that rhizopathic water stress enhance the disease. Many rhizopathic fungi (root destriong) cause wilt and other symptoms due to development of water stress in plant. It is however, not clear whether the soil moisture or the water reduction rate of plant decides the development

of disease. With a view to understand a role of plant water and soil water in relation to development of Fusarium wilt, a comprehensive study on plant water content was conducted.

4.4.1 Absolute water content (AWC) in basal stem portion and leaf portion

The two genotypes of safflower exhibited substantial depletion rate of water in stem as well as leaf portion in all the treatment days of observations. AWC was higher initially in the control and inoculated basal stem portions of the genotype. AWC reduced in all the genotype when they were subjected to inoculation and also inoculated parts as evident from the data. However, the rate of water decrement was higher in the inoculated plants of the leaf portion when compared to basal stem region. There was no mark difference in rate of water decrement in periodical measurement in the two genotypes in inoculated plant portions viz. basal stem and leaf portion. However, due to inoculation absolute water content (AWC) was markedly reduced in inoculated plants of stem and leaf portion up to 45 days indicating susceptible behaviour/reaction towards the development of disease.

After 60 days on wards even though there was a remarkable difference in absolute plant water content of the healthy and inoculated plant but the data on AWC in stem and leaf portion indicated that maximum and noticiable water deficits occured during 30 and 45 days of the plant growth.

4.4.2 Relative water content (RWC)

Relative water content did not vary substantially between healthy and inoculated plant portion of stem and leaf. However, low RWC were often observed in inoculated treatments. Similarly, the differences in RWC of two genotypes were also no much significant.

4.4.3 Water saturation deficits (WSD) :

In inoculated plants of the basal stem portion WSD increased marginally irrespective of genotype when compared to healthy. The same trend was noticed up to last sampling stage. It is interesting to note that WSD in healthy as well as inoculated plants of leaf portion did not altered in all the sampling, indicating negative role of WSD in leaf area(Table 5a and 5b).

Table 5a. Absolute water content (AWC), relative water content (RWC) and water stuaration deficit (WSD) of healthy and inoculated basal stem portion of safflower cultivars deffering in disease response.

Cultivars	Basal stem samples after in days	HEALTHY			INOCULATED		
		AWC	RWC	WSD	AWC	RWC	WSD
SSF-56	30	676.74	72.20	27.79	571.11	71.58	28.41
	45	416.32	67.77	32.22	377.66	66.66	33.39
	60	413.04	66.69	33.30	344.61	66.06	33.93
	75	393.93	65.00	35.00	334.01	64.34	35.65
	90	311.42	64.88	35.11	304.29	64.25	35.74
	105	261.29	64.28	35.71	259.45	64.00	36.00
JLSF-88	30	650.00	72.41	27.58	541.06	71.82	28.17
	45	398.00	69.09	30.90	366.66	67.90	32.09
	60	386.17	68.54	31.45	353.73	67.32	32.67
	75	382.30	65.39	34.60	351.72	65.05	34.94
	90	304.37	64.35	35.64	298.78	64.19	35.80
	105	255.31	64.28	35.71	255.26	64.06	35.93

Table 5b. Absolute water content (AWC), relative water content (RWC) and water saturation deficits (WSD) of healthy and inoculated leaf portion of safflower cultivar differing in disease response.

Cultivars	Leaf sample after in days	HEALTHY			INOCULATED		
		AWC	RWC	WSD	AWC	RWC	WSD
SSF-56	30	2815.15	88.98	11.01	2654.28	88.81	11.19
	45	1924.00	86.66	13.33	1842.30	86.61	13.38
	60	1833.96	86.47	13.52	1733.92	86.38	13.61
	75	1596.77	86.23	13.76	1569.84	85.92	14.07
	90	1435.71	85.53	14.46	1421.12	85.36	14.63
	105	1316.66	85.44	14.55	1290.00	85.28	14.71
JLSF-88	30	2752.94	89.91	10.08	2657.14	88.91	11.08
	45	1838.96	87.46	12.53	1807.54	87.40	12.59
	60	1730.35	86.90	13.09	1715.78	86.56	13.41
	75	1573.01	86.09	13.90	1548.43	86.02	13.97
	90	1485.29	85.44	14.55	1395.83	85.24	14.75
	105	1286.25	85.32	14.67	1276.54	85.17	14.82

Table 6. Rhizosphere soil moisture (RSM) and *Fusarium* wilt incidence.

Days after sowing	CONTROL				INOCULATED			
	JLSF-88		SSF-56		JLSF-88		SSF-56	
	Moisture (%)	Wilt (%)	Moisture (%)	Wilt (%)	Moisture (%)	Wilt (%)	Moisture (%)	Wilt (%)
30	43.38	4	42.73	12	42.90	20	45.06	32
45	44.45	12	43.01	24	43.37	36	44.62	56
60	41.17	16	41.53	28	42.55	56	41.89	68
75	39.80	20	41.51	32	40.29	62	40.05	76
90	40.82	20	40.10	32	40.81	66	41.80	80
105	38.38	20	40.74	32	39.67	66	38.86	84

* Based on 25 plants.

4.4.4 Rhizosphere soil moisture (RSM)

Rhizosphere soil moisture percentage was measured at 6 stages of safflower growth in two genotypes and in healthy as well as inoculated plants. Data presented in Table 6. Soil moisture percentage did not vary substantially in healthy as well as inoculated soil pots indicating, there were no soil water stress. However, disease incidence (wilting percentage) in the inoculated pots were recorded maximum up to 45 days. Considering the data on AWC, RWC, WSD and soil moisture percentage was indicative that low water content in the plant were due to inoculation and pathogen activity in the soil at the initial stages rather than soil water stress.

4.5 Site of infestation and parts affected in the seeds due to *Fusarium oxysporum* f.sp. *carthami*

It was observed from the Table 7 that the pathogen could infect the testa, integument, embryo (cotyledons + hypocotyl) region of the safflower seeds in all the tested three cultivars. This indicated external as well as internal seed borne naturo of the pathogen and it was also confermed in artificial inoculation and naturally infected seeds.

Table 7. Infective parts of the seed.

Cultivars	PER CENT SEED SHOWING INFECTION					
	Artificially inoculated seeds			Naturally infected seeds		
	Testa	Integument	Embryo	Testa	Integument	Embryo
JLSF-88	84	80	68	44	32	16
Tara	88	80	80	44	40	24
S-4	88	84	92	52	48	28

* Based on 25 seeds/treatment.

In artificial inoculation of seeds more or less all parts of the seeds in all the cultivar were infected highly. In contrast in naturally infected seeds showed marked variation in infection of different seed parts. Maximum infection to testa (44 per cent) was noticed in case of JLSF-88 cultivar, while greater parts embryo region was affected in Tara and S-4 cultivar. Embryo infection was comparatively low in general than the other parts of the seeds irrespective of genotypes.

4.6 Saprophytic survival and determination of inoculum density of *Fusarium oxysporum* f. sp. *carthami* from sick and non-sick soils

It is evident from the Table 8 that sick soil content more inoculum from the beginning and it was decreased slowly at the end of last observation i.e. after 8 month. Recovery of fungal colonies from the non-sick soil was comparatively very low at the initial stages and it was reduced to zero per cent at the end of 7 months. The above data showed that saprophytic survival and viability of the fungus propagules seems to be period of 7 months in non sick soils.

Graph 1. Saprophytic survival of *Fusarium oxysporum* f sp. *carthami* in ■ → sick and ▨ → non-sick soil.

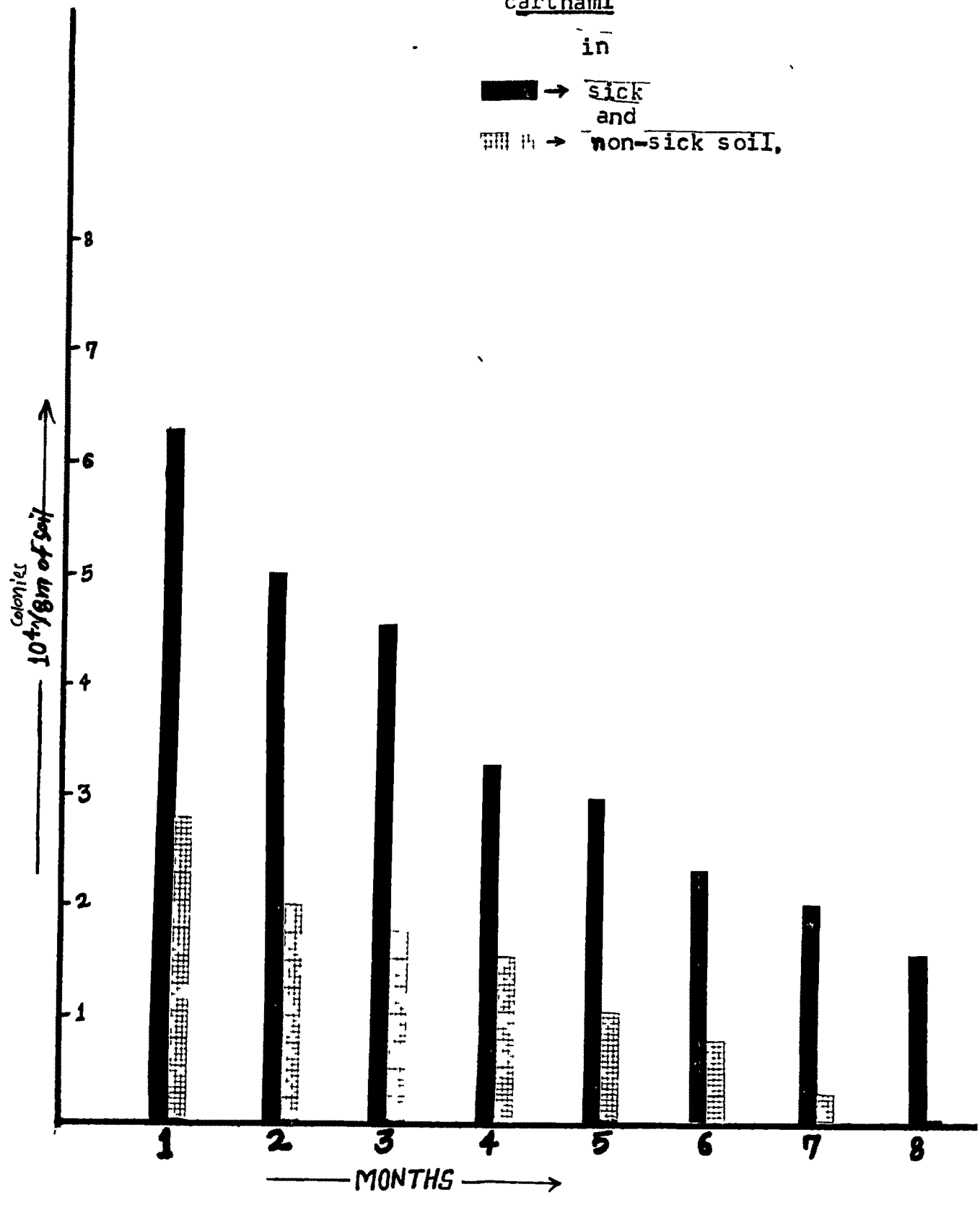


Table 8. Inoculation status of sick and non-sick soil.

Soil sampling in months after crop harvested	Mean number of fungal colonies per gm of soil at dilution 10^4	
	In sick soil	In non-sick soil
1	6.25	2.75
2	5.00	2.00
3	4.50	1.75
4	3.25	1.50
5	3.00	1.00
6	2.25	0.75
7	2.00	0.25
8	1.50	-

* Mean of 4 replications.

4.7 To study the association of Macrophomina phaseolina and Fusarium oxysporum f. sp. carthami in disease complex

Data presented in Table 9 revealed that Fusarium and Macrophomina in combination increased the severity of the disease to the tune of 82 per cent.

Wilt fungus (Fusarium oxysporum f. sp. cathami) recorded highest plant mortality (wilting per cent)

due to individual inoculation as compare to Macrophomina phaseolina. From the data it could stated that there were synergestic effect of the two pathogenic organisms.

Table 9. The asociation of Macrophomina phaseolina and Fusarium oxysporum f. sp. carthami in disease complex in Tara variety.

Per cent plant mortality due to		
Fusarium oxysporum f. sp. carthami	Macrophomina phaseolina	F.oxysporum f.sp. carthami and M. phaseolina
68	26	82

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Fig. 1 Showing cut parts of stem and leaves portions from non-inoculated and inoculated plants.

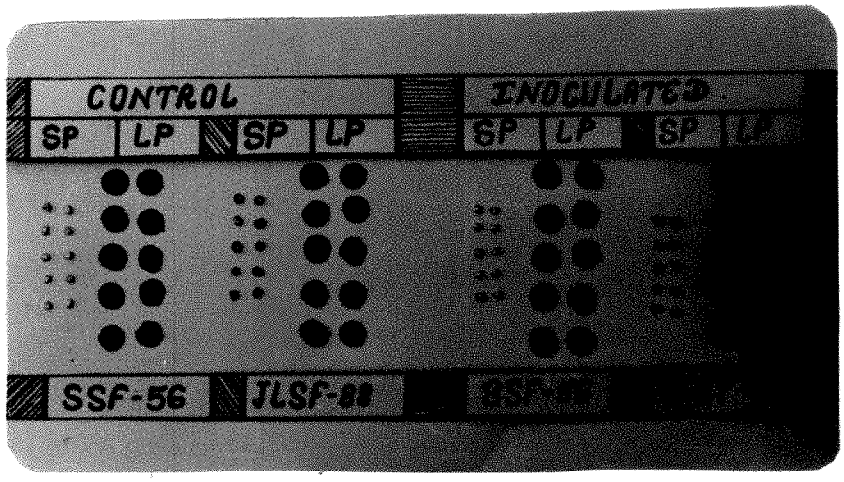
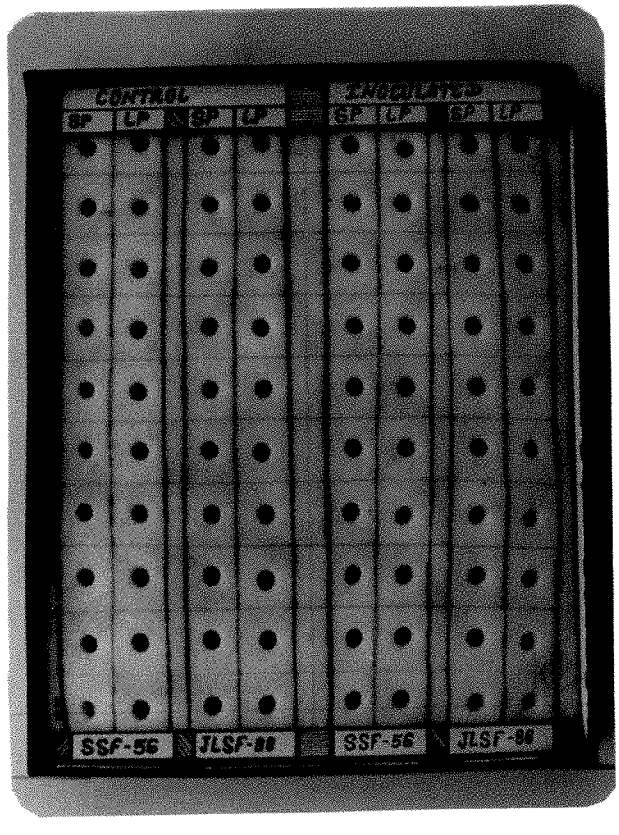


Fig. 2 Showing wells in the foamsheet for saturation of cut stem and leaf portion.



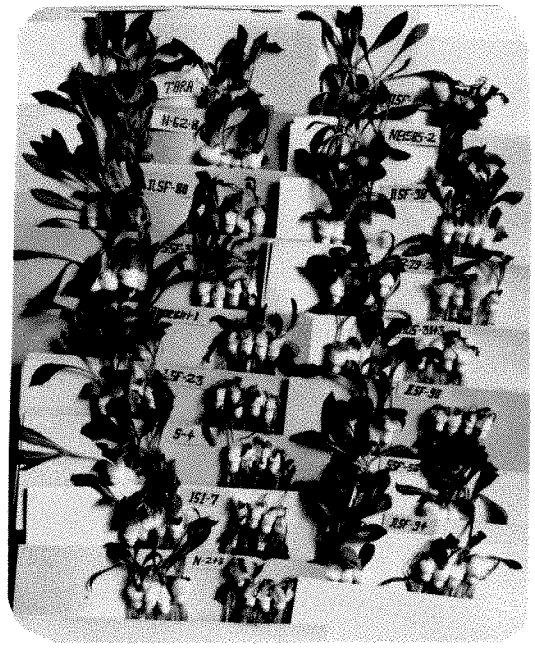


Fig. 3 Screening of safflower genotypes by water culture technique.

H - Healthy (Non-inoculated).

I - Wilted (Inoculated).

Fig. 4 Showing severe wilting symptoms in inoculated soil due to Fusarium oxysporum f. sp. carthami after 20 days.

1. Non-inoculated
2. Inoculated

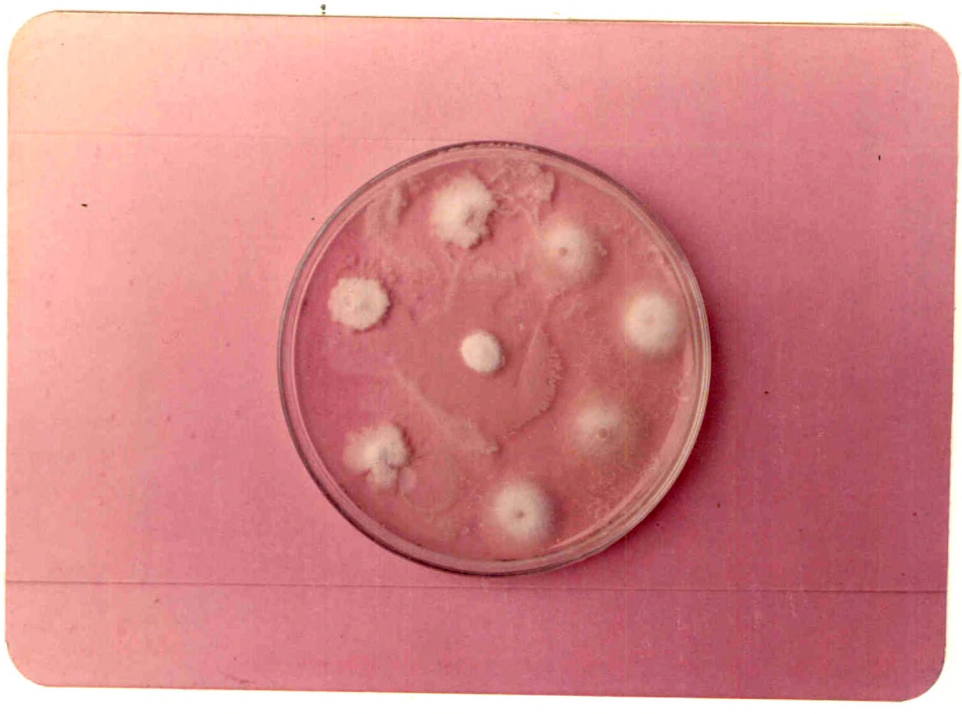


Fig. 5 Showing affected basal-stem cut portions with *Fusarium oxysporum* f. sp. *carthami* on agar plate.

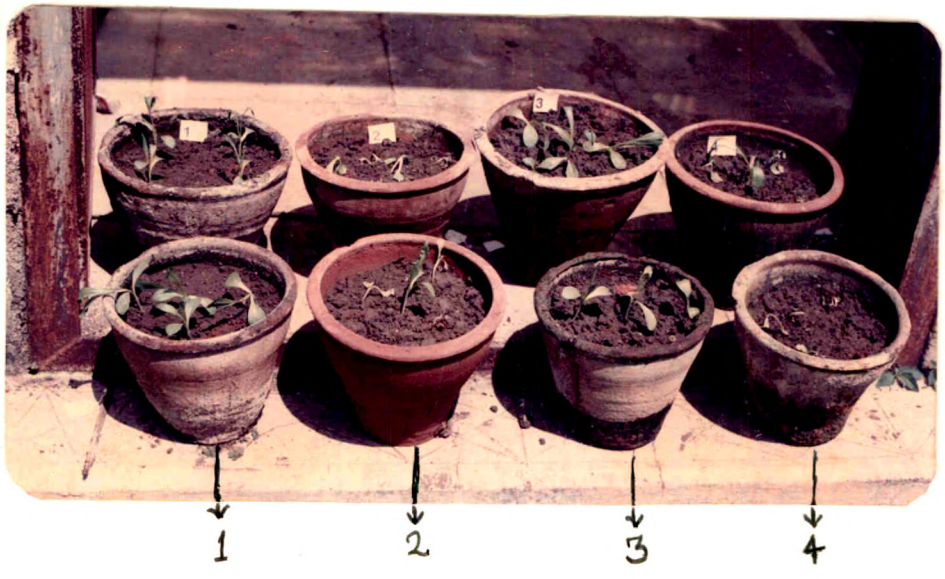


Fig. 6 Showing the effect of individual and mix inoculum of wilt and dry root-rot fungi after 15 days.

1. Control (Non-inoculated)
2. Inoculated with F. oxy. f. sp. carthami alone
3. Inoculated with M. phaseolina alone
4. Mix inoculum of F. oxy. f. sp. carthami and M. phaseolina.

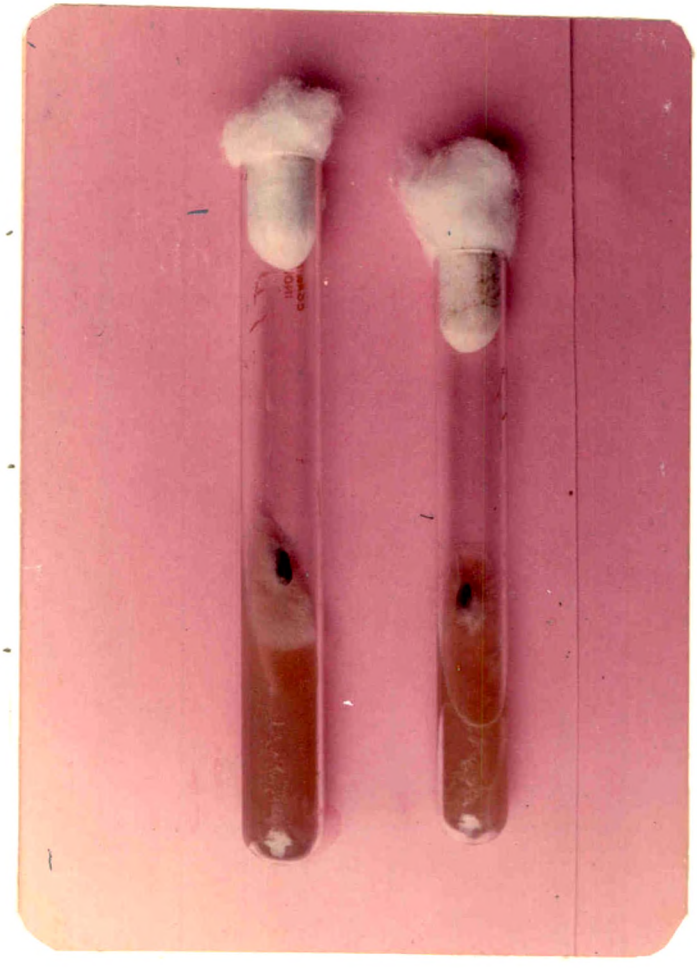


Fig.7 Showing appearance of colony of wilt fungus from affected and non germinated seeds from the inoculated soil.

********** CHAPTER - V

********** DISCUSSION

CHAPTER - V

DISCUSSION

Safflower (Carthamus tinctorius L.) is one of the major rabi oil seed crop of Marathwada region of Maharashtra State. Fusarium wilt of safflower caused by Fusarium oxysporum f. sp. carthami is one of the important and major fungal disease of this crop in this region. The disease is appearing every year and also increasing because of continuous use of same field and susceptible cultivars for safflower cultivation (Anonymous, 1985, 1986, 1987 and 1988). The disease occures in endemic form in many safflower growing areas of the world (Klisiewicz and Hoston, 1962; Zimmer et al., 1963; Zayed et al., 1980; Weiss, 1983 and Quilartan, 1985).

Fusarium oxysporum f. sp. carthami normally exists in the hyphal and chlamydospores form and the inoculum present in plant debries and soil. The main symptoms are unilateral yellowing of foliage followed by premature wilting. A brown discolouration of vascular bundles appears in one side of roots and plants with unilaterla top symptoms. Further, it may also cause roting of roots and rootlets (Klisiewicz and Houston, 1962).

This disease is reported as a wide spread disease and it causes considerable losses in safflower growing areas of the world and also in India and particularly in Marathwada region of Maharashtra State (Joshi, 1924; Klisiewicz and Hoston, 1962; Singh et al., 1975; Weiss, 1983 and Anonymous, 1989). Since the losses encountered were alarming, an investigation were directed so as to understand basic factors responsible for losses in yield and disease development, host resistance by adopting screening technique, management of the disease by knowing the effect of planting dates and role of plant water and soil water status in relation to disease development, etc.

Affected samples exhibiting symptoms of unilateral yellowing and premature wilting of plants were collected from the endemic areas perticularly from Hingoli and Partur taluka of Parbhani district. The wilt fungus was isolated and maintained on PDA. The pathogenicity of the isolate was proved and confirmed by sick soil method. Symptoms appeared after 15-20 days and exhibited similar wilt as that occur in nature.

The basic requirement of any resistant identification programme is meaning full only when an affective and quick screen technique is available. With this view, water culture technique for screening chickpea wilt as suggested by Nene et al. (1981) was tried.

A total of 34 promising safflower cultivars received from oilseed Research Station, Latur were screened using water culture technique. The results showed remarkable difference in wilting reaction when seedling were exposed to 14 days. None of the genotype was found free from wilt. However, tolerant susceptible and highly susceptible disease reaction were recorded. Cultivars JLSF-88, 708, CTV-9, N-887, NRS-209-75, CTV-88, BLY-652, N-62-8, JLSF-80, JLSF-98 were observed tolerant (40 per cent wilt mortality). Rest of the cultivars recorded either susceptible or highly susceptible disease reaction (Table 1 and 2).

Many workers employed various techniques like sick-soil in pots, developing sick plot in field or under natural conditions for screening the germplasm and elite cultivars. (Knowles et al. 1968; Klisiewicz and Thomas 1970b; Klisiewicz and Thomas, 1970c; Klisiewicz, 1975; Thomas and Hill, 1977; Klisiewicz and

Urie, 1982; Nirmal, 1985; Mehetre, 1988). For quick and prelimanary screening Pedgaonkar and Mayee (1988) used water culture technique in laboratory and reported t that JLSF-88 and N-248 showed 20 and 40 per cent wilting as against 100 per cent wilting in all other tested genotypes. The present findings are contradictory to the above finding in respect of disease reaction as evident from Table 1 and 2. However, water culture technique for preliminary screening was found to be good and quick method as suggested. This technique is also useful in discarding highly susceptible variety at initial stages and also reduce the labourious work in field. It could be stated that prior to screening of various elite material in sick plot, the material should be screened by water culture technique in laboratory which would reduce the extra labourious work and managable research programme in the field. However, it needs further conformation by repeated experiments. To varify and the substantiate the results obtained in the water culture technique a superimposing observations regarding wilt incidence were recorded in a trial "Effect of sowing dates on different rabi crops" conducted under the Dryland Research Project, MAU, Parbhani. In the similar way a separate study was also

under taken under epiphytotic conditions to find out suitable and optimum date of sowing in relation to Fusarium wilt. Result revealed that sowing of safflower crop between 1st week of October was more suitable and optimum period, as the disease incidence was at minimum level. Response to very early sowing and advancing the date of planting beyond 1st week of October (after 10th October) resulted increase in percentage wilting gradually (Table 3).

The observation recorded in dry land experiments are nearly inconformative with the results of early experiment of effect of different planting dates under the artificial inoculation. Considering the results of planting dates experiment under artificial inoculation and natural conditions, sowing of safflower crop should not be extend beyond the 10th October as it will suffer from maximum disease incidence and also reduce the yield (Table 4).

It is evident from the data on both experiments that late sown crops could picked up the infection probably because of vulnerable stage of crop growth, favourable soil moisture, temperature and weather conditions. Results are inagriment with the results of (Chakrabarti and Basuchaoudharyl 1978); who also

concluded that age and stage of the crop was important factors in induction of wilt disease. Various workers also concluded and reported that Fusarium wilt disease incidence were increased and there by reduced the yield potentially in many crops like chickpea, soyabean etc. when sowing delayed beyond the October. Findings of (Ramanamurthy, 1985; Mehetre, 1988; and Pangarkar, 1989) also supported the present finding that, 1st week of October sowing is a more suitable and optimum period for sowing of safflower crop.

To examine plant and soil water content in healthy and inoculated plants in relation to disease reaction and behaviour of the pathogen, a comprehensive study was conducted. Water constitues more than 80 per cent of the most plant cells and tissue. In this region active metabolism activity is carried out. A little change of water content in plants resulted cessation of most growth process (Slatyer, 1967). To a large extent soil moisture determine the plant water potential to which pathogen in leaves, stems and fruits are subjected. Soil water deficits on plant growth and disease development has been extensively studied. But there are very few studies in relation to internal plant water deficits and its relation to pathogen

activity behaviour, kind and type of host genotype and disease development. Plant water deficit affects a number of processes and even change in a small balance influence host, parasite interaction (Schoeneweiss, 1986).

Fusarium wilt of safflower is usually associated with high soil temperature, drought stresses and senescence (Chohan, 1979). It is now well established fact that the water stress in plants is a predisposing factor to invasion by many soilborne and root-roting fungi particularly by Macrophomina phaseolina in many crops. Water deficits affected photosynthesis in plant and number of physiological processes (Boyer, 1976; Kozlowski, 1976; McCree and Davis 1974) which ultimately affected plant water balance and it favours to invasion of pathogen (Dodd, 1980; Papendick and Compbell, 1981; Duniway and Gordon, 1986).

The results of present investigation showed that actual plant water potential are determined by the host genome even at uniform soil moisture level. Results on AWC revealed that two genotypes of safflower exhibited uniform reduction rate of water in inoculated basal stem as well as in leaf portions in all the treatments when compared to healthy plants.

The WSD is considered as a useful indicater of the water status of the plant, mainly because it expresses, the absolute amount of water which the plant requires to reach full saturation. WSD is the amount of water required by plant or plant part to attain full turgor or saturation. The WSD increased substantially in inoculated plants of the basal stem portion irrespective of the genotypes when compared to healthy, indicating water stress occured in the basal stem portion only; which ultimately help to wilt pathogen for invasion. However, the effect of water stress on host predisposing to wilt fungi appears to be complex (Scheoneweiss, 1986).

Very interesting results are obtained in case of WSD in healthy as well as inoculated plants of leaf portions. It is evident from the results (Table 5a and 5b) that there was negative role of water saturation deficits in leaf area. These results suggested that WSD of basal portion had prime importance than the leaf portion in Fusarium wilt disease development. However, it will be premature to conclude on the basis of present results and needs further conformation by repeated experiments.

Soil moisture studies showed that there were no remarkable difference in soil moisture percentage in healthy as well as inoculated soil pots. However, disease incidence (wilting percentage) increased in the inoculated pots up to 45 days to a great extent and there was no appreciable increase in wilting percentage in the latter stages of sampling.

The present results can be interpreted in general that low water content or water deficit in the plant was due to inoculation and pathogen activity in the soil and plant at initial stages rather than the water deficit in soil.

In the present investigation some basic studies of the pathogen in disease development were also conducted and reported.

Fusarium oxysporum f. sp. carthami has also been reported to be a seed borne (Klisiewicz, 1963). Detection of inoculum in various parts of the seed viz. testa, integument, embryo was positive and inconformative with the results of (Klisiewicz, 1963; Anilumar and Padaganur, 1978; Nyvall, 1979; Chakrabarti and Basuchaudhary, 1984). Seed borne nature was varified by artificial inoculation of seed as well as in the naturally infectes seeds. Results

revealed that (Table 7) in artificial inoculation all the parts of seeds were affected badly. In contrast, infection to various seed parts varied with the genotypes in naturally infected seeds. Findings indicated that seed borne inoculum has a greater role in transmission and establishment of disease in new area through the susceptible cultivars.

Saprophytic survival ability and inoculum density from the stick and non-sick soil were also studied up to 8 months. The inoculum of Fusarium oxysporum f. sp. carthami reported to be persist in the soil for a longer period as a dormant chlamydospore and mycelium (Tarr, 1972). Results in the present investigation showed that saprophytic survival ability of the fungus found to be 7 months in non-sick soil as evident from the recovery of fungal conolonies as against in the sick soil (Table 8). Saprophytic sarvival ability of Fusarium and Fusarium spp. were studied by number of worker by using dilution plate technique and reported that it varied with the type of surviving structure, type of soils,texture of soil, pH of the soil etc. (Thomas, 1959; Gorrett, 1970; Smith, 1970; Ghosal et al., 1977; Tarr, 1972 and Kulkarni, 1987). The present results did not inagreement of the results of the

Chakrabarti (1980) who reported that Fusarium oxysporum f. sp. carthami could survive in soil and plant debries for a period of one year. The probable reason would be the existance of Fusarium wilt pathogen races (Klisiewicz and Thomas, 1970 a; Klisiewicz, 1975).

To ascertain the role of Macrophomina phaseolina (Rhizoctonia bataticola) in association with Fusarium oxysporum f. sp. carthami in disease complex was studied by conducting a pot experiment in screen house. Result show (Table 9) that disease severity increased substantially due to mix inoculum of wilt pathogen and root rot pathogen (82 per cent). In single inoculation Fusarium wilt was comparatively high (68 per cent) than the dry root rot caused by Macrophomina phaseolina (26 per cent). Wilt symptoms became apparent 15-20 days after sowing and then increased in mortality a month later. Dry root rot (Macrophomina phaseolina) appeared after about 3 months when soil temperature exceeded above 30°C. The present findings indicated that there were synergestic effect of the two pathogens which ultimately enhanced severity of the disease.

Disease complex of wilt (Fusarium oxysporum f.sp. ciceri) and root rot fungus (Macrophomina phaseolina) in chickpea crop under different cropping system is reported and disease situation is inferred that in open

condition, Fusarium wilt was found to be the predominant pathogen and not Macrophomina phaseolina (Anonymous, 1986). However, in association of Fusarium wilt and Macrophomina phaseolina fungi enhanced the disease intensity depending up on the initial inoculum population in the field. Current findings are also provides supportive evidence that Fusarium wilt fungus initially infected safflower crop and Macrophomina phaseolina colonizes latter stages of growth and the disease severity increased due to associative effect the two pathogen.

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CHAPTER – VI

SUMMARY

CHAPTER - VI

SUMMARY

Wilt caused by Fusarium oxysporum f. sp. carthami is one of the important fungal diseases of safflower in the Marathwada region of the Maharashtra State. It expresses in the form of unilateral yellowing followed by premature wilting. The disease appeares generally 15 to 20 days of sowing and severity increases after 2 to 3 months. The disease is basically soil borne in nature and practically nothing can be done to prevent the damage. Continuous use of same field and use of susceptible cultivars, the disease is appearing every year and spreading fast. Therefore, present investigation was undertaken with a view to understand basic factors responsible in disease development and to know the host resistance and develop suitable management practices.

Wilt fungus was isolated from the affected samples and pathogenicity was proved. Total 34 elite and promising genotypes were screened using water culture technique in laboratory. None of the genotypes was found free from wilt. Cultivars JLSF-88, 708, CTV-9, N-887, NRS-209-75, CTV-88, BLY-652, N-62-8, JLSF-80

were recorded tolerant type of reaction (40 per cent wilt mortality). Rest of the cultivars were either susceptible or highly susceptible. Water culture technique was found suitable method for quick and preliminary screening of large germplasm and cultivars.

Effect of planting date experiments (artificial as well as natural conditions) showed that planting of safflower crop, beyond 10th of October badly affected with the Fusarium wilt disease and also reduced substantially yields.

The role of water potential of basal stem and leaves was examined in the development of wilt disease. It is revealed that differences in absolute water content (AWC), relative water content (RWC) and water saturation deficit (WSD) in basal cut stem portion existed within the genotypes. However, negative role of WSD noticed in cut leaf portion. Soil moisture studies in the healthy and inoculated soil pots indicated that appearence of disease to considerable extent and there was negative role of soil moisture and soil water stress.

Seed borne nature of the wilt fungus was varified by artificial inoculation to seeds as well as from the naturally infected seeds using component

plating method. Results revealed that in both methods seed parts were affected with varying degress. Moreover, wilt fungus (Fusarium oxysporum f. sp. carthami) detected maximum in testa region of the seed than the others.

Saprophytic survival ability and inoculum density of the wilt pathogen in the sick and non-sick soil was examined using soil dilution and plating technique for upto 8 months. Findings showed that fungus (Fusarium oxysporum f. sp. carthami) could survive seven months in natural and non-sick soil.

Pot experiment studies on role of association of wilt (Fusarium oxysporum f. sp. carthami) and dry root-rot fungus (Macrophomina phaseolina) showed that there was associative and synergestic effect of two pathogens and there by the disease severity increased in combine or mix inoculation.

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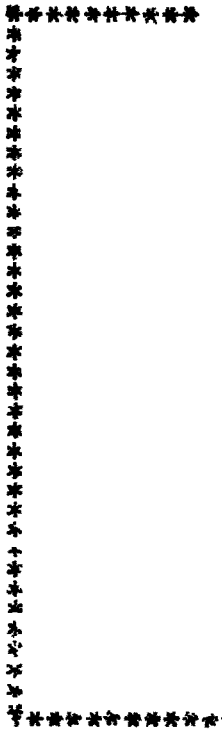

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*Original not seen.

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APPENDIX - I

Constituents of different culture media :

Potato dextrose agar medium	Peeled sliced potato	200.00	g
	Dextrose	20.00	g
	Agar	20.00	g
	Distilled water	1000	ml
Potato dextrose broth	Peeled sliced potato	200.00	g
	Dextrose	20.00	g
	Distilled water	1000	ml
Special media for <u>Fusarium</u> (Kerr 1963)	Agar	20.00	g
	Sodium nitrate	10.00	g
	Pot.Phosphate (KH_2Po_4)	5.00	g
	Pot. chloride	0.02	g
	$MgSo_4$	2.50	g
	Sucrose	50.00	g
	PCNB	100	ppm
	Streptomycin	50	ppm
	Rose bengal	50	ppm

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