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BACTERIAL LEAF SPOT DISEASE OF SUNFLOWER (*Helianthus annuus* L.) INCITED BY *Xanthomonas phaseoli* (E. F. SMITH) DOWSON

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BY

MALLIKARJUN VEERSHETTY BIRADAR

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CANDIDATE'S DECLARATION

I, hereby declare that the entire work embodied in this dissertation or part thereof has not been previously submitted by me for a degree of any University.

PARBHANI

DATED : 15th July, 1982

M.V.Biradar

(M.V. Biradar)

Dr. S.S. Kore,
M.Sc.(Agri), Ph.D.,
Associate Professor of Bacteriology,
Marathwada Agricultural University,
PARBHANI (Maharashtra)

C E R T I F I C A T E

Shri. Mallikarjun Veershetty Biradar has satisfactorily prosecuted his course of research for a period of not less than two years and that the dissertation entitled " A Bacterial leaf spot disease of sunflower (Helianthus annuus L.) incited by Xanthomonas phaseoli (E.F. SMITH) DOWSON" submitted by him is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the dissertation or part thereof has not been previously submitted by him for a degree of any University.

Date : 15th July, 1982

(Dr. S.S. Kore)
Research Guide

CERTIFICATE

This is to certify that the dissertation entitled "A BACTERIAL LEAF SPOT DISEASE OF SUNFLOWER (Helianthus annuus L.) INCITED BY Xanthomonas phaseoli (E.F. SMITH) DOWSON" submitted by Shri. Mallikarjun Veershetty Biradar to the Marathwada Agricultural University in partial fulfilment of the requirements for the degree of Master of Science (Agriculture) in Plant Pathology has been approved by the student's advisory committee after oral examination in collaboration with the external examiner.

External Examiner

(Dr. S.S. Kore)
Guide

Associate Dean
and Principal,
College of Agriculture,
Parbhani

1. (Dr. V.R. Mali)
2. (Prof. K.J. Bindu)
3. (Dr. V.D. Patil)

Advisors:

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DATE : 15th July 1982.

MVBiradar

(M.V. Biradar)

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INTRODUCTION

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INTRODUCTION

Sunflower (Helianthus annuus L.) localy known as suryaful, surajmukh, or suryakanti. It is a oil yielding crop of the world. It was used for food at Roanoke Island in (1586) but nobody would have thought that sunflower seeds with its oil content of 45 to 50 per cent would be used to wipeout the edible deficit in diet (Krishnarajan, et al., 1972).

Though sunflower is a native of North America, it is seldomly grown there for oil because of its low oil content of 20-30 per cent. In Russia after its introduction scientiests were able to evolve varieties yielding more oil (45-50 per cent) and now edible oil in Russia is extracted from sunflower seeds (Vyahalkar, 1972).

The world production of sunflower is estimated to be about 6 million tonnes grown on an area of 6-8 million hactres with average yield of 877.5 kg/ha. Sunflower has been recently introduced in India and now it is becoming popular as oil seed crop. It is a short duration crop and can be grown throughout the year inheavy to light soil with less moisture. In India total area under sunflower crop is about four lakh hactares in the states of Tamil Nadu, Karnataka, Maharashtra, Uttar Pradesh,

Andhra Pradesh, West Bengal, Orissa and Punjab (Sindagi,1981). The Maharashtra occupies an area of about 63,000 hactares (Gaikwad, 1981).

Sunflower has become a crop of dry land Agriculturist which plays important role in the economy of common farmer. The biggest single factor to its popularity is its potentiality to yield large quantity of top quality oil per unit area (Shukla, 1972).

Sunflower oil is classed as semidrying and is used in the manufacture of paints, soaps and varnishes. Besides oil content seed has got an equal quantity of meal the / meal has about 30 per cent protein, which is well balanced in aminoacids and makes a good feed for animals and birds specially to dairy animals, poultry and pigs. It has also been used in human consumption and oil contains fair quantities of vitamin D and E. Threshed sunflower heads are employed for manufacturing a good quality protein.

During Kharif, 1981 at Central Farm, Marathwada Agricultural University, Parbhani, a bacterial leaf spot disease of sunflower was found. The incidence was to the tune of 6 to 8 per cent. A literature review revealed that there is no report of bacterial leaf spot diesase of sunflower caused by Xanthomonas sp. in Maharashtra

Considering the increasing area under sunflower crop in Maharashtra State and severity of disease, the investigation was under taken with a view to get a detailed information on pathogenicity, perpetuation and spread of disease, host range and identification of pathogen.

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CHAPTER - II

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

The *Xanthomonas phaseoli* (E.F. Smith) Dowson is of wide occurrence since a long age. Beach (1892) was the first record the bacterial blight of French bean. Smith (1897) reported its occurrence for the first time on number of leguminous plants producing leaf spot disease and he named it as *Bacillus phaseoli* (Smith). Again in 1901 he named it as *Pseudomonas phaseoli* (Smith). However, in 1905 Smith renamed it as *Bacterium phaseoli* (Smith). Bergy *et al.* (1923) referred it as *Phytomonas phaseoli* (E.F. Smith) Bergy. Dowson (1939) referred it as *Xanthomonas phaseoli* (Smith), Dowson.

Burkholder (1930) was the first to describe the fuscous blight of bean caused by *Bacterium phaseoli* var. *fuscous* (Burk.) Starr and Burk.

Zaumyer (1930) carriedout detailed studies on epidemology and mode of spread of *Bacterium phaseoli* and stated that infection in influenced by moisture under proper temperature, in dry conditions disease is less. He further stated that primary infection is through plant debries and dissemination is by biological agents. He also found that "Refugee" type of bean varieties are tolerant to this disease.

Gross (1940) reported the relation of temperature for the development of common bean blight disease. He observed that Xanthomonas phaseoli (Smith) Dowson produced blight disease at the temperatures 16, 20 and 32°C and produced symptoms after 27, 23 and 7 days respectively. The transfer of plants from lower to higher temperature influenced disease development.

Uppal et al. (1946) created a new variety of Xanthomonas phaseoli (Smith) Dowson on kidney bean (Phaseolus vulgaris L.) and he named it as Xanthomonas phaseoli var. indicus. The pathogen produced minute water soaked spots on lower surface of leaves. The spot enlarge in size and become angular and turn dark brown in colour and at later stage leaves blighted completely.

Patel et al. (1949) observed occurrence of of Xanthomonas phaseoli (Smith) Dowson on Kulthi (Dolichos biflorus L.) and symptoms resembled to soybean bacterial blight. Symptoms were characterised by the presence of numerous minute specks which coalesce to form lesions measuring 1 to 2 mm spots and were raised, rough and found on both surface.

Elliot (1951) worked on several species of Xanthomonas. The chief bacterium studied by him was Xanthomonas phaseoli (Smith) Dowson.

Bhatt et al. (1954) reported the occurrence of Xanthomonas phaseoli (Hedges) Starr and Burk. on soybean. (Glycine Max (L.) Merr.) in India. The pathogen produced reddish, brown pustules like spot on both surface of leaves. Mature spot appear dark brown in colour and angular in shape and coalesce to form large dry patches which may result into defoliation. He further stated that organism enters through stomatal opening. Under humid condition organism spread rapidly through wind, splash rain. Plant debries, seed and soil are the chief sources of primary infection. In field observation, they observed that seed is the main source of disease.

Bhatt et al. (1954) they recorded 38 phytopathogenic bacterial from India, out of which 34 were belonging to Xanthomonas and 16 of them incite diseases on members of leguminosae only. They also found Xanthomonas species can grow well at pH 7, but did not grow at 3.1 and 10.1 pH, all cultures liquefy gelatin, produce hydrogen sulphide but did not reduce nitrate and they utilize most of the carbon sources.

Bhatt et al. (1956) described the occurrence of Xanthomonas phaseoli (Smith) Dowson on Phaseolus trilobus Ait. from Poona. The pathogen produced minute water soaked spots on leaf surface which enlarge in size and became dark brown

in colour. Since they found that the pathogen was host specific so they named it as Xanthomonas phaseoli trilobi (Smith) Dowson.

Dye (1958) reported that the identification of Xanthomonas species by laboratory procedure is yet impossible and therefore host specificity is only critarion for identification.

Rangaswami and Prasad (1959) described the occurrence of Xanthomonas species on bengal gram (Cicer arietinum L.). Further they identified the organism as Xanthomonas cassiae. In artificial inoculation test, they observed that organism could infect phaseolus mungo, Phaseolus aureus and Vigna catjang.

Patel (1962) observed that due to infection of Xanthomonas phaseoli (Smith) Dowson, there is increase in concentration of histidine, asparagine, glutamine, ornithine, phenylalanine, isoleucine and valine in the leaf.

Patel and Walkar (1963) studied the relation between air temperature, age and nutrition of host for the development of common and halo blight of bean. The older leaves were more tolerant and the younger leaves were more susceptible. The halo development was greater at 10°C day and 28°C night temperatures. The lesions were small at 10°C and largest at 20°C, 24°C and 28°C. While very low and high levels of N ,

P and K retarded the severity of halo and common blight and high K and high P enhanced the common blight development. Further they concluded that use of healthy seeds, crop rotation and seed treatment reduced the disease intensity.

Rangaswami and Gowda (1963 a) reported many bacterial diseases from Madras State. They reported the occurrence of Xanthomonas phaseoli (E.F. Smith) Bowson for the first time on urd bean (Phaseolus mungo L.) from Annamalainagar South Arcot district in Tamilnadu. They found that the pathogen produces circular to irregular water soaked spots on leaves. Lateron such spots appear, raised and become dark brown in colour. Several such spots coalesce and cause blight symptoms. In case of severe infection the spots also occur on stem but rairly on pods. In artificial inoculation test they found that this pathogen also infect to Dolichos lablab, Phaseolus radiatus, Phaseolus vulgaris and Vigna catjang.

Rangaswami and Gowda (1963 b) reported the occurrence of Xanthomonas phaseoli (Smith) Dowson on garden bean. They found that pathogen produced round to irregular, water soaked spots on leaves. The spots become brown with age and in case of sever infection defoliation is also seen. They also found that the primary infection is through seeds and plant debries and they concluded that use of disease free seeds and sanitation are useful for reducing disease intensity.

Fang and Chen (1964) studied the occurrence of genus Xanthomonas on leguminous plants in China. They obtained 18 cultures of Xanthomonas from seven leguminous plants and reported the occurrence of Xanthomonas phaseoli (Smith) Dowson on mung bean (Phaseolus aureus) for the first time in China. Further they found that isolates are similar in cultural and physiological characters and serological reactions but they differ in pathogenicity which is regarded as character for determining species. They found that host range was common for all isolates but each species was more virulent to its natural host. The author considers that culture from Phaseolus vulgaris, Phaseolus aureus, Phaseolus calcaratus though specialised in pathogenicity are regarded as the strain or race of Xanthomonas phaseoli.

Walkar and Patel (1964) reported that during 55 to 60 days period from planting to harvesting time for processing of snab beans in Wisconsin, diseased plants appeared up to the height of 85 ft. from source of inoculum provided for planting infected seeds. Under those conditions as few of 12 infected seeds per acre could bring about a severe epidemic. Splash dispersal as directed by prevailing wind during rainfall period appeared to be the chief agent of dissemination under Wisconsin condition.

Macarthy (1970) reported occurrence of Xanthomonas phaseoli (Smith) Dowson on navy beans. He stated

that bacterium was seed borne and often cause discolouration of seeds. Sowing of disease free seeds and frequent spray with bactericide reduce the disease intensity.

Cinaro and Rudolph (1971) reported significance of Xanthomonas phaseoli var. fuscans in Germany. They observed in field study that sowing of naturally infected bean seeds lead to serious out break of disease under favourable weather conditions. Infection spreads rapidly when average day and night temperature was above 19°C for several days.

Patel et al. (1971) made survey in 1968 and 1969 at elven location in nine different states, they observed ten diseases on seed legumes, among these leaf spot disease on urd bean (Phaseolus mungo L.) caused by Xanthomonas phaseoli (Smith) Dowson was of regular occurrence at pantnagar, Delhi, Ludhiana and Jabalpur. Further they stated that the pathogen involved was transmitted through seeds and by way of infected seeds which were disseminated through out the country.

Patel and Jindal (1972 a) reported occurrence of fuscous and common blight caused by Xanthomonas phaseoli on bean (Phaseolus vulgaris). They found that both pathogens produced small water soaked spots on leaves and cause typical blightening. They also found that both the pathogens give yellow colonies on nutrient agar plate. The organism could grow well at temperature from 25 to 30°C for 3 - 5 days. They

also reported that both the pathogens were gram negative, non capsulated, non sporeformer non acid fast and motile with singal polar flagellum, liquifies gelatin, hydrolyzed starch, hydrogen sulphide and Amonia produced, Indole not produced, nitrate not reduced, milk became peptonized without coagulation and litmus was reduced. They also reported that var. Fuscan can utilize xylose and Mannitol as carbon source but var. indicus utilize only xylose but not mannitol and it produces brown pigment and can infect Dolichos lablab but var. Fuscans produce dark brown pigment and not pathogenic to Dolichos lablab.

Patel et al. (1972 b) reported that Xanthomonas phaseoli (Smith) Dowson parasiting moth bean / (Phaseolus aconitifolius). The pathogen produced small irregular brown necrotic spots on both surface of leaves. In artificial inoculation test it also infected Phaseolus vulgaris, Phaseolus atropurpurous but not to Phaseolus mungo, Phaseolus aureus and Dolichos lablab.

Patel and Jindal (1972 c) reported the occurrence of Xanthomonas phaseoli (E.F. Smith) Dowson in India for the first time on mung bean (Phaseolus aureus Roxb.). They found that pathogen produces characterstic symptoms on leaves, the presence of brown, dry, raised spots which appear superficially as eruptions and gradually develops through the entire leaf thickness and became corky or rough. In artificial inoculation test, the pathogen infected

Phaseolus vulgaris, Phaseolus lunatus, Phaseolus bracteatus, Dolichos lablab. but not Phaseolus mungo. This suggests that mung bean pathogen has different pathogenic behaviour. In fields also urd crop grown near severly infected mung was free of any bacterial infection. Chee (1972) reported bacterial spot of soybean caused by Xanthomonas phaseoli for the first time in Malaysia.

Diaz (1974) reported bacterial pustule of soybean incited by Xanthomonas phaseoli var. sojense in Venezuela and he further observed that pathogen is seed transmitted affecting germination of seed and the later development of surviving plants. It was not associated with any other soybean bacterium.

Signoret et al. (1975) reported for the first time soybean disease incited by Xanthomonas phaseoli var. sojense in southern of France.

Shipler (1976) reported bacterial disease of soybean incited by Xanthomonas phaseoli var. sojense in Moldavia.

Sood et al. (1976) reported a mung strain of Xanthomonas phaseoli. The seed borne infection was reduced by seed treatment with Captan (0.3%) and Bleaching powder (0.025%). In field experiments at Ludhiana and Gurdaspur three protecive sprays of Streptocycline (100 ppm), Zineb (0.3%) or Benomyl (0.2%) were effective incontrolling in secondary infection.

Misra (1977) carriedout the study *in vitro* on growth and nutrition of *Xanthomonas phaseoli* var. *sojense* the causal organism of bacterial pustule of soybeans. He further recorded that the organism grew best on Wakimoto's medium and the optimum temperature and pH for growth were 30°C and 6.8 respectively. Hexoses supported good growth while galactose was best carbon source. Among the nitrogen sources Ammonium compound supported good growth but not nitrates. Isoluecin was the best organic nitrogen source.

Albornoz (1978) reported soybean bacterial disease caused by *Xanthomonas phoseoli* var. *sojense* in cuba. The pathogen produced spots on leaves, pods in artifical inoculation test. Bogatsevska (1979) reported bacterial disease of soybean caused by *Xanthomonas phaseoli* var. *sojense* in Bulgeria and he further recorded different temperatures for growth of the organism. Optimum, maximum and minimum temperatures were 30, 39 and 10°C respectively.

Kore and Khandale (1979) reported bacterial leaf spot disease of bean (*Dolichos lablab*) caused by *Xanthomonas phaseoli* at Parbhani and they further reported that the host could only be infected up to the age of 60 days and disease intensity was more in younger plants. The pathogen could infect cowpea (*Vigna radiata*), bean (*Phaseolus vulgaris*), *Dolichos biflorus* and *Phaseolus lunatus*

but not to soybean. They tested seven fungicides and an antibiotic in vitro and reported that Agallol 6 and Agrimycin gave maximum growth inhibition of the pathogen.

Weller and Saettler (1980) reported seed borne of bean (Phaseolus vulgaris) incited by Xanthomonas phaseoli and Xanthomonas phaseoli var. fuscans. Bean seeds externally infested with the bacteria were a source of primary inoculum and 14 per cent of commercial seed lots were so contaminated. In the field symptomless seeds internally contaminated with bacteria were potential primary source of inoculum, seeds with visible symptoms were allways associated with visibly infected pods, pod infection from systemically borne bacteria often caused hairline structured lesions which are difficult to detect.

Lakara and Parashar (1980) studied resistance in bacterial leaf spot disease of sunflower and reported that sunflower variety SC-17 is highly resistant to two new bacterial leaf spot diseases caused by Pseudomonas helianthi and Xanthomonas sp. He further reported that disease incidence was maximum when crop was sown in July than in April. High humidity and comparatively low temperature favoured disease development. Both the pathogens were found to be seed borne, plant debries may also play a role in perpetuation of the two pathogens.

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CHAPTER III

MATERIALS AND METHODS

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MATERIALS AND METHODS

During the year 1981 in kharif season a bacterial leaf spot disease of sunflower (Helianthus annuus L.) was observed on variety EC-68414 at Central Farm, Marathwada Agricultural University, Parbhani. The diseased leaves, and some damaged shrivelled grains from affected plants were collected and used for further studies.

3.1 Pathogenicity :

3.11 Isolation :

The infected samples of leaves and seeds of sunflower were used for isolation.

The leaf spot samples were thoroughly washed in tap water and after drying in air, affected portions of leaves were cut in two small pieces by sterilized razor blade. These pieces were then disinfected with 1:1000 solution of mercuric chloride for about 1 to 1 $\frac{1}{2}$ minute and washed in three changes of sterile water inorder to remove the traces of poison and then transferred to petridish containing few ml of sterile water and crushed with the help of sterilized razor blade. A loopful of suspension was transferred with the help of sterilized needle to sterilized petridish over which a liquified nutrient agar medium was poured.

In second isolation some damaged, discoloured and shrivelled seeds were surface sterilized in 1:1000 solution of mercuric chloride for about one to 1½ minute and washed in order to remove traces of poison then these seeds were transferred with the help of sterile forecep to sterile petridish over which a liquified nutrient agar medium was poured.

The petridishes were incubated at room temperature (27 to 30°C). After 2 to 4 days, a typical bacterial growth was noticed in all the petridishes. Transfers of bacterial colonies obtained from leaf spot and affected seeds were made separately on nutrient agar slants and cultures were maintained for further studies.

3.12 Pathogenicity :

The plants of sunflower variety EC-68414 were raised in earthenware pots, filled in with sterilized soil. Ten days old plants were used for inoculation. These plants were incubated in moist chambers for 24 hours prior to inoculation. Then they were taken out and bacterial suspension was sprayed on plants with the help of automizer. In second method leaves were injured with carborandum powder and inoculated the leaves with bacterial suspension with the help of cotton swab by hand.

Two pots for each method were inoculated containing four plants each. Inoculated plants were incubated in moist

chamber for 48 hours. They were subsequently removed from moist chambers and transferred to the benches of glass house. These inoculated plants were sprayed periodically with sterile water to maintain sufficient humidity for the development of disease. Adequate control was also maintained. Appearence of disease symptoms were recorded from 4 to 8 days after inoculation. The symptoms were compared with those obtained under natural conditions.

3.13 Re-isolation :

Re-isolation was made from artificially incoulated leaves of variety EC-68414. The culture obtained was identical with original culture.

MORPHOLOGY, STAINING REACTIONS, CULTURAL CHARACTERS AND GROSS PHYSIOLOGICAL CHARACTERS :

3.2 Morphology :

3.21 Shape :

Smear of 48 hours old culture grown on nutrient agar medium was taken and stained with Ziehl's carbol fuchsin and observed under oil immersion.

3.22 Size :

Using filar micrometer eye piece, size of stained organism was determined under oil immersion at 1500 x magnification.

3.23 Cell grouping :

Smear of 48 hours old culture grown on nutrient agar medium was taken, stained with Ziehl's carbol fuchsin and cell grouping was observed under microscope.

3.24 Motility :

The motility was studied by growing organism on King's B agar and also in semisolid agar (Colquhoun and Krikpatricks, 1932; Edwards and Ewing, 1955) having following composition.

Bacto-peptone - 10.00 g; Bacto gelatin 80.00 g; Bacto-agar - 4.00 g; Nacl - 5.00 g; Distilled water - 1000 ml composition of King's B agar :

Proteose-peptone - 20.00 g; Glycerol - 10.00 ml; Agar 20.00 g; K_2HPO_4 - 1.50 g; $MgSO_4 - 7H_2O$ - 1.50 g; Distilled water 1000 ml.

3.3 Staining reactions :

3.31 Gram Stain :

Culture was stained by Kopeloff and Beerman's modified method of Gram's staining.

3.32 Capsule staining :

Following Anthony's method with Tyler's modification as given in the Manual of Microbiological Methods (1957), organisam was stained for capsule.

3.33 Acid fast staining :

Culture was stained by Ehrilich's method as modified by Ziehl-neelsen (1883).

3.34 Spore staining :

Spore staining was done by Dorner's method.

3.35 Flagella staining :

Flagella staining was done following the method given by Blendon and Goldberg's (1965) (Methods in Plant Pathology, Kiraly et al., 1974).

3.4 Cultural characters :

The organism was grown in triplicate plates on different agar media, broths and agar slants at 29°C (± 1). Observations were recorded after 8 days of inoculation. Colour of colony was recorded following Ridgway's Colour Standard and Colour Nomenclature (Ridgway, 1912). Following medium were used :

3.41 Plate culture :

Nutrient agar, Potato dextrose agar, Beef peptone agar, Yeast extract mannitol agar and host extract agar.

3.42 Broth culture :

Nutrient broth, Potato dextrose broth, Yeast extract mannitol broth, Beef peptone broth.

3.43 Streak culture :

Nutrient agar, Potato dextrose agar, Yeast extract mannitol agar, Beef peptone agar and Host extract agar.

3.5 Gross physiological characters :

Gross physiological characters of organism were studied following Mannal of Microbiological Methods (1957) with slight modicications whereever felt necessary. The culture was incubated at 29°C (± 1) in all tests, unless otherwise mentioned.

3.51 Liquefaction of gelatin :

Gelatin liquefication was studied following Frazier's method modified by Smith (1946). After inoculation, plates were incubated for 7 days and flooded with an acidified solution of mercuric chloride and observations were recorded.

3.52 Production of ammonia :

The organism was grown in duplicate tubes containing King's 'B' broth. The production of ammonia was tested after 5 days. The red litmus paper strips were placed in the mouth of tubes and held in hot water bath for 10 minutes and observations were recorded.

3.53 Production of Hydrogen sulphide :

Production of hydrogen sulphide was studied in a liquid medium containing proteose peptone. Sterile filter

paper strips previously impregnated with lead acetate were placed over culture to detect the production of hydrogen sulphide as evidenced by darkening of filter paper strips and observations were recorded.

3.54 Reduction of Nitrate :

The culture was grown indduplicate tubes containing synthetic nitrate medium. These tubes were incubated for 7 days at 29°C. The reduction of nitrate was judged by additing a few drops of sulphanilic acid and ∝-naphthylamine and production of pink or red colour was noted.

3.55 Hydrolysis of starch :

The basal medium advocated by Stapp (1961) having following composition was used to know hydrolysis of starch by the organism.

Sodium nitrate 0.5 g; Potassium hydrogen phosphate 0.250 g; Magnessium sulphate 0.1000 g; Distilled water 100 ml; Starch 1 per cent.

Duplicate plates were poured, inoculated and incubated for 7 days at 29°C. After which they were flooded with Lugol's iodine solution and observation was noted.

3.56 Action on litmus milk :

The culture was inoculated in litmus milk in duplicate tubes and incubated for 30 days at 29°C. Production of acidity or alkalinity curdling, peptonization and reduction

of litmus milk was noted after 7, 13, 20 and 28 days of incubation.

3.57 IMVIC reaction :

3.571 Indole production

The ability of organism to produce indole was detected by growing in 1 per cent tryptophane broth recommended by Dowson (1899).

Oxalic acid paper test (Gnezda, 1897) was followed for detection of indole. Duplicate tubes were inoculated and sterilized filter paper strips previously soaked in concentrated oxalic acid were held over medium under asceptic conditions and observations were noted.

3.572 Methyl red test

Methyl red test was undertaken as per methods given in Manual of Microbiological Methods (1957). Tubes were inoculated in duplicate and incubated for four days at 29°C. Few drops of methyl red indicator used for detection of reaction and observations were recorded.

3.573 Voges-Proskauer test

The ability of organism to produce acetylmethyl carbinol was determined by noting crimson colour when 0.6 ml of five per cent α-naphthol and 0.2 ml of 40 per cent KOH were added and on the basis of colour indication reaction was noted.

3.58 Utilization of carbon compounds :

A synthetic carbohydrate medium recommended by Ayers, Rupp and Johnson (Manual of Microbiological Methods, 1957) was used. Stock solution of basal medium was prepared, and neutralised by adding N/10 sodium hydroxide solution. After adding bromothymol blue indicator, the solution was distributed in 200 ml lots in 500 ml Erlenmeyer flasks. To each of these flasks different carbon compounds were added on molecular weight basis to give the carbon content in 10 g of Glucose per litre.

The medium was distributed in test tubes in which Durham's fermentation tubes were placed in inverted position. The tubes were sterilized in Arnold's steam sterilizer for three successive days, inoculated and incubated for 7 days. Observations on production of acid and gas were recorded on 4th and 7th day. Following carbon compounds were tried.

1) Pentoses - Xylose
2) Hexoses - Fructose, Dextrose, Trehalose, Glucose, Galactose, Mannose
3) Disaccharides - Saccharose, Lactose, Maltose
4) Trisaccharides - Raffinose
5) Glucosides - Salicin
6) Deoxy sugars - Rhamnose,
7) Sugar alcohol - Mannitol

3.59 Utilization of introgen compounds :

The stock solution of Richard's medium without KNO_3 was prepared, distributed in 100 ml quantities in 250 ml

Erlenmeyer flasks. To each of these flasks different organic and inorganic nitrogen compounds were added in quantities calculated on molecular weight basis to give the nitrogen content in KNO_3. The pH of the medium was adjusted to 7 and after adding different nitrogen compounds the flasks were sterilized in autoclave at 15 lbs pressure for 15 minuts. Then flasks were inoculated with the isolate and incubated for 7 days at room temperature. Observations on the growth were recorded after 7 days. The nitrogen compounds were Amonium nitrate, Amonium chloride, Amonium sulphate, Barium nitrate, Magnesium nitrate, sodium nitrate, Potassium nitrate, Calcium nitrate, Asperagine, Glycine and Urea.

3.510 Relation to free oxygen :

Relation to free oxygen was studied by growing isolate on King's 'B' agar slants. Plugs were pushed in and pyrogallic acid was placed over them to fill up 1/3 of the gap and then 0.1/NaoH solution was added to pyrogallic acid. Tubes were plugged with rubber stopper; inverted and incubated for 7 days ang growth was recorded.

3.511 Relation of H-ion concentration to growth :

The relation of H-ion concentration of the medium to the growth of the organism was studied in nutrient broth adjusted to different pH levels ranging from 4.00 to 10.00 One hundred ml broth was added 250 ml Erlenmeyer Flasks and

sterilized. The organism was inoculated in duplicate flasks at each pH level keeping one flask as a control and incubated for 7 days at room temperature, after which the growth of the isolate to different pH levels were recorded.

3.512 Temperature and growth relation :

3.5121 Growth in relation to temperatures :

Organism was inoculated in petridish containing nutrient agar and incubated at different temperatures from 0 to 50°C for 7 days and observations were recorded.

3.5122 Thermal death point :

Duplicate test tubes of uniform thickness were used. These tubes were filled with 10 ml of nutrient broth medium, sterilized and inoculated with young culture. The inoculated tubes were maintained at each temperature for 10 minutes in hot water bath. One blank tube containing 10 ml of broth was kept to record the temperature. After exposure to each temperature for 10 minutes, tubes were immediately removed and placed in cold water. From the tubes one loopful of culture was then transfered to fresh nutrient agar slant, incubated and observed for growth.

3.513 Development of disease in relation to age of the plants:

The experiment was conducted with view to study the effect of age of the plant in relation to the disease development. The sunflower plants were raised in earthenware pots filled

in with sterilized soil. The leaves of the plants were inoculated from the age of 15 to 90 days keeping an interval of 7 days as described earlier. Inoculated plants were kept under glass house condition on benches. These inoculated plants were sprayed periodically with sterile water to maintain sufficient humidity for the development of disease. The observations on the disease development was recorded on the basis of percentage of leaf area infected and averages were calculated and each inoculated plants was classified following score card developped for this purpose.

3.514 Seed borne nature of the disease :

Experiment was carried out on variety EC-68414 to know the progress of bacterial leaf spot of sunflower from initial infection on cotyledons through seeds and its onwards spread on other plant parts. Fifty to sixty naturally infected shrivelled seeds were collected and sown in earthen ware pots filled in with sterilized soil. Seeds were germinated after 5 to 6 days and plants stand was 60 to 65 per cent after 12 days observations for appearence of symptoms on cotyledons and other plant parts was taken daily.

3.515 Varietal resistance study :

The object of this study was to find out the comparative resistance of some selected varieties of sunflower against leaf spot disease of sunflower. The seeds

of different varieties were obtained from the oil seeds research station, Latur and were sown in earthen ware pots filled in with sterilized soil. Two pots for each variety were used containing six plants. Ten to fifteen days old plants were inoculated and maintained in moist chamber for 24 hours before and 48 hours after inoculation. Uninoculated plants of each variety were served as control. Plants were sprayed with sterilized water periodically with help of automizer to ensure sufficient humidity for the development of disease. Observations were recorded after 20 days on the basis of percentage of leaf area affected. Average were calculated and each variety was classified following score card given below. The details of the score card and grades followed for classification of varieties are as under :

Percentage of leaf area affected	Grades
o per cent	HR or Free
1-10 per cent	R
10-20 per cent	MR
21-40 per cent	MS
41-60 per cent	S
61 and above per cent	HS

NOTE : HR = Highly resistant, R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible and HS = Highly susceptible

3.516 Host range study :

The present study was undertaken to determine the host specificity or otherwise of the isolate under study. For this experiment, seeds of following crops, were collected and surface sterilized in 1:1000 solution of mecuric chloride and were sown in earthenware pots filled in with sterilized soil. Ten to fifteen days old plants were used for inoculation. Leaves were injured by carborendum powder and bacterial suspension was sprayed with the help of automizer. Inoculated plants were maintained in moist chamber for 24 hours, before and 48 hours after inoculation. Then these pots were transferred on the benches of glasshouse and sprayed with sterilized water periodically to maintain sufficient humidity for development of diesase and observations were recorded after 10 days.

3.517 Control measure study :

In order to know the efficacy of different fungicides and antibiotics in vitro against the pathogen under study a method "poisoned food technique" was followed recommended by Nene and Thapliyal (1979). The principle involved in this technique is to "poison" the nutrient agar medium with a fungitoxicant. The efficacy of Agallol , B 'sten' Dithane M-45, Fytalon, Hexathir, Hexacap, were tested at 1000, 2000, 3000 ppm concentrations, while an Antibiotic streptocycline was tested at concentrations of 100, 200 and 300 ppm.

Suspension of bacterium was prepared from 48 hours growth on nutrient agar medium in sterile distilled water. Four mm diameter discs of filter paper were prepared by punching mechine and discs were sterilized and these discs were dipped in bacterial suspension and placed in a sterilized petridish containing different concentration of poisoned nutrient agar medium with the help of sterilized forcep. The petridishes were then incubated at room temperature for 48 hrs for zone inhibition. Growth measurement from the periphery of treated and untreated discs were taken after 48 hours and per cent growth inhibition was calculated following (Vincent, 1947).

$$I = \frac{100\ (\ C - T\ )}{C}$$

Where I = Inhibition of bacteria in per cent

C = Growth of bacteria in mm in control treatment

T = Growth of bacteria in mm in different fungicidal treatment

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CHAPTER - IV

EXPERIMENTAL FINDINGS

CHAPTER - IV

EXPERIMENTAL FINDINGS

4.1 Symptoms :

Naturally affected sunflower plant produced symptoms on leaves characterised by presence of small, round or irregular water soaked spots in the initial stage of the disease development and after wards turn dark brown with yellow haloes. In advance stage infection takes place on veins and veinlets. Interveining tissues turn dark brownish yellow, form lesions of dead tissues of various sizes and shapes. In severe infection such spots coalesce and leaves turn dark brown with yellow margins and suddenly drying of leaf takes place and thus a typical leaf blight phase was observed. The pathogen also infects petiole and lastly defoliation of leaf takes place.

In artificial inoculation test typical symptoms were developed within 5 to 7 days as observed on naturally affected leaves. The development of symptoms on injured leaves was earlier than on uninjured leaves. At first symptoms appeared on leaves as small, round, irregular water soaked spots, later on such spots appear raised and become dark brown in colour and after wards turn dark brown with yellow haloes with age of the plant. In advance stage the

symptoms were also found on veins, veinlets and petiole. In severe infection several such spots coalesce and form dead tissues of various sizes and shapes later on suddenly drying of leaf takes place and thus a leaf blight phase was observed which resulted in defoliation of leaves (Fig. 1).

The naturaly affected seeds were shrivelled, small in size with low volumetric weight such seeds were used for sowing. The symptoms were observed on cotyledons, leaves from from such affected seeds which were identical with naturally developed symptoms on seedlings and thus seed borne nature of sunflower caused by Xanthomonas sp. is proved.

MORPHOLOGY, STAINING REACTIONS, CULTURAL AND GROSS PHYSIOLOGICAL CHARACTERS:

4.2 Morphology :

4.21 Shape :

Isolate found tobe typical rod with rounded ends.

4.22 Size :

1.08 - 1.78 x 0.49 - 0.79.

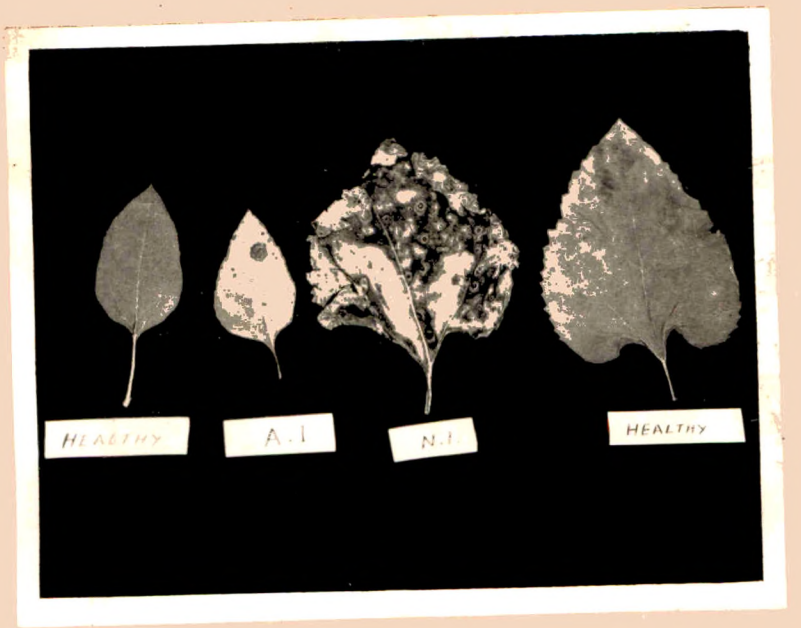
4.23 Cell grouping :

Isolate found to be arranged singly.

4.24 Motility :

Isolate found to be motile with polar flagellum.

FIG: 1



AI = DEVELOPMENT OF LEAF SPOT ON ARTIFICIA INOCULATED LEAF

NI = NATURALLY AFFECTED SUNFLOWER LEAF SHOWING LEAF BLIGHT SYMPTOMS

FIG: 2.

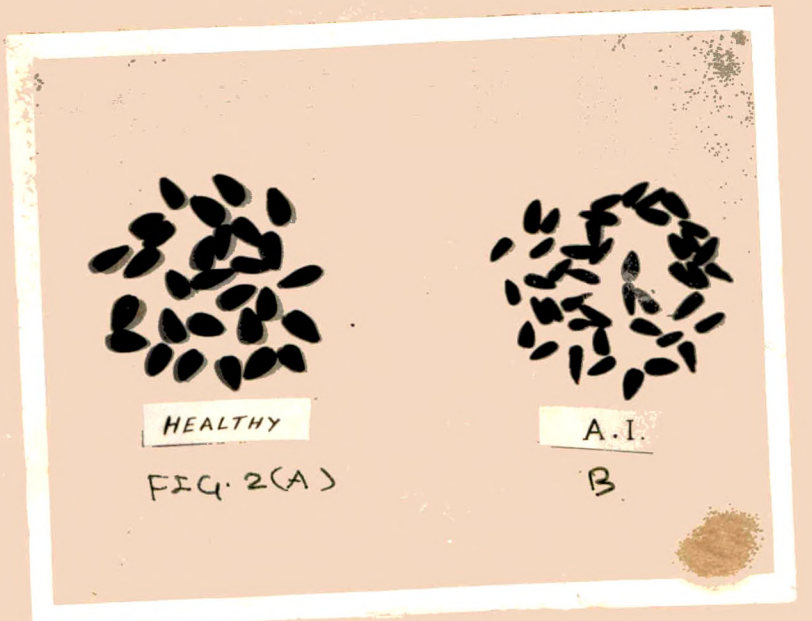


FIG. 2(A) B

Fig2. (A) HEALTHY
(B) AI = ARTIFICIALLY AFFECTED SEED

4.3 Staining reaction :

4.31 Gram stain :

Isolate found to be gram negative.

4.32 Acid fast stain :

Isolate found to be non acid fast.

4.33 Flagella stain :

Single polar flagellum.(Monotrichous).

4.34 Spore former :

Isolate found to be non spore former.

4.35 Capsule stain :

Isolate found to be non capsulated.

4.4 Cultural Characters :

Observations on plate culture, broth culture and streak culture are given in Table 1, 2 and 3 respectively. (Fig. II).

4.5 Gross physiological characters :

4.51 Liquefaction of gelatin :

Isolate found to liquefy gelatin.

4.52 Hydrolysis of starch :

Isolate found to hydrolyse starch.

4.53 Production of Hydrogen sulphide :

Hydrogen sulphide was produced.

4.54 Reduction of Nitrates :

Nitrate was not reduced.

4.55 Production of Ammonia :

Isolate produced Ammonia.

4.56 Action on litmus milk :

Peptonization become evident without coagulation.

4.57 IMVIC reaction :

4.571 Indole production

Isolate failed to develop pink colour on the litmus paper showing thereby isolate did not produce indole.

4.572 Methyl red test

Isolate was able to produce distinct red colour indicating positive reaction.

4.573 Voges proskauer test

Isolate did not give intense red or crimson colour indicating negative reaction.

4.58 Production of carbon compounds :

Observations on the production of gas and acidity was recorded on 4th and 7th days. Results given in Table 4 showed that isolate could utilize following compounds with

production of acid but not gas. Xylose, Lactose, Maltose, Galactose, Mannose, Saccharose, Raffinose, Mannitol, Trehalose, Dextrose, Fructose and Glucose but isolate failed to utilize Rhamnose and Salicin.

4.59 Utilization of nitrogen compounds :

Observation on the growth of the pathogen was recorded after 7 days. Results given in Table 5 indicated that isolate could utilize Ammonium nitrate, Ammonium chloride, Ammonium sulphate, Calcium nitrate, Potassium nitrate, Sodium nitrete, Magnesium nitrate, Glycin and Urea but did not utilize Barium nitrate and Asparagine.

4.510 Relation to free oxygen

Isolate failed to grow under aneerobic condition.

4.511 Relation to H-ion concentration

Observations on the growth of the isolate is recorded after 7 days. The results given in Table 6 indicate that isolate can grow at pH range from 5.2 to 9.0. However, the isolate grew well at pH 7.0 to 7.5, but isolate could not grow below 5.0 and above 9.0 pH. In general the isolate favoured neutral pH rather than acidic nor alkaline for maximum growth.

4,512 Temperature and growth relation

4.5121 Growth in relation to temperature

Observations on growth characters were recorded after

7 days. Results given in Table 7 indicate that the isolate had a maximum growth at 27 to 29°C and there is no growth below 5°C and above 37°C.

4.5122 Thermal death point

The thermal death point of the isolate is 51°C (± 1).

4.6 Development of disease in relation to age of plant :

The observations on the development of the disease are given in Table 8 reveales that isolate produced 60, 50, 40, 35, 25, 15, 10, 5 and 0 per cent disease at the age of 15, 20, 30, 40, 50, 60, 70, 80 and 90 days of plant respectively. The isolate found to be pathogenic to sunflower upto the age of 80 days while intensity of disease was more in younger plants than in older plants.

4.7 Varietal resistance :

Eighteen varieties were screened for resistance against the isolate under study and observations are recorded in Table 9. Result indicate that varieties EC-109802, EC-68414, L-local, X-B, were highly susceptible while SC-17 found to be highly resistant. Varieties Modern, N-50, EC-112141, X-E-36, RS-16, EC-20-2-70, EC-8910 were moderately susceptible and varieties EC-109209, EC-93164, EC-109294, EC-21992, EC-61077, EC-95266 were susceptible.

4.8 Host range studies :

The observations on the host range studies given

in Table 10 show that in artificial inoculation test isolate could infect French bean (Phaseolus vulgaris L.), Lima bean (Phaseolus lunatus L.), Common bean (Dolichos lablab L.), Green gram (Vigna radiata (L.) Wilczek.), Black gram (Vigna mungo (L.) Hopper.),and Sesamum (Sesamum indicum L.) while it could not infect (Arachis hypogae L.) Groundnut, Chilli (Capsicum annum L.), Pigeon pea (Cajanus cajan (L.) Millsp.), Bengal gram (Cicer arietenum L.), Sunhemp (Carotalaria juncea L.), Soybean (Glycine max (L.)Merr.), Cotton (Gossypium hirsutum L.), Rice (Oryza sativa L.), Bajra (Pennisetum typhoideum X. Pers.), Jowar (Sorghum bicolor L.), Wheat (Triticum vulgare L.) and Maize (Zea mays L.).

4.9 Efficacy of different fungicides and antibiotic *in vitro* against isolate under study

It is evident from the Table 11 that 100 per cent inhibition of isolate is observed with all concentrations of Agallol, while in an antibiotic streptocycline 100 per cent growth inhibition of isolate at the concentrations of 200 and 300 ppm is observed. The fungicides Dithane M-45, Fytolan, Hexacap had more inhibition than and Hexathir and B'stem.

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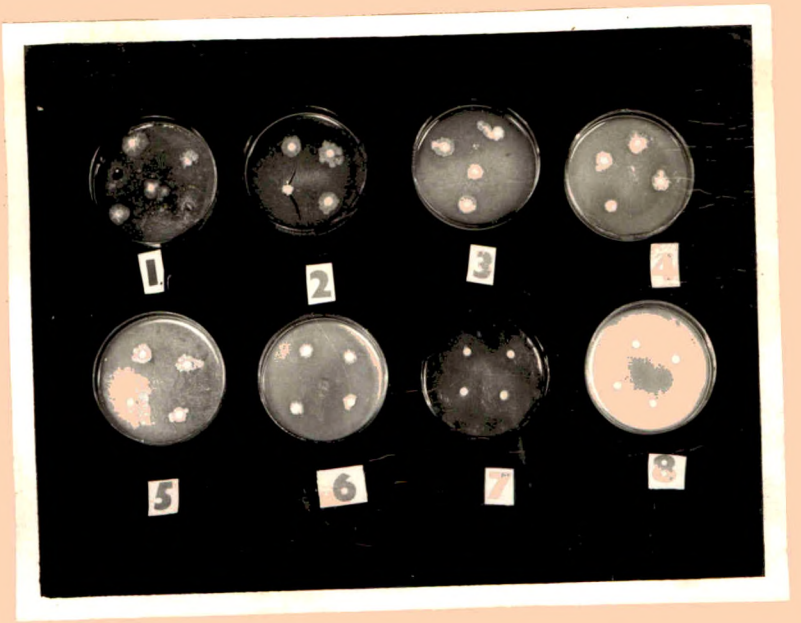
FIG. 3



GROWTH INHIBITION OF *Xanthomonas* sp due to fungicides and antibiotics

1) Control (without fugicide)
2) Hexathir (2000 PPM)
3) B'Stem (2000 PPM)
4) Hexacap (2000 PPM)
5) Dithane M-45 (2000 PPM)
6) Fytolan (2000 PPM)
7) Agallol-6 1000 PPM
8) Streptocycline (Antibiotic)

Table 1 : Growth and cultural characters of pathogen on different media

Nutrient agar	Potato dextrose agar	Beaf peptone agar	Yeast extract mannitol agar	Host extract agar
Colonies on nutrient agar plate were light yellow, then waxy yellow, glistening, raised to unbonate, secondary colonies begin to develop along the margin. Growth is abundant.	Colonies at first light yellow and then yellow, creamy, unbonate, round with entire margin. Secondary colonies begin to develop along the margin. Growth is abundant.	Colonies at first, translucent creamy, light, yellow, then yellow, unbonate round with entire to slightly undulated margin. Secondary colonies begin to develop along the margin. Growth is abundant.	Colonies at first slight yellow, then yellow raised with entire margin. Secondary colonies begin to develop along the margin and through the original colonies. Growth is abundant.	Colonies slight yellow and smooth secondary colonies begin to develop along with margin. Growth is moderate.

Table 2 : Growth and cultural characters of the isolate in different broths

Name of broth	Surface growth	Turbidity	Amount of growth	Type of sediment
Nutrient broth	Flocculent	Light cloudy	Moderate	Viscid on agitation
Beef peptone broth	Slight ring begin to form, flocculent particles and became irregular yellow ring	Dull	Scanty	Vscid
Yeast extract mannitol broth	Pellicle	Light cloudy	Moderate	Slightly viscid

Table 3 : Growth of the isolate on different agar slants

Nutrient agar	Potato dextrose agar	Beaf peptone agar	Yeast extract mannitol agar	Potato plugs	Host extract agar
Filiform, light yellow at first then yellow creamy. Growth is abundant.	Filiform, umbonate, light yellow then creamy yellow. Growth is moderate	Filiform, light yellow to waxy yellow, Growth is moderate	Filiform, creamy yellow, Growth is abundant.	Filiform, potato turn slight yellow and smooth. Growth is moderate glistening and deep creamy in colour	Filiform, light yellow and growth is moderate.

FIG: 4

GROWTH OF Xanthomonas sp ON SLANTS

1 = Nutrient Agar
2 = Potato Dextrose agar
3 = Beef peptone agar
4 = Yeast Extract mannitol agar
5 = potato plugs
6 = Host Extract agar

Table 4 : Utilization of carbon compounds by the isolate

Carbon compounds	Inoculation period (days)	Grade
Monosaccharides		
A) Pentoses		
Xylose	4	3 A
	7	3 A
B) Hexoses		
Glucose	4	3 A
	7	3 A
Galactose	4	2 A
	7	2 A
Mannose	4	2 A
	7	3 A
Fructose	4	2 A
	7	3 A
Dextrose	4	2 A
	7	3 A
Trehalose	4	2 A
	7	2 A

Contd....

Table 4 contd ...

Carbon compounds	Inoculation period (days)	Grade
Oligo saccharides		
A) Disaccharides		
Saccharose	4	2 A
	7	3 A
Lactose	4	2 A
	7	2 A
Maltose	4	3 A
	7	3 A
B) Trisaccharides		
Raffinose	4	A
	7	A
Deoxysugars		
Rhamnose	4	-
	7	-
Sugar-alcohol		
Mannitol	4	2 A
	7	2 A
Glucosides		
Salicin	4	-
	7	-

Note : A = Slight acidic, 2 A = Fair acidic, 3 A = Acidic

Table 5 : Utilization of inorganic and organic nitrogen compounds by the isolate

Sr.No.	Nitrogen compounds	Utilization
	Inorganic	
1	Ammonium nitrate	++
2	Ammonium sulphate	+++
3	Ammonium chloride	+
4	Barium nitrate	-
5	Calcium nitrate	+
6	Magnesium nitrate	+
7	Potassium nitrate	+
8	Sodium nitrate	++
	Organic	
9	Aspargine	-
10	Glycine	+
11	Urea	+

Note : - = No growth, + = Poor growth,
++ = Fair growth, +++ = Good growth

Table 6 : Relation of Hydrogen-ion concentration on growth of the isolate

pH after sterilization	Growth
4.0	-
4.5	-
4.9	-
5.2	+
5.5	++
6.0	++
6.5	+++
7.0	++++
7.5	++++
8.0	+++
8.5	++
9.0	+
9.5	-
10.0	-

Note : - = No growth, + = Poor growth,
++ = Fair growth, +++ = Good growth,
++++ = Excellent growth

Table 7 : Relation of temperature on the growth of the isolate

Temperature in ^{o}C	Growth
0	-
5	+
10	+
15	+
20	+
25	++
27	++++
29	++++
30	+++
35	+
37	+
40	-
45	-
50	-

Note : - = No growth, + = Poor growth,
++ = Fair growth, +++ = Good growth,
++++ = Excellent growth

Table 8 : Effect of age of the plants on the development of disease.

Age of plants (days)	Percentage of infection
15	60
20	50
30	40
40	35
50	25
60	15
70	10
80	5
90	0

Table 9 : Performance of sunflower varieties against isolate under study

Sr.No.	Name of variety	Reaction
1	EC-109802	HS
2	L-local	HS
3	X-B	HS
4	Modern	MS
5	N-50	MS
6	EC-112141	MS
7	X-E-36	MS
8	RS-16	MS
9	EC-8910	MS
10	EC-20-2-70	MS
11	EC-109209	S
12	EC-93164	S
13	EC-109294	S
14	EC-21992	S
15	EC-61077	S
16	EC-95266	S
17	SC-17	HR
18	EC-68414 (control)	HS

Table 10 : Reaction of different hosts against isolate under study

Common name of the host	Botanical name of the host	Reaction
Groundnut	(*Arachis hypogaea* L.)	-
Chilli	(*Capsicum annuum* L.)	-
Pigeon pea	(*Cajanus cajan* (L.) Millsp.)	-
Bengal gram	(*Cicer arietenum* L.)	-
Sunhemp	(*Crotalaria juncea* L.)	-
Common bean	(*Dolichos lablab* L.)	+
Soybean	(*Glycine max* (L.) Merr.)	-
Cotton	(*Gossypium hirsutum* L.)	-
Sunflower	(*Helianthus annuus* L.)	+
Rice	(*Oryza sativa* L.)	-
Limabean	(*Phaseolus lunatus* L.)	+
Frenchbean	(*Phaseolus vulgaris* L.)	+
Mungbean	(*Vigna radiata* (L.) Wilczek.)	+
Urdbean	(*Vigna mungo* (L.) Hopper.)	+
Bajra	(*Pennisetum typhoideum* X. Pers.)	-
Jowar	(*Sorghum bicolor* (L.) Moench.)	-
Sesamum	(*Sesamum indicum* L.)	+
Wheat	(*Triticum vulgare* L.)	-
Maize	(*Zea mays* L.)	-

Table 11 : Efficacy of different fungicides and antibiotic *in vitro* against the isolate

Sr. No.	Name of the fungicide	Chemical name	Percentage of inhibition		
			Concentration in ppm		
			1000	2000	3000
1	Hexacap	75% WP N-((trichloromethyl)thio)-4-cyclohexene-1,2-dicarboximide	60	72	100
2	Hexathir	75% WP bis (dimethyl-thiocarbamoyl disulfide)	52	60	68
3	B'Sten	50% WP 2-(Methoxy-carbonylamino-benzimidazole)	44	52	60
4	Dithane M-45	75% WP manganese ethyl bisdithiocarabamate	65	70	100
5	Fytolan	56-58% copper. Basic cupric chloride	64	68	100
6	Agallol	5% mercury as methyoxyelthyl mercuric chloride	100	100	100
	Name of the antibiotic		Concentration in ppm		
			100	200	300
1	Streptocycline	Mixture of streptomycine and chlorotetra cline hydrochloride	80	100	100
	Control (Without fungicide)	--	00.00	00.00	00.00

CHAPTER - V

D I S C U S S I O N

CHAPTER - V

DISCUSSION

During the year 1980-81, in Kharif season incidence of bacterial leaf spot of sunflower (Helianthus annuus L.) was found on variety EC-68414 at Central Farm, College of Agriculture, Parbhani. The incidence was to the tune of 6 to 8 per cent. The causal organism was successfully isolated on nutrient agar medium and proved pathogenic in artificial inoculation test.

In artificial inoculation test typical symptoms were developed with in 5 to 7 days after inoculation. Development of symptoms on injured leaves was earlier than on uninjured leaves. The symptoms were characterised by the production of small, round, irregular water soaked spots, later on such spots appeared raised and become dark brown in colour and after words turn dark brown with yellow haloes with age of the plant. In advance stage the symptoms were also found on veins, veinlets and petiole, in severe infection several such spots coalosce and form dead tissues of various sizes and shapes, later on suddenly drying of leaf took place, and thus a leaf blight phase was observed which resulted in defoliation of leaves. In present study the disease was found to be seed borne. The affected seeds were shrivelled, small in size with low volumetric weight (Lakara and Parashar, 1980).

Morphological and physiological characters and staining reactions were carried out following Mannual of Microbiological Methods (1957) and it was found that isolate is gram negative, rod shaped measuring 1.08 - 1.78 x 0.49 - 0.79 μ , non capsulated, non spore former, non acid fast, and motile with singal polar flagellum. Isolate liquefies gelatin, hydrolyse starch, hydrogen sulphide and amonia produced, nitrate not reduced, indole not produced, milk become peptonized and methyl red test is positive but voges proskauer test is negative.

In carbon compound study organism found to utilize carbon compounds like xylose, lactose, maltose, saccharose, galactose, mannose, raffinose, mannitol, dextrose, fructose, glucose but did not utilize Rhamnose and salicin. The pathogen utilized nitrogen compounds like ammonium nitrate, ammonium sulphate, ammonium chloride, potassium nitrate, sodium nitrate, calcium nitrate, magnesium nitrate, glycine and urea, but not barium nitrate and asparagine.

Cultural characters study showed that pathogen grew well in nutrient and yeast extract mannitol agar. On nutrient agar the colonies at first appeared light yellow, then, slight yellow and later waxy yellow, glistening raised to unbonate circular with entire margin and growth was abundant.

The pathogen strictly aerobic can be grow between pH range from 5.2 to 9.0. However, the isolate can grow well at pH from 7.0 to 7.5. In general the isolate favoured neutral pH rather than acidic nor alkaline for good growth. The isolate can grow between temp. 5°to 37°C. The good growth was at 27° to 29°C and there was no growth below 5°C and above 37°C. The thermal death point is 51°C. These results are agreement with those reported by Patel and Jindal (1972).

On the basis of cultural, morphological, physiological characters and biochemical reactions the organism under study is identified as Xanthomonas phaseoli (Smith) Dowson. The identification is also tallied with the results given in Burgey's Mannual of Determinative Bacteriology (1974).

Patel and Jindal (1972 a) reported Xanthomonas phaseoli (Smith) Dowson to be a bean common blight pathogen. Patel and Jindal (1972 c) reported mung bean leaf spot disease caused Xanthomonas phaseoli (Smith) Dowson. The results obtained by Patel and Jindal (1972) on the cultural morphological characters and host range studies closely agree with present studies thus identification also in confirmity with Patel and Jindal (1972 a and 1972 c).

In host range studies pathogen could infect Urd bean, Mung bean, Common bean, Lima bean, French bean and Sesamum

besides its own host. Similar results have been obtained by Patel and Jindal (1972 b and 1972 c) and thus the results and host range study are also closely in agreement with Patel and Jindal (1972 a and 1972 c).

In present studies the isolate did not infect Glycine max. Similar results are also reported by Hedges (1924) that Bacterium phaseoli (E.F. Smith) is very weakly pathogenic to soybean plants. Patel and Jindal (1972 a) reported that Xanthomonas phaseoli isolated from Phaseolus vulgaris L. was weakly pathogenic to Dolichos lablab L. but did not infected soybean. Patel and Jindal (1972 c) reported that mung bean pathogen did not infect urd bean. Further they also found that in fields Urd crop growing near severily infected mung bean, Urd crop was free from bacterial leaf spot disease. This indicated that the pathogen had different pathogenic behaviour.

Fang and Chen (1964) studied 18 cultures of Xanthomonas phaseoli from seven leguminous plants and stated that the isolates are similar in cultural physiological and serological reactions but they different pathogenicity which is regarded as a character for determining species. They found that host range was common for all isolates but each species was more virulent on its natural host. The authors considered that culture from

from Phaseolus vulgaris, Phaseolus aureus, Phaseolus calcaratus though specilized in pathogenicity they are regarded the strain on race of Xanthomonas phaseoli. Similarily in present studies the sunflower pathogen produced milder symptoms on bean (Dolichos lablab L.), Lima bean (Phaseolus lunatus L.), Urd bean (Vigna mungo (L.) Hopper.), French bean (Phaseolus vulgaris L.), Mung bean (Vigna radiata L.) and Sesamum (Sesamum indicum L.) but pathogen is having similar cultural, morphological characters as that of Xanthomonas phaseoli (Smith) Dowson and thus the pathogen under study is considered to be a new strain of the Xanthomonas phaseoli producing leaf spot disease of sunflower (Helianthus annuus L.).

The results of experiment on development of disease relation to age of the plant showed that bacterial leaf spot disease of sunflower could develop upto the age of 80 days of the plant. However, the disease intensity was morein younger plants than in older plants, similar results were reported by Patel and Walkar (1963).

The results on the varietal resistance experiment showed that varieties EC-109802, latur local, X-B, EC-68414 were highly susceptible, while variety SC-17 is found tobe highly resistant, varieties Modern, N-50, EC-112141, X-E-36, RS-16, EC-8910 were moderately susceptible and varieties EC-109209, EC-109294, EC-61077, EC-95266 were susceptible.

Efficacy of different fungicides and antibiotics *in vitro* was evaluated and it is found that Agallol inhibit 100 per cent growth of all isolate with all concentrations, while an antibiotic streptocycline inhibit 100 per cent growth of isolate at the concentration of 200 and 300 ppm. The fungicides like Dithane - M-45, Fytolan, Hexacap had more inhibition than Hexathir and B'Sten. Similar results were obtained by Sood *et al.* (1974) and Kore and Khandale (1979).

A systematic work on bacterial leaf spot of sunflower (*Helianthus annuus* L.) caused by new strain of *Xanthomonas phaseoli* was carried out for the first time in Maharashtra.

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CHAPTER - VI

S U M M A R Y

CHAPTER - VI

SUMMARY

A incidence of bacterial leaf spot disease of sunflower (Helianthus annuus L.) was reported for the first time at Central Farm, College of Agriculture, Parbhani, Maharashtra State. The incidence of disease was to the tune of 6-8 per cent during kharif, 1981. The causal agent (Xanthomonas phaseoli (Smith) Dowson) was isolated successfully on nutrient agar medium from naturally affected leaves of sunflower variety EC-68414 and proved pathogenic in artificially on sunflower variety EC-68414. Typical symptoms were developed in artificial inoculation test with in 5 to 7 days after inoculation on leaves. The symptoms were characterised by the production of small, round, irregular water soaked spots lateron suchspots appeared raised and become dark brown in colour and after wards turned dark brown with yellow haloes with age of the plant. In advance stage the symptoms were also found veins, veinlets, and petiole. In severe infection several such spots coalesced which resulted in defoliation of leaves. In present study the disease was found to be seed borne. The affected seeds were shrivelled small in size with low volumetric weight.

The temperature studies inrelation to growth of isolate revealed maximum growth at 27° to 29°C. There was no growth

below 5°C and above 37°C and growth was completely arrested at 40°C. The growth of the isolate was maximum at 7.0 to 7.5 pH while there was no growth below 5 and above 9.0 pH. In general isolate preffered neutral medium for growth. The thermal death point of pathogen was (51°C ± 1).

In host range studies pathogen infected French bean (Phaseolus vulgaris L.), Common bean (Dolichos lablab L.), Lima bean (Phaseolus lunatus L.), Mung bean (Vigna radiata (L.) Wilczeck), Urd bean (Vigna mungo (L.) Hopper.) and Sesamum (Sesamum indicum L.) besides its own host, but infection was not severe. On the basis of cultural, morphological, physiological characters and host range reactions the pathogen under study was identified as Xanthomonas phaseoli (Smith) Dowson, sunflower strain.

A experiment on relation between age of the plant and disease development showed that Xanthomonas phaseoli (Smith) Dowson was pathogenic to sunflower upto the age of 80 days only and younger plants were more susceptible than older plants.

In varietal resistance experiment, the variety SC-17 was found to be highly resistant while varieties EC-109802, Latur local, X-B, EC-68414, were highly susceptible. Six varieties modern, N-50, EC-112141, XE-36, RS-16, EC-8910 were moderately susceptible and varieties EC-109209, EC-109294, EC-61077, EC-95266 were found susceptible.

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Efficacy of different fungicides and antibiotics *in vitro* was seen against the pathogen under study and it was observed that there was 100 per cent inhibition of the pathogen in Agallol with all concentrations, while an antibiotic streptocycline inhibit 100 per cent of growth of isolate at the concentration of 200 and 300 ppm. The fungicides like Dithane M-45, Fytolan, Hexacap had more inhibition than Hexathir and B'Sten. A

A systematic work on bacterial leaf spot of sunflower (*Helianthus annuus* L.) caused by new strain of the *Xanthomonas phaseoli* was carried out for the first time in Maharashtra.

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*Originals not seen.

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