STUDIES ON SAFFLOWER RUST CAUSED BY *Puccinia carthami* Corda

BY

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CERTIFICATE

Mr.G.RAM REDDY has satisfactorily prosecuted the course of research and that the thesis entitled, STUDIES ON SAFFLOWER RUST CAUSED BY Puccinia carthami Corda submitted 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 thesis or part thereof has not been previously submitted by him for a degree of any University.

(Dr.A.G.RAMACHANDRA REDDY)
Chairman of the Advisory Committee

CERTIFICATE

This is to certify that the thesis entitled, STUDIES ON SAFFLOWER RUST CAUSED BY Puccinia carthami Corda submitted in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY IN AGRICULTURE of the Andhra Pradesh Agricultural University, Hyderabad, is a record of the bonafide research work carried out by Mr. G.RAM REDDY under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

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DECLARATION

I, G.RAM REDDY hereby declare that the thesis entitled, STUDIES ON SAFFLOWER RUST CAUSED BY *Puccinia carthami* Corda submitted to Andhra Pradesh Agricultural University for the Degree of DOCTOR OF PHILOSOPHY IN AGRICULTURE is a result of original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

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ABSTRACT

Laboratory and field experiments were conducted on safflower st caused by Puccinia carthami Corda during 1986-87 and 1987-88 rabi College of Agriculture, Rajendranagar, Hyderabad.

Safflower rust incited by P. carthami is confirmed as an itoecious macrocyclic species with all spore stages. Urediniospore .ability was more (40 days) when stored at laboratory conditions than ider natural conditions (25 days). Teleutospores remained viable ir 11 months under field conditions whereas 13 months under labora- ıry conditions. Teleutospores germinated profusely in November with ıximum germination in December.

Resting teleutospores carried with seed or in infected plant ebris oversummer and remain viable to bring about fresh infection in ıe following year. No collateral hosts were found although 15 weed lants and 2 cultivated plants were artificially tested.

Increase in per cent disease intensity of P. carthami on afflower was influenced by five environmental factors viz., MMXT, MIT, MMH, MEH and MSH. Multiple regression analysis indicated that nly MMXT had good correlation with disease development during 1986-87 hereas, MMXT, MEH and MSH during 1987-88 rabi. A combination of five ariables viz., MMXT, MMIT, MMH, MEH and MSH accounted for 49 per cent ıf the observed variation during 1986-87. Whereas, combination of MMXT ınd MMIT accounted for 67 per cent of the observed variation during .987-88 rabi. Elimination of MMH, MEH and MSH from analysis reduced :he efficiency of the regression equation slightly during 1987-88 :abi. However, the fact that a considerable amount of variation in data cannot be explained solely on the basis of meteorological factors studied. This indicates that some other micro-climate or biological parameters are important in influencing the development of rust disease under field conditions.

September sown crop escaped the disease while the disease was conspicuous right from flowering in the crop sown from October onwards. Disease appears right from seedling stage in December sown crop.

Out of 192 germplasm lines and cultivars screened 53 were identified as immune, 14 resistant, 17 moderately resistant, 32 moderately susceptible and 76 susceptible to the disease.

Bavistin 0.05 per cent and Dithane M-45 0.2 per cent were found to be highly effective in checking the disease and increased yields followed by Saprol 0.15 per cent and Topsin-M 0.05 per cent.

Per cent disease intensity of 22.08 and 18.92 was recorded in protected plots while 73.80 and 58.92 in unprotected plots during 1986-87 and 1987-88 rabi, respectively. Recorded yields of 13.05 and 13.24 q ha^{-1} from protected plots where as 8.76 and 10.15 q ha^{-1} from unprotected plots during 1986-87 and 1987-88 rabi respectively. Unprotected plots gave 49 and 30 per cent reduced yields over protected during 1986-87 and 1987-88 rabi respectively. On contrary protected plots gave 32 and 23 per cent increased yields over unprotected during 1986-87 and 1987-88 rabi respectively.

Thickness of cuticle cum epidermis, stomatal frequency, size of stomata and stomatal openings had no correlation with resistance.

Higher levels of total phenols, ortho-dihydroxy phenols and total amino acids in resistant cultivars had correlation with resistance whereas, higher amounts of sugars in susceptible cultivars had correlation with susceptibility. There was no correlation between ascorbic acid levels and resistance.

INTRODUCTION

CHAPTER I

INTRODUCTION

Safflower (Carthamus tinctorius L.) is one of the important oil seeds crops in India. The plant is used as fodder and the seed for extracting an edible oil which is of great commercial value. Safflower is cultivated in India over an area of 7.62 lakh hectares with a production of about 4.43 lakh tonnes. In Andhra Pradesh it is cultivated over an area of 19,200 hectares with a production of 5,800 metric tonnes (Directorate of Ecnomics and Statistics, New Delhi, 1991). In Andhra Pradesh it is grown mostly under rainfed situations as a rabi crop in black cotton soils of Rangareddy, Medak, Mahaboobnagar, Kurnool, Anantapur and Cuddapah districts. A number of diseases caused by fungi, bacteria and viruses were reported on safflower in India. Some are widely distributed, while others are localised in distribution and are not considered to be economically important as they are minor diseases.

Among the fungal diseases of safflower, rust caused by Puccinia carthami Corda is commonly present wherever safflower is grown. In India, safflower rust was first observed by Buttler (1931). Later it was recorded at IARI, New Delhi (Prasada, 1947; Prasada and Chothia, 1950); from Hyderabad, Andhra Pradesh (Vasudeva, 1958; and Kulkarni, 1961) and from Jabalpur (Singh and Singh, 1974; Sharma and Kulkarni, 1976; Reeti Singh, 1983). It has been worked in depth in USA, where 55-91 per cent loss in

stand was recorded in susceptible cultivars (Zimmer and Jensen, 1970). But in India no information on exact quantitative yield loss is available although it has been mentioned that it causes appreciable loss in the years of epiphytotics (Maiti, 1987).

Safflower rust has two distinct pathological phases, a seedling and foliage phase (Sackston, 1953; Zimmer, 1963b). The seedling phase usually occurs in North India while foliar phase is quite common in South India affecting yield losses. Pathogen mainly perpetuates through teleutospores which remain dormant on the seed or the crop debris (Conners, 1943; Prasada and Chothia, 1950). Prasada and Chothia (1950) and Vasudeva (1961) reported that the pathogen can also survive on Carthamus oxycantha in northen parts of India. Other Carthamus spp.viz., C.glaucus M.B., C. lanatus L., C. syriacus (Bioss) Dinson, and C.tenius (Bioss) Bornm, also appear as collateral hosts to P.carthami (Kolte, 1985).

The rust on cultivated crop appears in February or March (Prasada and Chothia, 1950). The distribution of rust of safflower in India has been largely determined, but the yield losses caused by this rust have not been quantitatively estimated. Although research on chemical control of rust was carried out and management recomendations have been made, latest fungicides remained to be tested for their efficacy.

However, the research on epidemiology of safflower rust has not recieved adequate emphasis in India. There is some

literature on viability of urediniospores and teleutospores and on the effects of various climatic factors on rust development in susceptible safflower cultivars under field conditions. However, information on the effect of rust on the crop sown at various periods and mechanism of rust resistance is lacking. Efforts have been made to evaluate sources of rust resistance which can be effectively used as donors in breeding programmes (Singh and Singh, 1974; Vyas and Prasad, 1985). All these aspects are considered essential to develop integrated disease management strategies for the control of the rust. In view of these facts investigations were carried out with the following objectives:

1. Survey of rust disease and its intensity in different safflower growing areas of Andhra Pradesh.

2. Study of different stages in the life cycle of Puccinia carthami.

3. Survival of pathogen and search for collateral hosts.

4. Effect of environmental factors on disease development.

5. Effect of different periods of sowing on disease incidence.

6. Screening of germplasm and cultivars against rust for identification of sources of resistance.

7. Evaluation of certain latest fungicides for the control of safflower rust.

8. Estimation of yield losses caused by safflower rust.

9. Investigations on the mechanism of rust resistance.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Relevant literature on geographical distribution, economic importance, symptoms, pathogen, survival, screening of germplasm, chemical control of safflower rust and inherent biochemical factors and morphological characters of leaf responsible for disease resistance is reviewed in detail in this chapter.

2.1 GEOGRAPHICAL DISTRIBUTION

Safflower rust caused by Puccinia carthami was first described by Corda in 1840 from Bohemia (Arthur and Bullaria, 1922). Occurrence of this disease was later reported from almost all the areas of commercial cultivation of safflower (Connerns, 1944; McGregor and Hay, 1952 from Canada; Nattrass, 1937 from Cyprus; Zacha, 1950 from Czechoslavakia; Castellani, 1940 from Ethiopia; Snell, 1923; Melchens, 1931 from Egypt; Bernaux, 1953; Darpoux 1946 from France; Punithalingam in 1970 from Germany; Bebhoudi, 1968 from Iran; Al-Beldawi and Walleed, 1978 from Iraq; Ashri, 1971; Minz, 1958 from Israel; Zazzerini and Capelli, 1982 from Italy; Gallegos and Rodriguez, 1966 and 1968 from Mexico; Classen et al., 1949; Classen, 1952 from United States of America).

In India, safflower rust was first observed at Pusa (Butler and Bisby, 1931). Subsequently it was recorded at the Indian Agricultural Research Institute, New Delhi (Prasada, 1947;

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Prasada and Chothia, 1950), from Hyderabad, Andhra Pradesh (Vasudeva, 1958; Chavan, 1961) and from Jabalpur (Singh and Singh, 1974; Sharma and Kulkarni, 1976; Reeti Singh, 1983).

2.2 ECONOMIC IMPORTANCE

2.2.1 Epiphytotics

Schuster and Christiansen (1952) reported severe epiphytotics of safflower rust in Nebraska during 1949 and 1950 after introduction of safflower crop.

Bernaux (1953) recorded a severe outbreak of safflower rust from South France in 1951.

Rust disease of safflower was considered as a limiting factor for successful production under irrigation in Great Plain areas of the U.S. Though the disease was prevalent in south western U.S., it seldom reached yield reducing intensities (Zimmer and Leininger, 1965).

Safflower rust is of particular importance in medium lands of plateau region of Bihar (Chota Nagpur), eastern Madhya Pradesh and extremly late sown conditions of south eastern Rajastan (Udaipur) and northern Andhra Pradesh. The disease may cause appreciable loss in the years of epiphytotics (Maiti, 1987).

2.2.2 Stand and yield loss

Under epiphytotic conditions, the susceptible line of safflower yielded 65 per cent of resistant line, while under rust

free conditions susceptible line yielded 95 per cent of resistant line (Thomas, 1956).

The major loss from safflower rust is the reduction in plant stand due to sowing of teliospore infested seed or planting where soil-borne teleutospores exist. Using artificially infested seed, the stand loss recorded was 98 per cent, while 20 per cent stand loss was reported by the use of naturally rust infested seed (Wilson and Ark, 1958).

Safflower rust incited by Puccinia carthami, caused an estimated five per cent loss amounting to 1 million dollars annually (United States Department of Agriculture, 1965).

Zimmer and Urie (1968) reported that in 1963, 1964 and 1966 heavy rust infection was limited to the lower leaves of unsprayed plots until after full bloom and did not significantly reduce seed yield and test weight. Heavy infection of both lower and upper leaves in the unsprayed plots occurred prior to full bloom in 1965 and significantly reduced yield of rust susceptible entries.

The rust of safflower is a serious disease in the Sanjoaquin valley of California causing an estimated annual yield loss of five to ten per cent (Zimmer and Urie, 1969).

Safflower infested entries viz., N-1-1-5, 6458-5, PI_195895-95 and PCA which were resistant to seedling rust by Puccinia carthami exhibited an average stand loss of 2.4, 8.2,

13.4 and 26.2 per cent respectively. Infested seeds of mode-
:ately resistant (ute) and highly susceptible entries (Gila, th-
10, th-5) recorded a stand loss of 55.9, 79.5, 95.7 and 97.7 per
:ent respectively and yielded significantly less than uninfected
:eed lots (Zimmer and Jensen, 1970).

Aliza Halfon-Meiri (1981) observed that safflower rust
ncited by Puccinia carthami caused a significant reduction of
lant stand.

.3 SYMPTOMS

Initial symptoms showing pycnia and primary uredia
)pear on cotyledons, leaves and tender stems. At later stages
redopustules were observed mainly on leaves and branches
)llowed by telial stages in the same sori. Teleutospores are
)rmed generally when host comes to maturity and environmental
)nditions unfavourable for the propagation of uredinial stages
'rasada and Chothia; 1950).

The disease damaged both seedlings as well as older
.ants. Earliest symptoms were noted at 6 to 8 leaf stage of
.ants as slightly yellow discolouration of leaves accompanied by
'ooping and wilting. Seedlings often die without exhibiting
)ove ground symptoms. In older plants, girdling of invaded
eas due to the collapse of tissues is a very charecteristic
mptom (Schuster and Christiansen, 1952).

Safflower rust has two distinct phases, a seedling phase and foliage phase. The seedling phase is a result of infection of hypocotyls by seed-borne or soil-borne teleutospores. The foliage phase may cause a substantial reduction in the yield of safflower if rust develops early in the growing season (Zimmer, 1963b).

Singh and Singh (1974) observed that urediniospores first infect the leaves and produce uredinial stages in abundance which are scattered on the entire leaf surface. The adjacent pustules coalesce to form bigger rust pustules leading to large scale premature defoliation. Many seedlings die without exhibiting the symptoms of the disease on the plant parts above ground level. In such cases, the underground parts found to be infected leading to death of the plants.

Safflower rust attacks the plants through seed, infects the seedlings near collar region which may result in death of the seedlings. Whereas, foliar phase pustules on leaves causing reduction in effective leaf area and yields (Sharma and Kulkarni, 1976).

Safflower rust was known as a foliar disease in Israel till 1979. Laboratory and field experiments carried out revealed that teleutospores infect seedlings during seed-germination, developing pycnia and pustules of urediniospores on cotyledons and hypocotyl tissue resulting in wilting and death of many seedlings and old plants. Under field conditions the infected

seedlings serve as a primary so urce for foliar infection (Alizon Halfon-Meiri, 1981).

During last two months of crop growth, the rust produced mainly telia on the infected plants which might be due to high temperatures during this period that encouraged the production of teleutospores (Aliza Halfon-Meiri, 1983).

Safflower rust has two distinct pathological phases, a seedling phase and a foliage phase. The seedling phase mainly develops because of infection of emerging seedlings by basidiospores resulting from germination of seed or soil-borne teleutospores. Initially orange yellow spots representing pycnia appear on cotyledons. The colour of such spots later changes due to development of uredinial aecidia called primary uredinia. Many such uredinia develop as pustules, and adjacent pustules coalesce to form large rust pustules. The foliar phase of disease is characterised by appearence of uredinial pustules on leaves and flower buds. The uredinia remain scattered, erumpent on leaves and these have a chestnut-brown colour. The teleutospores are formed in the urediniopustules when the safflower plant matures (Kolte, 1985).

According to Reeti Singh et al. (1988) safflower rust produces symptoms on seedlings as well as on foliage. It attacks cotyledons, young leaves, tender stem and even the tap and lateral roots. On seedlings the disease appear as slight yellow discolouration on cotyledons and on hypocotyls. The spots

elop orange colour due to formation of pycnia, later turn dark wn due to formation of uredinoid aecidia. When the host plant ures telia are formed in uredinial pustules, which are covered the host epidermis for long time without bursting. The diniospores and teleutospores are also formed on tap root and eral roots.

PATHOGEN

.1 Life cycle stages

Conners and Savile (1944) reported that safflower rust s recorded in Canada, which is a brachyform type of rust :king aecial stage in its life cycle. Evidence was obtained ıt teleutospores on the seed infect the young crop.

When safflower plants grown asceptically in sterile water ıtaining teleutospores of the fungus, the teleutospores were ɔosited on the cotyledons, which produced spermagonia and two three days later urediniospores. Inoculation with these ɛdiniospores gave rise to urediniospores and teleutospores of ɛcinia carthami (Darpoux, 1946).

Puccinia carthami overwinters mainly as the teleuto-ɔres on safflower seeds. In the spring these teleutospores rried away on the seed, germinate soon after sowing and the sidiospores infect the cotyledons of young plants. A week ter, orange spots appear consisting of spermagonia and after o to three days primay uredinia develop around them (Darpoux, 48).

Prasada and Chothia (1950) observed that when safflower plants inoculated with teleutospores during November, produced pycnia after 10 days of inoculation, while uredinia are formed three days later. Similar results were obtained in December, January and February, but inoculations made towards the end of March, when the temperature was above 26°C, gave negative results.

According to Thomas (1952), pycnia developed on the hypocotyls and cotyledons of plants after 10-12 days of planting followed by uredinia.

Siddiqui and Prasada (1959) reported that usually 7 to 8 days after inoculation with germinated teleutospores, pale green flecks appeared on the leaves which proved to be pycnia. In another 3-4 days uredinia appeared either on one or both surfaces of leaves. In some leaves, however, it was observed that uredinia were not formed even after fortnight and these centres of infection remained sterile.

Germinating teleutospores of Puccinia carthami, produced short, stout, four celled promycelia with each cell producing short sterigmata and a single sporidium. Sporidia are discharged violently and produced germtubes which penetrate the host directly. Microscopic studies of microtome sections of pustules resulting from teleutospore, aeciospore and urediniospore inoculations showed that Puccinia carthami is an autoecious, macrocyclic species with subepidermal parphysate spermagonia (appear

10-12 days after inoculation) and uredinoid aecia (3-4 days after appearence of spermagonia). Gross morphology of aecia and aeciospores is identical with uredinia and urediniospores. Pustules referred to here as aecia, although uredinoid in character, occupy the aecial stage in the life cycle (Zimmer, 1963).

Reeti Singh (1983) observed that safflower rust produces pycnial, uredinoid aecia , uredinial and telial stages in its life cycle.

2.4.2 Pycnia and Pycniospores

Pycnia were invariably sub-epidermal, flask shaped or spherical, and measures 80-100 u in diameter with a large number of flexuous hyphae protruding out of the ostioles. The pycnia also produce numerous pycniospores which were seen oozing out through the ostiole (Siddiqui and Prasada, 1959).

Zimmer (1963a) while studying the spore stages and life cycle of Puccinia carthami described that the spermagonia produced by this fungus were flask-shaped, sub-epidermal and paraphysate.

2.4.3 Uredinoid aecia and uredinoid aeciospores

Siddiqui and Prasada (1959) reported that the uredinoid aecia are amphigenous and chestnut brown. Uredinoid aeciospores were round or spherical, measuring 21-25 u in diameter, with perfectly smooth walls unlike normal urediniospores and borne terminally on a thick hyaline pedicel arising from dikaryotic

cell. Aecia were amphigenous, chestnut brown, producing globoid echinulate spores singly on hyline pedicels (Zimmer, 1963a).

2.4.4 Uredinia and Urediniospores

According to Prasada and Chothia (1950), the urediniosori are amphigenous, scattered, erumpent and chestnut brown. The urediniospores are globoid or broadly ellipsoid, their walls are light chestnut brown, 1.5u to 2.0 u thick, and finely echinulate. The urediniospores measure 21-28 x 19-25 u and have 3-4 equitorial germpores.

Reeti Singh et al. (1988) observed that uredinia are amphigenous, scattered, erumpent and chestnut-brown in colour and 0.33 to 0.46 mm in diameter.

2.4.5 Telia and teleutospores

Reeti Singh et al. (1988) reported that the telia are formed in uredinial pustules when the host plant matures. They are hypophyllous, scattered, often in groups and 0.6 to 0.9 mm in diameter. The telia remain covered by the epidermis for a long time without bursting. The teleutospores are uniseptate, globoid to broadly ellipsoid, chestnut-brown, finally verrucose with umbonate caps and fragile stalk, 25-28 x 30 um in diameter. The two cells of teleutospores are not equal, the lower one was smaller than that of upper one.

2.4.6 Urediniospore germination

Fresh urediniospores start germinating in 4 hours at 18°C, kept in a moist chamber over a temperature range of 8° to 35°C. 60-70 per cent of the spores germinate at 8°-10°C, 70-80 per cent at 12°C, 80-90 per cent at 18°-20°, 5 per cent at 29°-30°C, and only traces germiante at 35°C, while no germiantion has been observed at 40°C (Prasada and Chothia, 1950).

2.4.7 Teleutospore germination

Prasada and Chothia (1950) observed that the best germination of teleutospores was between 12° and 18°C, and a few spores germinated at 20°-22°C, but no germination was observed at 7°-8°C and at 26°C and above. It has been found that small percentage of spores germinate in April and May soon after formation, while others require a resting period.

2.5 SURVIVAL OF PATHOGEN

2.5.1 Perpetuation and disease cycle

Studies showed that P. carthami overwinters mainly as teleutospores on safflower seed. In the spring these teleutospores carried on the seed, germinate soon after sowing and basidiospores infect the cotyledons of young plants (Darpoux, 1948).

Prasada and Chothia (1950) while studying on safflower rust in India discussed that resting teleutospores that oversummer and remain viable to bring about fresh infection in the

following season. Inoculum may come from plant debris lying in the soil or it may be carried with the seed in the form of tiny bits of infected tissue. Teliospores from wild safflower (Carthamus oxycantha Bieb.) may infect cultivated species directly or may attack the wild plants first since the rust has been observed earlier on the wild plants than on the cultivated plants.

Thomas (1952) from U.S. reported that certain isolated fields of N8 safflower at Kansas, Colorado and California, which were previously planted with safflower, showed light to heavy rust infection. It appeared that this infection probably came from seed-borne inoculum.

Clavert and Thomas (1954) recorded 50 per cent infection of rust at 20° to 25°C and 90 per cent at 5° to 15°C in green house tests. Rust infection on the seedlings of N8 cultivar produced from seeds heavily inoculated with teleutospores and planted in steamed soil but negative results were obtained with urediniospores. Inoculated seed of the same cultivar was grown in steamed soil at 5, 10, 15 and 20°C for a week, after which all the temperatures were raised to 22°C for a month. The average infection at original temperatures was 96.1, 76.2, 67.3 and 29.3 per cent respectively.

In India safflower rust is commonly seen on wild safflower C. oxycantha Bieb. and it gets infected a month earlier than the cultivated safflower. Besides, viable teleutospores have

been observed on this wild safflower during the off-season suggesting a potential source of pathogen (Vasudeva 1961). Other Carthamus spp., viz., C. glaucus M.B., C. lanatus L., C. syriacus (Bioss) Dinson, and C. tenuis (Boiss) Bornm., also appear to act as collateral hosts to P. carthami (Kolte, 1985).

Gallegos and Rodriguez (1967) reported that P. carthami, the causual agent of rust on safflower, is seed-borne. Sharma and Kulkarni (1976) evaluating systemic fungicides against safflower rust reported that P. carthami attacks safflower plants through seed, infecting seedlings near collar region which may result in the death of seedlings.

Local seeds which were grown in different parts of the country carried a great number of teleutospores as well as urediniospores of the fungus. The teleutospores infecting seedling during seed germination, develop pycnia and pustules of urediniospores on cotyledons and hypocotyl tissue. Under field conditions the infected seedlings serve as a primary source of inoculum for foliar disease and they also enrich the soil with spores of the fungus (Aliza Halfon-Meiri, 1981).

Teleutospores of the fungus lodged on the safflower seed coat mainly during mechanised harvesting, served as initial inoculum for seedling disease. The rough structure of the pericarp provided shelter for the spores. They were frequently observed within the area of scarred tissue of achene ends. Spermagonia and *pustules of urediniospores on the above ground parts of seedlings*

served as the primary source of infection for the foliar phase of the disease in the field (Aliza Halfon-Meiri, 1983).

2.5.2 Viability of urediniospores

Urediniospores remained viable for over a year when they were stored dry at 8° to 10°C, whereas they did not germinate after 3 weeks in April at room temperature of 35°C. When infected plants were exposed to high temperatures, the urediniospores remain viable for 3 weeks at 30°-31°C, 20 days at 36°-37°C, 13 days at 40° and 41°C, 4 days at 45° to 47°C, and only 3 days at 52° to 55°C (Prasada and Chothia, 1950).

The epidemiological studies were conducted in 1981-82 and 1982-83 by Reeti Singh et al. (1990) with regards to survival of urediniospores and reported that urediniospores on exposed dried infected leaves in the field lost viability within 30 days. Whereas, in dried infected leaves stored at room temperature, the viability was prolonged. Viability of urediniospores at the time of storage was 84 per cent. At 8° and 24°C urediniospores remained viable only upto 60 and 40 days respectively. While at 40°C lost viability within 5 days. Urediniospores in the dried infected leaves remain viable upto 28 days while it was improved when the harvested plants were protectively stored at room temperature but the survival was longest at 8°C.

2.5.3 Viability of teleutospores

Prasada and Chothia (1950) reported that the small percentage of teleutospores germinate in April and May soon

after formation. After 5-6 months no germination is observed, but in November the spores germinate profusely from November to January approximately 60-80 per cent teleutospores germinate but from February to March there is gradual decline in germination. Exposure to summer heat in Delhi (which reached 40 to 45°C during May and June 1948) does not kill all the teleutospores, but storage at low temperature is more conducive to the retention of viability.

Infested safflower straw exposed to natural conditions contained viable teleutospores for 12 months but not 21 months. P. carthami is incapable of surviving 2 winters in the field in Nebraska, although a small percentage of teleutospores remain viable for atleast 45 months when stored at 5°C. Teliospores did not survive for 21 months in the field regardless of whether the infected straw was kept on the surface or mixed in with the soil (Schuster, 1956).

Aliza Halfon-Meiri (1983) observed that infectivity of teleutospores on seeds harvested in 1979 dropped sharply after 6-12 months of storage at room temperature (20-25°C). The spores from cultivars 'US-10', 'Gila' and 'Car mex' lost their infectivity completely after 18 months, while in AC-1 a few infected seedlings developed in the field after 20 months of harvest. In 1980, the infectivity of spores dropped slowly after 6 to 12 months of storage at room temperature (20-25°C). At 3°C the teleutospores maintained their infectivity at rather high level for 18 months.

2.6 EFFECT OF WEATHER FACTORS ON DISEASE DEVELOPMENT

During 1981-82 and 1982-83 safflower rust appeared in November and its intensity remained low in December. The maximum development of disease occurred during January and February and remained constant in March and April indicating that temperatures above 20°C were quite favourable for rust development. A relative humidity of more than 80 per cent favours the disease and as the relative humidity decreased the disease development is checked. The rust was severe in 1981-82 as compared to 1982-83 because of frequent rains and high temperature which prevailed during crop season (Reeti Singh et al., 1990).

2.7 SCREENING OF GERM PLASM AND VARIETIES AGAINST RUST OF SAFFLOWER

Safflower lines viz., PI 253931-5 and PI 253914-7 were highly resistant to all the cultures of P. carthami and may serve as source of rust resistance (Zimmer, 1963).

Out of 1200 plant introductions fifteen safflower lines were found to be resistant to foliage as well as seedling phases of the disease viz., PI 170274-100, PI 193764-66, PI 199882-82, PI 220647-98, PI 220647-55, PI 250601-109, PI 253759-62, PI 253911-25, PI 253912-9, PI 253914-5-108, PI 253914-26, PI 257291-68, PI 253913-5-72, PI 253913-5 and PI 253914-7. Safflower entries with high level of resistance to the foliage phase exhibited less than 5 per cent seedling death.

Whereas, less resistant and susceptible entries showed higher levels of seedling mortality (Zimmer and Leininger, 1965).

Zimmer and Jensen (1970) reported that the seedling rust resistance of N-1-1-5, PCA, PI 195895 and 6458-5 is sufficient to prevent significant yield losses, when planted in area of rust infestation. Unless new races of rust developed, the resistance of these lines should be sufficient to allow continuous cultivation of safflower without yield loss from seedling rust.

Of 2000 lines tested 547 were resistant to P. carthami (Ashri, 1971). Zazzerini and Capelli (1981) reported that out of 6 cultivars tested, saffola 202 appeared to be most resistant.

Safflower cultivars viz., APRR-1, APRR-2, APRR-3, APRR-4, APRR-5 and EC 11972 found to be immune while B-3-1-10 and JSF were found to possess moderate resistance under artificial epiphytotic conditions against P. carthami (Vyas and Prasad, 1985).

2.8 CHEMICAL CONTROL

Spraying with Plantvax (0.1%) and Campogran M (0.1%) failed to protect the plants from rust in the field and also under controlled conditions (Sharma and Kulkarni, 1976).

Chauhan and Muheet (1978) reported that Dithane M-45, Vitavax, Plantvax, Benlate and Flit 406 completely inhibited the rediniospore germination at concentrations of 1500, 1000, 500, 200 and 100 ppm in vitro, while ceresan wet, sandoz seed dressing

6335 and Ziram inhibited spore germination at all concentrations except 100 ppm. And in vivo Cuman-L, Vitavax, Dithane Z-78, Ferbam and Benadanil did not differ significantly among themselves. Whereas Dithane M-45 proved to be significantly superior and effective in checking the disease intensity. As regards to the yield, all chemicals were found significantly superior over control except Benadanil and Dithane M-45 dust. Cuman L, Vitavax, Dithane Z-78 and Ferbam did not differ significantly among themselves but Dithane M-45 proved to be most effective and gave highest yield.

2.9 MECHANISM OF RESISTANCE

Relevant literature regarding important inherent anatomical characters and biochemical constituents of leaf responsible for disease resistance as well as susceptibility among different crops are presented below.

2.9.1 Morphological resistance

Pool and Mckay (1916) reported that germtubes of Cercospora beticola were not able to push their way through closed stomata and could enter only through open stomata of sugarbeet leaves.

Potato tubers resistant to Pythium debaryanum have a tough epidermis with higher fibre content than susceptible varieties (Hawkins and Harvey, 1919).

Mclean (1921) showed that the resistance to canker in

mandarin orange (Citrus nabilis) was due to broad cuticular lips covering stomata and the susceptibility in grape fruit (Citrus grandis) was due to absence of these particular stomatal structures.

Melander and Craigie (1927) found that species of Barberis were not susceptible to black rust (Puccinia graminis) and resistant to sporidial invasion, because of thick walls, even when leaves are young. Thus, plants with tough and thick epidermal walls are not attacked by given pathogen and display resistance to penetration.

The stomata of some wheat varieties are closed much of the time and the stem rust (Puccinia graminis var tritici), cannot usually force its way through closed stomata, which was termed as "functional resistance". The delayed opening of stomata in the morning in resistant wheats prevents the entry of stem rust until the moisture on the plant was evaporated, thus exposing the delicate germtubes to dessication and death (Hart, 1929).

Sharvelle (1936) reported that in varieties of flax, susceptible to rust, the epidermis lacks a well developed cuticle. The individual epidermal cells are rectangle rather than isodiametric, and the hypodermis is usually absent. Consequently, resistance may be correlated to a well developed cuticle, shape of the individual epidermal cells and the presence of hypodermis. The varieties of flax, resistant to rust

(Melampsora lini) possessed a tougher epidermis than susceptible varieties.

Subramanyam et al. (1982) working with rust disease of groundnut observed that the rust resistance was not correlated with the number of stomata present on the leaf surface. Irrespective of whether a genotype was immune, resistant, or susceptible, the urediniospores germinated on the leaf surface and fungus entered the leaf through stomata.

According to Bishop and Cooper (1983), the hyphae of Verticillium sp. could penetrate the tissue of both resistant and susceptible varieties of peas and tomato. The epidermis does not have any relevance to resistance and susceptibility of the cultivars.

Brahmachari and Kolte (1983) working with Cercospora leaf spot of groundnut observed smaller leaflet size (83 to 160 sq.cm) and lesser number of stomata (11 to 12 sq.mm leaf area) in the resistant varieties while in susceptible varieties leaflet area was large (136 to 160 sq. cm) and number of stomata was high (13 to 15 per sq. mm leaf area). Significantly thicker palisade layer was observed in resistant varieties as compared to the susceptible varieties.

Resistance was correlated with hypodermic width and number of hypodermal cell layers, but not with epidermal cell wall thickness in winter wheats with reference to Pseudocercosporella herpotricoides (Murray and Bruehl, 1983).

Although the cuticle constitutes a mechanical protective barrier against several factors including pathogens of the host, thickness of cuticle *per se* did not contribute to the immune reaction of the leaves *in vitro* in the plants of resistant varieties against *Pyricularia oryzae* in rice (Veera Raghavan, 1983).

Begum (1984) while studying anatomical investigations into pearl millet with reference to downy mildew observed that the thickness of the epidermis of leaf does not have a significant role to play in resistance or susceptibility to downy mildew. The number of stomata present on leaf surface does not have direct relationship with resistance or susceptibility to downy mildew of pearl millet.

Leaflets of rust susceptible genotypes of groundnut had more and larger stomata per unit leaflet area than that of resistant genotypes. Palisade tissues were more compact and thicker in the resistant genotypes than in the susceptible genotypes (Sokhi *et al.*, 1985).

Ashok Krishna *et al.* (1987) while studying the thickness of cuticle and epidermis in relation to slow powdery mildewing in pea found that the cultivars IPS-293, IPS-77, IPS-207, IPS-198 and IPS-214 with slow mildewing possessed a thick cuticle layer and thin epidermis in comparison to other fast mildewers. Thick cuticle had delayed the formation of mature appressoria or penetration of infection hyphae thereby reducing the rate of development.

The resistant varieties of mulberry against leaf rust (Cerotilium fici) had higher thickness of cuticle-cum-epidermis and less number of stomata per sq.mm. than susceptible varieties. There was not much significant variation in the size of the stomata between the resistant and susceptible varieties, though stomata in susceptible varieties were slightly higher (Tomy Philip et al., 1991).

2.9.2 Biochemical resistance

Biochemical factors are known to play an important role in the defence mechanism of plants against plant disease. Several workers have studied various biochemical factors and correlated their presence to resistance against pathogens.

2.9.1.1 Phenols: Among biochemical factors the role of phenolic sustances has been extensively studied in many crops diseases. Phenolics comprises of anthocyanins, leucoanthocyanins, anthoxanthins, hydrobenzoic acids, glycosides, sugar esters of quinic acids, shikimic acids, esters of hydroxycinnamic acids and coumarin derivatives. Previously the compounds were referred to as tannins and recently as "polyphenols" (Goodman et al., 1967).

The phenolic substances are widely distributed both in healthy and diseased plants but the quantity of phenolics is more in resistant plants than in susceptible ones.

Walker et al. (1935) demonstrated that onion varieties resistant to Celletotrichum circinans accumulated flavones,

anthocyanins and simple phenolics such as catechol and protocatechuic acid in yellow or red pigmented bulb scales. The chemical sustances being water soluble diffused into the infected drop from the scales and inhibited germination and prevented penetration of fungi.

Abbott (1938) reported that sugarcane varieties showing resistance to redrot disease registered a higher phenolic content in the juice than the susceptible ones. The bottom internodes of resistant variety had approximately 50 per cent more phenolic content than that of susceptible variety.

Lee and Le tourneau (1958) reported that potato varieties resistant to Verticillium infection contained higher amounts of chlorogenic acid in roots than in the susceptible varieties as determined by paper chromatography and ferric chloride test.

Kuc and his associates (1959) demonstrated that infusion of young apple plants with D- and DL- phenyl alanine could induce resistance in an apple variety susceptible to Venturia inaequalis. The resistance was correlated with the increased amounts of phenols like phloretin and phloretic acid. It was also demonstrated that phenolic compounds were synthesised from phenyl alanine. It was further observed that several of the phenolics like phoridizin, chlorogenic acid, quarcetin glucosides were synthesized by plants from aromatic amino acid pool.

According to Kiraly and Farkas (1962) 'khapli' wheat resistant variety to most rust races contained more phenol content than the susceptible varieties.

Jaypal and Mahadevan (1968) working with leaf spot disease of banana reported that resistant varieties contained slightly more total phenols and O.D phenols than susceptible varieties. Sugarcane redrot resistant varieties had more native phenolic content than susceptible varieties (Rao et al., 1968).

Bhatia et al. (1972) reported that total phenols, total tannins and flavonols were higher in resistant varieties of tomato than in susceptible varieties to early blight of tomato.

Gangopadhyay and Wyllie (1973) while studying the comparison of biochemical constituents in soyabean varieties observed that resistant varieties to Macrophomina phaseolina had greater quantities of total phenols than that of susceptible varieties.

Vir and Grewal (1974) reported that there was no significant difference in phenolic contents of resistant and susceptible chickpea varieties prior to Ascochyta rabiei inoculation.

Resistant varieties of gram against Operculella padwickii had more total phenols, flavonols and tannins as compared to those of susceptible varieties (Gurdip Singh and Bedi, 1976).

Mathur and Vidhyasekaran (1978) observed that there was no correlation between phenols and disease resistance while studying the physiology of resistance to rust in sunflower.

Sathiyanathan and Vidhyasekaran (1981) while studying the role of phenols in disease resistance to brown spot of rice observed that resistant varieties had more quantities of total phenols than susceptible varieties.

According to Rama Mohan (1983), resistant varieties of chickpea contained more amount of O.D.Phenols and total phenols than the susceptible varieties against Fusarium oxysporum f.sp. ciceri.

Sharma et al. (1983) reported that the level of phenolic compounds was slightly higher in the resistant inbred CM-104 as compared with susceptible cultivar CM-600 in corn against leaf blight disease.

Sohi and Rawal (1983) working with rust disease of cowpea observed that no correlation could be established between the phenol contents in different varieties and their disease resistance against Uromyces phaseoli var. vignae.

Resistant cultivars of sunflower against Puccinia helianthi contained higher amounts of total phenols when compared to susceptible cultivars. On the contrary, the susceptible cultivars contained more than twice the amount of tannins as that of resistant cultivars (*Theertha Prasad and Shambulingappa, 1986*).

Chauhan (1987) reported that moderately resistant varieties of tobacco against Helminthosporium spiciferum contained higher amounts total phenols than highly susceptible varieties.

Resistant genotypes of groundnut against tikka leaf spot disease had higher level of total phenols and O.D. phenols as compared to susceptible genotypes (Sindhan and Jaglan, 1987).

According to Subash Chander and Chandravadana (1990), resistant lines of peas contained 3.5 to 3.75 times more phenols than the susceptible lines against rust and powdery mildew.

2.9.2.2 Sugars: Barnett and Lilly (1958) noted an increase in resistance with increased sugar concentrations, while Llyes et al. (1959) reported that wheat variety resistant to rust had higher sugar levels than the susceptible one.

Raghunathan et al. (1966) reported that Kattu vazai a banana variety resistant to many fungal diseases contained more sugars than the susceptible variety Monthan.

Jaypal and Mahadevan (1968) while working with leaf spot of banana found that resistant varieties contained higher amounts of reducing sugars, non-reducing sugars and total sugars than susceptible cultivars.

Mathur and Vidhyasekaran (1972) working with sunflower rust disease observed that there was no correlation between reducing, non-reducing and total sugars to disease resistance.

However, resistant leaves contained significantly more starch than the susceptible leaves.

Grapevine varieties susceptible to anthracnose contain higher levels of total sugars than resistant varieties (Mohanraj et al., 1972).

Gangopadhyay and Wyllie (1973) reported that susceptible varieties of soyabean against Macrophomina phaseolina had higher amounts of total soluble sugars than that of resistant varieties.

Resistant varieties of Itallian millet (Setaria italica L.) contained less amount of total sugars, particularly less amount of sucrose and glucose than the susceptible varieties against Uromyces setaria-italica (Vidhyasekaran et al., 1974).

Sugars were higher in the healthy tissues of susceptible cultivars than in the tissues of resistant cultivars of gram against Operculella padwickii (Gurdip Singh and Bedi, 1976).

Sati and Grewal (1982) working with gram blight incited by Ascochyta rabiei reported that the susceptible varieties contained higher contents of reducing and non-reducing sugars when compared to the resistant varieties.

According to Rama Mohan (1983), resistant varieties of chickpea contained significantly more reducing sugars and total sugars than the susceptible varieties against Fusarium oxysporum f.sp. ciceri.

Singh and Saksena (1983) while studying on biochemical basis for resistance in peas against powdery mildew found that resistant cultivars contained less amount of total sugars and total nitrogen in comparison with susceptible cultivars.

Level of reducing sugars and total sugars was low, whereas non-reducing sugars was high in resistant genotypes of groundnut against tikka leaf spot disease (Sindhan and Jaglan, 1987).

2.9.2.3 Aminoacids: Hadwiger and Hall (1963) reported that most of the aminoacids and specially cystine was in higher concentrations in resistant variety of watermelon to Colletotrichum lagenarium than the susceptible variety.

Jaypal and Mahadevan (1968) while working with leaf spot disease of banana observed that susceptible varieties had more amounts of aminonitrogen than resistant varieties.

Resistant cultivars of redgram against Fusarium wilt contained larger concentrations of aminonitrogen than the susceptible cultivars (Murthy and Bhagyaraj, 1974).

Mathur and Vidhyasekaran (1978) reported that leaves of sunflower rust resistant varieties contained more aminonitrogen than the leaves of susceptible varieties.

Theertha Prasad and Shambulingappa (1986) working with the sunflower rust disease reported that the resistant cultivars

contained higher amounts of free aminoacids (90.2±14.6) than the susceptible (37.4±9.1) cultivars.

Highly susceptible tobacco varieties to Helminthosporium spiciferum had relatively lesser amounts of amino acids than in moderately resistant varieties (Chauhan, 1987).

2.9.2.4 Ascorbic acid: According to Pilgrim and Futrell (1957), no consistent correlation of ascorbic acid concentration with resistance or susceptibility to stem rust of wheat was observed. The mechanism of resistance may not be the same in all resistant wheat varieties.

Kiraly and Farkas (1962) working with wheat stem rust found that there was no marked correlation between the ascorbic acid content of wheat seedlings and disease resistance.

Rama Mohan (1983), while studying with chickpea wilt disease incited by Fusarium oxysporum f.sp. ciceri recorded that resistant cultivars contained more amount of ascorbic acid than the susceptible cultivars. However, the difference between the varieties is not statistically significant.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 SURVEY OF SAFFLOWER RUST AND ITS INTENSITY IN ANDHRA PRADESH

Survey of important safflower growing areas of Andhra Pradesh was undertaken during February 1987 and 1988, with a view to assess the intensity of rust disease (Puccinia carthami Corda). The survey was conducted by adopting standard roving method in four major safflower growing districts of Andhra Pradesh viz., Mahaboobnagar, Ranga Reddy, Medak and Kurnool. Five fields were selected at random in each village of respective districts. In each field observations were made at 4-6 places at random on the diagonal line of the field (Singh, 1984). For estimating the intensity of the disease, a five square metre area was marked in each place of the field. Five plants were randomly selected in each of the marked areas to record the severity of the disease using 0-9 scale which was devised by Mayee and Datar (1986) is furnished below

0 No disease symptoms on leaf.

1 Small urediniopustules covering 1 per cent or less leaf area.

3 Chestnut brown urediniopustules covering 1-10 per cent of the leaf area.

5 Typical rust pustules covering 11-25 per cent of the leaf area.

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7 Typical rust pustules covering 26-50 per cent of the leaf area.

9 Rust pustules covering 51 per cent or more of leaf area followed by drying up of leaves.

Similarly based on per cent leaf area damaged, the disease intensity was calculated by adopting the following formula as suggested by Horsfall and Heuberger (1942).

$$\text{Per cent disease intensity (PDI)} = \frac{\text{Sum of individual ratings} \times 100}{\text{Number of leaves assessed} \times \text{Maximum number of rating}}$$

3.2 STUDY OF DIFFERENT STAGES IN LIFE CYCLE OF P.CARTHAMI

To study different stages in the life cycle of the pathogen, 15-20 days old safflower seedlings were inoculated with teleutospores possessing good germination. Rust infected safflower leaves bearing telia were collected from the crop in April during 1986-87 rabi and stored at room temperature ($28\pm1^{o}C$) for six months to avoid dormancy of teleutospores. A few pieces of these leaves were transferred on to cavity glass slides kept in moist chambers at $18-20^{o}C$ in dark for ninety six hours.

Since good germination was observed during December, inoculations were carried out on 15-20 days old safflower seedlings raised in six inches diameter pots. Before inoculation the pots were kept in humid chambers and gently sprayed with water by an automizer. Germinated tlelutospores were transferred

Plate 1: Infected plant showing uredinia pustules

Plate 2: Teleutopustules on leaf

Plate 3: Severely infected plant showing rust pustules on upper leaves

Plate 4: Teleutopustules on bracts of flower

directly on to the upper surface of leaves with the help of a loop of needle in the evening. The inoculated pots were further incubated in moist chambers for 18 hours before they were put on greenhouse bench, and observed daily for formation of different stages of life cycle of the pathogen. Leaves of bearing pustules of the pathogen at different stages of development were fixed in formalin - alcohol - acetic acid. Free hand sections were taken and semi-permanent slides were prepared in lactophenol. These slides were examined under microscope for the identification of different stages in the life cycle of the pathogen.

3.3 SURVIVAL OF PATHOGEN

3.3.1 Viability of urediniospores

To test the viability of urediniospores, experiments were conducted by storing the safflower leaves consisting of uredinia on the soil surface, at 10 cm depth in the soil and under laboratory conditions. Uredinispore per cent germination was tested by standard slide germination test before the experiment.

3.3.1.1 On soil surface

Rust infected safflower leaves possessing uredinia were collected from the field during second week of February, 1987 (82 DAP) and spread on the soil surface in an area of 2 m^2 and left exposed to natural weather variables in a protected area in the field. At 5-day intervals till the urediniospores fail to

germinate, 1 g of leaf sample was collected form the stored leaves and soaked for 15 minutes in 10 ml of sterile distilled water, crushed and made into a suspension. One or two drops of the suspension were placed on cavity galss slides and incubated in dark at 18°C in petriplates lined with filter papers. Percentage germination was recorded after 24 hours of incubation. Three replicates were maintained and 200 spores were counted for each replication.

3.3.1.2 At 10 cm depth in the soil

Three pits of 10 cm deep with 25 sq cm area each were dug in the field. Rust infected leaves were collected and buried in the soil by spreading uniformly in layers over the bottom of the pits and covered with soil. At 5-day intervals till the urediniospores fail to germinate, 1 g of buried leaf sample was removed and the viability of urediniospores was estimated as described under 3.3.1.1.

3.3.1.3 Under laboratory conditions

Rust infected leaves were shade dried and subsequently wrapped in butter paper and stored in the laboratory at room temperature (28±1°C). At 5-day intervals, 1 g of leaf material was taken out and the viability was estimated as described under 3.3.1.1 till urediniospores fail to germinate.

3.3.2 Viability of teleutospores

To test the viability of teleutospores, experiments were conducted by storing the dried safflower leaves bearing telia which were collected on the day of harvest in 1986-87 rabi season on the soil surface, at 10 cm depth in the soil and under laboratory conditions. Per cent viability of teleutospores was estimated by standard slide germination test.

3.3.2.1 On soil surface

Dried rust infected safflower leaves bearing telia were collected on the day of harvest in 1986-87 rabi season and stored on soil sruface as described under 3.3.1.1. At 30-day intervals, 1 g of sample was collected from the stored leaves till the teleutospores fail to germinate. These samples were soaked for 15 minutes in 10 ml of sterile distilled water, crushed and made into a suspension. One or two drops of the suspension were placed on cavity glass slides and incubated in dark at 18^{O}C in petriplates lined with filter papers. Percentage germination was recorded after ninety six hours of incubation. Three replicates were maintained and 200 spores were observed for germination under each replication.

3.3.2.2 At 10 cm depth in the soil

Rust infected leaves were stored as described under 3.3.1.2. At 30-day intervals till the teleutospores viable, 1 g of buried leaf sample was removed and the viability of teleutospores was estimated as described under 3.3.2.1.

3.3.2.3 Under laboratory conditions

Rust infected leaves were wrapped in butter paper and stored in the laboratory. At 30-day intervals till the teleutospores remain viable, 1 g of leaf material was taken out and the viability was estimated as described under 3.3.2.1.

3.3.3 Mode of transmission

Two experiments were conducted during 1987-88 rabi season in order to find out the mode of transmission of rust disease of safflower viz., seed transmission and soil transmission.

3.3.3.1 Seed transmission

To estimate teleutospore load per seed, an experiment was conducted with five replications. Twenty seeds of Manjira obtained from rust infected crop during rabi 1986-87 crop season were shaken for 8-10 minutes in a test tube containing 4 ml of distilled water. The contents were filtered through double layered cheese cloth and from the filterate, 20 drops were placed on cleaned plain glass slides using 1 ml pipette. Later slides were examined under a stereoscope microscope at 40x and the teleutospores were counted in each replicate. Number of spores per seed for one replicate and average number of teleutospores

per seed per seed lot were estimated by the following formulae given by Aliza Halfon-Meiri (1983).

$$1)\ K_1 = \frac{K_0 . W}{W_0 . N}$$

$$2)\ K_x = \frac{K_1 + K_2 + K_3 + K_4 + K_5}{5}$$

Where,

K_0 = No. of spores inthe drops examined

W = Total water volume

W_0 = Water volume of drops examined

N = Number of seeds per tube

K_1 = Number of spores per seed

K_x = Final average number of spores per seed per seed lot

Later to find out seed transmission, an experiment was carried out with one hundred seeds of Manjira carrying 128 teleutospores on an average per seed from the above seed lot. Seeds were sown during December 1987 in pots containing autoclaved soil. Three replicates were maintained with suitable controls. Surface sterilised seed with 0.1 per cent mercuric chloride for 2 minutes sown in pots containing autoclaved soil served as control. Data were recorded regarding the number of seedlings infected with rust upto 40 DAS starting from appearance of the disease with 3 days intervals and percentage of infected plants were estimated.

3.3.3.2 Soil transmission

Soil was collected from the experimental field immediately after harvest of the crop during rabi 1986-87 season where severe infection of rust was recorded (i.e., more than 50 per cent disease intensity) and filled in pots. Surface sterilised seeds with 0.1 per cent mercuric chloride for 2 minutes were sown in pots filled with above soil during the month of December 1987. One hundred seeds were sown in each replication consisting of 20 pots. Three replicates were maintained with suitable controls where in one hundred surface sterilised seeds were sown in pots containing autoclaved soil. Disease infected plants were recorded upto 40 DAS starting with appearance of rust at an interval of 3 days and percentage of plants infected were calculated.

3.3.4 Study on collateral hosts of rust pathogen

To study the possible collateral hosts, seeds of fifteen weed plants viz., Xanthium strumarium, Celotia argentia, Tridax procumbens, Taraxacum sp., Chrysanthemum sp., Legasca mollis, Parthenium hysterophorus, Euphorbia hirta, Abutilon indicum, Cleome viscosa, Cynodon dactylon, Eragrostis major and Cyperus rotundus growing in and around safflower fields at college farm, Rajendranagar and two cultivated crops viz., sunflower and niger belonging to compositae were collected and sown in earthern pots filled with autoclaved soil. Thirty days after sowing, the leaves of five plants of each species were moistened by spraying

water and dusted with 1:10 mixture of urediniospores and talc. High humidity was maintained for 48 hours by covering the pots with polythene sheets to initiate infection. Disease incidence was recorded after 30 days of inoculation on all hosts. Similarly inoculated safflower (cv. Manjira) plants served as control.

3.4 FIELD EXPERIMENTS

Field experiments were conducted at the college farm of Andhra Pradesh Agricultural University (APAU), Rajendranagar, Hyderabad during 1986-87 and 1987-88 rabi. The soil was sandy loam with good drainage facility. The farm is situated at a latitude of 17.27^{O}N, longitude of 78.28^{O}E and at 608 m MSL. The climatic conditions and field operations for all the experiments were identical throughout which are furnished below:

Climatic conditions

The climate of experimental site (Hyderabad, India) is tropical and semi-arid. The meteorological data recorded at Agricultural Research Institute, Rajendranagar (1.5 kms away from experimental site), during experimental period was collected and tabulated in Annexure I.

Preparatory cultivation: The experimental field was thoroughly ploughed with tractor drawn disc plough. The soil was brought to required tilth by tilling and harrowing. Levelling was done with cattle drawn levelling plank. The individual plots and irrigation channels are demarcated by bunding with bund former.

Fertilizer application: Fertilizers were applied in the form of urea and single superphosphate at the rate of 25 kg nitrogen and 25 kg phosphorus per hectare. Half of nitrogen and whole amount of phosphorus was applied as basal dose at the time of final ploughing. Remaining half of nitrogen was applied after 25 days of sowing.

Sowing: The seeds were sown by dibbling method in plots of 4 x 3 meters size by adopting a spacing of 50 cm between the rows and 20 cm within the row. An optimum plant population of [illegible] 140 was maintained in each plot.

Irrigation: The field was irrigated after sowing and thereafter as and when required to maintain optimum moisture conditions.

Other cultural practices: The plants were kept weed free by resorting to hand weeding.

Plant protection measures: Insecticide sprays of Ekalux 25 EC (2.0 ml per litre of water) were undertaken to control common insect pest like safflower aphid.

3.4.1 Effect of environmental factors on disease development

3.4.1.1 Layout

An experiment was laid out with Manjira variety which is susceptible to rust disease sown in six plots to observe the progress of the disease in relation to meteorological parameters like temperature, relative humidity, sunshine hours and rainfall.

The experiment was conducted during rabi 1986-87 and 1987-88. In both the years sowing were taken up on 24th November.

3.4.1.2 Disease assessment

Observations on the disease progress were recorded with disease appearance at 2 days interval starting from 4th January to 15th February during 1986-87 rabi and from 10th January to 15th February during 1987-88 rabi. Five plants were selected at random in each plot in order to evaluate the severity of the disease. The disease severity was scored on 0-9 scale which was devised by Mayee and Datar (1986). Based on leaf area damage, the disease intensity was calculated by using the formula given under 3.1.2.

3.4.1.3 Statistical analysis

Data on atmospheric temperature (Ta), relative humidity (RH), sunshine hours (SSH) and rainfall (Rf) were obtained from meteorological observatory and the effect of these weather variables on disease progress was estimated by using linear multiple regression analysis (MRA) with the prediction equation.

$$Y = b_0 + b_1x_1 + b_2x_2 \cdots\cdots b_px_p$$

where,

Y = Increase in disease severity

b_o, b_1, $b_2 \cdots b_p$ are regression parameters and

x_1, $x_2 \cdots\cdots x_p$ are independent weather variables

The fit of overall multiple regression equation was tested by using analysis of variance procedure. The significance of partial regression coefficients were tested by using 't' test. The five weather variables utilised in the study were mean maximum temperature, mean minimum temperature, mean relative humidity at 07.30 hours, mean relative humidity at 14.30 hours and mean sunshine hours. Arcsin transformed values of per cent disease intensity were used in analysis.

3.4.2 Effect of different periods of sowing on disease incidence

To ascertain the severity, spread and progress of the disease in realtion to different periods of sowing, studies were undertaken during rabi 1986-87 and 1987-88 crop season in the experiments laid out by Breeding Department of safflower unit, APAU, Rajendranagar. Observations were recorded on the Manjira variety sown at every 15 days interval starting from first fortnight of September to the last fortnight of December. The disease severity was recorded at different crop growth stages viz., seedling, stem elongation, branching, flowering, seed formation and seed maturity and per cent disease intensity was calculated as described under 3.1.2.

3.4.3 Screening of germplasm and varieties against *P. carthami*

A field experiment was laid out during 1986-87 and 1987-88 rabi with one hundred and eighty nine germplasm lines and three varieties obtained from Agricultural Research Institute,

Rajendranagar and Directorate of Oilseeds Research, Rajendranagar to screen against P. carthami.

3.4.3.1 Experimental details

Ten seeds of each germplasm line or variety were sown in a single row at a distance of 20 cm within the row and 50 cm between rows. For every 10 rows, a single row of Manjira (susceptible check) was utilised as an infector row. Further, to increase the inoculum pressure or load, two border rows of Manjira were also raised all around the entire experimental field. To create additional disease pressure, artificial epiphytotics were created by dusting urediniospores (1:10 mixture of urediniospores and talc) on moistened leaves in the evening (17.00 - 17.30 hrs) at 50th and 60th day of planting. Rust reaction was evaluated after 85 days of planting based on per cent leaf area damaged and were categorised into five groups according to Zimmer and Leinger (1965) which were as follows:

Immune	= No disease at all
Resistant	= 5 per cent (1-2 pustules) leaf area damage
Moderately resistant	= 6-20 per cent leaf area damaged
Moderately susceptible	= 21-50 per cent leaf area damaged
Susceptible	= More than 51 per cent leaf area damaged

3.4.4 Feild evaluation of certain fungicides against *P. carthami*

3.4.4.1 Layout and Design:

A randomized replicated field experiment was laid out with five treatments and four replications. The experiment was conducted during rabi 1986-87 and 1987-88 with Manjira variety which was sown on 25th November each year. To avoid chemical drift and also to avoid interplot spread of urediniospores, wheat crop was sown thickly in between two sub-plots which served as gaurd rows.

3.4.4.2 Experimental details:

The experiment was conducted with four fungicides at field recommended doses viz., Bavistin 0.05 per cent, Dithane M-45 0.20 per cent Saprol 0.15 per cent and Topsin-M 0.05 per cent for the control of rust. Three foliar sprays of each fungicide at 15 days interval, starting from disease appearance were given with the help of knapsack sprayer in both the years. Control plots were sprayed with water. Details of fungicides employed are given in Table 1.

3.4.4.3 Disease assessment:

Observations on disease severity of rust were recorded before first spray, second spray, third spray and final after 20 days of third spray. In each plot five plants were randomly selected to record the severity of rust throughout the season.

Table 1: Details of fungicides employed in the investigation

S.No.	Trade name	Common name	Chemical name	a.i. of the formulation	Source of supply
1.	Bavistin	Carbendazim	Methyl-IN-benzimidazole 2 YL carbomate	50% WP	BASF, India Ltd., Bombay
2.	Dithane M-45	Mancozeb	Manganese ethylene bis dithio carbamate plus zinc	70% WP	Indofil Chemicals Ltd., Bombay
3.	Saprol	Saprol	Trifornie [N,N-[1,4 - Piperazinediyl - bis - (2,2,2 - trichloroethylidene)] - bis - formamidel].	15 EC	E. Merck (India) Ltd., Bombay
4.	Topsin-M	Thiophanate methyl	1,2 bis (3 - methyl carbonyl - 2 thiouredio benzene	70% WP	Motilal Pesticides (India) Pvt. Ltd., Mathura

The disease severity was estimated on 0-9 scale as devised by Mayee and Datar (1986). Based on per cent leaf area damaged the intensity was calculated by using the formula given under 3.1.2.

3.4.4.4 Harvesting:

Harvestiang was done after 120 days of sowing. The produce of each plot was harvested and dried spearately.

3.4.4.5 Yield:

Yield obtained from each plot was recorded and expressed in quintals per hectare.

3.4.4.6 Statistical analysis:

The data on per cent disease intensity and the crop yield were subjected to statistical analysis. The data on PDI were transformed into Arcsin transformed values. Analysis of variance procedure as applied to RBD was used to identify the significant difference between the treatments.

3.4.5 Estimation of yield loss due to rust disease in safflower

A field experiment was conducted during rabi 1986-87 and 1987-88 to estimate the yield loss caused by rust in Manjira variety sown on 2nd December during both the years.

3.4.5.1 Layout and Design:

The design employed was paired-plot technique with six plots per block each of 4m x 3m size. The blocks were separated by gaurd rows of thickly sown wheat. One block was inoculated with urediniospores dust thrice starting from 45 DAS at 10 days interval which is unprotected and served as control. While another block was protected by spraying Dithane M-45 (2.5 g/litre of water) at an interval of 10 days starting from frist January till 21st February.

3.4.5.2 Disease assessment:

Observations on disease severity of rust were recorded at 50, 65, 80 and 95 DAS. In each plot five plants were randomly selected to record the severity of rust. Severity of the rust was estimated by using 0-9 scale devised by Mayee and Datar (1986). Per cent disease intensity was calculated as described under 3.1.2.

3.4.5.3 Harvesting:

The crop was harvested after 120 days of sowing.

3.4.5.4 Yield:

Yield obtained from each plot was recorded and expressed in quintals per hectare.

3.4.5.5 Statistical analysis:

Yields recorded from protected and unprotected blocks were subjected to statistical analysis.

3.5 MECHANISM OF RUST RESISTANCE

To find out the possible role of disease resistance to safflower rust, morphological and biochemical resistance mechanisms operated in the susceptible and resistant cultivars of safflower were studied. Safflower cultivars which were identified as resistant and susceptible in the screening trials were utilised for studying the mechanism of rust resistance throughout the study.

3.5.1 Morphological resistance:

Under morphological resistance mechanism, the role played by thickness of cuticle-cum-epidermis, stomatal frequency, size of stomata and size of stomatal opening in PI 307062, PI 199932 (resistant) and Manjira, Bhima (susceptible) cultivars were studied.

3.5.1.1 Thickness of cuticle cum epidermis:

The leaf material of resistant and susceptible safflower cultivars of same age and size were harvested from 65 days old potted plants and preserved in formalin - acetic acid - alcohol (FAA). Later the leaves were dehydrated with butyl alcohol and transferred the material to a mixture of equal parts of paraffin

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oil and teritiary butyl alcohol, in which it is kept for one hour. Fill a vial three fourths full with melted parawax and allowed parawax to solidify. Leaf material was placed on solidified parawax, and covered with a thin layer of butyl alcohol - paraffin oil mixture. The vials were transferred to an oven and kept at 60°C till the material sinks to the bottom of the vial. Poured off the contents of the vial and replaced with pure melted parawax. Repeated the process twice during 6 hours, discarding each change of parawax. Finally replaced with a good quality of melted paraffin and then material is arranged in proper order in previously prepared paper blocks containing melted liquid paraffin which is cooled quickly. Now the material is ready for taking sections with microtome. The sections of leaves with 18 µm thickness were mounted on glass slides consisting of adhesive on the surface of slides which was applied earlier. Application of adhesive helps in preventing the washing off of sections during staining process. Sections are stained with safranin and crystal violet as described below:

1. Stain in safranin and differentiate with 95 per cent alcohol saturated with picric acid, then complete dehydration with a change of alcohol.

2. Dip into a solution of equal parts of absolute alcohol and xylol for several seconds, then into another composed of 25 parts of absolute alcohol and 75 parts of xylol.

3. Stain in crystal violet solution consisting of 6 to 8 drops of a saturated solution of the dye in equal parts of absolute alcohol and clove oil. Leave the slide in the stain for a few seconds and differentiate in mixture of equal parts clove oil and xylol.

4. Wash thoroughly in xylol, pass through a change of pure xylol and mount in balsam.

Measurements of thickness of cuticle-cum-epidermis were recorded by using occular micrometer at ten locations in cross sections of each leaf.

3.5.1.2 Stomatal frequency, size of stomata and stomatal opening:

The seeds were grown in earthern pots and after 65 days of sowing, leaves of same age from bottom were applied with the paste prepared by mixing 1 ml of Benzene + 1 ml of toluene + 2 g of thermocol on both sides of the leaves in the afternoon hours when there will be maximum stomatal openings. After 30 minutes when the paste dried up, leaf peelings were removed gently and placed in between two sterilised glass slides. Later observations were recorded for the stomatal number, size of stomata and size of stomatal opening under compound microscope with a magnification of 40x. Stomatal number was counted from ten microscopic fields of both upper and lower surfaces of leaf in five peelings of each variety for each replication. Three replicates were maintained. The frequency of stomata per square millimetre was calculated. The length and breadth of 100 stomata and their

pening were measured by using occular micrometer on both the urfaces of five leaf peelings of each variety for each eplication. Three replicates were maintained. Stomata and tomatal opening was calculated and expressed in terms of icrons.

3.5.2 Biochemical resistance

Different biochemical constituents like reducing sugars, otal sugars, total amino acids, phenols, O.D.phenols and ascorbic acid were estimated in two resistant and two susceptible cultivars of safflower at four stages of crop growth viz., 45, 60, 75 and 90 days of sowing.

3.5.2.1 Quantitative estimation of reducing sugars, total sugars, total phenols, O.D.phenols and total amino acids:

3.5.2.1.1 Extraction of plant material: The plant material was extracted in ethanol by the procedure followed by Chandramohan et al. (1967).

Leaf samples were cleaned with water and pressed gently between folds of filter paper to remove excess water. Exactly 3 g of material was cut into small pieces and plunged into 12 ml of boiling 80 per cent ethanol, extracted for 5 minutes on a boiling water bath and cooled in running tap water. The material was homogenized by grinding in a porcelain mortar with pestle and squeezed through two layers of cheese cloth. The residue was transferred back to three millilitres of boiling 80

per cent ethanol and re-extracted for 5 minutes cooled and filtered through Whatman No.1 filter paper. Then, a filter paper was washed with a jet of hot ethanol. The final volume was adjusted to 15 ml with 80 per cent ethanol.

The ethanol extracts were used to estimate total sugars, reducing sugars, total phenols, O.D.phenols and total amino acids.

3.5.2.1.2 Preparation of reagents

3.5.2.1.2.1 Nelson's reagent: (Nelson, 1944)

Copper reagent: 25 parts of reagent 'A' + 1 part of reagent 'B'.

Copper reagent 'A': In 800ml of distilled water 25 g of anhydrous sodium carbonate, 25 g of Rochelle salt (sodium potassium tartarate), 20 g of sodium bicarbonate and 20 g of anhydrous sodium sulphate were dissolved and volume was made upto one litre with distilled water, filtered and stored in glass stoppered brown bottle.

Copper reagent 'B': 15 per cent solution of copper sulphate containing one or two drops of concentrated sulphuric acid per 100 ml was prepared and stored in a glass stoppered brown bottle.

3.5.2.1.2.2 Arsenomolybdate: In 450 ml of distilled water 25 g of ammonium molybdate was dissolved and 21 ml of concentrated sulphuric acid was added and stirred. Three grams of sodium arsenate dissolved in 25 ml of distilled water was added to the

solution and thoroughly mixed. The reagent was incubated at 37°C for 48 hrs and stored in a glass stoppered brown bottle.

3.5.2.1.2.3 Arnow's reagent: (Johnson and Schaal, 1952)

The reagent was prepared by dissolving 10 g of sodium nitrate and 10 g of sodium molybdate in 100 ml of distilled water.

3.5.2.1.2.4 Folin - Ciocalteu reagent: (A.O.A.C., 1960): In 70 ml of distilled water 100 g of sodium tungstate and 25 g of sodium molybdate were dissolved and 50 ml of 80 per cent ortho-phosphoric acid was added followed by 100 ml of concentrated hydrochloric acid. This solution was refluxed for 10 hrs. Then 150 g of lithium sulphate in 50 ml distilled water and a few drops of bromine water were added. The solution was boiled exactly for 15 minutes to remove the excess bromine. After cooling the volume was made upto one litre, filtered and stored in an amber coloured bottle. The reagent was diluted with equal volume of distilled water just before use.

3.5.2.1.2.5 0.2 M citric acid buffer : pH 5.0:

Twenty grams of citric acid was dissolved in 200 ml of 1 N sodium hydroxide in a 500 ml volumetric flask and the volume was made upto 500 ml with glass distilled water.

3.5.2.1.2.6 Ninhydrin reagent:

In 500 ml of 0.2 M citrate buffer at pH 5.0, 800 mg of hydrate stannous chloride was dissolved. The solution was mixed

with 20 g recrystalised ninhydrin dissolved in 500 ml methyl cellosolve. Fresh reagents were used for determination.

3.5.2.1.2.7 Diluent solution:

Equal volumes of glass distilled water and n-propanol were mixed.

3.5.2.1.3.1 Reducing sugars:

Reducing sugars were estimated by following the procedure of Nelson (1944).

To one millilitre of ethanol extract in 25 ml marked test tube, 1 ml of fresh reagent prepared by mixing 25 parts of reagent 'A' with one part of reagent 'B' was added. The solution was mixed and heated for 20 minutes in boiling water bath, cooled in running tap water and one millilitre of arsenomolybdate reagent was added. The volume was raised to 25 ml with distilled water and intensity of blue colour was read in Bausch and Lomb colorimeter at 495 nm wavelength. Reagent blank was maintained with one millilitre of distilled water in the place of ethanol extract. Standard curve was prepared from glucose was used to calculate the unknown.

3.5.2.1.3.2 Total sugars:

Total sugars in the plant extracts were estimated by following the procedure of Nelson (1944). For estimating the total sugars, hydrolysis of non-reducing sugars to reducing

sugars was done by adding one millilitre of 0.5 N hydrochloric acid to one millilitres of extract in 25 ml marked test tube and heating on a boiling water bath for 30 minutes. Later the acid in the hydrolysate was neutralized by adding 1 ml of 1 N sodium hydroxide and total sugars were estimated by the method adopted by Nelson (1944). Reagent blank was maintained with one millilitre of distilled water in place of ethanol extract. Standard curve was prepared from glucose was used to calculate the unknown.

3.5.2.1.3.3 Total Phenols:

Total phenols of ethanol extracts were estimated by following the method of Bray and Thorpe (1954).

To a 25 ml marked test tube 1 ml of the extract and 1 ml of folinciocalteu reagent were added, followed by 2 ml of 20 per cent sodium carbonate. The mixture was kept in a boiling water bath for exactly one minute and cooled. The blue coloured solution was diluted to 25 ml with distilled water and read in a Bausch and Lomb Spectronic-21 colorimeter at 725 nm. Reagent blank contained 1 ml of distilled water in place of the extract. From a standard curve prepared using catechol, the unknowns were calculated and expressed as catechol equivalents.

3.5.2.1.3.4 O.D.phenols:

To one millilitre of the ethanol extract in 25 ml marked test tube, one millilitre of 0.5 N hydrochloric acid, 1 ml of

Arnow's reagent, 10 ml of distilled water and 2 ml of 1 N sodium hydroxide were added. Soon after the addition of the alkali, pink colour appeared. The volume was raised to 25 ml with distilled water and read in Bausch and Lomb spectronic -21 colorimeter at 520 nm wave length. Reagent blank was maintained with one ml of distilled water in the place of extract. Standard curve was prepared from catechol was employed to calculate the unknowns.

3.5.2.1.3.5 Total aminoacids:

Ninhydrin method of Moore and Stein (1948) was employed to determine the amino groups in the extract.

To one millilitre of the extract in test tube, 1 ml of ninhydrin reagent was added. The mixture was thoroughly shaked and heated for 20 minutes on a boiling water bath covering the mouth of the test tube with an aluminium cap to minimise evaporation. Five millilitres of diluent solution was added to the cooled mixture and shaken for one minute upon which the solution turned purple. The volume was raised to 25 ml with diluent solution and the intensity of purple colour was read in a Bausch and Lomb spectronic-21 colorimeter at 570 nm wave length. Reagent blank was maintained with 1 ml of distilled water in the place of extract. Aminoacid content of the unknown was calculated from standard curve prepared with glycine and expressed as glycine equivalents.

3.5.2.2 Estimation of Ascorbic acid:

Ascorbic acid content of plant tissue was estimated by the visual titration method based on the reduction of 2, 6-dichlorophenol indophenol dye (Roe, 1954).

3.5.2.2.1 Extraction of plant material: Two grams of plant tissue was crushed thoroughly in a porcelain mortar with pestle with 8 ml of 0.4 per cent oxalic acid and a pinch of acid washed sand filtered through two layers of cheese cloth. The volume was made upto 10 ml with 0.4 per cent oxalic acid so that five ml of extract represented every g of tissue. The extract was centrifuged at 2,100 rpm for 20 minutes. The clear supernatant was used for the estimation of ascorbic acid.

3.5.2.2.2 Preparation of reagents:

Ascorbic acid standard solution: To a 250 ml volumetric flack, 50 mlo of 0.4 per cent oxalic acid and 50 mg of ascorbic acid were added and the volume was raised to 250 ml with 0.4 per cent oxalic acid. Each millilitre of this solution contained 0.2 mg of ascorbic acid and fresh solution was used to standardise the indophenol reagent.

Indophenol reagent: Fifty milligrams of sodium 2, 6-dicholoro-phenol indophenol was added to 150 ml distilled water and warmed gently on a water bath until the dye dissolved and 42 mg of sodium bicarbonate was added to it. The mixture was cooled and the volume raised to 200 ml in volumetric flask with distilled

water. The reagent was stored in darkness at 2^{o} C and was used within one week.

3.5.2.2.3 Standardisation:

The indophenol reagent was standardised before use. Five millilitres of the standardised ascorbic acid solution containing 0.2 mg per ml was taken in a white porcelain dish and titrated against the indophenol dye until the solution changed into pink colour which persisted at least for 15 seconds.

3.5.2.2.4 Estimation:

Five millilitres of oxalic acid extract was placed in a white porcelain dish and titrated against the standardised indophenol reagent until the pink colour persisted for 15 seconds. Ascorbic acid content of the tissue was calculated by employing the formula:

$$I \times S \times \frac{D}{A} \times \frac{1}{W} = \text{mg of ascorbic acid per gram of tissue}$$

Where,

I = millilitres of indophenol reagent used in the titration

S = milligrams of ascorbic acid reacting with 1 ml of reagent

D = Volume of extract in millilitres

A = Aliquots titrated in millilitres

W = Weight of the sample in grams.

RESULTS

CHAPTER IV

RESULTS

4.1 SURVEY OF SAFFLOWER RUST AND ITS INTENSITY IN ANDHRA PRADESH

Survey of safflower rust was conducted in four districts of Andhra Pradesh and the per cent disease intensity (PDI) was calculated which is presented in Table 2.

In Mahaboobnagar district, ten villages were surveyed during 1986-87 and 1987-88 rabi which recorded a per cent disease intensity of rust ranging from 6.40 to 11.88 and 4.96 to 9.15, respectively. Maximum per cent disease intensity of 11.88 and 9.15 was recorded in Kodangal during 1986-87 and 1987-88 rabi respectively, while least disease intensity was recorded in Kadtal and Rajapur with a PDI of 6.40 and 4.96, respectively. Twelve villages were surveyed in Ranga Reddy district which recorded a PDI ranging from 11.88 to 16.38 during 1986-87 and 8.28 to 11.59 during 1987-88 rabi, respectively. Maximum disease intensity was recorded at Agricultural Research Institute, Rajendranagar with a PDI of 16.38 and 11.59 during both the years. Whereas, least per cent disease intensity was recorded in Kankal during 1986-87 and Goutapur during 1987-88 rabi with 11.38 and 8.28, respectively. In Medak district, out of six villages surveyed, Sadasivpet recorded maximum per cent disease intensity during both the years with 14.75 and 10.28, respectively. Out of three villages surveyed in Kurnool district, maximum per cent

Table 2: Intensity of rust on safflower in Andhra Pradesh

Place of survey	Per cent disease intensity	
	1986-87 rabi	1987-88 rabi
I. MAHABOOBNAGAR (Dist)		
1. Kandukur	10.52	7.17
2. Kadtal	6.40	6.63
3. Rachalapally	7.88	5.30
4. Midjil	9.34	5.71
5. Gollapally	10.20	5.62
6. Vattem	7.65	5.00
7. Rajapur	8.94	4.96
8. Ranga Reddy gudem	9.68	6.32
9. Kosgi	11.67	8.04
10. Kodangal	11.88	9.15
II. RANGA REDDY (Dist)		
1. ARI, Rajendranagar	16.38	11.59
2. Moinabad	13.55	8.80
3. Chevella	13.88	9.62
4. Manneguda	14.39	9.48
5. Chittempally	13.73	9.76
6. Vikarabad	14.93	11.06
7. Tandur	15.49	10.85
8. Goutapur	12.53	8.28
9. Allapuram	12.95	8.92
10. Pargi	12.23	9.40
11. Kankal	11.68	9.27
12. Karankot	13.60	10.30
III. MEDAK (Dist)		
1. Mominpet	13.82	9.82
2. Sadasivapet	14.75	10.28
3. Sanga Reddy	11.84	8.58
4. Duddeda	10.53	7.32
5. Siddipet	11.40	7.80
6. Chandhrapur	10.43	7.19
IV. KURNOOL (Dist)		
1. Nandikotkur	10.15	6.39
2. Nandyal	10.17	6.38
3. Yemmiganur	9.28	7.06

disease intensity was recorded in Nandyal (10.17) during 1986-87 rabi and Yemmiganur recorded 7.06 during 1987-88 rabi (Table 2).

Among the districts, Ranga Reddy recorded maximum per cent disease intensity followed by Medak, Mahaboobnagar and Kurnool districts (Table 2).

4.2 STUDY OF DIFFERENT STAGES IN LIFE CYCLE OF *Puccinia carthami* Corda

After initiation of disease symptom (s), hand sections were taken from different type of pustules that are produced on the leaves. The results of study comprising pustule types appeared and corresponding stages of life cycle are presented in Table 3.

Upon germination, teleutospores produced a short, stout, 4 celled promycelia with each cell bearing a single kidney shaped sporidium(basidiospore) borne on sterigmata (plate 5).

Ten to thirteen days after inoculations, orange yellow spots appeared on cotyledons and leaves representing spermagonia. Spermagonia were formed usually in groups, subepidermal and flask shaped. A large number of flexuous hyphae were observed protruding out of ostioles (plate 6).

Masses of spermorgonia accompanied with chestnut brown pustules appeared four to seven days later comprising primary uredia which are presently referred as uredinoid aecia. They are small, saucer shaped sori without peridium and paraphyses.

Table 3: Life cycle stages of Puccinia carthami produced under artificial conditions

S. No.	Life cycle stage	Description of pustules
1.	Spermagonium	Orange-yellow spots developed on cotyledons and leaves
2.	Urediñoid aecia	Orange-yellow spots accompanied with cluster of small chestnut brown pustules developed on leaves
3.	Uredia	Scattered chestnut brown pustules without orange spots developed on leaves
4.	Telia	Black coloured pustules developed on leaves

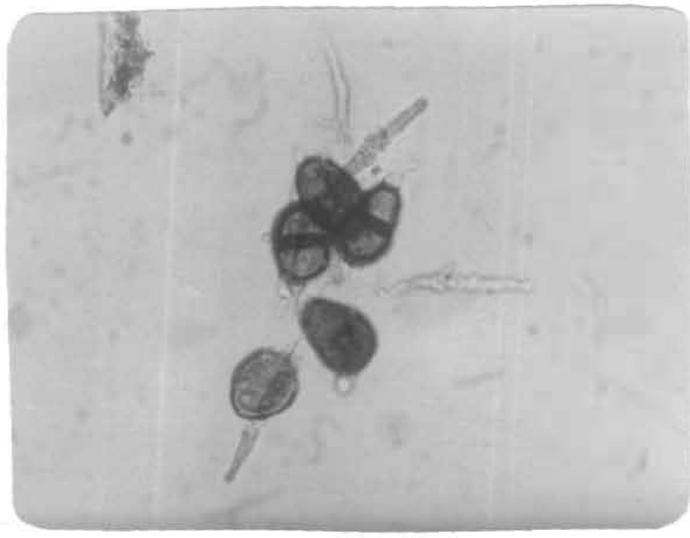


Plate 5: Germinated teleutospores with promycelium and sterigmata (x 200).

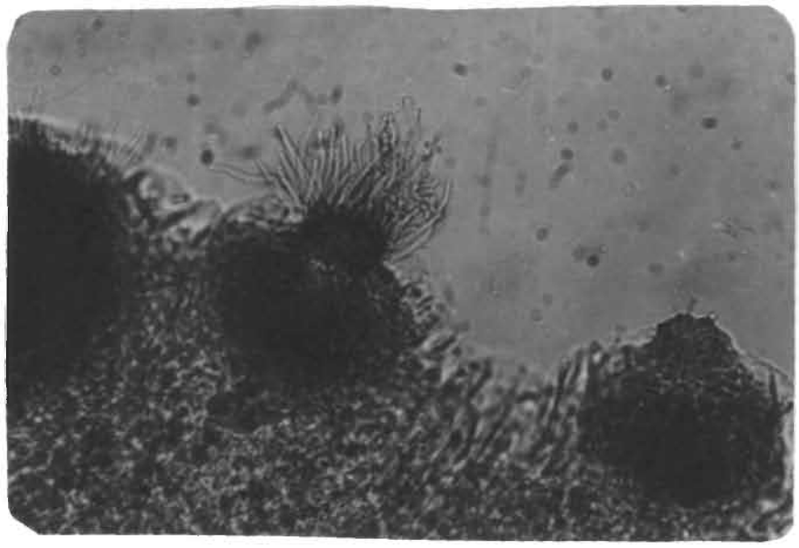


Plate 6: Sub-epidermal paraphysate spermagonium (x 100).

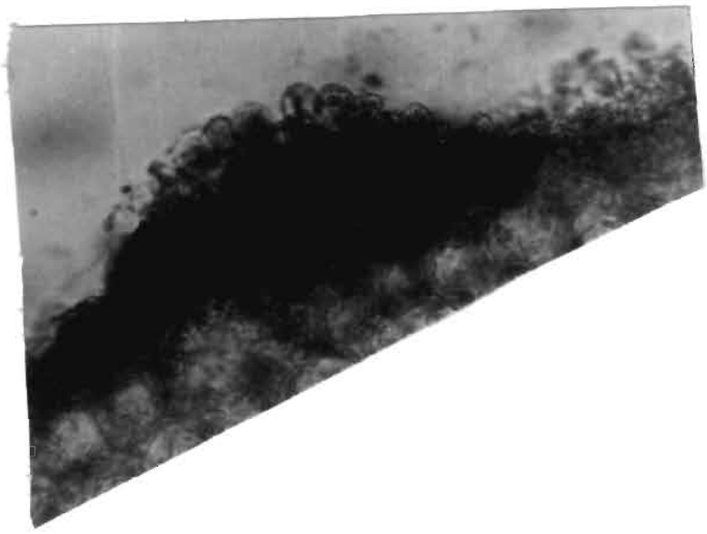


Plate 7: Aecium (x 100).

Plate 8: Close-up view of aecium with aeciospores borne on pedicels (x900).

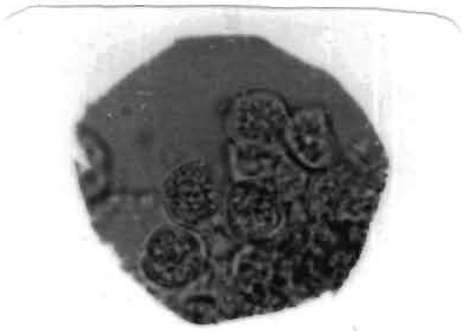


Plate 9: Aeciospores (x 200).

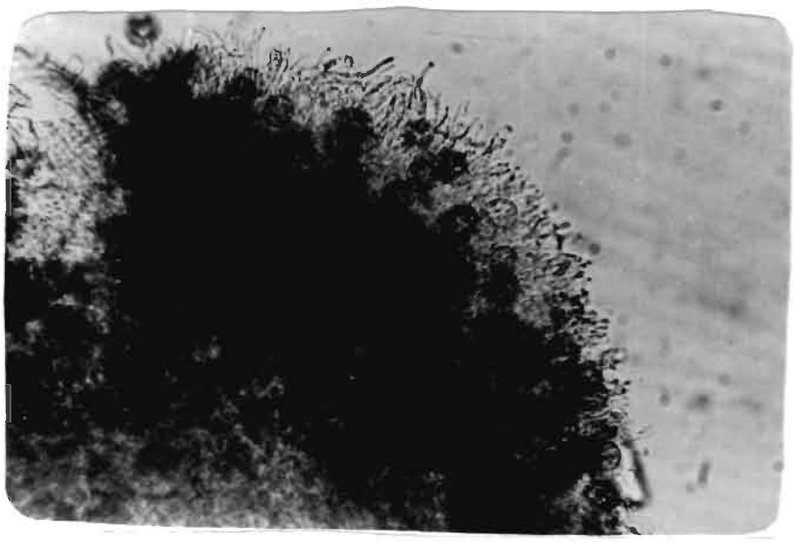


Plate 10: Urediniosori (x 100).

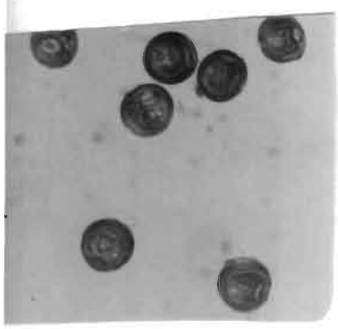


Plate 11: Urediniospores (x 200).

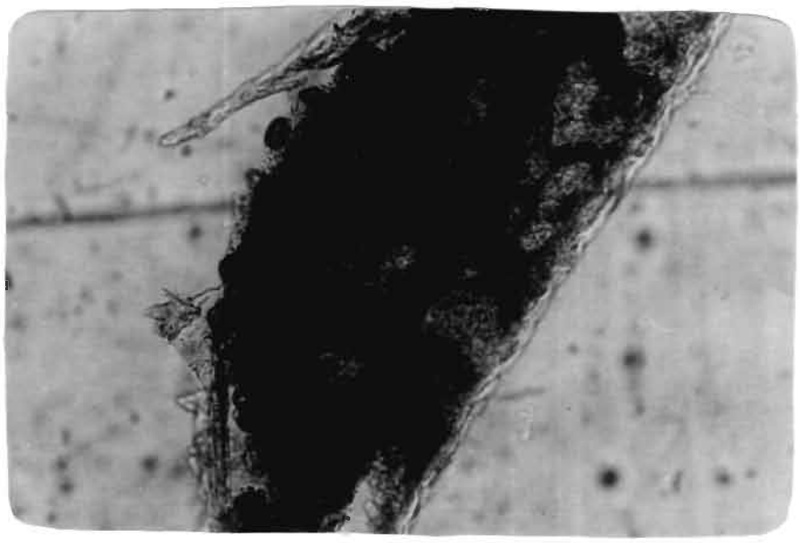


Plate 12: Teleutosori (x100)

In these sori, round or spherical sopres with smooth walls unlike normal urediniospores were seen and borne terminally on thick hyaline pedicels (Table 3 and Plate 7, 8 & 9).

Scattered chestnut brown pustules representing uredinia appeared after 20 DAI. Sori were amphigenous, scattered, erumpent, and chestnut brown. Sori were large with full of paraphyses. Urediniospores were globoid or spherical and borne singly on pedicels (Plate 10 & 11).

Black coloured pustules appeared after 55 DAI along with uredinia pustules, representing telia. They were hypophyllous seattered and often in groups. The teleutospores were chestnut brown, two celled ellipsoid, slightly constricted at septa (Plate 12).

4.3 SURVIVAL OF PATHOGEN

4.3.1 Viability of Urediniospores

The viability of urediniospores in the infected leaves stored under different conditions was determined and results are presented in Table 4.

A perusal of Table 4 indicated that the urediniospores were found viable upto 25 days in infected leaves when stored on soil surface and at 10 cm depth in the soil, whereas spores remained viable for 40 days under laboratory stored conditions. Percentage of viable spores was more under laboratory conditions as compared to other two treatments at different sampling times.

Table 4: Viability of urediniospores under different storage conditions

Storage Condition	[1] Per cent viable urediniospores									
	Days of storage									
	0	5	10	15	20	25	30	35	40	45
Soil surface	84.70 (66.93)	67.30 (55.12)	47.10 (43.34)	33.78 (35.54)	15.83 (23.44)	7.12 (15.48)	0.00 (0.00)	-	-	-
10 cm depth in the soil	85.83 (67.49)	65.85 (54.24)	45.24 (42.24)	31.25 (33.99)	13.74 (21.75)	6.25 (14.48)	0.00 (0.00)	-	-	-
Laboratory	84.66 (66.94)	72.36 (58.22)	58.96 (50.16)	46.72 (43.12)	30.28 (33.39)	24.25 (29.50)	14.36 (29:50)	8.10 (16.54)	6.42 (14.68)	0.00 (0.00)
F test	NS	Sig	Sig	Sig	Sig	Sig	-	-	-	-
S.E.	1.22	1.06	1.11	1.37	1.27	1.28	-	-	-	-
C.D. (0.05)	-	2.94	3.08	3.80	3.53	3.55	-	-	-	-

1 Average of three replications
Figures in parenthesis are arcsin transformed values

No germination was observed after 30 days of storage in two treatments viz., on soil surface and 10 cm depth in the soil while, under laboratory conditions after 45 days of storage. The initial viability of urediniospores before storage ranged from 84.66 to 85.83 which gradually decreased with increased period of storage. The percentage germination of urediniospores was statistically significant between laboratory conditions and other two treatmentsviz., on soil surface and 10 cm depth in the soil.

However, no significant differences in percentage germination of urediniospores were observed between soil surface and 10 cms depth in the soil (Table 4).

4.3.2 Viability of teleutospores

To test the viability of teleutospores, dried leaves of safflower bearing telia were collected and stored under three storage conditions. The percentage germination was recorded at an interval of 30 days and the results are presented in Table 5.

Teleutospores remained viable for 11 months when stored on soil surface and at 10 cms depth in the soil, while for 13 months under laboratory conditions. There was no germination upto 4 months of storage in all the three treatments. The germination started from 5th month onwards reaching maximum on 8th month and thereby it gradually decreased. Maximum viable spores were observed when stored under laboratory conditions, 73.50 per cent, followed by soil surface and at 10 cms depth in the soil

Table 5: Viability of teleutospores under different storage conditions

Storage conditions	*Per cent viable teleutospores														
	MONTH OF STORAGE														
	April 1987	May 1987	June 1987	July 1987	Aug. 1987	Setp. 1987	Oct. 1987	Nov. 1987	Dec. 1987	Jan. 1988	Feb. 1988	March 1988	April 1988	May 1988	June 1988
Soil surface	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	5.94 (14.11)	18.62 (25.56)	34.84 (36.17)	57.62 (49.36)	42.18 (40.30)	26.34 (30.80)	11.72 (20.02)	0.00 (0.00)	-	-
10 cm depth in the soil	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	4.68 (12.50)	16.08 (23.64)	33.48 (35.36)	55.12 (48.51)	40.25 (39.38)	24.48 (29.66)	9.86 (18.30)	0.00 (0.00)	- (0.00)	- (0.00)
Laboratory	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	9.84 (18.28)	23.74 (29.16)	42.25 (40.54)	73.50 (59.02)	54.78 (47.74)	38.74 (38.49)	21.76 (27.70)	13.68 (21.70)	7.10 (15.41)	0.00 (0.00)
F - test	-	-	-	-	-	Sig	Sig	Sig	Sig	Sig	Sig	Sig	-	-	-
S.E.	-	-	-	-	-	0.85	0.81	0.86	1.34	1.05	0.98	0.92	-	-	-
CD (0.05)	-	-	-	-	-	2.36	2.25	2.39	3.72	2.92	2.72	2.56	-	-	-

* Average of three replications
Figures in parenthesis are arcsin transformed values

after 8 months of storage. Teleutospore germination under laboratory conditions was significantly superior over that of soil surface and 10 cms depth in the soil. Whereas, percentage viable teleutospores stored on soil surface and at 10 cms depth in the soil was on par with each other. No germination was observed upto August but, teleutospores germinated profusely from November onwards with maximum in December. Whereas from January onwards there was a gradual decline in spore viability (Table 5).

4.3.3 MODE OF TRANSIMISSION

4.3.3.1 Seed transmission: To determine the teleutospore load and seed borne nature of the disease, estimations were undertaken and the data are presented in Table 6. The results showed that the average number of teleutospores per seed per seed lot was 128.66 (Table 6).

The data showed that 9.66, 17.00, 23.16, 27.80, 31.33, 32.40, 33.00 and 33.20 per cent of plants were infected after 18, 21, 24, 27, 30, 33, 36 and 40 days of sowing respectively (Table 7). The disease first appeared after 18 days of sowing and per cent infection of plants increased upto 30 days, but thereafter increase was not much compared to healthier plants in control (Fig.1).

4.3.3.2 Soil transmission: The data on soil transimission presented in Table 8 revealed that rust infection first appeared after 18 days of sowing and per cent infected plants after 18, 21, 24, 27, 30, 33, 36 and 40 days were 1.33, 7.25, 11.66, 14.33,

Table 6: Teleutospore load on harvested seed lot (K_x) of 1986-87 rabi crop

Replications	No.of seeds per tube (N)	Total water volume (W)	Water volume of drops (W_o)	No.of spores in drops observed (K_o)	No.of spores per seed (K)
1	20	4	0.6	48	160.00
2	20	4	0.6	36	120.00
3	20	4	0.6	42	140.00
4	20	4	0.6	28	93.33
5	20	4	0.6	39	130.00
				Average (K_x) =	128.66

Table 7: Seed transmission of *P. carthami*

S. No.	Treatment	[1] Per cent plants infected							
		Days after sowing							
		18	21	24	27	30	33	36	40
1.	Autoclaved soil + infested seed	9.66	17.00	23.16	27.80	31.33	32.40	33.00	33.00
2.	Autoclaved soil + surface sterilised seed (control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

1 Average of three replications

Table 8: Soil transmission of *P. carthami*

S. No.	Treatment	[1] Per cent plants infected							
		Days after sowing							
		18	21	24	27	30	33	36	40
1.	Infested soil + surface sterilised seed	1.33	7.25	11.66	14.33	16.00	17.86	18.45	18.45
2.	Autoclaved soil + surface sterilised seed (control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

1 Average of three replications

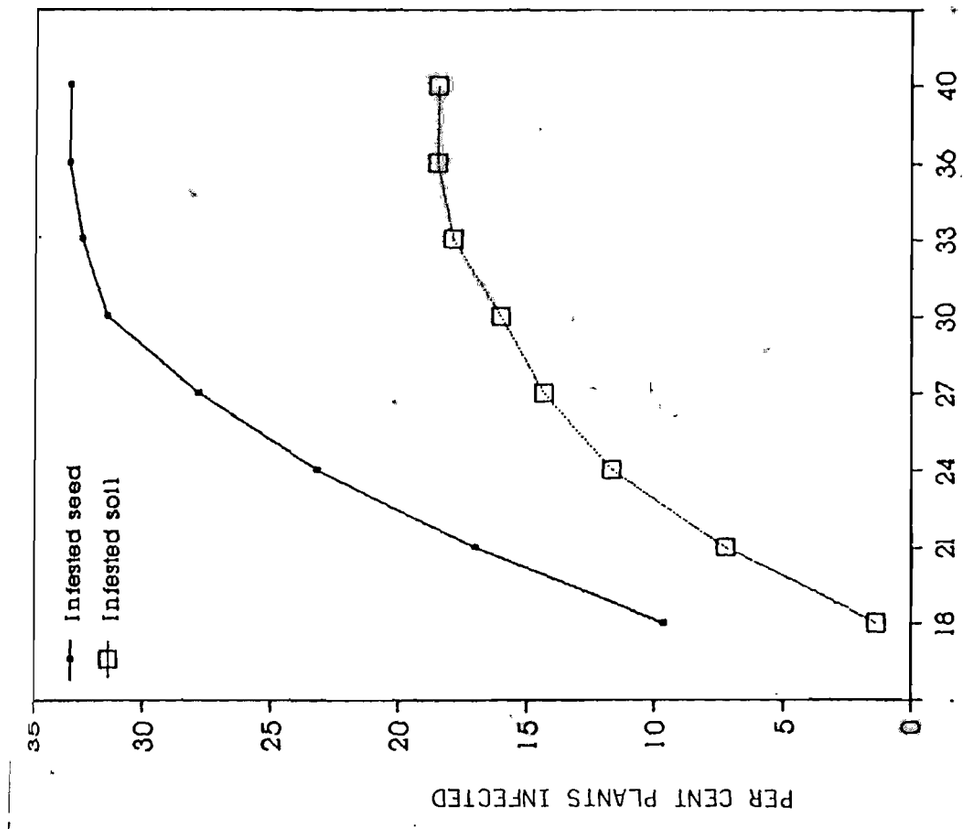


Fig.1: SAFFLOWER RUST INFECTION DUE TO INFESTED SEED AND SOIL

16.00, 17.86, 18.45 and 18.45 respectively. The per cent plant infection gradually increased upto 36 DAS without further increase, while plants in control remained healthy (Fig.1).

4.3.4 Study on collateral hosts of rust

To study the host range, fifteen weed plant species and two cultivated crop species were inoculated with urediniospores in the green house. None of them developed the disease, while the safflower (natural host) under similar conditions developed the disease (Table 9).

4.4 FIELD EXPERIMENTS

4.4.1 Effect of environmental factors on disease development

An experiment was conducted to study the effect of environmental factors on disease development during 1986-87 and 1987-88 rabi. The disease intensity recorded at different intervals (Table 10 & 13) was correlated to the environmental factors. Data were analysed statistically and correlation coefficients and prediction equations were developed which are presented in Tables 11, 12, 14 and 15.

1986-87 rabi

The periodic and cumulative increase in per cent disease intensity of rust in relation to environmental factors over a period of 43 days is shown in Fig. 2. Visual observation of Fig.2 reveals that mean maximum temperature (MMXT) and mean minimum temperature (MMIT) had great influence on the progress of

Table 9: Rust reaction of different plant species

Plant species	Rust reaction
I. WEED PLANT SPECIES	
1. *Xanthium strumarium*	-
2. *Celotia argentia*	-
3. *Tridax procumbens*	-
4. *Taraxacum* sp.	-
5. *Chrysanthemum* sp.	-
6. *Lecasca mollis*	-
7. *Parthenium hysterophorus*	-
8. *Euphorbia hirta*	-
9. *Acalypha indica*	-
10. *Indigofera glandiosa*	-
11. *Abutilon indicum*	-
12. *Cynodon dactylon*	-
13. *Cleome viscosa*	-
14. *Eragrostis major*	-
15. *Cyperus rotundus*	-
II. CROP PLANTS	
1. *Helianthus annuus*	-
2. *Guizotia abyssinica*	-
3. *Carthamus tinctorius* (natural host)	+

- No disease
+ Disease developed

Table 10: Periodic, cumulative and predicted per cent disease intensity of safflower rust over period of 42 days of crop growth (1986-87 rabi)

Date of Observation	Per cent disease intensity		
	Cumulative	Observed (periodic)	Predicted
1. 4th Jan	0.98 (5.68)	0.98 (5.68)	0.84 (5.27)
2. 6th "	1.20 (6.29)	0.22 (2.690)	0.44 (3.80)
3. 8th "	1.56 (7.20)	0.36 (3.44)	0.51 (4.08)
4. 10th "	2.69 (9.44)	1.13 (6.08)	1.90 (7.95)
5. 12th "	3.33 (10.50)	0.64 (4.59)	0.92 (5.50)
6. 14th "	3.75 (11.75)	0.42 (3.72)	0.32 (3.25)
7. 16th "	4.73 (12.56)	0.98 (5.68)	1.40 (6.79)
8. 18th "	5.99 (14.16)	1.26 (6.39)	1.40 (6.79)
9. 20th "	7.36 (15.74)	1.37 (6.70)	2.04 (8.16)
10. 22nd "	9.01 (17.46)	1.65 (7.38)	1.75 (7.59)
11. 24th "	11.04 (19.40)	2.03 (8.17)	0.92 (5.50)
12. 26th "	13.49 (21.54)	2.45 (9.024)	1.92 (7.98)
13. 28th "	15.73 (23.36)	2.24 (8.606)	3.94 (11.439)
14. 30th "	19.51 (26.22)	3.78 (11.122)	3.05 (10.05)

Contd..

Date of Observation	Per cent disease intensity		
	Cumulative	Observed (periodic)	Predicted
15. 1st Feb.	27.83 (31.82)	8.32 (16.760)	3.25 (10.25)
16. 3rd "	32.89 (34.96)	5.06 (12.842)	2.42 (8.914)
17. 5th "	35.57 (36.60)	2.68 (9.48)	2.15 (0.46)
18. 7th "	38.42 (38.30)	2.85 (9.738)	2.53 (9.17)
19. 9th "	40.18 (39.32)	1.76 (7.65)	1.46 (6.94)
20. 11th "	41.58 (40.14)	1.40 (6.84)	3.55 (10.85)
21. 13th "	43.26 (41.11)	1.68 (7.438)	2.05 (8.19)
22. 15th "	44.28 (41.70)	1.02 (5.79)	2.24 (8.58)

Figures in parenthesis are arcsin transformed values

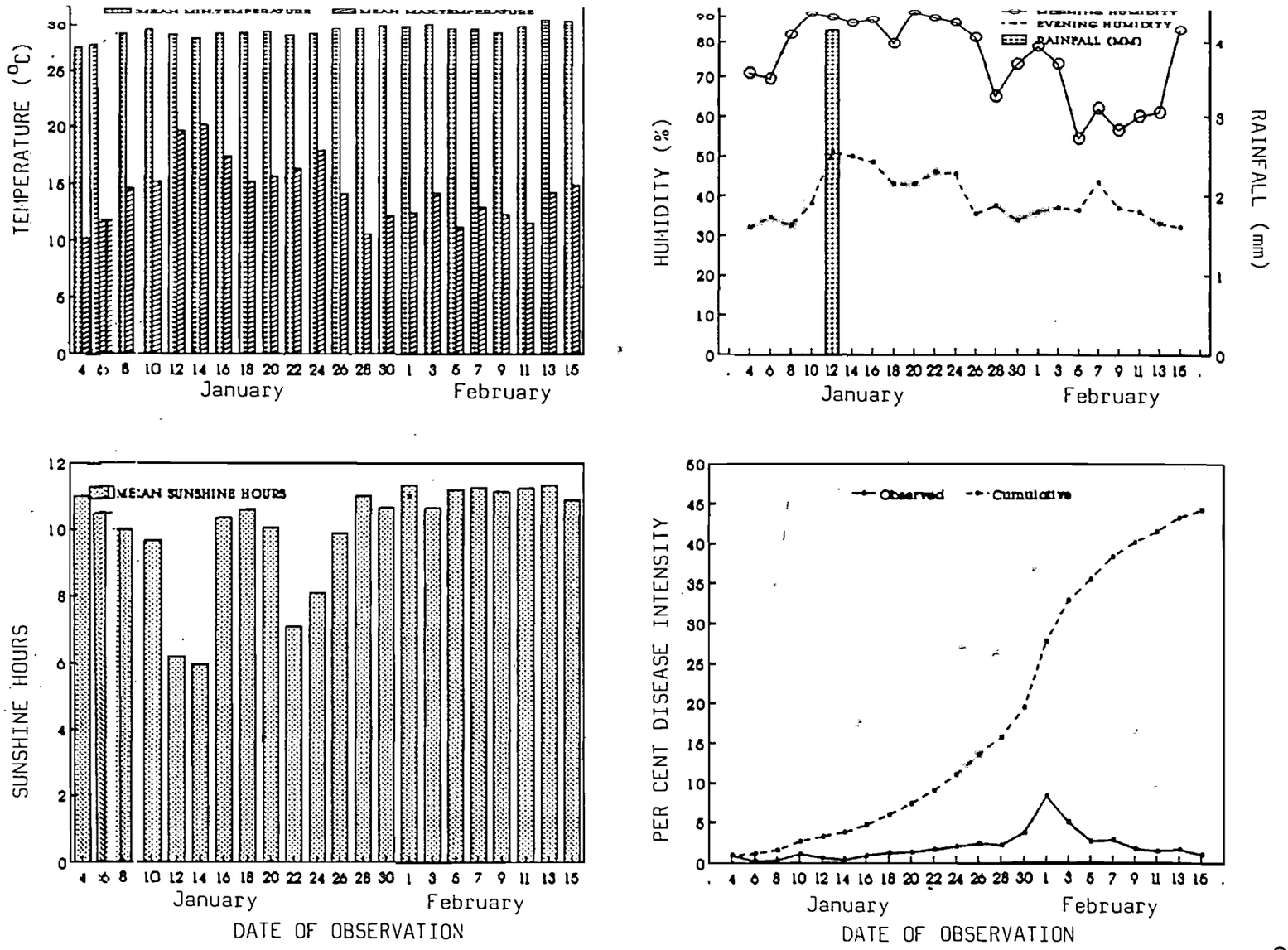


Fig.2: PERIODIC AND CUMULATIVE INCREASE IN PER CENT DISEASE INTENSITY OF SAFFLOWER RUST IN RELATION TO ENVIRONMENTAL CONDITIONS (1986-87 rabi)

Table 11: Correlation coefficients of meteorological factors with progress of safflower rust (1986-87 rabi)

S.No.	Environmental factors	'r' value
1.	Mean Maximum Temperature (MMXT)	0.5329*
2.	Mean Minimum Temperature (MMIT)	-0.3431
3.	Mean Morning Humidity (MMH)	-0.2533
4.	Mean evening humidity (MEH)	-0.1753
5.	Mean Sunshine Hours (MSH)	0.3769

* Significant at 0.05 level of probability

Table 12: Prediction equations developed for the progress of safflower rust (1986-87 rabi)

S.No.	Prediction equations	R^2 value
1	Y = -63.3143 + 2.4965 (MMXT) - 1.2417 (MMIT) + 0.0825 (MMH) + 0.3201 (MEH) - 0.2376 (MSH) (3.095**) (-2.292*) (1.115) (1.922) (-0.368)	0.4964*
2.	Y = -42.3697 + 1.8580 (MMXT) - 0.2376 (MMIT) (2.662)* (-1.473)	0.3574*
3.	Y = -51.0285 + 2.0024 (MMXT) (2.8167*)	0.2840*

Figures in parenthesis are 't' values of partial regression coefficients

* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

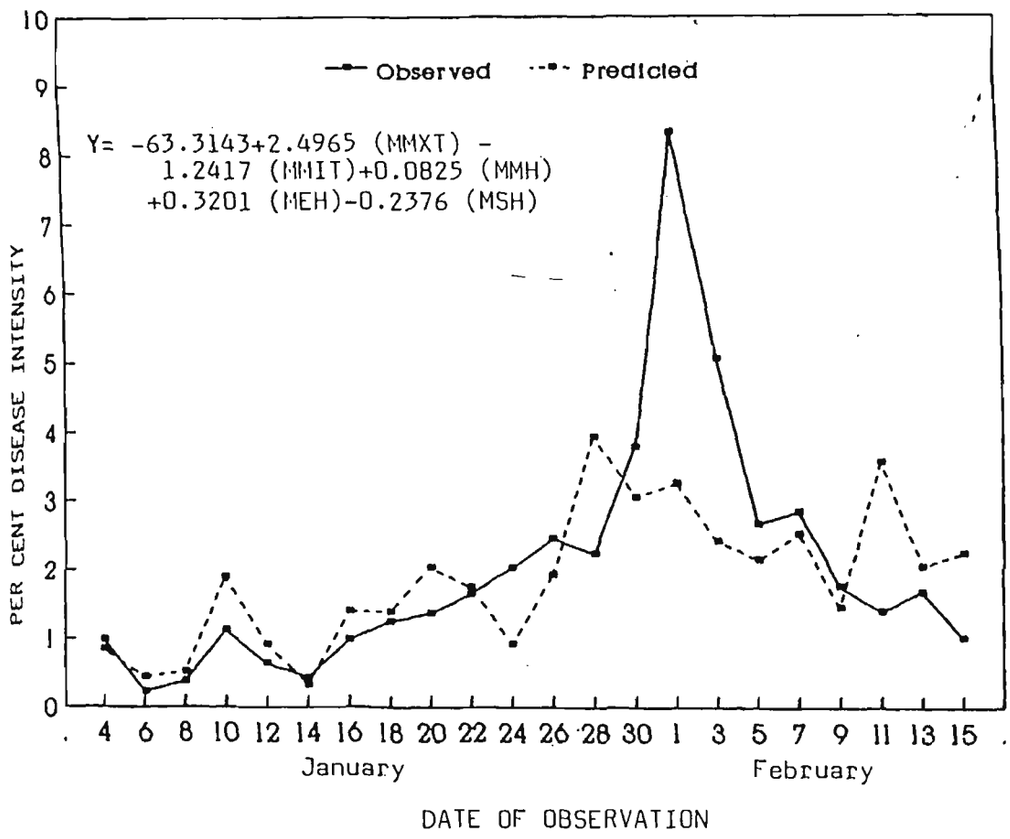


Fig.3: OBSERVED AND PREDICTED DISEASE INTENSITY OF P.carthami OF SAFFLOWER INFLUENCED BY ENVIRONMENTAL FACTORS (1986-87 rabi)

the disease. Correlation coefficients (r) of environmental factors revealed that the progress of the disease had positive and significant correlation with MMXT. Whereas, the relation with other factors such as MMIT, MMH, MEH was negative and non-significant. However, MSH had positive correlation but non-significant (Table 11).

Multiple regression analysis was carried out with environmental factors as independent variables for predicting the disease development. The analysis indicated taht the relationship consisting of environmental factors viz., MMXT, MMIT, MMH, MEH and MSH turn out to be of best fit (Table 12, equation 1, R^2=0.49). This relationship indicated that 49 per cent of variation in disease development can be estimated on the basis of environmental factors included in the relationship. Further, the partial regression coefficients reveal that out of five environmental factors only MMXT and MMIT had signficant impact on the dependent variable. In particular MMXT has significantly positive impact while MMIT has significantly negative impact on the disease development. It can be seen from correlation analysis (Table 11) that except MMXT no other environmental factors had any significant correlation with disease development. In this context the significant impact of MMXT has an indirect impact on the disease development. To understand the behaviour, further regression equation developed with MMXT and MMIT as independent variables since their partial regression coefficients in equation 1 (Table 12) were significant. The relationship obtained after

deletion of non-significant variables indicated that the fit of equation is relatively low (Table 12, Equation 2, R^2=0.35). In this relationship, the partial regression coefficients of only MMXT turn out to be significant (Table 12, Equation 2). Hence, a 3rd equation developed after deleting non-significant variable i.e. MMXT. This resulted further reducing the fit of the equation (Table 12, equation 3, R^2=0.28). Hence considering the fit of the equations and the significance of partial regression coefficients it can be concluded that equation 1 can be applied for predicting the disease development (Table 12). The corresponding graph of the observed and predicted values of disease development for the period is presented in Fig.3.

1987-88 rabi

The periodic and cumulative increase in per cent disease intensity of rust in relation to environmental factors over period of 37 days is shown in Fig.4. Visual observation of Fig.4 reveals that MMXT and MMIT had great influence on the progress of the disease. Correlation coefficients (r) of environmental factors with the disease development revealed that disease development had positive and significant correlation with MMXT and MSH whereas, the relation with MMIT and MMH was negative and non-significant. But with MEH the relation was negative and significant (Table 14).

The regression equations developed to estimate disease development on the basis of environmental factors revealed that

Table 13: Periodic, cumulative and predicted per cent disease intensity of safflower rust over period of 36 days of crop growth (1987-88 rabi)

Date of Observation	Per cent disease intensity		
	Cumulative	Observed (periodic)	Predicted
1. 10th Jan.	0.58 (4.37)	0.58 (4.37)	0.48 (3.96)
2. 12th "	0.70 (4.80)	0.12 (1.99)	0.198 (2.525)
3. 14th "	0.89 (5.41)	0.19 (2.53)	0.24 (2.806)
4. 16th "	1.61 (7.28)	0.72 (21.87)	1.05 (5.914)
5. 18th "	2.45 (9.01)	0.84 (5.26)	0.61 (4.50)
6. 20th "	3.31 (10.48)	0.86 (5.32)	1.05 (5.96)
7. 22nd "	3.99 (11.52)	0.68 (4.73)	1.30 (6.57)
8. 24th "	5.41 (13.46)	1.42 (6.84)	0.75 (4.95)
9. 26th "	6.39 (14.63)	0.98 (5.68)	1.90 (7.53)
10. 28th "	7.71 (16.14)	1.32 (6.60)	2.12 (8.38)
11. 30th "	9.47 (17.90)	1.76 (7.66)	1.44 (6.87)
12. 1st Feb.	10.79 (19.18)	1.32 (6.52)	2.16 (8.49)
13. 3rd "	12.37 (20.60)	1.58 (7.75)	1.32 (6.61)
14. 5th "	14.33 (22.25)	1.96 (8.04)	3.80 (11.24)

Contd..

Date of Observation	Per cent disease intensity		
	Cumulative	Observed (periodic)	Predicted
15. 7th "	20.17 (26.68)	5.84 (14.016)	3.24 (10.43)
16. 9th "	23.37 (28.98)	3.20 (10.31)	2.47 (9.02)
17. 11th "	25.22 (30.15)	1.85 (7.76)	1.55 (7.179)
18. 13th "	26.48 (30.95)	1.26 (6.49)	1.11 (6.03)
19. 15th "	27.37 (31.54)	0.89 (5.41)	0.59 (4.41)

Figures in parentheses are arcsin transformed values

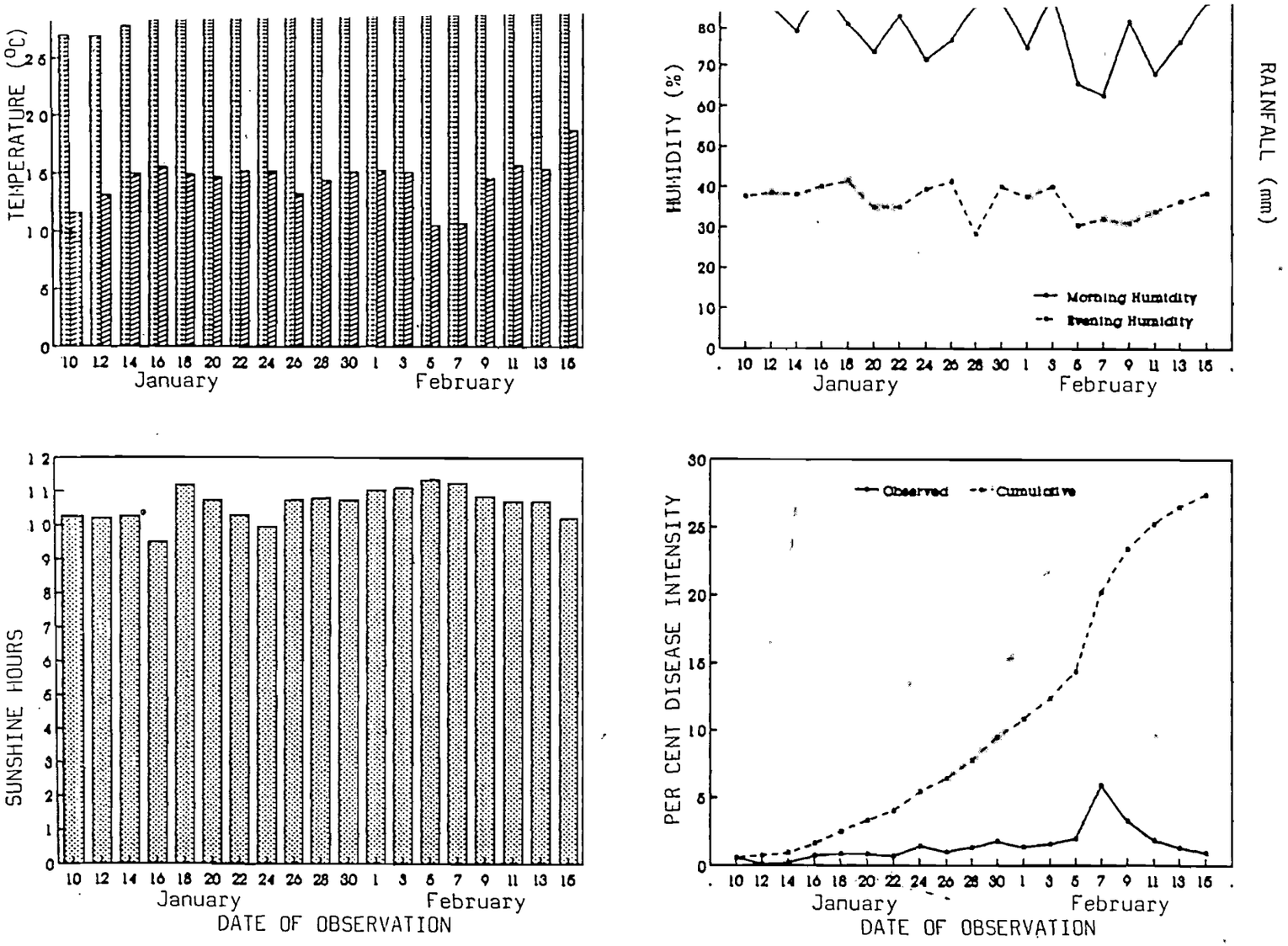


Fig.4: PERIODIC AND CUMULATIVE INCREASE IN PER CENT DISEASE INTENSITY OF SAFFLOWER RUST IN RELATION TO ENVIRONMENTAL CONDITIONS (1987-88 rabi)

Table 14: Correlation coefficients of meteorological factors with progress of safflower rust (1987-88 rabi)

S.No.	Environmental factors	'r' value
1.	Mean Maximum Temperature (MMXT)	0.6435**
2.	Mean Minimum Temperature (MMIT)	-0.3392
3.	Mean Morning Humidity (MMH)	-0.4343
4.	Mean evening humidity (MEH)	-0.5426*
5.	Mean Sunshine Hours (MSH)	0.6253**

* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

Table 15: Prediction equations developed for the progress of safflower rust (1987-89 rabi)

S.No.	Prediction equations	R^2 value
1	Y = -34.6919 + 1.2510 (MMXT) - 0.5524 (MMIT) + 0.00097(MMH) - 0.0427 (MEH) + 1.2576 (MSH) (2.748)* (-1.62) (-0.155) (-0.287) (0.988)	0.6973**
2.	Y = -29.0534 + 1.5371 (MMXT) - 0.7809 (MMIT) (5.222)** (3.590)**	0.6728**
3.	Y = -32.6716 + 1.2857 (MMXT) (3.466)**	0.4140**

Figures in parenthesis are 't' values of partial regression coefficients

* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

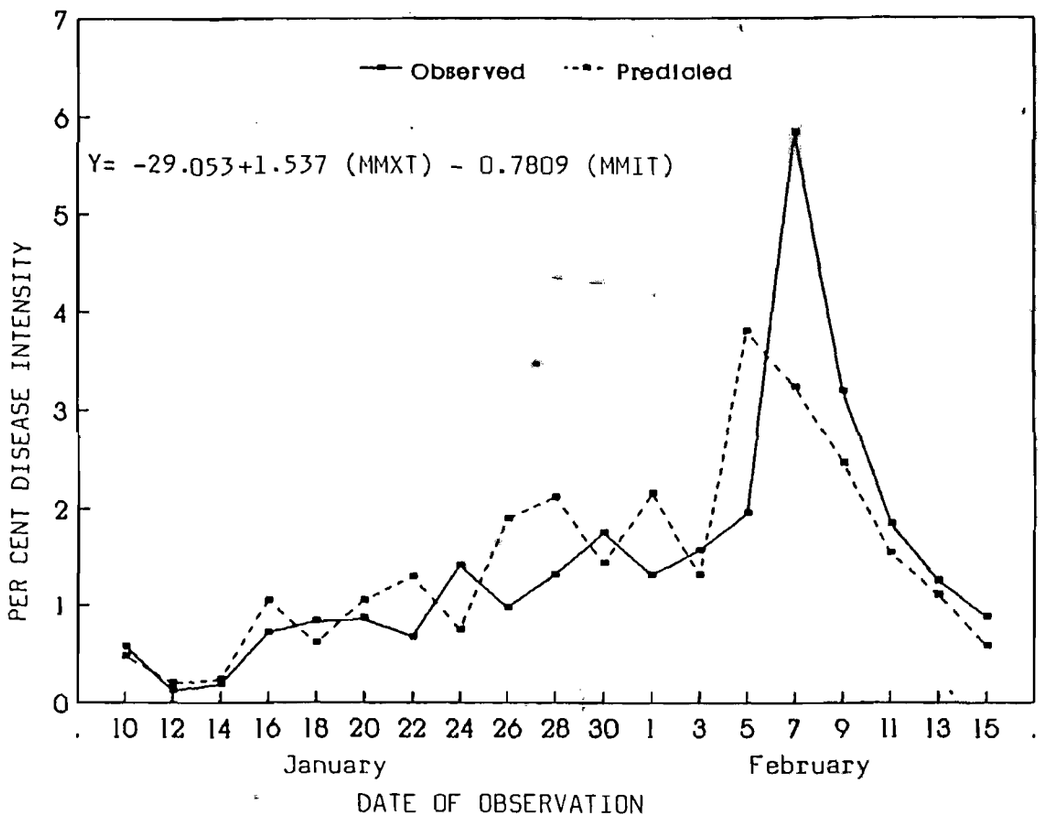


Fig.5: OBSERVED AND PREDICTED PER CENT DISEASE INTENSITY OF *P.carthami* OF SAFFLOWER INFLUENCED BY ENVIRONMENTAL FACTORS (1987-88 rabi)

the MMXT is the major environmental factor which influenced the disease development. However, when MMXT was included along with other factors such as MSH and MEH (which were having a significant correlation with the disease development), the influence of these factors as measured in terms of partial regression coefficients was found to be non-significant (Table 15, equation 1). The R^2 of the regression equation revealed that the fit of equation 1 was 0.69 (R^2) indicating 69 per cent variation in disease development (Y) can be explained by the environmental factors viz., MMXT, MMIT, MMH, MEH and MSH included in this equation. The partial regression coefficients of this equation indicated that every unit increase in MMXT leads to corresponding increase in disease development by 1.25 units, when other variables in equation are at constant levels. Since the partial regression coefficients of only MMXT was significant in the above relationship (Table 15, equation 1), the data were further subjected to regression analysis by deleting the variables having relatively low and non-significant 't' values for the partial regression coefficients. Thus, the relationship obtained with MMXT and MMIT as independent variables indicated that 67 per cent of the variation in the dependent variable can be explained with these two independent variables (Table 15, equation 2). Further it can also be observed that the partial regression coefficients of these two variables are significant at 1 per cent level. Another noteworthy feature of this equation 2 is that MMIT whose partial regression coefficient was not

significant in equation 1 turn out to be highly significant. Finally based on the relatively high 't' values of the partial regression coefficients in equation 2, a relationship was developed with MMXT as independent variable. The fit of this relationship was very low as compared to other two equations (Table 15, equation 3, R^2=0.41). Considering the relative fit of the relationships it can be seen that equation 2 had only a marginal decrease (0.02) in the R^2 as compared to equaiton 1. Hence, it can be inferred that the variable which were deleted in equation 2 had negligible impact on the dependent variable. Further, all the partial regression coefficients in equation 2 are highly significant. Therefore equation 2 can be considered for predicting the disease development (Table 15). The corresponding graph of the observed and predicted values of the disease development for the period is presented in Fig.5.

A comparison of the relationships of the two seasons data indicate that the disease development can be effectively predicted with MMXT and MMIT.

4.4.2 Effect of different periods of sowing on disease incidence

To find out the effect of varied sowing periods on the disease intensity, studies were conducted during 1986-87 and 1987-88 rabi season. The results are depicted in Tables 16 and 17.

Table 16: Disease intensity at different crop growth stages in varied periods of sowing

S.No.	Crop stage	Per cent disease intensity (PDI)							
		First fortnight of Sept. 1986	Second fortnight of Sept. 1986	First fortnight of Oct. 1986	Second fortnight of Oct. 1986	First fortnight of Nov. 1986	Second fortnight of Nov. 1986	First fortnight of Dec. 1986	Second fortnight of Dec. 1986
1.	Seedling	-	-	-	-	-	-	1.06	2.78
2.	Stem elongation	-	-	-	-	-	1.82	14.85	21.62
3.	Branching	-	-	-	-	1.72	16.54	37.43	49.25
4.	Flowering	-	-	1.02	2.38	14.65	38.75	50.26	64.76
5.	Seed Formation	-	0.08	6.52	17.84	32.26	46.40	57.84	69.62
6.	Seed maturity	0.52	2.05	10.65	22.56	34.86	48.09	59.76	69.75

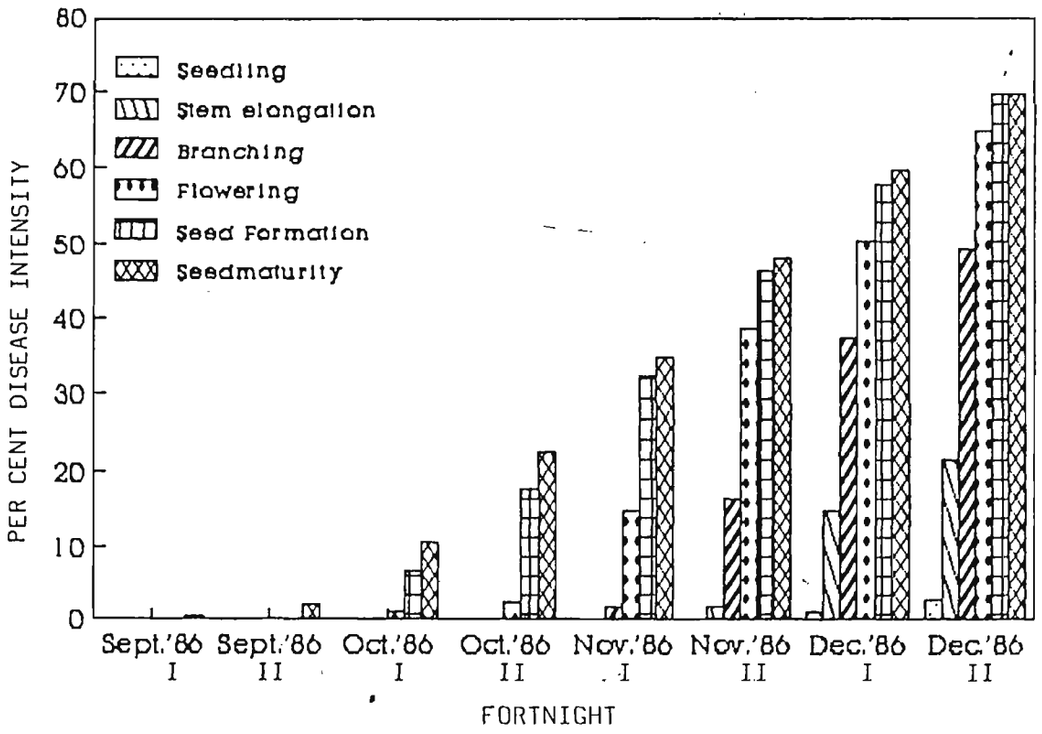


Fig.6: DISEASE INTENSITY AT DIFFERENT CROP GROWTH STAGES IN VARIED PERIODS OF SOWING (1986-87 rabi)

During 1986-87 rabi, the disease was conspicuous right from flowering to seed maturity stage in the crop sown in first fortnight of October onwards. Practically there was no disease in seedling, stem elongation and branching stages in majority of the sowings. The disease intensity gradually increased with time of sowing and was found highest with 69.75 per cent in December second fortnight sown crop at seed maturity stage, unlike in September and October sowings (Fig.6). Minimum disease was observed at seed maturity stage in September sown crop with disease intensity of 0.52 to 2.05 per cent (Table 16).

Similar trend was observed in the rabi 1987-88 season too. But maximum disease intensity was only 58 per cent in December sowings which was about 20 per cent less than previous year (Table 17). However, the crop sown in first fornight of September was completely free from rust disease (Fig.7).

4.4.3 Screening of germplasm and varieties against P.carthami

The reaction of three varieties and 189 germplasm lines totally 192 were assessed under field conditions during 1986-87 and 1987-88 rabi season. The observations were recorded as per cent leaf area damaged. Based on which these were categorised into five groups and the results are presented in Table 18.

Out of 192 germplasm lines and varieties tested 53 were identified as immune, 14 resistant, 17 moderately resistant, 32 moderately susceptible and 76 susceptible to rust disease (Table 18).

Table 17: Disease intensity of rust at different crop growth stages in varied periods of sowing (1987-88 rabi)

S.No.	Crop stage	Per cent disease intensity (PDI)							
		First fortnight of Sept. 1986	Second fortnight of Sept. 1987	First fortnight of Oct. 1987	Second fortnight of Oct. 1987	First fortnight of Nov. 1987	Second fortnight of Nov. 1987	First fortnight of Dec. 1987	Second fortnight of Dec. 1987
1.	Seedling	-	-	-	-	-	-	-	1.25
2.	Stem elongation	-	-	-	-	-	0.72	5.87	14.86
3.	Branching	-	-	-	-	0.44	7.85	26.75	37.65
4.	Flowering	-	-	-	1.08	3.20	24.80	38.46	49.78
5.	Seed Formation	-	-	2.54	10.62	18.29	32.46	43.24	52.24
6.	Seed maturity	-	0.68	5.67	12.34	20.08	33.74	44.08	58.00

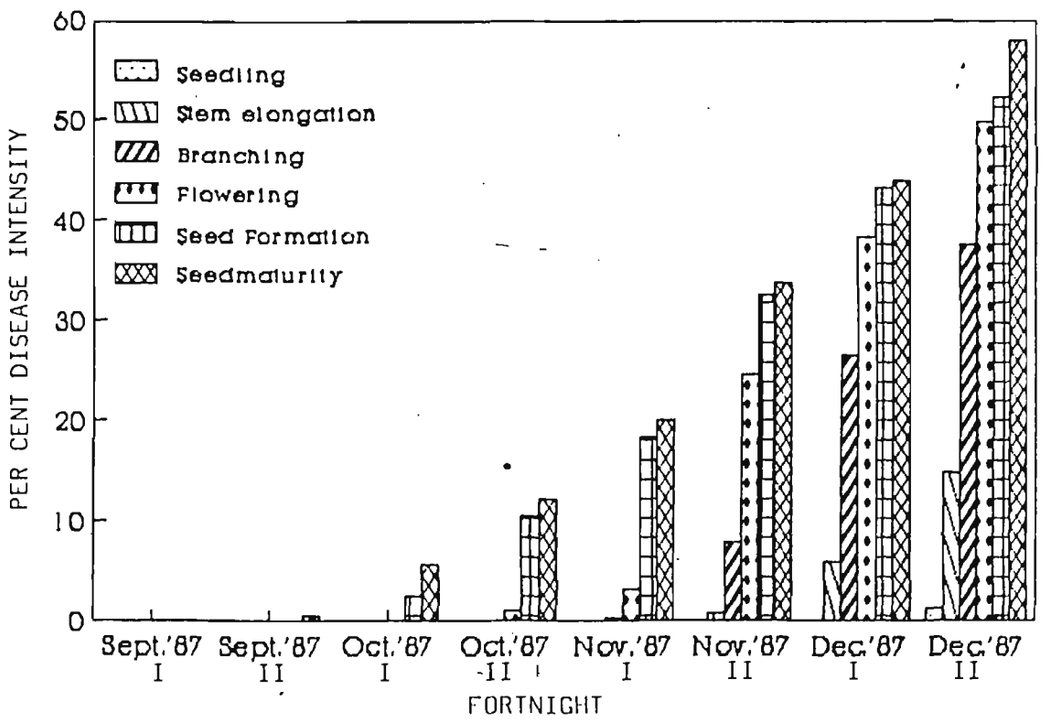


Fig.7: DISEASE INTENSITY OF DIFFERENT CROP GROWTH STAGES IN VARIED PERIODS OF SOWING (1987-88 rabi)

Table 18: Reaction of germplasm lines and varieties against *P. carthami* Corda

S. No.	Germplasm/ variety	1986-87 rabi		1987-88 rabi	
		% leaf area damaged	Reaction	% leaf area damaged	Reaction
Germplasm					
1.	HUS-1	0.00	I	0.00	I
2.	HUS-30	17.95	MR	13.64	MR
3.	HUS-60	0.00	I	0.00	I
4.	HUS-71	4.24	R	2.94	R
5.	HUS-81	70.16	S	64.35	S
6.	HUS-138	68.65	S	56.70	S
7.	HUS-151	0.00	I	0.00	I
8.	HUS-156	0.00	I	0.00	I
9.	HUS-152	59.62	S	50.96	S
10.	HUS-159	69.18	S	62.12	S
11.	HUS-160	0.00	I	0.00	I
12.	HUS-161	35.70	MS	26.95	MS
13.	HUS-174	0.00	I	0.00	I
14.	HUS-175	40.67	MS	32.74	MS
15.	HUS-176	68.95	S	62.75	S
16.	HUS-212	0.00	I	0.00	I
17.	HUS-215	0.00	I	0.00	I
18.	HUS-224	62.10	S	55.02	S
19.	HUS-227	65.76	S	58.10	S
20.	HUS-252	65.25	S	58.70	S
21.	HUS-261	7.10	MR	4.98	R
22.	HUS-262	45.76	MS	36.94	MS
23.	HUS-263	16.03	MR	9.60	MR
24.	HUS-269	6.18	MR	4.26	R
25.	HUS-278	0.00	I	0.00	I
26.	HUS-285	0.00	I	0.00	I
27.	HUS-287	0.00	I	0.00	I
28.	HUS-294	34.60	MS	26.25	MS
29.	HUS-308	19.00	MR	1.16	MR
30.	HUS-452	71.25	S	65.60	S
31.	HUS-453	35.12	MS	28.30	MS
32.	HUS-472	68.10	S	56.00	S
33.	HUS-483	0.00	I	0.00	I
34.	HUS-484	69.20	S	61.34	S
35.	HUS-488	58.75	S	52.05	S

contd...

Table 18 contd...

S. No.	Germplasm/ variety	1986-87 rabi		1987-88 rabi	
		% leaf area damaged	Reaction	% leaf area damaged	Reaction
Germplasm					
36.	HUS-500	38.12	MS	31.86	MS
37.	HUS-507	72.16	S	64.70	S
38.	HUS-509	43.97	MS	38.26	MS
39.	HUS-515	41.08	MS	34.10	MS
40.	HUS-518	59.06	S	52.76	S
41.	HUS-519	0.00	I	0.00	I
42.	HUS-522	0.00	I	0.00	I
43.	HUS-533	43.50	MS	37.65	MS
44.	HUS-541	65.36	S	56.07	S
45.	HUS-548	62.10	S	52.65	S
46.	HUS-552	71.25	S	58.70	S
47.	HUS-556	46.97	MS	40.16	MS
48.	HUS-560	73.16	S	60.75	S
49.	HUS-277	0.00	I	0.00	I
50.	HUS-566	53.76	S	46.94	MS
51.	HUS-568	59.92	S	52.10	S
52.	HUS-673	27.86	MS	21.78	MS
53.	HUS-675	36.80	MS	30.05	MS
54.	HUS-676	0.00	I	0.00	I
55.	HUS-677	0.00	I	0.00	I
56.	HUS-678	56.70	S	48.90	MS
57.	HUS-689	61.75	S	56.70	S
58.	HUS-690	59.30	S	53.25	S
59.	HUS-691	60.62	S	53.00	S
60.	HUS-692	67.63	S	60.35	S
61.	HUS-695	53.86	S	46.70	MS
62.	HUS-696	59.60	S	51.75	S
63.	HUS-697	62.18	S	54.00	S
64.	HUS-699	3.96	R	3.46	R
65.	HUS-701	4.08	R	3.05	R
66.	HUS-702	16.75	MR	11.84	MR
67.	HUS-703	19.00	MR	14.35	MR
68.	HUS-704	16.50	MR	12.96	MR
69.	HUS-706	0.00	I	0.00	I
70.	HUS-708	69.12	S	60.35	S

contd...

Table 18 contd...

S. No.	Germplasm/ variety	1986-87 rabi		1987-88 rabi	
		% leaf area damaged	Reaction	% leaf area damaged	Reaction
Germplasm					
71.	HUS-709	57.18	S	50.96	S
72.	HUS-712	57.92	S	51.02	S
73.	HUS-714	65.70	S	58.65	S
74.	HUS-716	62.90	S	56.70	S
75.	HUS-717	0.00	I	0.00	I
76.	HUS-721	4.25	R	3.18	R
77.	HUS-748	19.08	MR	16.34	MR
78.	HUS-752	6.24	MR	4.00	R
79.	HUS-792	0.00	I	0.00	I
80.	HUS-794	34.56	MS	28.15	MS
81.	HUS-801	0.00	I	0.00	I
82.	HUS-802	56.40	S	49.75	MS
83.	HUS-810	61.70	S	54.76	S
84.	HUS-813	41.95	MS	36.62	MS
85.	HUS-818	4.50	R	3.45	R
86.	HUS-827	0.00	I	0.00	I
87.	HUS-829	0.00	I	0.00	I
88.	HUS-831	67.85	S	60.35	S
89.	HUS-835	0.00	I	0.00	I
90.	HUS-842	46.84	MS	39.96	MS
91.	HUS-853	0.00	I	0.00	I
92.	HUS-861	61.70	S	52.00	S
93.	HUS-872	62.50	S	50.75	S
94.	HUS-887	0.00	I	0.00	I
95.	HUS-902	0.00	I	0.00	I
96.	HUS-908	3.70	R	2.83	R
97.	HUS-921	59.62	S	51.40	S
98.	HUS-926	56.10	S	45.70	MS
99.	HUS-934	62.23	S	54.60	S
100.	HUS-947	0.00	I	0.00	I
101.	HUS-950	56.10	S	43.70	MS
102.	HUS-954	52.32	S	38.30	MS
103.	HUS-955	34.00	MS	25.40	MS
104.	HUS-957	4.26	R	3.65	R
105.	HUS-959	0.00	I	0.00	I

contd...

Table 18 contd...

S. No.	Germplasm/ variety	1986-87 rabi % leaf area damaged	1986-87 rabi Reaction	1987-88 rabi % leaf area damaged	1987-88 rabi Reaction
Germplasm					
106.	HUS-960	59.92	S	52.13	S
107.	HUS-961	68.05	S	58.74	S
108.	HUS-963	0.00	I	0.00	I
109.	HUS-964	30.20	MS	21.80	MS
110.	HUS-965	3.75	R	2.46	R
111.	HUS-967	0.00	I	0.00	I
112.	HUS-968	0.00	I	0.00	I
113.	HUS-969	0.00	I	0.00	I
114.	HUS-977	0.00	I	0.00	I
115.	HUS-980	0.00	I	0.00	I
116.	HUS-1001	0.00	I	0.00	I
117.	HUS-1085	0.00	I	0.00	I
118.	HUS-1146	3.12	R	2.08	R
119.	HUS-1157	0.00	I	0.00	I
120.	HUS-1994	0.00	I	0.00	I
121.	HUS-3040	38.866	MS	32.68	MS
122.	HUS-3042	13.02	MR	8.65	MR
123.	HUS-3043	47.50	MS	40.50	MS
124.	HUS-3047	9.56	MR	6.32	MR
125.	HUS-3057	4.65	R	3.47	R
126.	HUS-3060	0.00	I	0.00	I
127.	HUS-3066	0.00	I	0.00	I
128.	HUS-3072	0.00	I	0.00	I
129.	HUS-3076	0.00	I	0.00	I
130.	HUS-3078	0.00	I	0.00	I
131.	HUS-3085	4.05	R	3.20	R
132.	HUS-3090	0.00	I	0.00	I
133.	HUS-3100	0.00	I	0.00	I
134.	HUS-3103	0.00	I	0.00	I
135.	HUS-4039	0.00	I	0.00	I
136.	PI 408	44.95	MS	38.75	MS
137.	PI 262-24	60.76	S	54.84	S
138.	PI 2457	47.03	MS	41.30	MS
139.	PI 19991-5	16.24	MR	13.18	MR
140.	PI 199923	62.10	S	56.07	S

contd...

Table 18 contd...

S. No.	Germplasm/ variety	1986-87 rabi		1987-88 rabi	
		% leaf area damaged	Reaction	% leaf area damaged	Reaction
Germplasm					
141.	PI 199929	39.26	MS	30.96	MS
142.	PI 199932	3.02	R	1.60	R
143.	PI 220283	32.50	MS	24.75	MS
144.	PI 253912	3.96	R	2.03	R
145.	PI 262449	48.85	MS	42.80	MS
146.	PI 271139	72.08	S	60.05	S
147.	PI 279051	59.00	S	52.68	S
148.	PI 279053	38.42	MS	31.96	MS
149.	PI 283740	61.93	S	55.65	S
150.	PI 283757	67.03	S	58.12	S
151.	PI 283790	63.18	S	56.30	S
152.	PI 306825	39.65	MS	33.52	MS
153.	PI 306828	13.00	MR	9.97	MR
154.	PI 306831	67.70	S	56.10	S
155.	PI 306899	69.98	S	61.70	S
156.	PI 306917	63.16	S	57.25	S
157.	PI 306925	58.30	S	50.65	S
158.	PI 306926	61.38	S	54.16	S
159.	PI 306934	59.85	S	52.03	S
160.	PI 306941	65.10	S	56.68	S
161.	PI 306944	60.95	S	53.12	S
162.	PI 306967	58.32	S	50.98	S
163.	PI 306983	68.80	S	59.10	S
164.	PI 306984	32.98	MS	27.30	MS
165.	PI 307033	10.75	MR	6.95	MR
166.	PI 307053	12.28	MR	8.05	MR
167.	PI 307060	0.00	I	0.00	I
168.	PI 307062	2.46	R	1.28	R
169.	PI 307077	46.07	MS	39.16	MS
170.	PI 307078	0.00	I	0.00	I
171.	PI 307079	46.96	MS	40.78	MS
172.	PI 307099	59.85	S	51.95	S
173.	PI 317061	65.50	S	58.96	S
174.	NO. 7	73.00	S	61.25	S
175.	NO. 144	58.30	S	50.86	S

contd...

Table 18 contd...

S. No.	Germplasm/ variety	1986-87 rabi % leaf area damaged	1986-87 rabi Reaction	1987-88 rabi % leaf area damaged	1987-88 rabi Reaction
Germplasm					
176.	NO.1021	45.95	MS	38.62	MS
177.	NO.1021-1	48.18	MS	40.75	MS
178.	B-20	61.05	S	52.32	S
179.	B-40	62.86	S	53.16	S
180.	B-144	62.35	S	54.03	S
181.	B.210	68.16	S	58.17	S
182.	SSF-13	35.58	MS	28.76	MS
183.	SSF-16	62.80	S	55.10	S
184.	SSF-40	0.00	I	0.00	I
185.	A-11-2	75.02	S	63.48	S
186.	A-21-3	49.00	MS	43.50	MS
187.	P-35	9.95	MR	7.60	MR
188.	NS-133	0.00	I	0.00	I
189.	TL-45	3.78	R	2.34	R
Varieties					
1.	APRR-3	0.00	I	0.00	I
2.	TARA	58.65	S	50.12	S
3.	BHIMA	72.56	S	61.05	S

I : Immune MR : Moderately resistant S : Susceptible
R : Resistant MS : Moderately susceptible

4.4.4 Field evaluation of certain fungicides against P. carthami

Four fungicides viz., Bavistin, Dithane M-45, Saprol and Topsin-M with three spray shedules were tested in the field during rabi 1986-87 and 1987-88 for their efficacy against rust disease. The results are presented in Table 19 and 20.

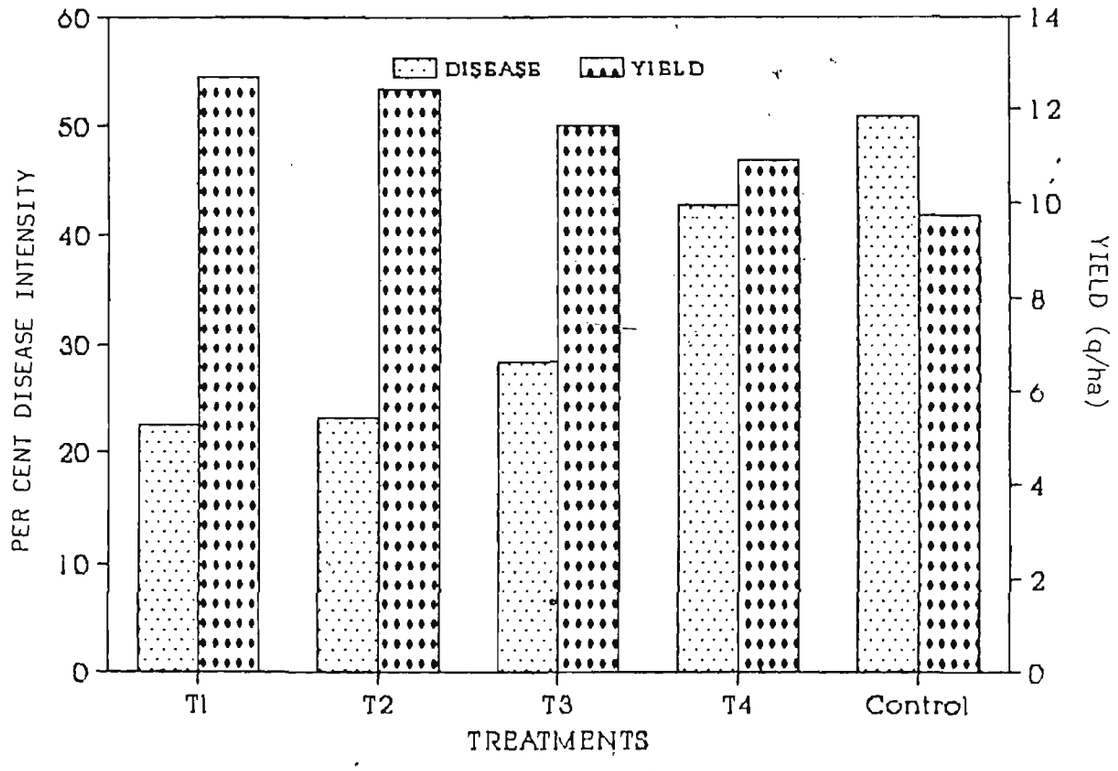
It is evident from the data that all the fungicides tested against rust were effective in reducing the disease intensity and significantly superior over control in both the years i.e., rabi 1986-87 and 1987-88 (Table 19 and 20). Bavistin and Dithane M-45 did not differ significantly and were on par with each other. However, Bavistin and Dithane M-45 proved to be highly effective in checking the disease intensity followed by Saprol and Topsin-M. Bavistin, Dithane M-45, Saprol and Topsin-M treated plots recorded a per cent disease intensity of 22.62, 23.24, 28.38 and 42.78 in 1986-87 and 16.84, 17.48, 21.75 and 30.72 in 1987-88 rabi respectively after 20 days of third spray. Whereas, control recorded 50.85, 35.68 per cent disease intensity during 1986-87 and 1987-88 rabi respectively. Per cent disease intensity before first spray ranged from 0.06 to 0.08 and 0.04 to 0.05 during 1986-87 and 1987-88 rabi, respectively (Tables 19 and 20). In all the treatments rust intensity increased gradually from the first spray onwards till the final assessment (Fig.8 and 9).

As regards yields, the effect of chemicals were found significantly superior over control. The effect of Bavistin and

Table 19: Effect of fungicides on the control of rust and yield of safflower (1986-87 rabi)

S. No.	Treatment and dose		[1]Per cent disease intensity: Before first spray	Before second spray	Before third spray	Final	Yield ($q\ ha^{-1}$)
1.	Bavistin	0.05%	0.08 (1.62)	2.26 (8.63)	10.03 (18.47)	22.62 (28.39)	12.68
2.	Dithane M-45	0.20%	0.07 (1.52)	2.35 (8.81)	10.78 (19.17)	23.24 (28.80)	12.42
3.	Saprol	0.15%	0.06 (1.40)	3.50 (10.28)	15.34 (23.06)	28.38 (32.19)	11.65
4.	Topsin M	0.05%	0.06 (1.40)	5.08 (13.02)	22.46 (28.29)	42.78 (40.84)	10.90
5.	Control		0.07 (1.52)	8.96 (17.42)	34.85 (34.97)	50.85 (45.49)	9.78
	F - test		NS	Sig	Sig	Sig	Sig
	S.E.		0.33	0.49	1.22	1.21	0.32
	CD (0.05)		-	1.06	2.65	2.62	0.69

[1] Averages of four replications
Figures in parenthesis are arcsin transformed values



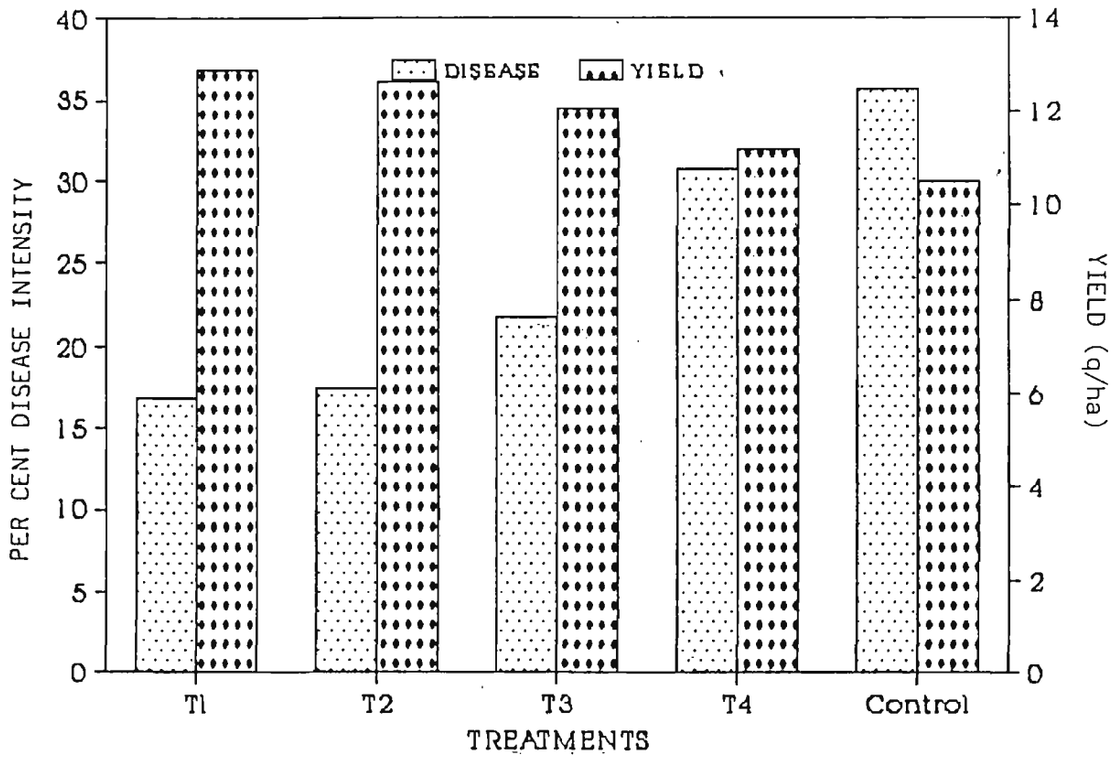
T1 - Bavistin; T2 - Dithane M-45; T3 : Saprol
T4 - Topsin-M;

Fig.8: EFFECT OF FUNGICIDES ON PER CENT DISEASE INTENSITY OF RUST AND YIELD OF SAFFLOWER (1986-87 rabi)

Table 20: Effect of fungicides on the control of rust and yield of safflower (1987-88 rabi)

S. No.	Treatment and dose		[1]Per cent disease intensity: Before first spray	Before second spray	Before third spray	Final	Yield ($q\ ha^{-1}$)
1.	Bavistin	0.05%	0.04 (1.15)	0.98 (5.68)	6.26 (14.49)	16.84 (24.23)	12.90
2.	Dithane M-45	0.20%	0.05 (1.28)	1.06 (5.87)	6.89 (15.22)	17.48 (24.72)	12.65
3.	Saprol	0.15%	0.05 (1.28)	1.90 (7.92)	9.95 (18.39)	21.75 (27.80)	12.05
4.	Topsin M	0.05%	0.04 (1.15)	3.02 (10.01)	16.26 (23.47)	30.72 (33.66)	11.20
5.	Control		0.05 (1.28)	5.62 (13.72)	24.86 (29.90)	35.68 (36.65)	10.52
	F - test		NS	Sig	Sig	Sig	Sig
	S.E.		0.21	0.85	0.89	0.93	0.29
	CD (0.05)		-	1.86	1.93	2.24	0.63

[1] Average of four replications
Figures in parenthesis are arcsin transformed values



T1- Bavistin; T2 - Dithane M-45; T3 - Saprol
T4- Topsin-M

Fig.9: EFFECT OF FUNGICIDES ON PER CENT DISEASE INTENSITY OF RUST AND YIELD OF SAFFLOWER (1987-88 rabi)

Dithane M-45 on yield did not differ significantly (Tables 19 and 20). Bavistin treated plots yielded highest (12.68 and 12.90 q ha^{-1}) followed by Dithane M-45, Saprol and Topsin-M during both the years. Whereas, control plots yielded 9.78 q ha^{-1} in 1986-87 rabi and 10.52 q ha^{-1} in 1987-88 rabi, respectively (Tables 19, 20 and Fig.8 and 9).

4.4.5 Estimation of yield loss due to rust disease of safflower

Yield losses caused by rust pathogen in safflower were estimated during 1986-87 and 1987-88 rabi by adopting paired-plot technique. The data on per cent disease intensity at 50, 65, 80 and 95 DAS and the corresponding yields in protected and unprotected plots are presented in Tables 21 and 22.

There were significant differences in disease intensity between protected and unprotected plots at different periods of crop growth during rabi 1986-87 and 1987-88 (Tables 21 and 22). The disease intensity recorded at 50 DAS was 0.86 per cent in protected and 8.46 per cent in unprotected plots during 1986-87 rabi and 0.08 per cent in protected and 2.10 per cent in unprotected during 1987-88 rabi respectively. Finally the per cent disease intensity recorded at 95 DAS was 22.08 and 18.92 in protected plots and 73.80 and 58.92 in unprotected plots during 1986-87 and 1987-88 rabi respectively (Fig.10 and 11).

On yield front, there was statistically significant difference between protected and unprotected treatments in both

Table 21: Disease intensity of P. carthami and yield loss (1986-87 rabi)

S.No.	Treatment	Per cent disease intensity at the age of				Yield	
		50 days	65 days	80 days	95 days	q ha^{-1}	% increase[i]/ decrease[d]
1.	Protected	0.86 (5.32)	4.75 (12.59)	15.92 (23.52)	22.08 (28.03)	13.05	32[i]
2.	Unprotected	8.46 (16.90)	42.18 (40.50)	45.15 (54.18)	73.80 (59.21)	8.76	49[d]
	S.E.	0.799	2.338	2.453	2.268	0.41	
	t-cal value	14.48**	11.99**	12.49**	13.47**	11.93**	

** Significant at 0.01 level of probability

Figures in parenthesis are arcsin transformed values

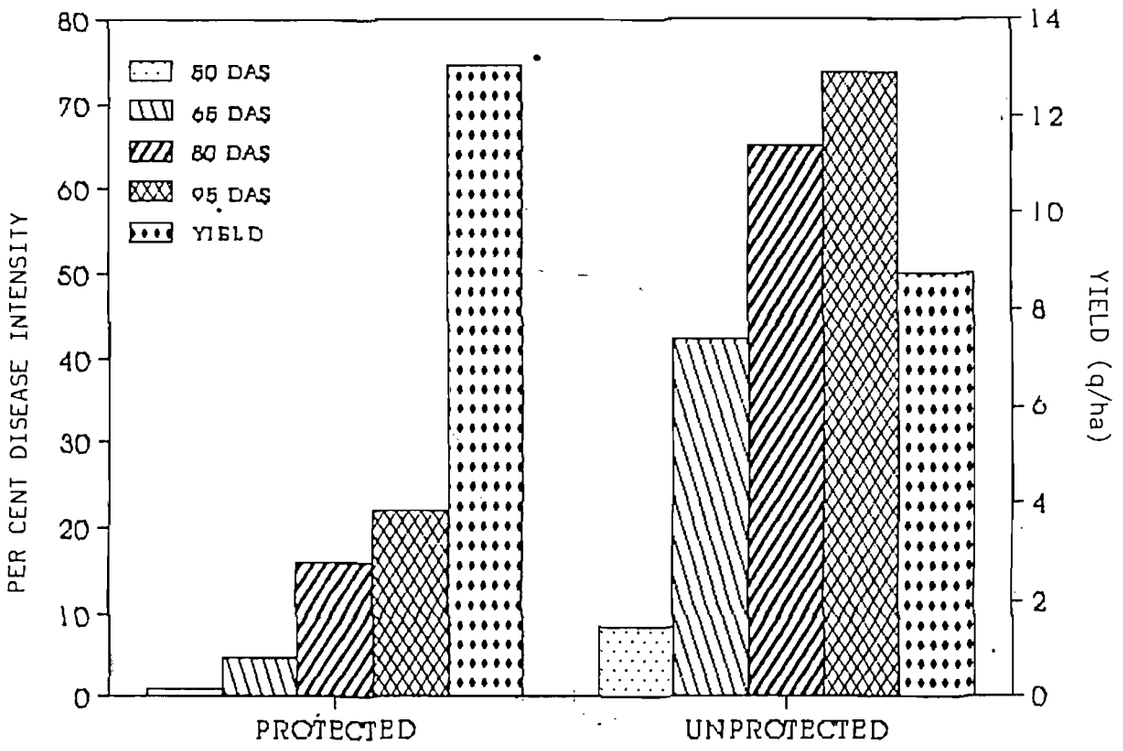


Fig.10: PER CENT DISEASE INTENSITY OF *P carthami* AT DIFFERENT AGES OF CROP GROWTH AND YIELD LOSS IN SAFFLOWER (1986-87 rabi)

Table 22: Disease intensity of P. carthami and yield loss (1987-88 rabi)

S.No.	Treatment	Per cent disease intensity at the age of				Yield	
		50 days	65 days	80 days	95 days	q ha^{-1}	% increase[i]/ decrease[d]
1.	Protected	0.08 (1.62)	2.75 (9.54)	12.65 (20.84)	18.92 (25.78)	13.24	30[i]
2.	Unprotected	2.10 (8.33)	27.61 (31.70)	42.75 (40.83)	58.92 (50.15)	10.15	23[d]
	S.E.	0.435	1.399	1.507	1.892	0.40	
	t-cal value	15.41**	12.96**	18.66**	15.59**	10.23**	

** Significant at 0.01 level of probability

Figures in parenthesis are arcsin transformed values

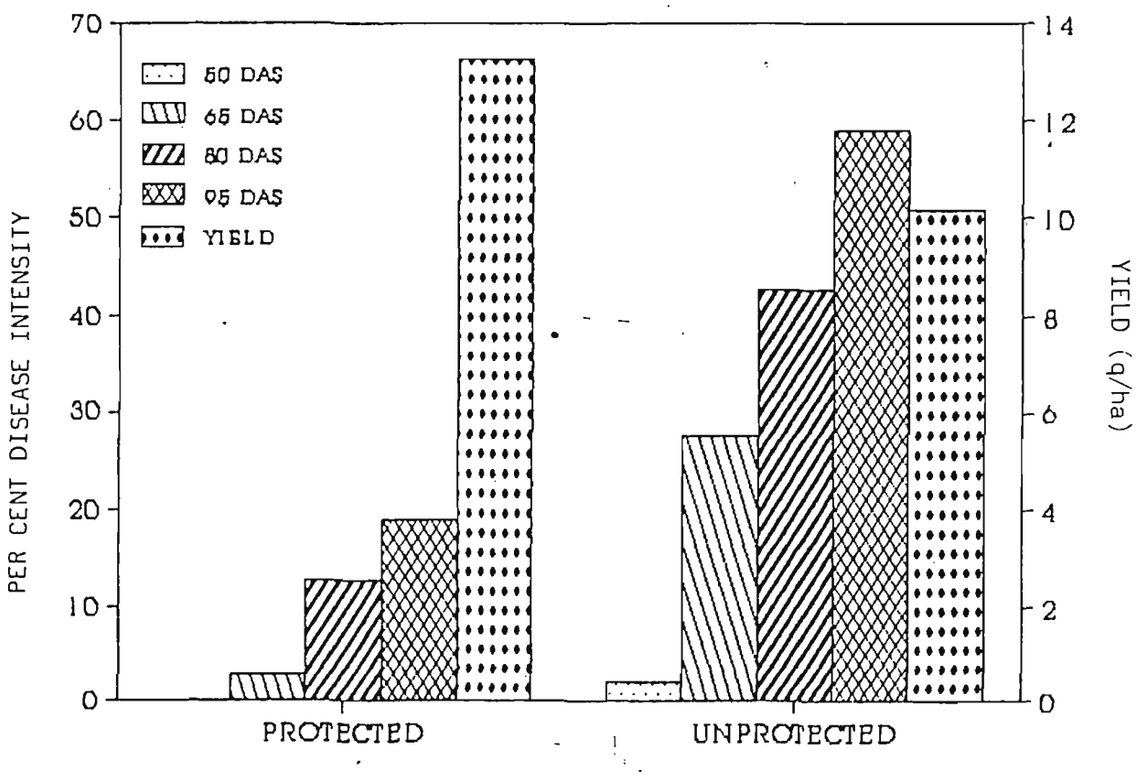


Fig.11: PER CENT DISEASE INTENSITY OF P. carthami AT DIFFERENT AGES OF CROP GROWTH AND YIELD LOSS IN SAFFLOWER (1987-88 rabi)

the years of experimentation (Tables 21 and 22). Yields recorded from protected plots were 13.05 and 13.24 q ha^{-1} whereas 8.76 and 10.15 q ha^{-1} in unprotected plots during 1986-87 and 1987-88 rabi respectively (Fig.10 and 11). Unprotected plots gave 49 and 30 per cent reduced yields over protected plots during 1986-87 and 1987-88 rabi respectively. On contrary, protected plots gave 32 and 23 per cent increased yields over unprotected plots during both the years of testing (Tables 21 and 22).

4.5 MECHANISM OF RESISTANCE

4.5.1 Morphological resistance

Anatomical characters like, thickness of cuticle cum epidermis, stomatal frequency, size of stomata and stomatal opening from both the surfaces of leaves of resistant and susceptible cultivars were observed and results are presented in Table 23.

The average thickness of cuticle cum epidermis on upper and lower surface of leaves in resistant and susceptible cultivars were 18.68 and 18.11; 18.60 and 18.00 µm, respectively. There was no significant difference in thickness of cuticle cum epidermis between these two reaction categories (Table 23).

The average number of stomata on upper and lower surface of leaves in resistant and susceptible cultivars were 370 and 488; 373 and 491 per mm^2, respectively. No significant differences were found between resistant and susceptible cultivars on both the surfaces of leaves (Table 23).

Table 23: Anatomical characters of resistant and susceptible cultivars of safflower to rust

Cultivar	Thickness of cuticle cum epidermis (µm)		Stamatal frequency per mm^2		Size of stomatal (µm) L x B		Size of stomatal opening (µm) L x B	
	Upper Surface	Lower Surface	Upper Surface	Lower Surface	Upper surface	Lower surface	Upper surface	Lower surface
RESISTANT								
1. PI 307062	18.74	18.24	369	487	29.47 x 15.45	27.06 x 14.08	19.64 x 8.36	17.85 x 7.62
2. PI 199932	18.62	17.98	371	489	29.20 x 15.75	27.82 x 14.50	19.38 x 8.43	18.02 x 7.82
Average	18.68	18.11	370	488	29.34 x 15.60	27.19 x 14.29	19.51 x 8.39	17.94 x 7.72
SUSCEPTIBLE								
1. Manjira	18.36	17.87	374	493	28.95 x 15.50	27.62 x 13.06	19.78 x 8.78	18.36 x 8.16
2. Bhima	18.84	18.12	372	489	29.35 x 15.56	27.90 x 14.25	19.52 x 8.70	18.21 x 7.96
Average	18.60	18.00	373	491	29.15 x 15.53	27.76 x 13.65	19.65 x 8.74	18.29 x 8.06
F - test	NS	NS	NS	NS	-	-	-	-
S.E.	0.98	0.82	3.45	3.70	-	-	-	-

The size of stomata on upper and lower surface of leaves in resistant and susceptible cultivars on an average were 29.34 µm x 15.60 µm and 27.19 µm x 14.29 µm; 29.15 µm x 15.53 µm and 27.76 µm x 13.65 µm, respectively. There was no marked difference between resistant and susceptible cultivars in size of stomata Likewise, there was no difference in the size of stomatal opening between the resistant and susceptible cultivars of safflower (*Table 23*).

4.5.2 Biochemical resistance

Biochemical constituents like reducing sugars, total sugars, total phenols, ortho-dihydroxy phenols, total amino acids and ascorbic acid were estimated quantitatively in resistant and susceptible cultivars of safflower at 45, 60, 75 and 90 days after sowing (DAS) and results are depicted in Tables 24, 25, 26 and 27.

Reducing sugars: Reducing sugars on an average at the age of 45 days in susceptible and resistant cultivars were, 10.74 and 7.28 mg/g of leaf *Sample*, respectively, which increased to 12.21 and 8.73 mg/g of leaf *sample*, respectively at 60 DAS. Later at 75 and 90 DAS the level of reducing sugars declined gradually in susceptible as well as in resistant cultivars (Fig.12). Susceptible and resistant cultivars contained 9.07 and 6.63; 8.51 and 5.30 mg/g of leaf *Sample* at 75 and 90 DAS respectively. Levels of reducing sugars between susceptible and resistant were found to be statistically significant at all stages tested (Table 24).

Table 24: Reducing sugars and total sugars of rust resistant and susceptible cultivars of safflower at different ages of growth.

Cultivar	Reducing sugars (mg/g)				Total sugars (mg/g)			
	45 DAS	60 DAS	75 DAS	90 DAS	45 DAS	60 DAS	75 DAS	90 DAS
RESISTANT								
1. PI 307062	7.24	8.82	6.52	5.24	17.78	18.96	16.26	14.78
2. PI 199932	7.32	8.64	6.74	5.36	17.66	18.92	16.34	14.92
Average	7.28	8.73	6.63	5.30	17.72	18.94	16.30	14.85
SUSCEPTIBLE								
1. Manjira	10.92	12.10	9.18	8.69	21.48	22.35	19.95	18.56
2. Bhima	10.56	12.82	8.96	8.33	21.32	22.60	19.87	18.74
Average	10.74	12.21	9.07	8.51	21.40	22.48	19.91	18.65
F - test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
S.E.	0.42	0.48	0.45	0.39	0.52	0.58	0.47	0.49
C.D.(0.05)	0.90	1.02	0.96	0.84	1.12	1.24	1.01	1.05

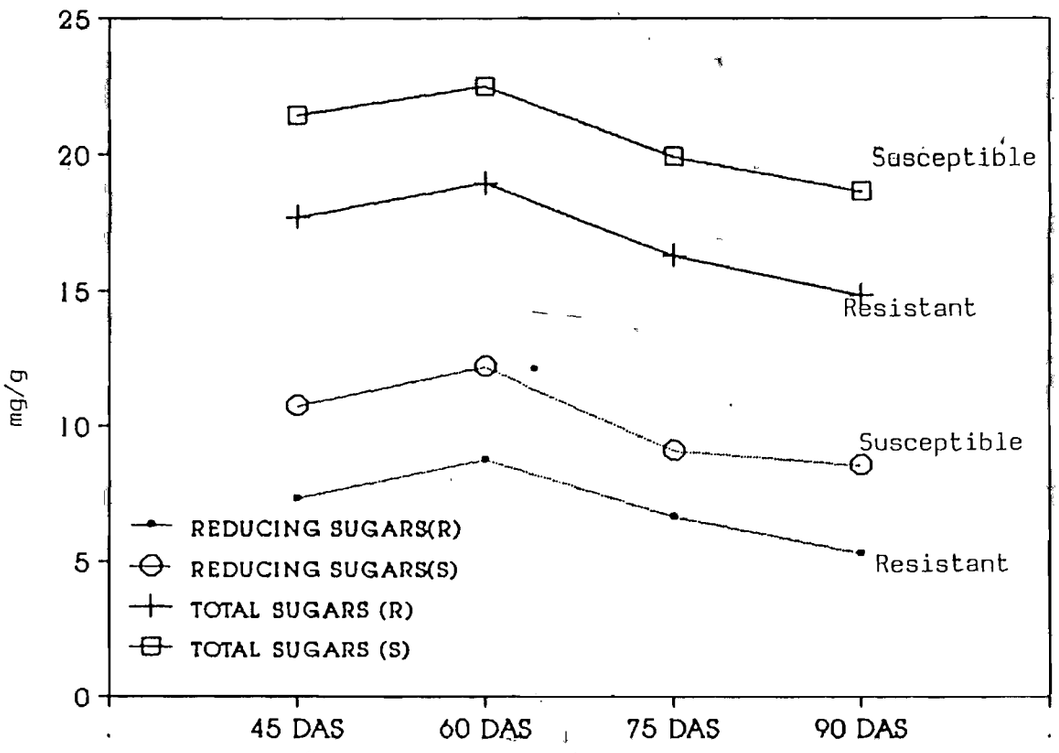


Fig.12: REDUCING SUGARS AND TOTAL SUGARS OF RESISTANT AND SUSCEPTIBLE CULTIVARS OF SAFFLOWER AT DIFFERENT AGES OF GROWTH

Total sugars: Susceptible cultivars on an average contained more amounts of total sugars at all stages tested i.e., 45, 60, 75 and 90 DAS which recorded 21.40, 22.48, 19.91 and 18.65 mg/g of leaf material respectively than resistant cultivars. Resistant cultivars recorded 17.72, 18.94, 16.30 and 14.85 mg/g of leaf material at 45, 60, 75 and 90 DAS respectively. Accumulation of total sugars increased from 45 DAS upto 60 DAS and thereafter declined gradually in all the cultivars under study (Fig.12). There was statistically significant difference in total sugars between resistant and susceptible cultivars at all stages tested (Table 24).

Total phenols: Resistant cultivars on an average contained higher amounts of total phenols than susceptible cultivars at all stages tested i.e., 45, 60, 75, and 90 DAS and recorded 22.10, 23.99, 25.00 and 25.54 mg/g of leaf material, respectively. Susceptible cultivars contained 14.48, 16.06, 16.95 and 18.00 mg/g of leaf material at 45, 60, 75 and 90 DAS respectively. Total phenolic contents increased in all the cultivars with the increase of plant age (Fig.13). There was statistically significant difference in total phenols between resistant and susceptible cultivar at all the stages tested (Table 25).

Ortho-dihydroxy phenols: Resistant cultivars contained higher amounts of O.D phenols at all the stages tested i.e., 45, 60, 75 and 90 DAS than susceptible cultivars and recorded on an average of 15.99, 17.19, 18.02 and 18.79 mg/g of leaf material respectively. Susceptible cultivars on an average contained 9.20, 10.53,

Table 25: Total phenols and O.D.Phenols of rust resistant and susceptible cultivars of safflower at different ages of growth.

Cultivar	Total phenols (mg/g)				O.D. Phenols (mg/g)			
	45 DAS	60 DAS	75 DAS	90 DAS	45 DAS	60 DAS	75 DAS	90 DAS
RESISTANT								
1. PI 307062	22.08	23.95	24.82	25.96	16.12	17.32	18.12	18.95
2. PI 199932	22.12	24.03	25.18	26.18	15.85	17.06	17.92	18.63
Average	22.10	23.99	25.00	25.54	15.99	17.19	18.02	18.79
SUSCEPTIBLE								
1. Manjira	14.33	15.98	16.86	17.92	9.02	10.72	11.26	11.72
2. Bhima	14.62	16.14	17.04	18.08	9.38	10.34	10.82	11.62
Average	14.48	16.06	16.95	18.00	9.20	10.53	11.04	11.67
F - test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
S.E.	0.56	0.59	0.52	0.60	0.48	0.54	0.52	0.49
C.D.(0.05)	1.28	1.26	1.12	1.28	1.02	1.16	1.12	1.05

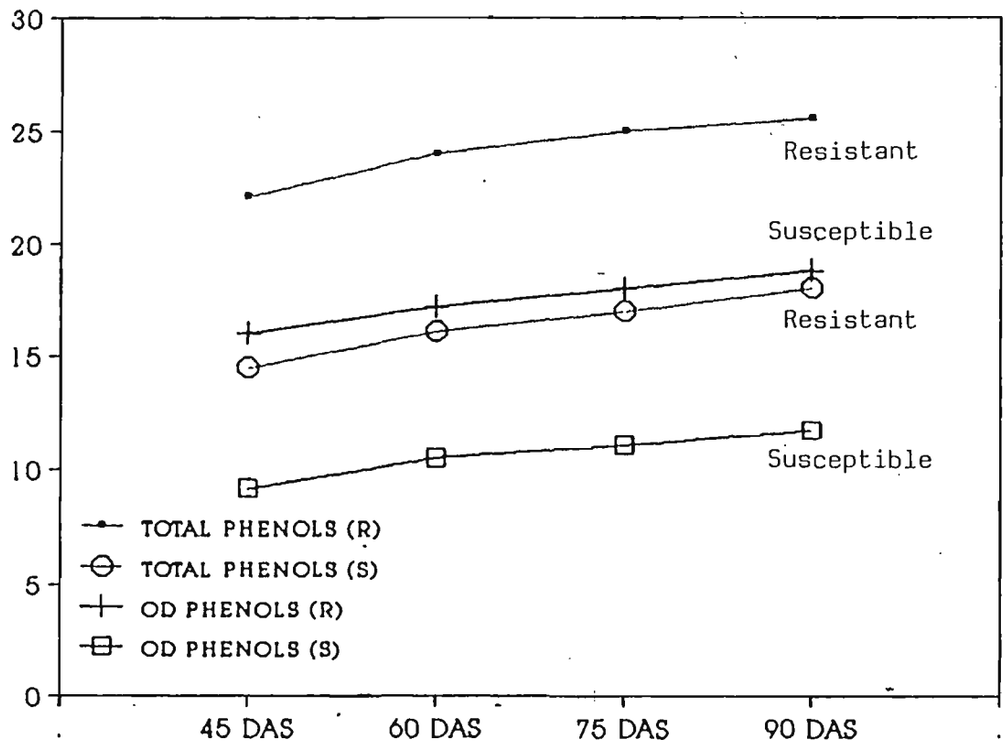


Fig.13: TOTAL PHENOLS AND O.D PHENOLS OF RUST RESISTANT AND SUSCEPTIBLE CULTIVARS OF SAFFLOWER AT DIFFERENT AGES OF GROWTH

11.04 and 11.67 mg/g of leaf material at 45, 60, 75 and 90 DAS respectively. Levels of O.D.phenols were increased with increased age of plants in all the cultivars under study (Fig.13). There was statistically significant difference in O.D. phenols between resistant and susceptible cultivars at all the stages tested (Table 25).

Total amino acids: Resistant cultivars on an average contained higher amounts of total amino acids at all the stages tested i.e., 45, 60, 75 and 90 DAS than susceptible which recorded 8.00, 7.15, 6.67 and 6.17 mg/g of leaf material respectively. Susceptible cultivars contained 6.21, 5.34, 4.70 and 4.20 mg/g of leaf material on average at 45, 60, 75 and 90 DAS respectively. The contents of total amino acids decreased gradually with increase of age of plants in all the cultivars (Fig.14). There was statistically significant differences in total amino acids between resistant and susceptible cultivars (Table 26).

Ascorbic acid: Resistant cultivars contained higher amounts of ascorbic acid at all the stages tested i.e., 45, 60, 75 and 90 DAS which recorded on an average of 5.36, 4.71, 3.98 and 3.78 mg/g of leaf material than susceptible cultivars respectively. Susceptible cultivars after 45, 60, 75 and 90 days of sowing on an average contained 5.17, 4.59, 3.83 and 3.62 mg/g of leaf material respectively. There was a gradual decline in ascorbic acid content with increased age of plants (Fig.15). No significant difference was found in ascorbic acid betwen resistant and susceptible cultivars (Table 27).

Table 26: Total amino acids concentration of rust resistant and susceptible cultivars of safflower at different ages of growth.

Cultivar	Total amino acids (mg/g)			
	45 DAS	60 DAS	75 DAS	90 DAS
RESISTANT				
1. PI 307062	8.04	7.28	6.75	6.20
2. PI 199932	7.96	7.02	6.58	6.14
Average	8.00	7.15	6.67	6.17
SUSCEPTIBLE				
1. Manjira	6.12	5.28	4.68	4.16
2. Bhima	6.30	5.40	4.72	4.24
Average	6.21	5.34	4.70	4.20
F - test	Sig	Sig	Sig	Sig
S.E.	0.29	0.31	0.29	0.34
C.D.(0.05)	0.62	0.66	0.62	0.72

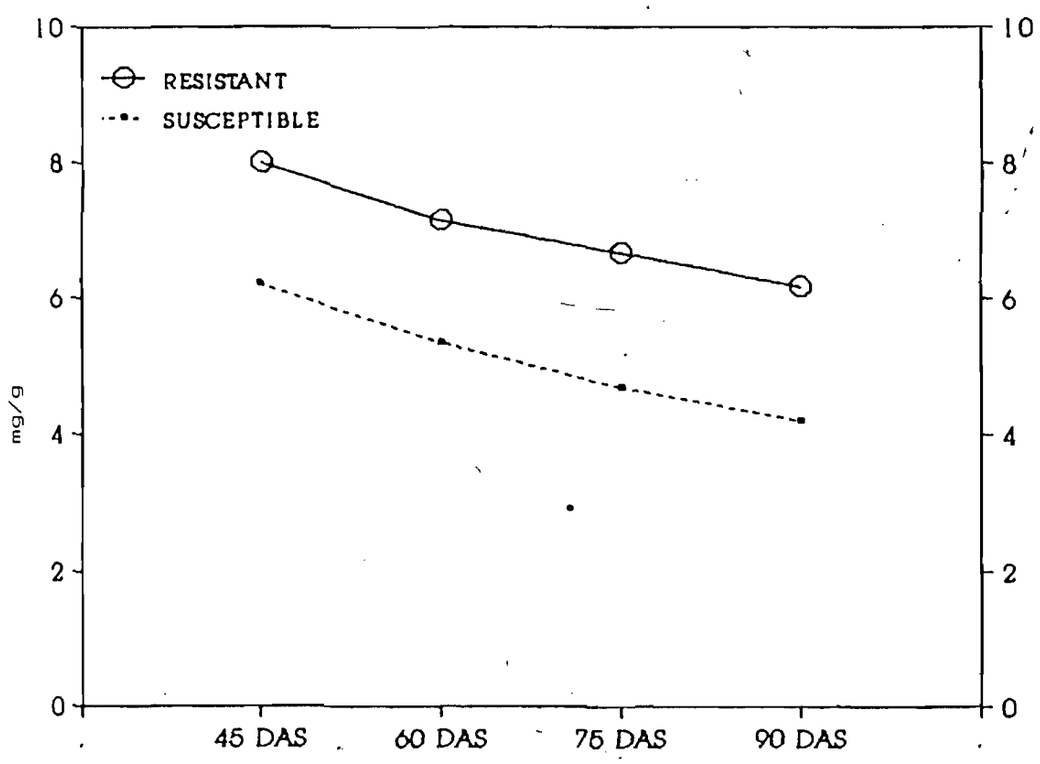


Fig.14: TOTAL AMINO ACID LEVELS OF RUST RESISTANT AND SUSCEPTIBLE CULTIVARS OF SAFFLOWER AT DIFFERENT AGES OF GROWTH

Table 27: Ascorbic acid concentration of rust resistant and susceptible cultivars of safflower at different ages of growth.

Cultivar	Ascorbic acid (mg/g)			
	45 DAS	60 DAS	75 DAS	90 DAS
RESISTANT				
1. PI 307062	5.42	4.75	4.04	3.78
2. PI 199932	5.30	4.64	3.92	3.72
Average	5.36	4.70	3.98	3.75
SUSCEPTIBLE				
1. Manjira	5.18	4.58	3.86	3.55
2. Bhima	5.16	4.62	3.80	3.68
Average	5.17	4.60	3.83	3.62
F - test	NS	NS	NS	NS
S.E.	0.31	0.32	0.34	0.30
C.D.(0.05)	-	-	-	-

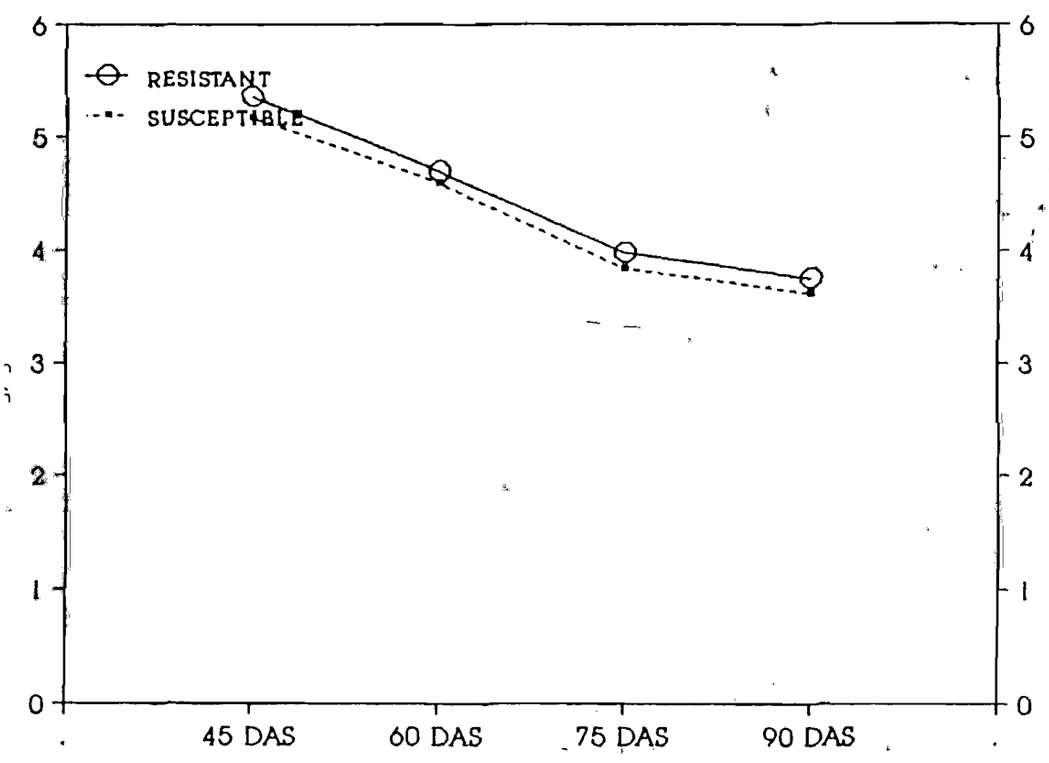


Fig.15: ASCORBIC ACID LEVELS OF RUST RESISTANT AND SUSCEPTIBLE CULTIVARS OF SAFFLOWER AT DIFFERENT AGES OF CROP GROWTH

DISCUSSION AND CONCLUSIONS

CHAPTER V

DISCUSSION AND CONCLUSIONS

Survey of safflower rust revealed that rust incidence was uniformly distributed and practically there was no difference among different villages within the district. However, there was difference in the intensity of disease between two years of survey which might be due to favourable environmental conditions that prevailed.

Teleutospores of Puccinia carthami germinate normally by production of 4-celled promycelia with each cell bearing short sterigmata and a kidney shaped sporidium (Prasada and Chothia, 1950; and Zimmer, 1963a). Likewise, in the present study also teleutospores on germination produced 4-celled promycelia with short sterigmata on each cell bearing kidney shaped sporidium (Plate 5).

Ten to thirteen days after inoculation with germinated teleutospores orange yellow spots appeared comprising of spermagonia which were sub-epidermal and flask shaped (Plate 6). After four to seven days small chestnut brown pustules mixed with spermagonia appeared consisting of small saucer shaped sori without peridium and paraphyses (Plate 7). In these sori smooth walled spherical spores unlike normal urediniospores were observed which were borne terminally on thick hyaline pedicels (Plate 8). Eventhough sori resemble uredinia they are not uredinia because peridium and paraphyses are absent and occupied aecial

stage. Likewise, spores are also not true urediniospores because they are smooth walled though they are produced on pedicels. Although aecium and spores exhibited the characters of aeciospores and urediniospores they referred to as aecia only because they satisfy all the requirements of aecia. After 20 days of inoculation scattered chestnut brown pustules appeared consisting of uredinia followed by telia. Arthur (1929) termed rusts with aecia resembling uredia as stylosporic or uredinoid. Cummins (1959) termed such sori as aecia urecia which are morphologically like uredinia but accompanied by spermagonia and occupying aecial position in the life cycle. Spermagonia of P. carthami accompanying uredinium like pustules have been termed as primary uredia (Darpoux, 1948; Prasada and Chothia, 1950; Schuster and Christiansen 1952; Schuster 1956). Zimmer (1963a) working with P. carthami reported that pustules accompanying spermagonia referred to as aecia although uredinoid in character, occupy the aecial stage in the life cycle and meet requirements of aecia.

The present investigation revealed that P.carthami produced all the spore stages in its life cycle and it is confirmed as an autoecious and macrocyclic rust.

Studies on viability of urediniospores under different stored conditions revealed that urediniospores remained viable for 25 days when stored on soil surface and at 10 cm depth in the soil, while they survived for 40 days under laboratory conditions at room temperature (Table 4). Urediniospores remained viable for longer periods stored under laboratory conditions than under

field conditions. However, the period of survival under field and laboratory conditions was short and urediniospores were not likely to be useful in perpetuation from one season to another. Similarly, Reeti Singh (1990) also observed that urediniospores in infected leaves were short lived (28 days) when exposed to weather conditions, while it was improved (45 days) when stored at room temperature. The short survival of urediniospores under field conditions might be due to prevalence of high temperatures.

Teleutospores in infected leaves survived for 11 months when stored on soil surface and at 10 cm depth in the soil, while 13 months under laboratory condition (Table 5). There was no germination upto 4 months of storage, while maximum germination was found in December followed by gradual decrease. Since the teleutospores survived for long periods (11 months) it is presumed that they are helpful in perpetuation of the disease. Prasada and Chothia (1950) also observed that teleutospores remain viable for 12 months under field conditions. No germination was observed upto 5-6 months after storage and they germinate profusely in November. Clavert and Thomas (1954) reported that teleutospores remain vialble upto 18 months when stored in an unheated building. Reduced germination and viability of teleutospores might be due to killing of some teleutospores by their exposure to summer months.

Transmission studies were conducted to findout role played by the teleutospores carried through seed or infected

plant debris lying in the field. The mode of transmission with two sources of inoculum i.e., seed and soil was similar. In both the cases infected seedlings were recorded 18 days after sowing but number of infected plants rapidly increased upto another 12 days. After 36 days of sowing no further increase was observed (Fig.1). Hence, it is presumed that either the resting teleutospores on seed or lying in the soil might cause fresh infection in the following season. Similarly, Prasada and Chothia (1950) reported that inoculum may come from plant debris lying in the soil or it may be carried with the seeds in the form of tinybits of infected tissue. Aliza Halfon-Meiri (1983) also made similar observations that inoculum may come from seed or soil. However, some of the workers concluded that it is a seed borne disease since teleutospores carried through seed cause fresh infection (Darpoux 1948; Clavert and Thomas, 1954; Gallegos and Roodriquez, 1966; Sharma and Kulkarni, 1976; Aliza Halfon-Meiri, 1981).

Prasada and Chothia (1950) reported that wild species Carthamus oxycantha act as collateral host of P.carthami and Kolte (1985) reported that besides C. orycantha other Carthamus species i.e., C. glaucus, C. lunatus, C. syriacus and C tenius also appear to act as collateral hosts to P. carthami. There was no authentic record of the occurrence of any collateral hosts of safflower rust out side the genus Carthamus. Hence, in the present investigation 15 weed plants and two compositae cultivated plants were examined as possible hosts of the safflower rust pathogen, but no infection was recorded on any of them (Table 9).

However, the above wild species of Carthamus were not tested in this investigation due to non occurrence of this hosts. In the absence of wild species of Carthamus and other collateral hosts in Andhra Pradesh, it is presumed that the Safflower rust perpetuated in the infested seed or soil.

The disease development and progress of safflower rust was monitered on Manjira cultivar (susceptible to rust) of safflower under natural conditions during 1986-87 and 1987-88 rabi at College of Agriculture, Rajendranagar. Rust pustules appeared one week early in 1986-87 than 1987-88 rabi safflower crop. The per cent disease intensity was also higher in 1986-87 than 1987-88 rabi. This difference in rust development between two years was probably due to differences in weather conditions prevailed during crop period i.e., from December to March in both the years. Total rainfall (121.2mm) and number of rainy days (6) were more during 1986-87 than 1987-88 rabi. During 1987-88, the total rainfall was 51.9 mm and number of rainy days were 4 days but no rainfall from December 12th to February 22nd with 71 days of dry period. During 1986-87 rabi there was rain for three days from December 28th to 11th January. The results of present investigation showed that the disease severity was more when there was rain after its appearence. And also it appeared that continuous dry period after its appearence reduced its severity. Rainfall and number of rainy days after appearence of disease were important factors on epidemic build-up. Krishnaprasad et al. (1979) reported that intermittant rainfall with mean relative humidity

above 87 per cent and optimum temperature of 23-24^{o}C were congenial for groundnut rust development in the field. Siddaramaiah et al. (1980) reported that continuous dry periods with a temperature of above 26^{o}C and relative humidity below 70 per cent delayed rust occurrence and severity in groundnut. Reeti Singh et al. (1990) reported that the rust disease severity was more when there were frequent rains and high temperature during cropping season in safflower. The results of the present investigation showed that climatic conditions during rabi 1987-88 were not favourable for rust disease development.

Studies on periodic increase in disease severity under field conditions during rabi 1986-87 and 1987-88 showed that mean maximum temperature had considerable influence on disease development (Fig.2 & 4). During 1986-87 there was mean maximum temperature (MMXT) only had significant correlation with the disease development (Table 11). Whereas, during 1987-88 in addition to mean maximum temperature (MMXT), mean evening humidity (MEH) and mean sunshine hours (MSH) also had significant correlation (Table 14). Correlation coefficients of two years can be attributed to the effect of rainfall during 1986-87 rabi. Other environmental factors which were non-significant but nevertheless for overall development of rust under field conditions their contributions can not be under estimated during both the years.

Multiple regression equations built up from different combinations of variables for the development of the disease with significant R^2 values explained variation ranging from 28.4 to

49.6 per cent during rabi 1986-87 (Table 12). Whereas, it was 41.4 to 69.7 per cent during 1987-88 rabi (Table 15). Eventhough, the disease intensity was more in 1986-87 rabi the R^2 values with tested variables showed less than that of rabi 1987-88. This difference might be due to rainfall of 4.2 mm in one day during the period of disease development in 1986-87 rabi. However, there was no rainfall during disease developmental period (1987-88 rabi). In rabi 1987-88 whatever the disease observed varied with variables tested. Rainfal which occured during 1986-87 rabi was not included as independent variable in the regression analysis because out of 22 observations there was only one observation with rainfall. This might be the cause for low R^2 values with the tested variables in 1986-87 rabi. In the same season the disease severity was more because of early onset of the disease and one day rainfall during disease developmental period and increased temperature. These results are in confirmity with Reeti Singh (1990). In 1987-88 rabi the disease severity was less because of late onset of the disease, no rainfall during disease developmental period, eventhough there was increased temperature.

It could be finally concluded that rainfall, number of rainy days and temperature are important factors for progress and development of the disease. Further rainfall will also help in carrying the urediniospores along with rain splash resulting in the rapid spread of the disease.

Different periods of sowing can also influence the disease incidence. To findout the optimum period of sowing an

experiment was conducted during 1986-87 and 1987-88 rabi with fortnightly intervals. In September sowings of both the years disease was either negligible or completely absent at different stages of crop growth observed. Later the disease progressed with October sowings till December with maximum PDI in December second fortnight sowings of both the years.. In December sown crop the disease appeared from seedling stage itself in both the years (Table 16 and 17). September sown crop escaped disease as disease appears usually in the month of January by which time the crop reached the seed maturity stage. Prasada and Chothia (1950) reported that the disease normally appears in the month of February or March thereby resulting in disease escape in early sowings. Since the teleutospores carried on seed or in the plant debris germinate with maximum in the month of December, the late sown crop was more infected than early sown crop. The severity of disease was more in 1986-87 than in 1987-88 rabi which might be due to early onset of disease and occurrence of rainfall after its appearance. Maiti (1987) reported that the rust of safflower appeared only in late sown crops and its severity also more in late sown than early sown crop.

It is evident from the studies that disease could be avoided by adopting early sowings i.e., in September second fortnight because the susceptible stage of the plant did not coincide with the profuse germination of teleutospores. By the time teleutospores start germinating profusely the early sown crop reaches maturity stage hence avoids disease.

Several sources of rust resistance have previously been reported in safflower (Zimmer and Leininger, 1965; Zimmer and Uric, 1967; Zimmer and Jensen, 1970; Vyas and Prasad, 1985), but these have rapidly succumbed to new or previously undetected races of rust (Thomas, 1952 and 1956; Zimmer, 1963b). Therefore, the present study was undertaken to identify and evaluate sources of resistance among cultivated safflower.

The results revealed that out of 192 germplasm lines and varieties of safflower tested, 53 were identified as immune, 14 resistant, 17 moderately resistant, 32 moderately susceptible and remaining 76 susceptible to rust (Table 18). Similar observations were also reported by Zimmer and Lieninger (1968) and Vyas and Prasad (1985). Therefore, the resistant cultivars identified could be effectively used as donors for breeding resistant cultivars of safflower against this disease.

In the present study attempts were made to findout the fungicide for effective control of safflower rust. Bavistin 0.05%, Dithane M-45 0.2%, Saprol 0.15% and Topsin-M 0.05% were tested against safflower rust during 1986-87 and 1987-88 rabi. All the fungicides tested were significantly effective over control in reducing the incidence of safflower rust with a consequent increase in yield. However, Bavistin and Dithane M-45 were more effective than Saprol and Topsin-M in checking the disease during both years of testing (Table 19 and 20). Similarly, Chauhan and Muheet (1978) reported that Dithane M-45 was most

effective in reducing the intensity of safflower rust with maximum yields.

However, none of the fungicides tested could completely control the disease and protect or cure the plants from rust. Similar observations made by Sharma and Kulkarni (1976) working with safflower rust.

Increased yields obtained from Bavistin and other fungicidal treatments over control might be due to decreased incidence of disease and consequent defoliatiaon. Particularly in Bavistin treated plots increased yields could be attributed partly due to kinetin like behaviour. Similarly, Thomas (1974) reported that number of benzimidazole fungicides are known to possess cytokinin like activity. Andortha (1976) working with groundnut rust reported that this increased yield in carbendazim treated plots might be partly due to kinetin like behaviour of the carbendazim. Wang et al. (1961) have reported that benzimidazol fungicides control biosynthesis of wheat leaves, so an increase in chlorophyll content of treated plants could be attributed to cytokinin like activity of these chemicals.

Two year yield study was conducted to determine the influence of rust on yield of safflower. The intensity of rust varied from year to year with the highest intensity during 1986-87 than 1987-88 rabi when environmental conditions were more conducive to rust. The recorded per cent disease intensity from flowering to seed setting ranged from 42.18 to 65.15 and 27.61 to

42.75 in unprotected, while from 4.75 to 15.92 and 2.75 to 12.65 in protected plots during 1986-87 and 1987-88 rabi respectively. Recorded yields from protected plots were 13.05 and 13.24 q ha^{-1} whereas, 8.76 and 10.15 q ha^{-1} in unprotected plots during 1986-87 and 1987-88 rabi respectively. Per cent reduced yields in unprotected plots were 49 and 30 during 1986-87 and 1987-88 respectively. While per cent increased yields in protected plots were 32 and 23 during 19867-87 and 1987-88 respectively (Tables 21 and 22).

It is assumed that severe intensity of rust predisposes plants to drying, to reduce amount of hull on seed and to lower the yield. Similar observations were made by Thomas (1956) and Zimmer and Jensen (1970).

Therefore the difference in per cent reduced yields observed between two years might be due to occurrence of severe intensity of disease during 1986-87 than in 1987-88 rabi.

It is evident that natural and induced defense mechanism operate in the host plants against different diseases. In nature majority of the plants exhibit resistance and the capacity or ability of a plant to defend itself against a pathogen is governed by its genetic constitution and the environmental conditions under which the genes operate. The characters of host plants which either reduce the chances of infection or the development of the pathogen are considered to be defense

mechanism. Such defense mechanisms operating in plants grouped into morphological and biochemical defense mechanisms.

The first line defense against pathogens is the surface barrier which a pathogen must penetrate before it can cause infection. The entry of the pathogens might be either through the epidermal cell walls directly or through natural openings in the epidermis. Certain external and internal structures of the epidermis might greatly affect the ability of the pathogen to penetrate or to invade a host plant. Such morphological defense structures might be present before penetration or might be produced afterwards as a result of interaction of the host and the pathogen. Ample evidence has been provided by several workers to this effect on disease incidence in several crops (McLean, 1921; Yoshii, 1936; Akai, 1959; Dickinson, 1960; Martin, 1964; Royle, 1976).

With a view to find out the correlation between the thickness of cuticle cum epidermis of leaves of safflower and rust resistance was studied in resistant and susceptible cultivars to P. carthami. There was no significant difference in the thickness of cuticle cum epidermis between resistant and susceptible cultivars of safflower (Table 23). Hence, the thickness of cuticle cum epidermis do not have a role to play in resistance or susceptibility of safflower to rust. Similarly, Murray and Bruehl (1983) reported that resistance was correlated with hypodermis width and number of hypodermal layers, but not with epidermal cell wall thickness in winter wheats in relation to Pseudocercosporella

herpotrichoides. Begum (1984) also observed that the thickness of leaf epidermis does not have any role to play in pearlmillet cultivars resistant and susceptible to downymildew. Irrespective of thickness of cuticle-cum-epidermis, the urediniospores upon germination are capable of penetrating the cuticle-cum-epidermis.

On contrary, Melander and Craigie (1927) observed that plants with tough thick epidermal cell walls are not attacked by a pathogen and displayed resistance in Barberis against black stem rust of wheat caused by Puccinia graminis. Likewise, Sokhi et al. (1985) and Tomy Philip (1991) while working with groundnut rust and mulberry leaf rust, respectively, reported that the thickness of cuticle cum epidermis in resistant genotypes was significantly higher than susceptible genotypes.

Stomata play an important role in causing infection, since urediniospores germinate and enter through stomata. Hence, stomatal frequency, size of stomata and stomatal openings were studied in relation to safflower rust resistant and susceptible cultivars. The results reveal that there was no marked difference in the number of stomata, size of stomata and stomatal openings between resistant and susceptible cultivars (Table 23). As such it is concluded that the number of stomata present on leaf surface do not have direct relationship with resistance or susceptibility of safflower rust. Subramanyam et al. (1982) reported that rust resistance was not correlated with the number of stomata present on the leaf surface in cultivated and wild peanuts.

Similarly, Begum (1984) observed that number of stomata on leaf surface of pearlmillet do not play a role in downymildew infection of resistant and susceptible cultivars.

Irrespective of whether a genotype was resistant or susceptible, the urediniospores germinated on the leaf surfaces and the fungus entered the leaf through stomata.

The studies on morphological characters of resistant and susceptible cultivars of safflower indicated that anatomical characters do not differ both in resistant and susceptible cultivars. Hence, it suggests that resistance/susceptibility in safflower to rust could be essentially chemical in nature.

Biochemical defence mechanism may consist of the presence or absence of a particular chemical substances which interfere with the growth and multiplication of the pathogen. The biochemical substances might be present before infection or produced by the interaction of the host and the pathogen. Phenolics, Sugars, amino nitrogen, flavonoids and alkaloids present in the host plant have often been implicated in resistance of the host plant to the pathogen. Ample evidence is provided by several workers to this effect on disease incidence in several crops (Martin et al., 1957; Lee and Le Tourneau, 1958; Claassen, 1961; Bhullar et al., 1972; Ingham, 1973).

Hence, present study was undertaken with a view to determine whether sugars, phenols, aminoacids and ascorbic acid contents present in resistant and susceptible cultivars of

safflower against rust play a role in resistance or susceptibility and the results are discussed here.

Soluble sugars of a host plant were found to be associated with resistance or susceptibility of the plant to various diseases (Trelease and Trelease, 1929; Yarwood, 1934; Raghunathan et al., 1966; Jaypal and Mahadevan, 1968; Reddy, 1969; Mohanraj et al., 1972; Prasad et al., 1972).

Horsfall and Dimond (1957) suggested that the disease could be classified into high sugar diseases and low sugar diseases. Several workers believed that there may be direct relationship between sugar content of plants and susceptibility of rusts and classified the rust as high sugar disease (Livington, 1953; Mclean et al., 1961; Vidyasekaran, 1974). In order to verify these leaves of rust resistant and susceptible cultivars of safflower were analysed for the levels of sugars present. The susceptible cultivars contained significantly higher levels of reducing and total sugars than resistant cultivars at all growth stages (Table 24). Further, a general reduction was noticed with increase in the age of plants (Fig.12). These results are in agreement with the findings of Mclean et al. (1961), Prabhu and Swaminathan (1968), Vidhyasekaran et al. (1974), Ramaswamy and Shanmugam (1977) and Siddaramaiah et al. (1980).

The results indicated that high sugar content is related to susceptibility and on the basis of this safflower rust might be

considered a high sugar disease and thus fall in line with wheat stem rust disease (Prabhu and Swaminathan, 1968) and Italian millet rust (Vidhyasekaran, 1974) which were also reported to be high sugar diseases. The higher level of sugars in susceptible genotypes can be correlated with susceptibility in safflower plants to rust because (P. carthami)is a high sugar pathogen and requires high sugars for pathogenesis and further development within the host.

In contrast, Mathur and Vidhyasekaran (1978) observed that there was no correlation between reducing, non-reducing and total sugars and disease resistance in sunflower rust.

Among various biochemical factors determining the resistance of disease, certain polyphenols have been recorded to play an important role. Several workers have correlated higher levels of total phenols and O.D phenols with resistance of the host plants in respect of several diseases in different crops (Walker, 1923; Kargopolva, 1937; Johnson and Schaal, 1957; Valle, 1957; Tomiyama, 1963; Rao et al., 1968; Schlosser, 1969; Singh and Chand, 1970; Bhatia et al., 1972; Prasad et al., 1972).

The data on total phenols and O.D phenols in resistant and susceptible cultivars indicated that resistant cultivars contained high level of these compounds at all growth stages tested (Table 25). Amounts of these constituents were increased with increase in the age of plant both in resistant and susceptible cultivars (Fig.13). These results are in agreement with

Murthy and Bhagyaraj (1974), Abbott (1938), Teertha Prasad and Shambhulingappa (1986). In contrast, Sohi and Rawal (1983) working with cowpea rust reported that no correlation could be established between the phenol contents in different varieties and their resistance.

Several workers have implicated that total phenols, O.D phenols as resistant factors (Oku, 1965; Kuc, 1966; Mahadevan, 1966; Sridhar, 1972), as they are highly reactive upon oxidation and may result in the formation of substances, highly toxic to pathogens or which inactivate enzymes including hydrolytic enzymes elaborated by plant pathogenic fungi (Byrde, 1963). There might be similar stimulation of active defense mechanism reaction by the oxidation of phenols in the resistant cultivars of safflower against rust. Therefore, the high phenol levels of a tissue certainly advantageous from the point of view of resistance. Evidently, however, it is only one of the factors involved and the parasitically induced rapid polyphenol synthesis and other unknown processes might some times be more important.

Kirkham (1954), Raghunathan et al. (1966), Jaypal and Mahadevan (1968), and Majerink (1965) have correlated the resistance with lower amounts of amino acids. In contrast to this Srikanta Murthy (1975), Ramachandra Reddy (1976), Murthy and Bhagyaraj (1974), Theertha Prasad and Shambulingappa (1986), and Chauhan (1987) reported that resistant cultivars contained higher amounts of amino acids than the susceptible cultivars. Hence, total amino acid contents present in rust resistant and

susceptible cultivars of safflower were determined. The resistant cultivars contain higher levels of total amino acids as compared to the susceptible cultivars at all stages of crop growth tested (Table 26). Further, the levels of total amino acids were decreased with increase in the age of the palnts both in susceptible and resistant cultivars (Fig.14).

Kirkham (1954) stated that large amounts of aminoacids in the resistant host plant helped in the beakdown of naturaly occurring phenols to toxic products which in turn would inhibit the pathogen.

Some theories have been proposed by various workers on the mode of action of plant aminoacids in disease resistance and these can be summarised as follow:

i) amino acids may act as fungicides per se (Kuc, et al. 1959; Moje et al., 1963; Papavizas and Davey, 1963).

ii) aromatic amino acids are precursors of phenyl propanoidal compounds which are fungi-toxic (Kosuge, 1969; Tomiyama, 1963; Towers, 1964; Richards, 1965; Van Andell, 1966).

iii) certain aromatic acids and their phenyl propane derivatives may control regulatory enzymes in he biosynthesis of their fungi-toxic substances (Varner, 1965; Fehrmann and Dimond, 1967; Lingen, 1968), and

iv) amino acids may combine with other fungitoxic compounds to form a highly toxic compounds (Clark et al., 1959).

Therefore, the presence of higher amounts of total amino acids in resistant cultivars either help in the break down of naturally occurring phenols into toxic substances which inhibit the growth and multiplication of pathogen or presence of amino acids in higher amounts act as fungicide.

It has been claimed to play a significant role in disease resistance and susceptibility in several host-parasite combinations by ascorbic acid which is an organic reducing agent because it reduces the toxic quinones to less toxic phenols (Szent-Gyorgyi and Vietrosiz, 1931; Uritani and Iechika, 1953). In plants, the level of ascorbic acid is generally influenced by the enzyme, ascorbic acid oxidase and hence the ascorbic acid-ascorbic acid oxidase system has recieved considerable attention (Kalyana Sundaram, 1952; Farkas and Kiraly, 1962; Sridhar and Mahadevan, 1968).

In the present study no significant difference was observed in ascorbic acid content between resistant and susceptible cultivars at all stages tested. Similar observations made by Rama Mohan (1983).

Mishra and Prasad (1964) observed that rice varieties resistant to H. oryzae contained greater amounts of ascorbic acid than the susceptible. It is probable that as suggested by Kalyana Sundaram (1952) high ascorbic acid levels in resistant

variety might be readily utilised for the synthesis of toxic substances against pathogen.

In contrast, Pilgrim and Futrell (1951) reported that no correlation was recorded between ascorbic acid concentration and resistance to stem rust in wheat. Similarly, no correlation between ascorbic acid content and resistance to safflower rust was observed in present findings.

SUMMARY

CHAPTER VI

SUMMARY

The results obtained in the "Studies on safflower rust caused by Puccinia carthami Corda" are summarised below:

1. Rust incidence was uniformly distributed in all the areas surveyed.

2. Safflower rust caused by P. carthami produced all the five spore stages and hence confirmed as an autoecious macrocyclic rust. Although aecium and aeciospores, resembled uredinia and urediniospores they were referred to as aecia only because they occupied aecial stage in the life cycle and satisfied all the requirements of aecia.

3. Urediniospores remained viable for 25 days when exposed to natural weather conditions, whereas under laboratory conditions they remained viable for 40 days. Since they are shortlived they help in the spread of the disease only but not in perpetuation.

4. Teleutospores remained viable for 11 months under natural conditons while under laboratory conditions they remained viable for 13 months. Since teleutospores remain viable for long periods they help in perpetuation of the disease from one season to other. Teleutospores germinated profusely in November with maximum in December.

5. The estimated average number of teleutospores per seed lot was 128.66 from highly infested fields. The teleutospores might lodge on the seed coat during the process of harvesting and threshing and they are carried through seed.

6. Teleutospores carried either through seeds or infected plant debris lying in the field oversummer and remain viable to bring about fresh infection in the following year.

7. No collateral hosts for rust were found.

8. Mean maximum temperature and mean minimum temperature had great influence on the progress of the disease during both the years. Only mean maximum temperature had positive correlation with disease during 1986-87 rabi. Whereas, in addition to mean maximum temperature, mean evening humidity and mean sunshine hours also had significant correlation with disease during 1987-88 rabi. Relationship consisting MMXT, MMIT, MMH, MEH and MSH turn out to be best fit during 1986-87 rabi, while, MMXT and MMIT turn out to be best fit during 1987-88 rabi. From this relationship 49 per cent variation in disease development can be estimated on the basis of environmental factors included in relationship during 1986-87 rabi, whereas 67 per cent during 1987-88 rabi. This difference in variation between two years is due to rainfall that occurred during 1986-87 rabi after disease appearance.

9. The disease severity was more in 1986-87 rabi than in 1987-88 rabi. Continuous dry period of 72 days from December 15th onwards during 1987-88 rabi resulted in less severity of disease.

10. September sowings escaped the disease as it appears usually in January by which time the crop reached seed maturity stage. The disease intensity gradually increased with advanced sowings and was found highest in December sowings. The rust appeared in late sown crops because resting teleutospores germinate profusely from November onwards which served as primary source of inoculum.

11. Out of 192 germplasm lines and cultivars tested 53 were identified as immune, 14 resistant, 17 moderately resistant, 32 moderately susceptible and 76 susceptible to rust.

12. Bavistin 0.05 per cent and Dithane M-45 0.2 per cent were found more effective in checking the disease resulting in increased yields.

13. Per cent disease intensity was more and less yields in unprotected plots than protected plots. Unprotected plots gave 49 and 30 per cent reduced yields over protected during 1986-87 and 1987-88 rabi respectively. Whereas, protected plots gave 32 and 23 per cent increased yields over unprotected plots during both the years respectively.

14. Thickness of cuticle-cum-epidermis, stomatal frequency, size of stomata and size of stomatal opening had no correlation with resistance.

15. Resistant cultivars contained higher levels of total phenols, ortho-dihydroxy phenols, total amino acids and ascorbic acid but lesser amounts of reducing and total sugars than susceptible cultivars.

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APPENDICES

APPENDIX I

Daily meteorological data from December - March during 1986-87 and 1987-88 rabi

Month	Date	Temperature (°C)		Relative Humidity (%)		Rain-fall (mm)	Bright sunshine hours
		Maximum	Minimum	07.30 hours	14.30 hours		
December							
1986	1	28.2	12.0	89	38	0	11.1
	2	29.4	10.2	97	39.4	0	11.3
	3	27.7	10.3	81	43	0	9.8
	4	28.4	9.5	62	33	0	11.4
	5	28.3	9.4	75	44	0	10.6
	6	28.9	9.4	75	30	0	11.2
	7	29.1	10.5	71	37	0	11.2
	8	28.1	10.9	81	33	0	9.5
	9	29.3	14.7	78	47	0	11.3
	10	30.1	16.2	87	44	0	9.8
	11	30.5	19.7	88	42	0	9.2
	12	31.1	20.1	90	44	0	9.8
	13	29.7	20.4	84	47	0	11.0
	14	31.1	19.2	91	38	0	9.0
	15	30.4	19.7	75	40	0	9.2
	16	30.1	16.8	71	30	0	8.5
	17	30.2	17.1	67	27	0	10.5
	18	30.2	14.2	90	31	0	10.1
	19	30.1	16.1	79	41	0	10.8
	20	29.1	15.6	74	40	0	10.7
	21	29.1	13.7	78	42	0	9.5
	22	29.7	14.0	79	35	0	10.4
	23	30.2	15.2	77	32	0	10.8
	24	29.9	17.5	91	40	0	10.0
	25	29.5	17.7	87	53	0	9.8
	26	26.8	17.5	82	50	0	7.3
	27	26.5	17.5	91	52	2.2	9.8
	28	25.7	15.3	90	40	6.4	4.3
	29	25.7	14.5	78	43	0	10.2
	30	26.1	12.6	93	44	0	8.9
	31	27.1	13.1	77	44	0	9.4
	Mean	28.9	14.8	82	40	-	9.6

contd....

Appendix contd...

Month	Date	Temperature (°C)		Relative Humidity (%)		Rain-fall (mm)	Bright sunshine hours
		Maximum	Minimum	07.30 hours	14.30 hours		
January							
1987	1	26.9	10.3	88	44	0	11.3
	2	27.0	9.4	72	28	0	11.3
	3	27.1	9.3	70	32	0	11.2
	4	27.6	11.0	72	32	0	10.8
	5	27.1	10.5	67	30	0	10.2
	6	28.3	13.1	72	39	0	10.8
	7	27.9	14.1	75	31	0	10.8
	8	29.2	15.0	90	34	0	9.7
	9	28.9	14.9	92	34	0	10.2
	10	29.9	15.5	91	42	0	9.1
	11	28.5	19.7	91	47	4.2	4.4
	12	29.1	19.6	88	55	0	8.0
	13	29.5	20.7	88	53	0	6.1
	14	27.2	19.7	86	47	0	5.8
	15	28.8	17.9	91	44	0	10.2
	16	29.1	17.0	86	53	0	10.5
	17	28.1	15.2	81	42	0	10.7
	18	29.8	15.2	78	44	0	10.5
	19	29.2	15.3	90	44	0	9.8
	20	29.0	15.9	94	72	0	10.3
	21	28.4	16.4	89	46	0	9.6
	22	29.0	16.3	89	46	0	4.6
	23	28.8	19.5	86	46	0	5.8
	24	29.0	16.4	88	45	0	10.4
	25	29.5	15.6	92	37	0	9.4
	26	29.6	12.7	71	34	0	10.4
	27	30.1	9.3	51	34	0	11.3
	28	29.0	11.8	79	41	0	10.7
	29	29.6	11.2	71	34	0	10.6
	30	30.2	13.1	76	34	0	10.7
	31	30.3	12.0	76	39	0	11.3
	Mean	28.7	14.6	82	40	-	9.5

contd....

Appendix contd...

Month	Date	Temperature (°C) Maximum	Temperature (°C) Minimum	Relative Humidity (%) 07.30 hours	Relative Humidity (%) 14.30 hours	Rain-fall (mm)	Bright sunshine hours
February 1987	1	29.4	12.9	81	33	0	11.4
	2	30.2	15.4	89	37	0	10.1
	3	30.0	13.0	58	37	0	11.2
	4	29.2	11.2	57	29	0	11.0
	5	29.8	11.1	52	44	0	11.4
	6	29.9	13.0	59	46	0	11.4
	7	29.0	12.9	67	41	0	11.1
	8	28.55	12.5	54	37	0	11.1
	9	29.85	12.0	59	37	0	11.2
	10	29.25	11.5	59	34	0	11.2
	11	30.5	11.6	61	38	0	11.3
	12	31.1	13.0	62	39	0	11.3
	13	30.5	15.5	60	27	0	11.4
	14	31.0	14.8	92	26	0	10.4
	15	30.2	15.0	76	38	0	11.4
	16	31.1	14.1	65	34	0	10.9
	17	31.5	16.2	75	27	0	11.1
	18	31.4	16.4	57	27	0	11.2
	19	31.9	18.4	67	35	0	11.4
	20	30.3	16.5	68	29	0	11.1
	21	32.3	18.5	69	33	0	11.3
	22	31.3	19.5	67	32	0	10.5
	23	31.7	20.1	62	29	0	11.4
	24	33.9	18.3	46	27	0	10.5
	25	34.5	16.3	50	26	0	10.9
	26	33.5	17.9	60	26	0	10.5
	27	33.5	13.4	61	26	0	10.1
	28	33.8	19.0	80	40	0	9.8
	Mean	31.0	15.2	64	33	-	10.9

contd....

Appendix contd...

Month	Date	Temperature (°C) Maximum	Temperature (°C) Minimum	Relative Humidity (%) 07.30 hours	Relative Humidity (%) 14.30 hours	Rain-fall (mm)	Bright sunshine hours
March							
1987	1	31.3	18.3	84	37	0.6	7.0
	2	31.3	17.2	82	26	0	10.8
	3	32.1	16.3	68	25	0	11.4
	4	32.4	17.6	62	27	0	11.1
	5	32.1	16.7	62	31	0	10.7
						0	
	6	32.4	17.5	72	34	0	11.5
	7	32.2	17.4	55	29	0	11.5
	8	33.2	20.0	49	19	0	10.2
	9	33.7	21.1	50	21	0	6.0
	10	34.0	21.1	49	19	0	11.0
						0	
	11	33.9	20.3	77	31	0	11.2
	12	31.4	16.3	50	29	0	7.4
	13	32.5	19.5	74	30	0	11.4
	14	33.4	19.6	61	33	0	11.6
	15	32.5	20.8	65	32	10.0	9.1
						0	
	16	33.0	22.9	53	25	0	9.3
	17	35.2	22.3	44	19	0	11.2
	18	36.4	24.6	44	19	0	9.8
	19	37.3	20.8	76	30	43.9	8.7
	20	36.2	21.1	68	39	0	11.0
	21	34.8	19.1	50	22	0	8.8
	22	36.2	20.0	50	27	0	11.8
	23	36.0	18.7	54	20	0	11.5
	24	36.2	20.1	48	20	0	11.4
	25	36.1	20.1	42	19	0	11.3
	26	36.0	20.3	47	14	0	10.9
	27	35.0	19.7	43	19	0	8.1
	28	35.9	20.6	51	19	0	11.2
	29	37.1	21.0	66	20	0	10.8
	30	37.6	19.1	75	18	0	10.9
	31	37.1	20.1	51	17	0	11.2
	Mean	34.4	19.0	58	25	-	10.3

contd....

Appendix contd...

Month	Date	Temperature (°C) Maximum	Minimum	Relative Humidity (%) 07.30 hours	14.30 hours	Rain-fall (mm)	Bright sunshine hours
December							
1987	1	28.2	9.4	82	37	0	10.7
	2	28.1	11.3	86	35	0	10.8
	3	28.4	10.5	88	32	0	9.9
	4	28.6	16.5	78	47	0	10.2
	5	29.2	18.7	78	41	0	6.3
	6	29.1	17.7	82	38	0	9.8
	7	28.6	13.8	88	42	0	10.0
	8	28.5	12.7	89	43	0	10.2
	9	28.2	16.3	64	61	0	10.6
	10	26.1	20.5	86	59	0	2.6
	11	24.9	20.9	89	46	1.7	0.7
	12	27.1	19.3	86	49	0	3.1
	13	28.3	19.1	94	47	0	4.7
	14	29.3	20.7	89	50	0	3.2
	15	28.2	14.0	87	59	0	5.5
	16	25.5	10.7	78	40	0	7.4
	17	27.6	8.5	84	40	0	11.3
	18	26.3	8.5	82	39	0	10.1
	19	26.8	7.8	81	34	0	9.6
	20	25.1	8.6	81	36	0	11.2
	21	25.5	8.6	73	39	0	11.3
	22	27.3	10.1	85	38	0	10.8
	23	27.9	17.3	90	51	0	9.6
	24	28.2	15.3	90	50	0	7.3
	25	28.1	18.2	96	53	0	10.3
	26	28.1	15.7	77	42	0	9.9
	27	28.4	12.4	70	43	0	10.8
	28	27.7	11.0	86	42	0	8.5
	29	26.7	10.5	83	39	0	10.8
	30	28.2	12.7	76	42	0	10.2
	31	27.0	10.7	88	40	0	10.9
	Mean	29.1	12.7	83	43	-	8.6

contd....

Appendix contd...

Month	Date	Temperature (°C) Maximum	Temperature (°C) Minimum	Relative Humidity (%) 07.30 hours	Relative Humidity (%) 14.30 hours	Rain-fall (mm)	Bright sunshine hours
January 1988	1	26.9	13.2	85	40	0	9.9
	2	27.5	12.5	90	47	0	11.0
	3	26.9	10.8	96	39	0	7.5
	4	26.3	12.2	89	39	0	9.9
	5	28.3	13.2	86	44	0	10.3
	6	28.4	12.2	82	45	0	9.7
	7	29.0	11.2	86	40	0	9.9
	8	27.7	11.0	84	37	0	10.3
	9	27.4	11.5	86	38	0	10.2
	10	27.3	11.6	88	39	0	10.9
	11	27.5	12.2	91	44	0	10.2
	12	26.9	14.0	83	33	0	10.2
	13	28.0	15.2	86	37	0	10.5
	14	28.9	14.7	72	39	0	10.0
	15	30.6	15.2	92	40	0	9.0
	16	30.7	15.9	96	40	0	10.2
	17	29.6	14.6	79	41	0	11.6
	18	29.7	15.2	83	42	0	10.8
	19	30.1	14.1	79	34	0	11.0
	20	30.4	15.3	68	36	0	10.5
	21	31.0	15.2	81	36	0	10.2
	22	30.8	15.2	86	34	0	10.4
	23	29.4	17.3	73	40	0	9.7
	24	30.3	13.1	70	39	0	10.2
	25	30.5	13.4	73	41	0	11.1
	26	31.0	13.0	80	42	0	10.4
	27	31.4	13.7	86	27	0	10.7
	28	31.9	15.0	88	30	0	10.9
	29	31.0	14.7	88	40	0	11.3
	30	31.4	16.1	88	40	0	10.2
	31	32.0	15.1	69	35	0	11.0
	Mean	29.3	13.8	82	38	-	10.3

contd....

Appendix contd...

Month	Date	Temperature (°C) Maximum	Temperature (°C) Minimum	Relative Humidity (%) 07.30 hours	Relative Humidity (%) 14.30 hours	Rain-fall (mm)	Bright sunshine hours
February							
1988	1	32.4	15.5	80	40	0	11.1
	2	31.1	15.3	90	41	0	11.1
	3	30.7	15.0	90	39	0	11.1
	4	31.1	11.3	67	36	0	11.5
	5	32.0	9.7	64	35	0	11.2
	6	31.8	8.3	61	36	0	11.6
	7	30.4	13.0	64	28	0	10.9
	8	32.2	14.1	76	31	0	10.8
	9	32.2	15.0	87	31	0	10.9
	10	30.1	15.6	70	33	0	10.6
	11	33.1	16.0	66	35	0	10.8
	12	31.2	15.3	71	37	0	10.8
	13	30.1	15.5	81	36	0	10.6
	14	30.7	17.3	88	38	0	10.4
	15	31.9	20.2	87	39	0	10.0
	16	32.7	20.5	79	34	0	10.2
	17	34.8	20.7	90	40	0	10.4
	18	33.9	11.9	86	41	0	10.2
	19	30.8	19.4	82	36	0	10.0
	20	33.6	23.5	78	34	0	10.8
	21	33.8	20.2	82	31	0	10.4
	22	34.9	18.3	93	15	27.2	10.0
	23	33.1	19.8	93	14	0	10.8
	24	33.3	19.7	93	41	0	10.7
	25	33.2	19.5	91	46	0	10.4
	26	32.3	20.6	88	39	0	9.2
	27	35.8	22.1	91	40	0	11.2
	28	32.2	22.3	81	47	0	10.1
	29	33.0	20.8	90	38	1.4	9.2
	Mean	32.5	17.4	81	36	-	10.5

contd....

Appendix contd...

Month	Date	Temperature (°C)		Relative Humidity (%)		Rain-fall (mm)	Bright sunshine hours
		Maximum	Minimum	07.30 hours	14.30 hours		
March							
1988	1	34.3	22.5	79	29	0	10.4
	2	34.3	17.3	60	29	0	10.2
	3	33.7	16.1	65	31	0	11.2
	4	33.4	21.3	88	30	0	10.6
	5	34.0	21.1	79	31	0	10.8
	6	34.8	21.3	71	34	0	9.6
	7	35.4	21.5	78	35	0	10.9
	8	35.2	21.7	75	40	0	10.0
	9	34.3	20.4	75	42	0	10.9
	10	34.1	20.5	79	30	0	11.4
	11	34.2	19.1	68	32	0	11.7
	12	34.3	17.8	77	32	0	12.0
	13	36.2	19.5	68	34	0	12.0
	14	36.7	19.3	85	34	0	12.0
	15	37.2	21.4	74	31	0	11.4
	16	37.0	21.9	58	34	0	11.8
	17	36.7	21.3	56	30	0	10.2
	18	37.7	24.3	58	32	0	12.0
	19	38.1	22.2	55	33	0	12.0
	20	36.4	20.0	58	37	0	11.8
	21	35.3	19.2	59	30	0	10.2
	22	37.0	20.2	50	34	0	11.1
	23	36.3	21.5	89	46	0	10.3
	24	35.3	22.8	83	46	0	9.1
	25	35.1	25.3	83	56	11.6	9.1
	26	33.7	23.3	71	36	0	4.4
	27	35.3	22.1	61	33	0	10.6
	28	35.7	20.1	57	32	0	10.8
	29	36.2	18.3	60	26	0	10.6
	30	37.0	18.0	52	27	0	10.0
	31	37.5	19.7	52	29	0	9.6
	Mean	35.5	20.6	68	34	-	10.6

APPENDIX II