

**STUDIES ON RACES, ECOLOGY, DETECTION AND CONTROL
OF *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye
CAUSING BACTERIAL SPOT OF TOMATO
(*Lycopersicon esculentum* Mill.)**



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DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE

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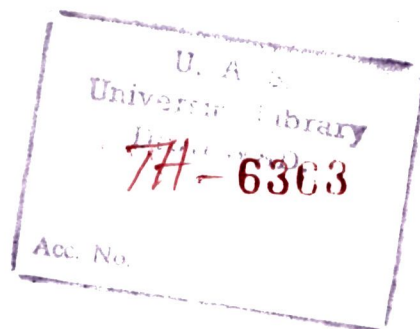
M. R. RAVI KUMAR



Thesis submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfilment of the requirements
for the award of the Degree of
Doctor of Philosophy
in
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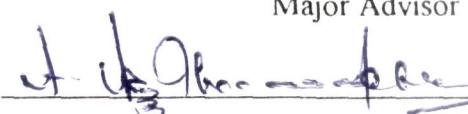
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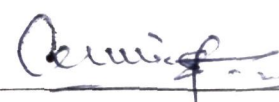
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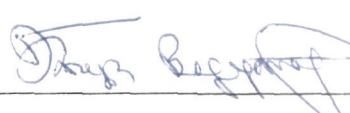
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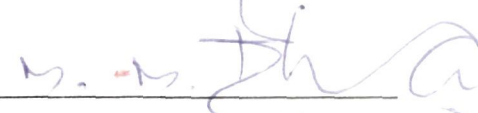
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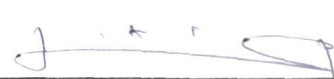
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INTRODUCTION

I INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the important vegetables grown through out the year in India. The fruits are very rich source of vitamins and minerals and has a high nutritive value. The fruits are often used for table purpose as a salad for making soups, ketchup and also in canning industry.

The total acerage under tomato cultivation in India is 3,10 lakh ha. with an annual production 46 lakhs tonnes, (FAO 1993-94). In Karnataka tomato is widely grown in almost all parts and occupies an area of 32.07 thousand ha with an annual production of 8.02 lakh tonnes (Anon., 1993-94).

Tomato crop is affected by large number of diseases incited by fungi, bacteria, viruses, phytoplasma, nematodes etc. Among the bacterial diseases bacterial wilt of tomato is one of the most wide spread and serious disease. However, in recent years bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* is assuming a very serious proportion particularly in the monsoon and summer crops, affecting fresh market tomatoes in Karnataka. The disease not only affects the yield but the quality of the fruits (Culled fruits) in severely affected plants. Reported losses in USA under heavy disease severity have been estimated to be as high as 52 per cent as measured by weight loss of marketable fruits (Pohronezny and Volin, 1983). While in pepper 16.6 per cent loss was reported by Shekhawat and Chakravarti (1976) from India.

Three groups of *X. c.* pv. *vesicatoria* have been distinguished on the basis of virulence on tomato and pepper. Further in each group, several races have been reported depending on the virulence of the strains of *X. c.* pv. *vesicatoria* on near isogenic lines of pepper, in pepper group and on the basis of infectivity to tomato genotypes in case of tomato group. In order to control the bacterial spot of tomato through resistant breeding, it is very essential to know the presence and distribution of races of tomato in different agroclimatic zones of the state. Information on this aspect of the pathogen is totally lacking in India. Control of bacterial spot with copper

and/or streptomycin sprays or resistant plants has been reported to be erratic because of variation in population of the bacterium in their resistance to the bacteriocides and in races that do not interact with resistance genes in the hosts. Genes for resistance to copper and streptomycin are reported to be plasmid-encoded in *X. c. pv. vesicatoria*. Survey of plasmid contents in strains has not been reported, even though this ecologically important traits in bacteria are plasmid encoded. Hence a strong need for a comprehensive study of plasmid profile in strains of *X. c. pv. vesicatoria* obtained from different agroclimatic zones was felt in order to know if a single plasmid or a plasmid profile was common in strains of *X. c. pv. vesicatoria* in Karnataka.

Bacterial spot of tomato is becoming a bottle neck to tomato production in Karnataka especially under the environmental condition existing in the state and under such favourable condition it is rather difficult to control the disease. Hence in order to get a good crop of tomato, effective management strategies for the disease, have to be developed. Therefore, it is very essential to have an understanding of the life cycle of the pathogen so as to exclude the pathogen or else a vulnerable link in the life cycle of a parasitic organism in which its population density is at its lowest ebb should be exploited. However the best control measures which reduce the disease losses are through exclusion of the pathogen; to achieve this it is necessary to determine the source of inoculum. Often bacteria are seed borne, previous workers have reported the association of *X. c. pv. vesicatoria* in both tomato / pepper seeds (Gardner and Kendrick, 1921). The survival of plant pathogenic bacteria in seed, has a significant economic importance both for seed industry and growers, thus it is essential to understand the survival of the bacterium on seeds, which would serve as principal source of inoculum.

Crop residue provides a means by which phytopathogenic bacteria over winter and over summer, thus this link in the survival or life cycle of the pathogen needs close examination under the sub-tropical climatic conditions.

Weeds, either as host or non-host and other non host crop plants have been shown to serve as reservoirs of bacterial pathogen which may help the bacteria to lead a resident life, at

the same time supply the inoculum to the nearby susceptible crop. Further the role of insect in the transmission of the pathogen especially in causing infection on fruit, is not known, hence its role in the epidemiology of the diseases needs to be ascertained.

Quick detection of *X. c. pv. vesicatoria* in the seeds is important in preventing dissemination of the bacteria. The conventional isolation, cultural and inoculation techniques used in identifying bacteria is time consuming apart from the other inherent defects in such diagnostics procedures. In recent years several sensitive serological techniques have been developed in the detection of plant pathogenic bacteria. It is essential to apply / standardize a serological technique which is effective, sensitive and economical in the detection of the pathogen in seeds.

Ever since the disease was reported by Doidge (1980) from South Africa, many workers have tried different chemicals for the control of bacterial spot of tomato, and there are conflicting reports about the success of the chemicals in controlling the disease. A spray schedule to control the disease using bactericides / antibiotics needs to be developed. Therefore, the present investigation were envisaged with the following objectives.

1. To study the prevalence of races in Karnataka.
2. To study the survival of the *X. c. pv. vesicatoria* in nature.
3. To study the environmental factors favouring the development of the disease.
4. To develop a rapid, simple and sensitive method of detection of *X.c. pv. vesicatoria* in seeds.
5. To develop a spray schedule using bactericides /antibiotics to control the disease.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Bacterial spot of tomato (*Lycopersicon esculentum* Mill.) incited by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye. Syn. *Xanthomonas vesicatoria* (Ex. Doidge) Vauterin, Hoste, Kersters and Swings, (1995) is one of the most serious diseases of tomato plants affecting yield and quality of fresh market tomatoes in the temperate and Sub- tropical climatic conditions of the world.

The literature pertaining to various aspects of the disease have been collected and summarised and presented in this chapter.

RACES AND STRAINS OF *Xanthomonas campestris* pv. *vesicatoria*

Xanthomonas campestris pv. *vesicatoria* (XCV) causes economically important losses on its hosts in warm and humid climatic conditions. Several strains / races have been recorded on the basis of the pathogenecity either on tomato, pepper or both.

Cook and Stall (1982) isolated *X. c.* pv. *vesicatoria* from naturally infected pepper and tomato obtained from different countries, and categorized all the cultures pathogenic on pepper as pepper strain race-1 (except one strain of race-2 from Guadeloupe). Further they stated that race-2 is widely present on pepper in Florida state in USA in U.S.A.

Reifshneider *et al.* (1985) reported three group of *X. c.* pv. *vesicatoria*. The tomato group was virulent only on tomato, pepper group was virulent only on pepper and both pepper and tomato were susceptible to the pepper - tomato group.

Maringoni and Kimati (1987) inoculated capsicum and tomato by leaf spray and leaf injection with 12 isolates of the bacterium, six from each host. They distinguished capsicum isolates from the tomato isolates on the basis of reaction on the plants producing the symptoms. Further they found ... the isolates were more virulent on their

original host. All the tomato isolates hydrolysed starch while those from capsicum did not.

Hartman *et al.* (1990) recorded races 1,2 and 3 of *X. c. pv vesicatoria* in Taiwan at rates of 71 per cent and 20 per cent and 82 per cent from capsicum and tomato respectively. The 3 races occurred at least once in 5 out of 8 of countries.

Hseu *et al.* (1990) collected isolates of *X. c. pv vesicatoria* from pepper and tomato from different parts of Taiwan, and on the basis of the reaction of the isolates on the differential hosts, they distinguished the isolates in 3 races and further they stated that 21 isolates belonged to race-1, 6 to race 2 and 31 isolates to race-3. However, no correlation was established between the 3 serovars and the 3 races.

Minsavage *et al.* (1990) identified 3 groups with in *X. c. pv vesicatoria* and several races of this pathogen using tomato and pepper host differentials. The pepper host cv. Early calwonder (ECW) and three near-isogenic lines derived from ECW contained the resistance genes BSI (ECW-10R), BS2 (ECW-20R) and BS3 (ECW-30R). Further they added that strains of tomato group (XCVT) are pathogenic only on tomato, those in the pepper group (XCVP) only on pepper and members of the pepper - tomato group (XCVPT) on both pepper and tomato. They distinguished the strain in XCVP and XCVPT groups further into races based on the reaction on the near-isogenic lines; viz. ECW 10R, ECW-20R, ECW-30R. Resistance to bacterial spot of pepper is conferred by single dominant gene-BS1, BS2 and BS3. Genes BS3 and BS1 offer resistance to race-1 and 2 respectively and BS2 gene restrict race 1-3.

Ritchie and Dittapongpitch (1991) found that, out of 65 strains pathogenic to pepper, cv. California wonder, 55, 31 and 12 per cent were race 1, 2 and 3 respectively. They isolated a strain that was pathogenic on Early calwonder (XCV-19), but elicited a hypersensitive response in the three nearisogenic lines of Early calwonder. This strain was designated as race-O.

Ward and O'Garro (1992) working on bacterial spot of pepper and tomato in Barbados found race 1 to 3 in commercial pepper and tomato fields. The race-1 strains were two to nine times more abundant than race-2 and 3. Strain isolated from seed were commonly race-1 or non-pathogenic and some tomato strains were virulent on tomato cultivar Hawaii 7998.

Ramesh chand *et al.* (1993) studied the distribution of 74 isolates of XCVPT pathogen group and races, on tomato and pepper. They collected totally 74 XCV from 63 fields of pepper and tomato and categorised these isolates into two pathogenic groups; 3.4 per cent belonged to tomato group (XCVT) and 96.6 per cent to pepper-tomato group (XCVPT). Among the XCVPT group they found that all the isolates belonged to Race-3.

Five races (PO-P4) of *X. c. pv. vesicatoria* causing bacterial spot on pepper were reported by Bouzar *et al.* (1994). They stated that three single dominant genes in pepper conferred resistance to these races. Gene BS-1 provide resistance to race P₀, P₂ and gene BS-2 to race P₀, P₂ and gene BS-3 to race P₀, P₁ and P₄.

O'Garro and Tudor (1994) reported the use of lesion size to assess virulence of races of XCV causing the bacterial spot of tomato and pepper fruits in the field. XCVP (pepper group) race-1 was seven times more prevalent than XCVT (tomato group) race-1 and 4.5 times more common than XCVPT (pepper-tomato group), race-2 strains of XCVP and XCVT race-1 strains were generally more virulent than XCVT race-3 strains. Further they noted that strains from tomato were virulent on pepper carrying BS2 resistant gene to bacterial spot, but no pepper strain was detected on tomato plants.

Jones *et al.* (1995) reported a third tomato race - T3 from Florida in USA. The strains of T3 race produced a compatible reaction on tomato genotype Hawaii 7998 and were amylolytic and pectolytic. However these strains produced a rapid hypersensitive reaction when infiltrated into tomato genotype. Hawaii 7981, P₁ 126932 and P₁ 128216 whereas strains of T2 produced a compatible reaction on these genotypes. When they

inoculated a low level of bacterial cells into the mesophyll of leaflets of P₁ 126932, PI 128216, Hawaii 7998 and cv. walter (compatible with all tomato strains), the internal population of the amylolytic and pectolytic strains were reduced in the first two genotypes only but not in the latter two genotypes. While the population of T1 strain was found to be reduced only in Hawaii 7998. Thus they designated these new strains from Florida as tomato race-3 (T3). When the T3 race strains were compared with T1 and T2 strains for fatty acid analysis and carbon substrate utilization patterns, the T3 strains clustered differently from the representative T1 and T2 strains.

Kousik and Ritchie (1995) reported two new races viz., race 4 and 5 belonging to pepper group. They isolated some strains of *X. c. pv. vesicatoria*, virulent on pepper near-isogenic line ECW-20 R containing resistant gene BS2, from tomato field in Barbados. Previously no field strains of XCV were reported to overcome the BS2 gene. Further they found that several XCV strains isolated from pepper fields elicited a hypersensitive reaction (HR) on ECW-30R (containing resistance gene BS3) but not on ECW-20R or ECW-10R (containing resistance gene BS-1), thus these strains were designated as P₄. Later in mid January 1995, they isolated strains of XCV that elicited a HR in ECW-10 R and a compatible reaction in ECW-20 R and ECW-30R. On the basis of current system of race designation as enunciated by Bouzar *et al.* 1994, the strains were designated as race P5.

Sahin and Miller (1995) reported another new race of pepper viz., race-6 of *X. c. pv. vesicatoria* from Ohio in U.S.A. Bacterial strains were identified based on reaction on pepper tomato differential lines, ability to degrade starch, and pectin, composition of fatty acid, reaction to a panel of monoclonal antibodies and sensitivity to copper and streptomycin. Of the 76 strains isolated during that period and identified as *X. c. pv. vesicatoria*, one strain isolated from pepper in Huron county, did not cause HR reaction on any of the three pepper race differential lines. This new strain of *X. c. pv. vesicatoria* was designated as pepper race-p-6.

AMYLOLYTIC AND PECTOLYTIC ACTIVITY OF *Xanthomonas campestris* pv. *vesicatoria*

Amylolytic activity has been described as a variable characteristics of *X. c. pv. vesicatoria* according to Dye (1964). In the original description of the causal agent in Pretoria, South Africa, Doidge (1921) indicated that the pathogen, *Bacterium vesicatorium* was able to hydrolyze 0.1 per cent starch in broth. However, at about the same time, Gardner and Kendricks (1921) reported that the bacterial spot on tomato in Indiana caused by *B. exitiosum* produced a halo in starch containing medium in just 5 days. Later Gardner and Kendricks (1923) compared representative strains of the two pathogen and concluded that the pathogen was identical and by priority role retained *B. vesicatorium*, however, they failed to report amylolytic activity, a key characteristic to differentiate strains within this taxon. (Bouzar *et al.* 1994 and Stall *et al.* 1994). Burkholder and Li (1941) reported that a strain isolated from tomato were strongly amylolytic and those from pepper were not. Another non-amylolytic bacterial spot pathogen of tomato was described by Sutic (1957) as *pseudomonas gardneri*, this pathogen has since been proposed as a pathovar of *X. campestris* and even as a synonym of *X. c. pv. vesicatoria* (Dye 1966).

Marigoni and Kimati (1987) characterized strains of *X. c. pv. vesicatoria* infecting tomato and capsicum and stated the strains infecting tomato and capsicum could be differentiated on the basis of symptoms and reaction on the host plants. The tomato strains hydrolysed starch while those from capsicum did not. In the same year, Gitatis *et al.* (1987) studied the pectolytic and amylolytic activity of *Xanthomonas* infecting tomato and pepper transplants and stated that *X. c. pv. vesicatoria* displayed strong starch hydrolysis and pectolytic activity in crystal violet pectate medium. Wang *et al.* (1990) isolated one strain from Brazil which was found to be both amylolytic and pectolytic in nature, which was designated as race-1, on the basis of its ability to cause disease in resistance tomato breeding line Hawaii 7998. Canteros (1990) reported that out of 522 strain of *X. c. pv. vesicatoria* tested, 104 were pectolytic and amylolytic, most of these were from Argentina and only one out of 331 strains from USA were

pectolytic and amylolytic. Two groups viz., non amylolytic and pectolytic and amylolytic and pectolytic groups have been recognised among the strains of *X. c. pv. vesicatoria* infecting tomato and pepper Beaulieu *et al.* 1991 and Jones *et al.* 1993). Stall *et al.* (1994) also identified two genetically diverse groups of strains among cultures of *X. c. pv. vesicatoria* isolated from pepper and tomato. Group "A" strain did not pit pectate gels or hydrolysed starch whereas group "B" strain were strongly positive for both reaction, further they observed that group "A" strain caused hypersensitive reaction in tomato breeding line Hawaii 7998, while group 'B' did not. These two groups of strains have different genetic back ground, but caused essentially the same disease on tomato and pepper. Bouzar *et al.* (1994a) reported that race (T1) strains from tomato were unable to hydrolyze starch (Amy-) and very few degraded pectate. (Pec-). In contrast, most race-2 strains were positive for amylase and pectate. Later Bouzar *et al.* (1994b) catagorized the worldwide collection of strains of *X. c. pv. vesicatoria* into A and B group on the basis of amylolytic activity and the presence of a and b protein bands. Further, they determined the race affiliation of the *X. c. pv. vesicatoria* A and B strains with tomato and pepper differential genotypes. On the basis of the reaction on tomato lines they distinguished the two groups as separate races, as 'A' strains belonging to tomato race-1 (T1) and B strain race-2 (T2). Further they stated that except 14T2P3 strains, pepper race strains were affiliated with the "A" group.

Jones *et al.* (1995) reported a new race-3 (T3) of tomato, the strains of which were both amylolytic and pectolytic and produced a compatable reaction on tomato genotype H7998 and HR on H7981 and P₁ 126932 and P₁ 28216. Sahin and Miller (1996) reported that of the 76 strains which were virulent on pepper and most of these (89%) caused infection in tomato also, 52 strains were non amylolytic and non pectolytic and the remaining 24 strains including 2 pectolytic and 22 pec- strains were amylolytic.

Occurrence of plasmids in *X. c. pv. vesicatoria*

Virtually all economically important plant pathogenic bacteria have been examined for plasmids, most of the reports, describe the presence of covalently closed

circular / plasmid DNA, and the vast majority of the plasmids in these bacterial species are still genetically cryptic, with no known function (Coplin, 1983).

The association between copper resistance and race-2 of the pepper strain of *X. c. pv. vesicatoria* was noted by Manco and Stall, (1983). Later, in 1986 Stall *et al.* were able to relate the resistance to copper in strain of *X. c. pv. vesicatoria* to a self transmissible plasmid. They obtained a transconjugate selected for copper resistance only from mating with strains avirulent on pepper breeding line ECW-10R. The loci for copper resistance and avirulence on pepper plant was linked and both were transferred with self transmissible plasmid.

Hender *et al.* (1988) recorded a 190 kb plasmid in Oklahoma strains, of *X. c. pv. vesicatoria* conferring copper resistance. The plasmid was designated as P X V 10A, and this could be efficiently transferred into cu^s strains of pathovars of *Xanthomonas campestris* viz., *vesicatoria*, *dieffenbachia manilota*, *vitians*, *nigromaculans*, *phaseoli*, *campestris*, and *pelargonii*. The study revealed that there is potential threat to disease control with copper spray, especially in nurseries where multiple pvs. of *X. campestris* may occur.

Ulaganthan and Mahadevan (1988) isolated a plasmid without chromosomal contamination from *X. c. pv. vesicatoria* by a modified method. The plasmid was found to be of 95 megadaltons.

Minsavage *et al.* (1990) cultured 548 strains of *X. c. pv. vesicatoria* from pepper and tomato on medium containing 100 µg / ml of streptomycin sulphate and the result indicated that only 55 per cent of the strains could grow on the streptomycin supplemented medium. They found direct correlation between resistance or sensitivity to streptomycin sulphate and the presence or absence respectively of a unique plasmid responsible for resistance. The streptomycin resistance was found to be on a plasmid of 68 kb.

Dittapongpitch and Ritchie (1993) recorded four sizes of miniplasmids from *X. c. pv. vesicatoria*. The estimated size of mini plasmids was 2.5, 2.28, 1.8 and 1.7 kbp. There were DNA homology between large mini plasmids that is 2.5 and 2.28 kbp and between the two small mini plasmids of 1.8 and 1.7 kbp but not between two size groups. They noticed only one size of miniplasmid with in bacterial strain.

Canteros *et al.* (1995) determined the plasmid profile in 522 strains of *X. c. pv. vesicatoria* and found that the number and size were diverse. They classified plasmids into 13 classes based on size ranging from 3-300 kbp. Ten strains had no detectable plasmid. The mobility of plasmid in field conditions using a donor and recipient was studied and found that diverse plasmid content in strains is the result of frequent transfer of plasmids between strains.

Plasmids in other phytopathogenic bacteria

Choi *et al.* (1989) isolated indigenous plasmid DNA which was reproducible in five of 122 Korean strains of *X. c. pv. oryzae*. The plasmid extracted were unique and the three candidate strains viz, 8604005, 8604136 and 8604143 had plasmid which were of 8.2 kbp, 7.5 kbp and 7.0 kbp respectively. The restriction fragment length polymorphism (RFLP) of the plasmid from strain 8604005 was distinctly different from plasmid from strains of 8604136 and 8604143. When digested with *Sau 3A*, plasmid reisolated had same molecular weight to that of parental strains. They found no correlation between the presence of indigenous plasmid and phenotypic characters such as virulence, antibiotic resistance, nutritional requirement, colony colour and mucosity.

Amuthan *et al.* (1992) collected 41 isolates of *X. campestris* belonging to 15 pathovars from different geographical regions in India and screened for plasmids, 25 contained 1-4 plasmid but in most isolates a single plasmid was detected.

Ezavin *et al.* (1992) detected a plasmid by alkaline extraction in *X. c. pv. vasculorum*. The plasmid DNA was detected using agarose gel electrophoresis and ultraviolet light.

Sathyanarayana and Verma, (1993) studied the plasmid profile of highly virulent race 32 of *X. c. pv. malvacearum* which could overcome multigene resistance of cotton blight in plants. They identified five plasmids of length viz., 60, 40, 10, 5.5 and 2.2 kbp. Curing plasmids with Mitomycin-C revealed loss of virulence in strains.

Amuthan and Mahadevan, (1994) screened 17 isolates of *X. c. pv. oryzae* from different geographical regions for the presence of plasmids. Out of 17 isolates 14 harboured plasmids, of which two isolates (X_oP_3 and X_oC_{26}) had two plasmids each, isolates (X_oR_{20}) had 3 plasmids. The remaining isolate had single plasmid of identical mobility. The RFLP pattern of each plasmid was distinct.

Sharma *et al.* (1994) obtained five isolates of *X. c. pv. glycine* from soybeans and only two of the five isolates consistently induced chlorotic lesions in susceptible plants. These two isolates contained 2 cryptic plasmids of 1.5 and 25 kb, where as no plasmids was found in non pathogenic isolates. SDS page profiles of soluble proteins showed distinct molecular weight bands between pathogenic and non-pathogenic isolates which could be of diagnostic value.

ECOLOGY

Long term survival of phytopathogenic bacteria on various sources such as seeds; crop residue, soil, and the epiphytic survival on weeds, host and non host plants has significant economic importance for both seed production companies and growers.

SURVIVAL OF *Xanthomonas compestris* pv. *vesicatoria*

Survival on seeds.

Often, bacteria are seed borne, survival of pathogenic bacteria with seed and propagative parts is wide spread and the source of serious epidemics of diseases of annual crop.

According to Gardner and Kendrick (1921) *X. vesicatoria* survived for 16 1/2 months on dry seeds, while, Bashan *et al.* (1982) observed that *X. c. pv. vesicatoria* survived on infected dried seeds for 10 years.

Survival on crop residue

Crop residue provides a means by which phytopathogenic bacteria over winter and over summers.

Takimoto (1939) found that *Bacterium vesicatorium* over wintered on diseased plant refuse. Peterson (1963) was able to detect *X. vesicatoria* in dead tomato stalks and also in the rhizospheres of dead tomato plants.

Jones *et al.* (1986) reported the survival of *X. c. pv. vesicatoria* for three months and six week on crop residues during May 1982 and 1983 respectively and the pathogen survived during the summer of 1982 and 1983, beyond 3 and 6 weeks respectively.

Survival in Soil

There is much evidence to show that plant pathogenic bacteria survive from season to season in soil. Peterson (1963) reported that the *X. vesicatoria* was eliminated within two weeks in laboratory soil held 25°C and he designated the bacterium as exochthonous since the bacterium lived for a short period in soil. Shekhawat and Chakravarti (1978) reported that *X. c. pv. vesicatoria* survived for 105 days in autoclaved soil with 75 per cent moisture at 8°C, but for only 15 days in unautoclaved soil held at similar conditions. Survival was reduced at 30-35°C.

Survival of other plant pathogenic bacteria

Survival on crop residue :

According to Israliski and Artemieva (1939) *Pseudomonas mori* survived in diseased mulberry leaves upto six days. Preston (1949) reported that *X. vignicola* remained viable in the plant debris for two years. While, Patel and Diwan (1950) found that bacterium survived in leaf debris upto 154 days at 13°C and upto 100 days at 0°C.

Kuibomila and Nakayama (1975) investigated the seasonal differences in total number of bacteria (*P. mori*) in dead leaves in the mulberry field under severe conditions, and observed that, the bacterium which had overwintered in the dead leaves in the mulberry field survived until the middle of May when the mulberry sprouted in Matsumato area. The bacterium occurred in great number before winter season and decreased gradually with the progress of time, even though the survival was calculated in the order of 10^2 - 10^3 per 10 mg of dry powder obtained from the dead leaves from the beginning of April through the end of May. The duration of existence of the bacterium was longer under the low temperature and dry conditions, while it was shorter under the high temperature and wet ones under laboratory conditions. The persistence of the bacterium in the dead leaves in the mulberry field was affected by the climatic conditions, such as temperature and moisture, during the winter to spring seasons. The young shoots of mulberry were affected with the bacterium, which passed through the winter season. This fact suggested that the overwintered bacterium was the source of the primary infection of the disease in the spring.

Sato and Takahashi (1975) studied the duration of survival of *P. mori* and its phage in the mulberry tissue and soil, and opined that *P. mori* in mulberry leaves and shoots which were packed in a paper bag was alive for more than 738 days kept in the refrigerator (5°C). The duration of survival of *P. mori* was shorter under room temperature condition than that in a refrigerator.

Shekhariah (1981) reported that *X. c. pv. vignicola* could survive for a period of two months in leaf debris mixed in soil under natural conditions.

Khan (1996) reported the role of seeds, crop residue and infested soil, in the survival of *X. c. pv. vignicola* causing bacterial blight of cowpea. The studies revealed that seeds play a very important role as a source of inoculum. The pathogen did not survive beyond two weeks in crop residue placed on the surface of soil or buried at a depth of 8 cm. The disease incidence which was as high as 73 per cent on zero day sown crop was reduced to 30 per cent on the seventh day sown crop. The pathogen could survive up to 3 weeks after the incorporation of infected crop residue in soil under field conditions, and the disease was not observed in crop sown after 3 weeks of incorporation of crop residues.

Srinivasachary and Khan (1996) found that the pathovar of *X. campestris* infecting mulberry survived beyond 11th week in the infected crop residue placed on the surface of the soil or uniformly mixed with the soil. The bacterium failed to survive beyond fourth and ninth weeks in the infected crop residue placed at a depth of 15 and 6 cm depth respectively.

Survival in soil

Sato and Takahashi (1972) reported that *P. mori* was isolated from diseased shoots but not from the soil suspension of diseased areas. Later in 1973, they found that the duration of survival of *P. mori* in sterilized soil was influenced by the size of soil particles, that is, the bacteria in a soil consisting of particles smaller than 3 mm in dia. survived for a longer period than on the particles smaller than 0.50 mm. Further, they stated that the number of bacteria decreased more rapidly in the non-sterilized soil than in the sterilized soil and this tendency was remarkable at 20°C or 30°C. The duration of survival in non-sterilized soil at 5°C was different from the bacterial concentrations added into the soil, indicating that it was 20 days when the bacterial suspension of about 10^8 cfu/ml was added, while it was 53 days when the bacterial suspension of about

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10^{10} cfu/ml was added. When the bacterial suspension was added into each supernatant of both homogenates, mulberry leaves and mulberry roots, the bacteria survived for 100 days in the later.

Epiphytic survival of *X. c. pv. vesicatoria*

Jones *et al.* (1986) isolated *X. c. pv. vesicatoria* from six weeds species viz; *Solanum americanum*, *Physalis pubescens*, *Ambrosia artemisifolia*, *Eclipta alba*, *Trifolium repens* and *Eupatorium capillifolium*. and populations of bacterium isolated from the weeds was very low.

Trujillo (1989) reported that *X. c. pv. phaseoli* grew epiphytically during the rainy period in Araguastate, Venezuela on the leaves of several non-host crop and weed species. Viable populations were recorded upto 30 days after bacteria were placed on the leaves of *Amaranthus sp.*, *Physalis sp.*, *Portulaca oleracea*, *Acalypha olopecuroides*, *Chamaesyce hypericifolia*, *Sida rhombifolia*, *Sorghum bicolor*, *S. halepense*, *Rucilia tuberosa* and *Cyperus rotundus*, while viable populations were recovered upto 20 days from Cassava and *Flaveria triniervia*.

Bogatzevska and Deneva (1996) reported the epiphytic survival of *X. c. pv. vesicatoria* on *Amaranthus retroflexus*, *Datura stramonium*, *Solanum nigrum* and *Portulaca oleraceae*.

Khan (1996) isolated *X. c. pv. vignicola* from five weeds i.e. *Parthenium hysterophorus*, *Tylophora indica*, *Tephrosia purpurea*, *Lucas aspera* and *Legascea mollis*. Further he reported the epiphytic population of 10^3 cfu/ml on *Parthenium hysterophorus* and *Tephrosia purpurea*.

Transmission of plant pathogenic bacteria through insect

Plenty of literature has accumulated in the recent year regarding the role of insect in the transmission of bacterial pathogens. The literature point out that insects also

assist in bacterial survival, dissemination and penetration into the hosts. Dissemination apparently ranges from intimate association to accidental contamination. Intimate association would include transmission of bacteria restricted to a small group of insects.

Harrison *et al.* (1977) reported that *Erwinia carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* were isolated from some dipterous insects and suggested that contaminated insects could readily transmit the pathogen to damaged areas in the aerial part of potato plants. They suggested that potato stocks freed from these bacteria by the stem cutting procedure could become recontaminated by insect from infected dumps.

Beer (1979) reported that the secondary dissemination of *Erwinia amylovora* is facilitated by the pollinating insects. The pathogen may be carried to new blossoms in infected nursery stock in association with vectors such as insects, birds or man or by wind.

Kloepper *et al.* (1979) found that 10 genera of insects belonging to 9 families were contaminated with *E. carotovora* var. *atroseptica* than with *E. c.* var. *carotovora*. They suggested that insect played a potentially competent part in the epidemiology of black leg and soft rot diseases, especially in its reintroduction into field free from the bacterium. Later in 1981, they observed that adult fruitfly (*Drosophilla melanogaster*) readily transmitted *E. c.* var. *carotovora* and *E. c.* var. *atroseptica* under artificial inoculating conditions. The injured inoculated plants developed symptoms when maintained at high relative humidity.

Surkov and Yu (1981) listed the vectors of bacterial diseases of millet *Panicum miliaceum* viz., *P. syringae* pv. *syringae* is transmitted by *Trigonotylus ruficornis*, *Lygus pratensis*, *Phyllotreta vittula*, *Calligypana marginata* and *Cicadella viridis*. *Xanthomonas campestris* pv. *holicola* by *Lygus pratensis* and *Phyllotreta vittula* and *E. carotovora* sp. *carotovora* was transmitted by *Trigonotylus ruficornis*, *Macrostelus laevis* and *Calligypania viridis*.

Guevara *et al.* (1985) found that *E. carotovora* and *E. herbicola* were carried by insects viz., *Ceratitis capitata*, *Antiteuchus tripterus* and *Anastrepha*, *Alabama*, *Aulacaspis* and *Acteca* spp. all of these insects settle on mango and play a major role in spread of the pathogen.

Broadbent and Matteoni (1991) reported that the adult of *Liriomyza trifolii* were able to acquire and transmit *Pseudomonas cichorii* both from *invitro* cultures of the bacteria and *insitu* from infected to non-infected chrysanthemum.

De-Wael *et al.* (1992) worked on the life cycle of *E. amylovora* on apples, pears and other Rosaceae and the transmission of *E. amylovora* and its vectors, particularly pollinating insects.

Eden-Green *et al.* (1992) showed that the tube-building cereopoids, *Hindola fulva* and *H. striata* were natural vectors of Sumatra disease of clovers, caused by *Pseudomonas syzygii*.

Alonso *et al.* (1993) established the role of *Loxa* sp. and *Hypothenemus* sp. in the transmission of *Erwinia*.

Lomer *et al.* (1993) carried out studies in Indonesia on the biology of four species of *Hindola* found on clove trees, viz., these were *H. striata*, *H. fulva*, *Hinda* sp. *H. Striata* and *Hindola* sp. from Bengkulu, played an important role in transmission of xylem limited bacterial disease caused by *P. syzygii*.

Host range

Doidge (1921) in their first report on *Xanthomonas vesicatoria* observed that the bacterium infected *Solanum nigrum*, *Physalis minima* and *Datura stramonium* var. *tatula*. Higgins (1992) reported that *Bacterium vesicatorium* infected pimento, tobacco, chilli and bell peppers.

Hayward and Waterston (1964) reported that the *X. vesicatoria* infected cape gooseberry (*Physalis peruviana*) and wild gooseberry (*P. minima*). Kishun *et al.* (1977) stated that *X. c. pv. vesicatoria* infected *physalis peruviana* in India.

X. c. pv. vignicola incitant of bacterial blight of cowpea infected Redkidney bean (*Phaseolus vulgaris*) (Burkholder, 1944). Patel and Diwan (1950) found that *X. vignicola* infected *Vigna catjang*, *Vigna sesquipedalis* and several varieties of *Phaseolus vulgaris*. Patel *et al.* (1951) reported that *X. vignicola* besides its own host also infected *P. vulgaris*.

X. c. pv. campestris infected *Brassica* spp. including broccoli (*B. oleracea* var. *italica*), Brussels sprouts (*B. oleraceae* var. *gemmifera*), cabbage (*B. oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), turnip (*B. rapa* var. *rapa*), radish (*Raphanus sativus*), shepherdipurse (*Capsella bursa-pastoris*) and stock (*Malthiola incana*), *Boerhavia erecta*. (Hayward and Waterston, 1965 ; Simmonds, 1966 and Young, 1969).

Leyns *et al.* (1985) reported that Genus *Xanthomonas* infected at least 124 monocotyledonous and 268, dicotyledonous plant spp. None has been reported on gymnosperms or lower plants.

EPIDEMIOLOGY

Understanding the epidemic nature of plant diseases and the factors influencing the spread, dispersal and deposition of pathogen propagules will help in formulating suitable management practices based on the incidence and spread of disease in a given geographical region, it also helps to constitute models for disease forecasting.

Gorlenko and Voronkevich (1950) reported that both under experimental and field conditions, fairly high temperature combined with high humidity appeared to be most

conducive for the development of bacterial spot of tomato disease. Further they stated that the time just before and upto beginning of fruit maturity (June and July) being the most favourable in the field.

Basu (1966) found RH ranging from 87-97 per cent and temperature between 23-28°C with pre-inoculation host injury (by gentle rubbing to break epidermal hairs) and an inoculum concentration of 1×10^8 cfu/ml were most favourable for infection. *Xanthomonas vesicatoria* caused small greenish-yellow to brown spots, later large yellow blotches becoming necrotic and blighting of the leaves and light brown streaks on hycocotyl. On cotyledons it caused minute, white, flaky spots with greenish centers, some time coalescing to wrinkle the cotyledon surface.

Shekhawat and Chakravarti (1976) found that the high RH and temperature in the range of 23-34°C were necessary for the development of bacterial spot of pepper caused by *X. c. pv. vesicatoria* while Docea and Marinescu (1978) found temperature of 26-27°C with 85-95 per cent RH is favourable for the development of bacterial spot of tomato.

DETECTION OF PLANT PATHOGENIC BACTERIA

Several methods have been described for testing seeds for the presence of plant pathogenic bacteria in / on the seed (Schaad, 1979).

Ercolani (1968) compared the two phase plaque count methods for detecting the bacteria in tomato seed. The most accurate results were obtained with plaque counts on homogenates of seedlings grown from seeds in a sterile environment. This method showed that the incidence of bacteria was three per cent in seed from naturally infected tomatoes extracted by fruit pulp fermentation, while the seeds extracted without fermentation, 43 per cent contained *X. c. pv. vesicatoria*.

Cruz and Fernandez (1979) reported that the seeds and plants of tomato, chilli, cabbage and bean were inoculated with suspensions of *X. c. pv. vesicatoria*, (for the first two) *X. c. pv. campestris* and *X. c. pv. phaseoli* respectively, infection was detected with uv in an optical microscope using antibodies against the bacteria and antibodies labelled with fluorescein isothiocyanate.

Shekhawat and Chakravarti (1979) confirmed the seed borne nature of *X. c. pv. vesicatoria* by cotyledon test in 10 samples out of 18 tested and one sample by agar plate method. Karaca and Demir (1988) detected *X. c. pv. vesicatoria* on tomato and pepper seeds by using selective medium.

Kritzman (1989) described a method of detecting *Pseudomonas syringae* pv. *tomato*, *P. corrugata* *X. c. pv. vesicatoria* and *Corynebacterium michiganense* sub sp. *michiganense*. The sensitivity of the method was found to be high, as 10 cfu/g of tomato seeds could be detected by this method.

Kritzman (1991) developed a method of detection and quantitative estimation of tomato seed borne pathogenic bacteria. The method employed was dry grinding, weighing, bacterial extraction and quantitative calculation on selective or semi selective medium. It enabled detection in a 7g tomato seed samples, of as few as 10 cfu/g seed. The following seed borne pathogens of tomato; *P. s* pv. *tomato*, *P. corrugata*, *X. c. pv. vesicatoria* and *Clavibacter michiganense* sub. sp. *michiganense* were detected by this method.

Wilson (1938) soaked bean seeds in a selective medium to extract *Pseudomonas phaseolicola* and then inoculated bean pods to identify the pathogen. Wharton (1967) showed that the bean seeds infected with *P. phaseolicola* tended to fluoresce under ultraviolet light and used this character to select seeds for further testing. Taylor (1970) developed a routine dilution plate method for isolation of *P. phaseolicola* from seed extracts of bean. This method could detect low levels of seed-borne infection.

Chambers and Merriman (1975) isolated *Pseudomonas syringae* pv. *tomato* from mechanically harvested tomato seeds extracted from infected fruits but not from seed extracted by the fermentation or acid extraction.

Direct plating of seeds on a selective medium has been used to detect *X. campestris* in crucifer seeds (Schaad and Kendricks, 1975). Recovery of *X. phaseoli* from bean seeds is accomplished by soaking the seeds in liquid media (Trigalet and Bidaud, 1978; Trujillo and Saettler, 1979). Extraction of bacteria by the liquid soak method required 24 to 48 hours and is often adversely affected by contaminant bacteria which hinder the isolation of specific pathogens. The problem of contaminant bacteria was avoided through the use of selective media for *X. phaseoli*, and *X. phaseoli* var. *fuscans* (Trujillo and Saettler, 1979 and Trujillo and Saettler, 1980).

Gupta and Chakravarti (1982) from India failed to detect the presence of *X. c.* pv. *vignicola* in cowpea by directly plating surface sterilized and unsterilized seeds on nutrient agar, yeast extract glucose chalk agar and on plain agar medium till germination. However, they were able to demonstrate the transmission of this pathogen through the seeds by raising the plants from artificially inoculated and uninoculated cowpea seeds under green house conditions. Thus they stated that the bacterium is carried externally as well as internally along with the seeds which might serve as a primary source of inoculum. Gitaitis and Nilakhe (1982) devised a method for detecting *X. c.* pv. *vignicola* in cowpea seeds. They infused samples of 100 seeds with 100 ml sterile water by the sudden release of 680 mm mercury vacuum, the seeds were agitated at 150 rpm for 2 hours on an orbital shaker and the suspension was centrifuged at 4000 g for 30 min. The pellets were resuspended in 5 ml of supernatant and the mixture was infiltrated with a syringe and a needle into healthy cowpea leaves. The bacterial cells were recovered from lesions that developed in 5-20 days and identified as *X. c.* pv. *vignicola*. They stated that incidence of bacterial blight in field plots was significantly greater in cultivars that were grown from heavily infected seeds. Thind and Soni (1983) carried out studies on the seed borne nature of *X. c.* pv. *vignicola* and reported that both external infestation and internal infection play an important role in the disease development. They stated

that a minimum of 10^3 cfu/ml was needed in the inoculum used for soaking the seeds to get successful seed transmission. The pathogen was recovered from individual seeds collected from artificially inoculated and naturally infected pods by streaking the seed suspension on potato sucrose peptone agar rifampin aureofugin medium and by inoculating the seed suspension on susceptible host cultivars respectively. Further they were able to detect as low as 0.1 per cent infected seeds present in the seed sample by double sowing test.

Van vuurde *et al.* (1983) extracted *Pseudomonas syringae* pv. *phaseolicola* from bean seeds by soaking the seeds in water at 5°C for 24 hour and isolated by dilution plating onto King's B medium.

Kuan *et al.* (1985) developed a method of detection of *X. c.* pv. *carotae* in carrot seeds by extracting the pathogen in cold (5°C) stationary aqueous soak for 18 hrs in darkness followed by vigorous shaking of seeds in water containing Tween-20, and concentrating of the bacterial cell by centrifugation and finally plating of dilution series of these cells onto a modified Kado and Heskett's D-5 medium. They stated that one naturally infected seed in 10,000 seeds could be detected by this method.

Soni and Thind (1987) detected *X. c.* pv. *vignicola* in the seeds by traditional growing on test. They stated that the incubation growing test is better than traditional growing on test for the detection of bacterium.

Venette *et al.* (1987) developed a method of detection of seed borne bacteria. Seeds are soaked in water and the soaked bean infusion is vacuum infiltrated into pre germinated seeds from the same lot and symptoms were observed on seedlings grown under high humidity. In tests of 233 lots of beans seeds, all of the lots produced atleast some seedlings with water soaked spots on primary leaves, intensity of water soaked spots varied among seed lots. Pathogens, which were isolated from lesion on plants from 118 of 139 lots and were characterized by biochemical, physiological, morphological and pathogenecity tests are *Pseudomonas syringae* pv. *syringae*, *P. s.* pv. *phaseolicola* and *X. c.* pv. *phaseoli*

Khan (1989) detected *X. c. pv. vignicola* using selective and semi selective media. He found that SIBU agar and NSCA media were found to be efficient in the detection of pathogen in naturally infected cowpea seeds.

DETECTION OF SEED BORNE BACTERIA BY SEROLOGICAL TECHNIQUES

The use of serology in the detection and identification of bacterial pathogens has been extensively reviewed (Trigalet *et al.*, 1978 and Schaad, 1979).

Agglutination and Agar double diffusion test

Agglutination and agar double diffusion tests have been most commonly used to identify *Pseudomonas phaseolicola*, *X. phaseoli* and *X. phaseoli* var. *fuscans* from bean seed leachates (Guthrie *et al.*, 1965 Saettler, 1971 and Trujillo and Saettler, 1979).

Immunofluorescence test

Marton (1965) compared three serological techniques for detecting *X. c. pv. vesicatoria* in the leaves of pepper (*Capsicum frutescens* L.). He succeeded in detecting the presence of the pathogen in the diseased leaves by immunofluorescence staining technique, slide agglutination technique and modified slide agglutination technique in which bentonite clay particle stained with methylene blue were immunised by purified r-globulin obtained from antiserum developed against *X. c. pv. vesicatoria*. He found that all the three methods were specific and no reaction was obtained when healthy leaf material and several other bacterial species were tested.

Cruz and Fernandez (1979) developed immunofluorescence technique in the detection of *X. c. pv. vesicatoria* and *X. c. pv. campestris* and *X. c. pv. phaseoli* infecting pepper, cabbage and beans respectively. The detection of bacterium is carried out using

immunoserum specific to the bacteria derived from the rabbit and labelled fluorescein isothiocyanate.

Immunofluorescence microscopy (IF) is now successfully used for the routine testing for pathogenic bacteria in beans (*Phaseolus vulgaris*) (Trigalet and Bidaud, 1978). A complete schedule of operations for the *in-situ* location and identification of *Erwinia amylovora* the causal agent of fire blight of apple and pears, in the thin section of host tissue by means of fluorescent antibody technique was described by John HockenHull (1979).

Dinesen (1981) used immunofluorescence microscopy and ELISA for the detection of latent infection of *E. chrysanthemi* in the cuttings of *Dieffenbachia* sp. and *Kalanchoe blossfeldiana*. He reported that in the undiluted plant extract or a dilution of 1:10 from weakly attacked plants, the immunofluorescence method was as good as the ELISA method.

A comparison of two serological method viz., immunoflorescence microscopy (IF) and enzyme linked immunosorbent assay (ELISA), with the dilution plating for the detection of *P. s. pv. phaseolicola* in bean seeds, demonstrated the potential of IF for reliable and inexpensive routine application (Van Vuurde *et al.*, 1983). They stated that IF had a high sensitivity and a lower subjectivity of sample judgement as compared to dilution plating. Further IF also offered a good prospect for the detection of *P. s. pv. phaseoli* (fuscan strain) in the bean seed extract. Malin *et al.* (1983) applied indirect immunofluorescence staining for the detection and identification of *X. c. pv. phaseoli* in the naturally infected bean seeds. They detected an infection level as low as 0.01 per cent and stated that IF provides a reliable method for rapid screening of bean seed lots for the detection of the pathogen. Kulik (1984) also advocated the use of indirect immunofluorescence technique in the detection of *X. c. pv. phaseoli* in bean seeds.

Khan (1996) compared three serological techniques viz; Indirect immunofluorescence microscopy (IF), enzyme-linked immunosorbent assay (ELISA)

and immunosorbent dilution plating (ISDP) for the detection of *Xanthomonas campestris* pv. *vignicola*, in naturally infected cowpea seeds (*Vigna unguiculata*). He demonstrated the potential of indirect IF for reliable, inexpensive and quick application in the detection of seed borne pathogenic bacteria and reported that the sensitivity of the indirect IF was comparable with that of dilution plating. In one seed sample belonging to cv.C- 152, he was able to detect 32 and 26 fluorescent cells per microscopic field, which correlated well with 1.5×10^4 and 9.66×10^3 cfu / ml of seed extract detected by dilution plating.

Enzyme linked immunosorbent assay (ELISA)

Lazarovits *et al.* (1987) reported the usefulness of enzyme linked immunosorbent assay on nitrocellulose membrane (dot-ELISA) for diagnosis and identification of plant pathogenic bacteria including *X. c* pv. *vesicatoria*. They detected bacterial population of 1000-2000 cfu / spot (Ca 0.3 cm²), and stated that the extract of lesions from tomato and pepper leaves infected with *X. c* pv. *vesicatoria* reacted positively with the antiserum produced against this pathovar but not produce against *X. c* pv. *translucence*.

Maringoni and Kimati (1987) used agar double diffusion test to differentiate capsicum and tomato strains of *X. c.* pv *vesicatoria*. They also found that the capsicum strain reacted with homologous antiserum, while all tomato strains reacted with heterologous antiserum.

The usefulness of ELISA was tested for diagnosis of plant pathogenic bacteria and in particular for detection of *E. carotovora* sub sp. *atroseptica* and *X. pelargonii* infecting potato and pelargonium respectively. On comparing the sensitivity of ELISA with immunofluorescence it was found that it can compete rather well with IF and that it has also certain advantages above IF method (Vruggink, 1978). Application of ELISA for the detection of *P. phaseolicola* resulted in the detection 10^6 cfu / ml (Weaver and Guthrie, 1978). Recent studies have revealed that this technique is more promising and is equal in sensitivity to the dilution plate method but less affected by the presence of

saprophytic bacteria (Van Vuurde and Bovenkemp, 1981). Wesley (1982) reported that ELISA could be used in the detection of *Corynebacterium michiganense* in tomato seeds. He reported that single seed ELISA was effective in determining infection rates of one per cent or more, whereas batches seed ELISA was able to detect atleast 0.06 per cent infection rate. Dinesen (1981) used IF and ELISA in the detection of *E. chrysanthemi* infecting *X. blossomfieldiara* and *Dieffenbachia sp.* The sensitivities of the two methods were equal, and further he stated that the results are easy to record, even when the plants were weakly attacked.

Barzic and Trigalet (1982) have demonstrated the use of ELISA in the detection of seed borne *P. s. pv. phaseolicola* in beans. Van Vuurde *et al.* (1983) compared the sensitivity of ELISA, immunofluorescence with dilution plating and found that ELISA was comparable with that of dilution plating in the detection of *P. s. pv. Phaseolicola*, *X. c. pv. phaseoli* and *X. c. pv. phaseoli fuscus* (strain) in the bean seeds. Further ELISA was found to be less sensitivity than IF.

Sheppard (1983) reported that ELISA, radioimmuno assay and IF are rapid and required a small amount of reagents and often these tests can be completed in a single day and strongly advocated the use of these techniques in the detection of seed borne bacteria of bean. Alvarez and Lou (1985) developed a soluble antibody sandwich (DAS) ELISA technique to detect *x. c. pv. campestris* in the leaf disks sampled from cabbage in the field. The technique permitted the processing of large number of samples and facilitated identification of the pathogen in seed beds, on weeds and on cabbage plants that showed unusual symptoms.

Muratore *et al.* (1986) used ELISA and IF techniques for the detection of latent infection of *E. chrysanthemi* and *P. caryophylli* in carnation. They reported that ELISA had a highest theoretical sensitivity threshold about 10 times over IF and dilution plating.

Further Khan, 1996 modified and applied ELISA in the detection of *X. c. pv. Vignicola* in naturally infected cowpea seed. he used for the first time a different enzyme

substrate system in ELISA for the detection of seed borne pathogenic bacteria by using horseradish peroxidase conjugate and diphenylendiamine-dihydrochloride substrate system. The sensitivity of ELISA was comparable with dilution plating. The ELISA extinction values of 0.125 and 0.129 compared well with the population of 2.0×10^6 and 6.0×10^6 cfu/ml of seed extract of cv. C-152 detected by dilution plating. He also compared the sensitivity of indirect IF with ELISA and dilution plating and stated that indirect IF was more sensitive and showed a better correlation with dilution plating than ELISA.

Immunosorbent dilution plating (ISDP)

Van vuurde and Henten (1983) developed solid phase immunosorption method, namely, immunosorbent dilution plating (ISDP) for selective concentration of bacteria. Experiments with ISDP showed that immunosorption of *P. s. pv. phaseolicola*, *C. michiganense* pv. *michiganense* and *X. c. pv. phaseoli* from the mixed culture on to inoculation sticks, prior to dilution - plating is promising as an alternative to selective medium. They stated that ISDP might prove useful in solving some of the problems associated with the development and evaluation of routine serological methods for the detection of pathogenic bacteria like *C. m. pv. michiganense* of tomato and *X. c. pv. campestris* of cabbage, which are known to be specifically difficult to detect. Khan (1996) applied ISDP in the detection of *X. c. pv. vigninica* in cowpea seeds. He found the technique to be simple efficient and sensitive.

Serotypes of *X. c. pv. vesicatoria*

Charudattan *et al.* (1973) catagorised serotypes, among 72 isolates of *X. c. pv. vesicatoria* on the basis of presence or absence of certain precipitin bands in gel-diffusion plates. The two types were distinguished by their specific agglutination reaction. The fast diffusable components of type I and II were associaed with soluble bacterial surface components. However, they found no correlation between serology and pathogenicity of *X. c. pv. vesicatoria* isolates. The tomato and pepper isolates included

both serotypes, and the tomato and pepper isolates could not distinguished on the basis of serological tests and neither the serotypes had any correlation with the biochemical characters of the isolates such as ability to hydrolyse starch and development of resistance to streptomycin.

Maringoni and Kimati (1987) reported that serologically, *X. c. pv. vesicatoria* pepper strains were different from tomato strains, however, within the pepper group the strains differentiated, while there was no variations among the tomato strains.

Hseu *et al.* (1990) from Taiwan grouped 58 isolates of *X. c. pv. vesicatoria* obtained from pepper and tomato in 3 sero groups, but the sero groups have no relevance to the three races designated on the basis of the reaction on host differentials. Later in 1996, Sahin and Miller. grouped 106 isolates from pepper into 6 serogroups using monoclonal antibodies in an ELISA. They found that 39.5 per cent of the strains did not react with any of the monoclonal antibodies and could not be assigned to a serovar.

CONTROL OF *Xanthomonas campestris pv. vesicatoria* :

Biological control

Shekhawat and Chakravarti (1977) reported actinomycetes antagonistic to *X. c. pv. vesicatoria*. A black tinged, yellow bacterium associate with bacterial lesions on fruit skin and leaves of the *capsicum* cv. NP-46A, was antagonistic under *in vitro* condition as, no disease developed from inoculum prepared from *X. c. pv. vesicatoria* mixed the antagonistic bacterium in the following ratio 1:16, 1:32 and 1:64.

Colin *et al.* (1984) reported that 25 isolates of fluorescent *Pseudomonas spp.* exhibited various degree of antagonism towards *X. c. pv. vesicatoria*. There was no correlation between antagonistic activity and concentration of fluorescent pigment expressed as siderophore units.

Tudor-Nelson *et al.* (1995) reported that tomato race 3 (T3) strain of *X. c. pv. vesicatoria* were antagonistic to all of 25 strains of the A group and two to six strains of the B group of *X. c. pv. vesicatoria*. All of 10 strains of T3 produced antagonism to strains of the A group. Antagonism occurred to five of 14 other pathovarns of *X. campestris* and antagonism did not occur to four other genera of plant pathogenic bacteria.

Chemical control

Falicode - Alcaraz (1973) stated that the bacterial spot caused by *X. c. pv. vesicatoria* is effectively controlled by spraying streptomycin sulphate.

Firsov and Kurbatov (1976) reported that Pencillin sprayed at 0.001 per cent gave the best control of bacterial black spot of tomato and increased yield by 16.8 per cent.

A cyclic compounds BF-6 tested against *X. c. pv. vesicatoria* was found to be very effective. When roots were soaked for two hour in a 0.01 per cent solution before planting, leaf and fruit infection were reduced and yield increased by 28-32.7 per cent (Basymybekov and Firsov, 1978).

Kishun and Sohi (1982) reported that three application of agrimycin - 100 were effective in reducing the intensity of infection by *X. c. pv. vesicatoria* and increasing yield.

Takimoto (1939) reported good control of *Bacterium vesicatorium*. Seed treatment with 1:1000 to 1:3000 mercuric chloride solution in water followed by two to three applications of 3-2-75 or 3-2-90 Bordeaux mixture prior to the appearance of the first symptoms gave good control of the disease.

Beattie *et al.* (1944) stated that bacterial spot (*X. vesicatoria*) can be controlled by soaking the seed for not more than two minutes in a 1:2000 mercuric chloride solution followed by thorough washing in water and immediate drying in shade.

Haskell (1944) recommended seed treatment against seed borne disease of tomato. The diseases partly controlled by seed treatment were, bacterial spot (*X. vesicatoria*) and bacterial canker (*Corynebacterium michiganense*). Seed were treated by immersing in hot water at 122°F for 25 min or for five minutes in mercuric chloride (1:2000).

Gorlenko and Voronkevich (1950) reported that *X. vesicatoria* could be controlled by changing the soil and disinfecting the wood work of the frames in which the disease has been observed and by disinfection of seeds obtained from infected areas and deep ploughing of all plant debris in the autumn.

Ercolani (1968) reported that the incidence of *X. vesicatoria* and *Corynebacterium michiganense*, was reduced to 19 and 16 per cent respectively, by spraying agrimycin-100 and by phenyl mercuric acetate to 8 and 13 per cent, by acetic acid to 8 and 2 per cent respectively. Mercuric chloride from 0 and 14 per cent and hot water to 0 and 6 per cent.

Burykhina (1972) stated that seed treatment of tomato with chloramine at 1.0 per cent for 5 min was as effective as mercurials and copper fungicides against *X. vesicatoria*.

Butenets and Musaev (1976) obtained best control of *X. vesicatoria* by spraying 0.5 and 0.75 per cent zineb and 0.5 per cent phygon.

Docea and Marinescu (1978) stated that Dithane - Cupromix 0.5 per cent and 0.05 per cent cryptonal gave the best control of bacterial disease of tomato.

Marinescu (1978) found that 0.5 per cent Micram (Copper oxychloride + Zineb), Dithane M-45 at 0.2 per cent, Dithane-cupromix 0.5 per cent were most effective against *X. vesicatoria*.

Conover and Gerhold (1981) stated that basic copper sulphate + Maneb or basic copper sulphate + Mancozeb were more effective against *X. c. pv. vesicatoria* on tomato.

Jones and Jones (1986) compared many copper based formulations and antibiotics for controlling bacterial spot of pepper caused by *X. c. pv. vesicatoria*. Addition of mancozeb at 1.5 lb/100 gal to the cu-bactericides improved their efficacy. Copper hydroxide + Mancozeb resulted in significantly better control.

Kucharek *et al.* (1986) found that control of *X. c. pv. vesicatoria* was best when Maneb + Zinc sulphate was added to a copper hydroxide (2.1 kg/cm²) and sprayed twice per week.

Simons (1987) reported that *X. c. pv. vesicatoria* was controlled with Maneb and Zinc sprays supplemented on 3 occasions by addition of liquid copper.

Maringoni and Kurozawa (1988) stated that thiram at 500 ppm or more and captan at 12.5-50 ppm reduced disease severity.

Panagopoulos and Biris (1988) reported that the control methods included for *X. c. pv. vesicatoria* are the use of disease free seeds, ploughing in plant debris as soon as possible after harvest, avoiding irrigation with sprinklers and spraying with antibiotics or copper.

Hernandez and Medina, (1991) advocated seed treatment of tomato with sodium hypochlorite and chlorohydric acid at 2 and 3 per cent for 20 min. to effectively control the bacterial spot of tomato.

Mandge *et al.* (1992) from India reported that *X. c. pv. vesicatoria* was effectively controlled with 3 sprays of Agrimycin plus copper oxychloride plus carboryl.

Jones *et al.* (1993) also observed suppression of population of *X. c. pv. vesicatoria* in the leaves sprayed with cupric hydroxide plus Mancozeb, while Mancozeb alone or copper ammonium carbonate had no effect on the population build of pathogen.

Koushik *et al.* (1994) reported that bacterial spot of pepper could be effectively control by weekly spray of copper plus carboryl.

Ravi kumar and Khan (1995) reported that, the seed treatment with streptomycin sulfate or streptocyclin for 120 min. at 300, 400 and 500 ppm eliminated the *X. c. pv. vesicatoria* from the seeds.

Thirumalachar *et al.* (1956) reported that chloramphenicol, tetracyclin, aureomycin and streptomycin sulphate inhibited *X. c. pv. vignicola* under in-vitro conditions in the order of efficiency.

Valkhov *et al.* (1974) reported that bean seeds treated with Streptomycin solution at 5 ml/ml for one hour was most effective against *X. phaseoli*.

Chakravarti *et al.* (1976) stated that bacterial blight of cowpea could be controlled by spraying agrimycin-100 at 250 ppm thrice at an interval of 10 days. They recorded up to 35 per cent increase in yield over control.

Balaraman and Rajagopalan (1978) reported the inhibition of *X. oryzae* under in-vitro test by using antibiotics like tetracycline hydrochloride which gave the highest inhibition zone followed by terramycin, ledermycin, erythromycin and chloramphenicol. In pot culture tests, the best control was seen in erythromycin, tetracycline hydrochloride, ledermycin treated plants.

Chauhan and Vaishnav (1980) obtained control of *X. c. pv. Oryzae*, under *in vitro* conditions and in the field by applying streptocyclin or copper containing compounds.

Shekaraiah (1981) obtained better control of bacterial blight of cowpea by spraying paushamycin at concentration of 500 ppm at 10 days interval as compared to Streptomycin sulphate and teramycin.

Mary and Mathew (1983) found pencillin at 500 ppm was most inhibitory to the growth of bacterial blight of rice pathogen in *in vitro* conditions, when pencillin or agromycin-100 at 250ppm applied as post inoculation sprays, they were equally effective in reducing disease intensity on rice.

Tsvetkov and Doner (1984) stated that, treatment of *Phaseolus vulgaris* plants at the beginning of anthesis with TB, Phytoderma and Streptomycin reduced infection by *X. c. pv. phaseoli* and increased the yield by 14.4 per cent.

Takahi and Shirahagen (1985) developed a new systemic bactericide, tecloftalam for the control of rice bacterial leaf blight. It was very effective in inhibiting the bacterial multiplication in rice plants.

Mary *et al.* (1986) stated that spraying of penicillin, paushamycin and streptomycin controlled *X. c. pv. oryzae*. Hu *et al.* (1987) stated that the application of cytosine antibiotic 16A6 gave 70 per cent control of bacterial blight of rice with increased yield of 10 to 20 per cent.

Xia *et al.* (1987) found spraying of thioldiarole bactericide at 200 to 500 ppm once or thrice at an interval of 10 to 15 days controlled *X. c. pv. oryzae* and this compound inhibited the growth of the pathogen under *in vivo* and *in vitro* conditions.

Honda *et al.* (1980) reported that, n-(2,3- dichlorophenyl) and n(1-rapthyl) 3,4,5,6- tetrachloro- phthalamic acid was most effective against *X. c. pv. oryzae*. Verma *et al.* (1980) found Dithane C-90 (Copper + Maneb) reduced bacterial blight of rice.

Marinescu (1982) found that the infection of *Phaseolus vulgaris* seeds by *X. c. pv. phaseoli* was reduced by Kocide- 50 at 0.25 per cent and Kocide-101 at 0.5 per cent, when the seeds were vacuum infiltrated with the chemicals at 80 torr.

Lu *et al.* (1983) stated that bacterial blight of rice could be controlled by the application of F-48 at 750 ppm. It gave 85.77 per cent control compared with phenazin applied at 500 ppm.

Gitaitis and Bell (1984) reported that bacterial leaf blight due to *X. c. pv. vignicola* was reduced significantly when copper ammonium carbonate was sprayed at 0.65 kg a.i./ha. The disease severity was 1.5 per cent in treated plots over 38.5 per cent in untreated plots.

Gossele *et al.* (1984) found that formaldehyde applied at a concentration of 0.0001 per cent and 0.0005 per cent were inhibitory to the growth of *X. c. pv. oryzae*.

Hoa *et al.* (1984) obtained better control of bacterial blight of rice by spraying ammonium sulphate five day before glass house inoculation. The disease intensity decreased in moderately resistant and susceptible cultivars and was more effective than other treatments.

Gitaitis *et al.* (1986) stated that spray of copper and copper + Maneb mixtures controlled leaf blight and stem canker symptoms of cowpea and further significantly increased the yields.

Harman *et al.* (1987) found that species of *Brassica* seeds treated with a mixture containing 10:3:90 Nyolate solution (containing sodium chloride, lactic acid and water) completely eradicated *X. c. pv. campestris* from most of the seed lots tested and greatly reduced the detectable level of the pathogen in the remaining lots, Nyolate promised to be an alternative to hot water seed treatment of *Brassica* species.

Combination of antibiotics and chemicals in the control of bacterial spot of tomato

Singh and Dilip Monga (1985) reported that seed treated with a combination of blitox (Copper oxychloride) and terramycin (Oxytetracyclin) effectively eliminated *X. c.* pv. *Oryzae*.

Thapliyal and Duby (1986) reported that combination of a antibiotic with a copper fungicide generally gave improved control of *X. c.* pv. *sojense* on soybean and the highest yields were obtained with agrimycin-100 + blitox -50 (Copper oxychloride).

MATERIAL AND METHODS

III MATERIAL AND METHODS

3.1 ISOLATION OF *Xanthomonas campestris* pv. *vesicatoria* FROM INFECTED TOMATO LEAVES AND FRUITS

Leaves and fruits of Tomato (*Lycopersicon esculentum* Mill.) showing typical symptoms of bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge), were collected. The symptoms appeared on leaves as small greenish-yellow to dark brown minute spots. Several spots on leaves caused blighting of leaves. On fruits, the symptoms appeared as sunken brown spots with irregular lobed margins with water soaked halos (Plate 2). The infected tomato leaves and fruits showing the symptoms were collected during the Kharif seasons of 1994 and 1995 from different agro-climatic zones of Karnataka as shown in Table 1.

The bacterium was isolated by extracting the ooze in sterile distilled water followed by dilution plating on nutrient agar.

Small tissue pieces of infected fruits and leaves were cut aseptically from the edge of typical spots along with a little portion of healthy tissue. The infected bits were surface sterilized in 70 per cent alcohol and washed in three series of sterile water to remove traces of alcohol. The infected bits were then suspended in a small test tube containing 3 ml. sterilized distilled water for 10 min. When water became slightly turbid due to oozing of bacterial cells from the the cut ends of the diseased tissue, the bacterial suspension was serially diluted in 9 ml sterile distilled water. One ml of the diluted bacterial cell suspension was added into each sterilized petriplates and melted nutrient agar and nutrient broth glucose yeast extract agar was poured separately. The plates were rotated gently in clockwise and anti-clockwise direction, so as to distribute the bacterial cell suspension uniformly in the plates and to obtain well separated bacterial colonies. The inoculated plates were incubated at 28°C for 72 hr. Observations were made for development of well separated white, convex, small bacterial colonies on the N.A medium.

Table 1. Collection of bacterial spot affected tomato fruits / leaves from different agro-climatic zones of Karnataka during kharif 1994 and 1995.

Zone	Area	Host
East dry zone	Bangalore (GKVK)	Tomato
East dry zone	Bangalore (Hoskote)	Tomato
Central dry zone	Chitradurga	Tomato
Central dry zone	Daravagere	Tomato
Southern dry zone	Hassan	Tomato
East dry zone	Kolar	Tomato
Hilly zone	Mudigere	Tomato
Southern dry zone	Mysore	Tomato
Southern transition zone	Shimoga	Tomato
East dry zone	Tumkur	Tomato
East dry zone	IIHR-1(Bangalore)	Tomato
East dry zone	IIHR-2(Bangalore)	Tomato
Coastal zone	Mangalore	Tomato
Southern dry zone	Mandya	Tomato
East dry zone	IIHR(Bangalore)	Pepper
-	Himachal Pradesh	Pepper

3.1.2 PURIFICATION OF BACTERIAL CULTURES

The suspected bacterial colonies were picked up with the help of sterilized inoculated loop and streaked onto the surface of yeast extract dextrose calcium carbonate agar (YDCA, Schaad and Stall 1988) contained in sterilized petriplates. The inoculated plates were incubated at 28°C for 72 hours and observations were made for the development of well separated light yellow coloured bacterial colonies. The purified bacterial colonies was streaked on nutrient agar slants and stored at 5°C in refrigerator and also in sterile distilled water taken in small culture tubes, by suspending 2-3 loop full of the bacterial culture, for future use and the 16 isolates were designated according to location from where the respective disease specimens were obtained viz., Bangalore 1 and 2 for GKVK and Hoskote respectively, Chitradurga, Davanagere, Hassan, Kolar, Mangalore, Mandya, Mysore, Mudigere, Shimoga, Tumkur, IIHR-1 and IIHR-2 (Bangalore).

Two strains of *X. c. pv. vesicatoria* isolated from pepper were obtained from Dr. Vasanthakumar, sunseeds corporation, Bangalore and Dr. Rupali Singh Bacteriologist, IIHR, Bangalore respectively.

3.3 Identification of causal organism

3.3.1 Morphological, physiological and biochemical studies

The light yellow colonies of *Xanthomonas* were studied with reference to their morphology, physiological, biochemical characteristics as per Schaad and Stall (1988).

3.3.2 Hypersensitive reaction on tobacco

The test was carried out to find out if the 16 bacterial strains are pathogenic or not. The 14 tomato and one pepper strain of *X. c. pv. vesicatoria* obtained from

different agroclimatic zones of Karnataka state, and a pepper strain from Himachal Pradesh were multiplied in nutrient broth. A loopful of bacterial culture of each isolate was inoculated to 100 ml sterilized nutrient broth contained in Erleyenmayer flask separately. The inoculated flasks were incubated at 28°C. for 72 hours.

The bacterial cultures were centrifuged at 335000 g in high speed refrigerated centrifuge for 10 min. The supernatant was discarded and pellets of the sixteen bacterial strains were collected separately and suspended in sterile distilled water. The concentration of cell suspension was adjusted to 2×10^8 cfu/ml in spectrophotometer at wave length of 480nm. (Spectronic 20D. Milton and Roy, USA). The bacterial suspensions were injected into intercellular spaces of intact tobacco (*Nicotiana tabacum*) var. samsun leaves using hypodermic needle. The injected plant were kept in glass house at $25 \pm 2^\circ\text{C}$. Leaves injected with sterilized water served as control. Plant were observed for the development of confluent necrotic lesions in injected leaves during next 24 hours.

3.3.3 Pathogenecity on tomato and pepper plants

Pathogenicity test was carried out to find out if isolated bacteria are capable of producing typical symptoms of bacterial spot under artificial inoculation condition on tomato and pepper plants.

Tomato seedling cv. pusa Ruby and pepper seedlings cv. California wonder were raised in sterilized soil in nursery in small plastic boxes. Twenty five days old seedlings were transplanted to sterile soil contained in six inches plastic pots for the purpose of inoculation of the bacterial isolates.

The 16 strains of *X. c. pv. vesicatoria* from tomato and pepper were multiplied seperately in nutrient broth taken in Earlyenmayer flask by inoculating a loopful of bacterial culture to nutrient broth. The inoculated flasks were incubated at 28°C for 72 hours. Bacterial suspensions was prepared by adjusting cell concentration to 5×10^7 cfu/ml in spectrophometer at 480 nm. (spectronic 20D, Milton and Roy, USA). The

preinjured leaves of tomato plants were sprayed with bacterial suspensions separately. The inoculated plants were kept in the plastic tent for two days in which high humidity was maintained by spraying sterile water inside the tent. The plants were taken out from the plastic tent and kept open in glass house. Observation were made for the development of symptoms of bacterial spot. Plants similarly injured and sprayed with sterile water constituted control.

3.4 STUDIES ON THE PREVALENCE OF RACES OF *X. c. pv. vesicatoria*.

Broadly three groups have been identified within *X. c. pv. vesicatoria*; within each group different physiological races have been reported. Hence present investigation were under taken to findout the prevalence of these group and races within the each group under climatic conditions prevailing in Karnataka State.

3.4.1 Differentiation of races

The sixteen strains *X. c. pv. vesicatoria* isolated from tomato and pepper plants were multiplied on YDC for 72 hours at 28°C and the cells were harvested in sterile distilled water. The concentrations of cells were adjusted to approximately 10^8 cfu / ml. in sterile distilled water. The bacterial suspensions were infiltrated into fully expanded leaves of tomato cv. Bonny Best, Hawaii 7998, Hawaii 7981 and six other popular tomato hybrids, viz; Rashmi, Roopali, Rajani, Naveen, Vyshali, NS-386 and NS-815 and pepper cv. California Wonder and the three near isogeniclines viz; ECW-10R, ECW-20R and ECW-30R, by spraying the bacterial suspension to pre-injured leaves at 6-8 leaf stage as per the methods described by Hartman *et al.*(1990). The inoculated plants were incubated in polythene tent at 25-30°C for 72 hours under green house condition. The reaction of the bacterial strains on the tomato and pepper differential and cultivars was recorded on alternate days.

3.5 Physiological characterization of the strains of *X. c. pv. vesicatoria*

3.5.1 Amylolytic activity :

The ability of sixteen strains of *X. c. pv. vesicatoria* including the two pepper strains to hydrolyse starch was tested by supplementing one per cent soluble starch to nutrient agar. The starch supplemented nutrient agar was poured into sterilized petriplates and the sixteen bacterial strains were inoculated in the centre of the plates on the medium separately. The plates were incubated at 28°C for 5 days. Hydrolysis of starch by the strains of *X. c. pv. vesicatoria* was revealed by production of colourless zone surrounding bacterial growth in the medium flooded with lugol's iodine solution.

3.5.2 Pectolytic activity

The pectolytic activity of the sixteen strains of *X. c. pv. vesicatoria* was tested as per the method described by Beaulieu *et al.* (1991) using modified crystal violet pectate (CVP) medium with crystal violet and thallium nitrate (Schaad and Stall, 1988). The 16 strains of *X. c. pv. vesicatoria* were streaked separately on the surface of the medium and the inoculated plates were incubated for two weeks at room temperature. A depression developing in the medium surrounding the growth of bacterium indicated the pectolytic activity of the strain.

3.6 DETECTION OF PLASMIDS IN THE STRAINS OF *X. c. pv. vesicatoria*

3.6.1 Development of resistance to Streptomycin sulphate and copper sulphate

Resistance to streptomycin sulphate and copper was widespread among strains of *X. c. pv. vesicatoria* as per the literature and this has been implicated to be plasmid borne trait. Investigation were undertaken to find out the presence of plasmids in 16 strains of *X.c. pv. vesicatoria* by developing resistance to streptomycin sulphate and copper sulphate under *in vitro* conditions.

Fresh stock solution of streptomycin sulphate were prepared in sterile distilled water, filters sterilized through 0.22 μm pore size millipore filter and appropriate concentrations of 500, 600, 800, 1000, 2000, 4000, 5000, 6000, 7000, 8000, and 10,000 ppm were added to nutrient agar after autoclaving the medium and cooling it to 45°C before pouring in petriplates.

Bacterial strains were grown on YDC agar plates for 72 hours, and the cells were harvested in sterile distilled water. The concentration of cell was adjusted to 10^8 cfu /ml. 100 μl of the bacterial suspension was spread on plates containing nutrient agar amended with 500, 600, 800, 1000, 2000, 4000, 5000, 6000, 7000, 8000, and 10,000 ppm of streptomycin sulphate and 0.4, 0.5, 0.7, 0.8, 0.9 and 1.0 Per cent copper sulphate. The plates were incubated at 28°C for 5 days and observations were made for the development of the colonies of *X. c. pv. vesicatoria* on the medium.

A couple of colonies developed on medium containing 6000 ppm streptomycin sulphate indicating that the resulting colonies are the ones which have developed resistance to the antibiotic and in case of copper sulphate, 2-3 colonies were found to develop on medium supplemented with 8000 ppm of copper sulphate. As several reports are available in the literature on the development of resistance to antibiotics such as streptomycin sulphate. Hence, for the purpose of isolating plasmids from the strain of *X.c.pv. vesicatoria* the 14 strains showing resistance to streptomycin sulphate were selected. The two strains of *X. c. pv. vesicatoria* isolated from Bangalore (Hoskote and IIHR) were sensitive to all the concentrations of the antibiotics tested and did not show any resistance, hence deleted in the experiment.

3.6.2 Isolation of plasmid DNA.

The plasmid DNA was isolated from the 14 strains of *X. c. pv. vesicatoria* showing resistance to streptomycin sulphate.

The indigenous plasmid profiles /plasmid of the strains of *X. c. pv. vesicatoria* were determined by agarosegel electrophoresis. The native plasmid DNA was extracted by the following three procedures

- (i) Lysis by boiling method (Holmes and Quigley, 1981).
- (ii) Alkaline lysis method of Birnboim and Doly (1979), and modified by Ish-Horowicz and Burke (1981).
- (iii) Rapid plasmid isolation method (wheat Croft and Williams 1981).

i. Protocol for Lysis by boiling method of Holmes and quigley (1981)

1. Transfer a single bacterial colony in to 2ml of NA medium containing 6000 ppm streptomycin sulphate appropriate antibiotic in a loosely capped 15ml tube. Incubate the culture over night at 28°C with vigorous shaking.
2. Pour 1.5 ml of the culture in to a microfuge tube, centrifuge at 12,000g for 30 seconds at 4°C in a microfuge, store the remainder of the culture at 4°C
3. Remove the medium by aspiration, leaving the bacterial pellet as dry as possible.
4. Resuspend the bacterial pellet in 350 µl of STET
5. Add 25µl of a prepared solution of lysozyme (10mg / ml in 10 mM TrisCl (PH 8.0). Mix by vortexing for 3 seconds.
6. Place the tube in a boiling-water bath for exactly 40 seconds
7. Centrifuge the bacterial lysate at 12,000g for 10 min.at room temperature in a microfuge.

8. Remove the pellet of bacterial debris from the microfuge tube with a sterile tooth pick.
9. Add to the supernatant 40ml of 2.5 M Sodium acetate (PH 5.2) and 420ml of isopropanol. Mix by vortexing and store the above for 5 min. at room temperature.
10. Recover the pellet of nucleic acids by centrifugation at 12,000g for 5 min. at 4°C in a microfuge.
11. Remove the supernatant by gentle aspiration. Stand the tube in an inverted position on a paper towel to allow all of the fluid to drain away. Remove any drops of fluid adhering to the walls of the tube.
12. Add one ml of 70% Ethanol and recentrifuge at 12,000g for 2 min. at 4°C in a microfuge.
13. Again remove all of the supernatant by gentle aspiration as described in step 11. Remove any beads of ethanol that form on the sides of the tube. Store the open tube at room temperature until the ethanol has evaporated and no fluid is visible in the tube (2-5 min.).
14. Redissolve the nucleic acid in 50µl of TE (PH 8.0) containing DNAase free pancreatic RNAase (20 µg /ml) vortex briefly, store the DNA at -20°C.

ii Protocol for Alkaline lysis method.

This protocol is a modification of the methods of Birnboim and Doly (1979) and Ish-Horowicz and Burke (1981).

1. Transfer a single bacterial colony into 2 ml of LB medium containing 6000ppm Streptomycin sulphate in a loosely capped 15 ml tube, Incubate the culture overnight at 37°C with vigorous shaking.

2. Pour 1.5 ml of the culture into microfuge tube, centrifuge at 12,000g for 30 seconds at 4°C in a microfuge, store the remainder of the culture at 4°C.
3. Remove the medium by aspiration, incubate the bacterial pellet as dry as possible.
4. Resuspend the bacterial pellet in 100 µl of ice-cold solution I by vigorous vortexing.
5. Add 200 µl of freshly prepared solution II, close the tube tightly, and mix the content by inverting the tube rapidly five times. Make sure that the entire surface of the tube comes in contact with solution II. Do not vortex, store the tube in ice.
6. Add 150 µl of ice-cold solution III

The resulting solution is 3M with respect to potassium and 5 M with respect to acetate, close the tube and vortex it gently in an inverted position for 10 seconds to disperse sol. III through the viscous bacterial lysate. Store the tube on ice for 3-5 minutes.

7. Centrifuge at 12,000 g for 5 min. at 4°C in a microfuge, transfer the supernatant to a fresh tube.
8. Add an equal volume of phenol, mix by vortexing. After spinning at 12000 for 2 min. at 4°C in a microfuge, transfer the supernatant to a fresh tube.
9. Precipitate the double-stranded DNA with 2 volumes of ethanol at room temperature. Mix by vortexing, Allow the mixture to stand for 2 min. at room temperature.

10. Centrifuge at 12,000 for 5 min. at 4°C in a microfuge.
11. Remove the supernatant by gentle aspiration, stand the tube in an inverted position on a paper towel to allow all of the fluid to drain away. Remove any drops of fluid adhering to the walls of the tube.
12. Rinse the pellet of double-stranded DNA with 1 ml of 70 per cent ethanol at 4°C. Remove the supernatant as described in step 11 and allow the pellet of nucleic acid to dry in air for 10 min.
13. Redissolve the nucleic acid in 50 µl of TE (PH 8.0) containing DNAase, free pancreatic RNAase (20 µg / ml) vortex and store the DNA at -20°C.

(iii) Protocol Rapid plasmid isolation method (Wheat craft and Williams 1981)

1. Transfer a single bacterial colony in to 5 ml of LB medium containing the appropriate antibiotic 6000 ppm of streptomycin sulphate in a loosely capped 15 ml tube. Incubate the culture overnight at 37°C with vigorous shaking.
2. Transfer 1.5 ml of the overnight bacterial culture into Eppendorf tube and pellet the cells by centrifuging at 8000 rpm for 1 min.
3. Suspend the bacterial pellet in 100 µl of solution by gentle vortexing.
4. Add 25 µl of solution B, mix by inverting for couple of times. Then vortex the viscous solution.
5. Add 125 µl of phenol : Chloroform solution, mix thoroughly by vortexing.
6. To break the emulsion, centrifuge at 12,000 rpm for 3-4 min. Use the aqueous supernatant directly for agarose gel electrophoresis.

Samples of 14 strains of *X.c.pv. vesicatoria* were electrophoresed through 0.4% agarose gel in TAE buffer (0.04M Tris-acetate, 0.001M EDTA, PH 8.0) isolated with Ethidium bromide (2.5µl/50 ml) for one hr. Plasmid DNA was subjected to electrophoresis until a bromphenol blue front had moved 10 cm from the well. The electrical current was 5 V cm⁻¹ for gels in mini-sub cell and Lambda Hind III was as a marker in the gel.

3.7 STUDIES ON THE SURVIVAL OF *X. c. pv. vesicatoria*

3.7.1 Survival of *X.c. pv. vesicatoria* on tomato seeds:

Well ripened naturally infected fruits showing typical symptoms of bacterial spots were collected from fields.

Infected tomato fruits were crushed mechanically by hand in sterile distilled water. The pulp and flesh of the fruit were removed. The crushed fruit juice was passed through a sieve. The extracted seeds were washed once in distilled sterile water. The seeds were dried on blotting paper overnight at room temperature and then 400 seeds per bag were packed in two layered butter paper bag. Three bags containing the seeds were stored at 5°C and another set of three bags containing the same number of seeds was stored at room temperature (27±3°C).

The bacterium was detected by drawing seed samples from respective storage condition at fortnightly intervals.

3.7.1.2 Extraction of bacterium of these seeds

Each sample consisting of 100 tomato seeds were added to 10 ml sterilized distilled water taken in 50 ml Erleymayer flasks, the flask were incubated at 5°C over night.

3.7.1.3 Isolation of *X. c. pv. vesicatoria* on semi selective Tween - 80 medium.

One hundred microlitres of the seed extract was pipetted out with the help of one ml sterile pipette from the flask. The seeds extract so obtained was poured onto the surface of Tween - 80 medium contained in petriplate and the extract was spread with the help of a spreader. The inoculated plates were incubated at 28°C for 72 hours. Observations were made for the development of colonies *X. c. pv. vesicatoria* and population of bacterial cells detected in the seed extract of the seed stored at 5°C and room temperature condition was recorded.

3.7.2 Survival of *X. c. pv. vesicatoria* crop residue :

Crop residue provides a means by which plant pathogenic bacteria survive in the soil. The present investigation was undertaken to know wheather crop residue serves as a source of primary inoculum.

Tomato foliage and stem tissues belonging cv. PusaRuby were collected from severely affected bacterial spot plants during the rainy season (June and October 1995.) for over wintering study. The infected foliage and stem tissues were cut into small bits and were used in the following treatments.

Treatment: 1. 25 gm of diseased crop residue were placed on the surface of 5kg sterilized and unsterilized soil contained in plastic pots seperately.

Treatment :2. 25 gm of diseased crop residue were burried at a depth of 7.5 cm in 5kg sterilized and unsterilized soil contained plastic pots separately.

Treatment :3 25 gm of diseased crop residue were mixed uniformly with soil to a depth of 15cms in 5kg sterilized and unsterilized soil contained in plastic pots separately.

All the treatments were replicated thrice.

All the plastic pots were placed in the green house at $25\pm 3^{\circ}\text{C}$. The soil moisture in the plastic pots was maintained at 50% water holding capacity by careful watering. At an interval of 15 days 10 gm of soil sample was drawn from each plastic pot at each depth.

The soil samples were assayed for the recovery of *X. c. pv. vesicatoria* by serial dilution plating using semi selective Tween - 80 medium and the number of colonies of the bacterium developing on the Tween-80 medium after 72 hours incubation at 28°C was recorded.

3.7.3 Survival of *X. c. pv. vesicatoria* in soil :

The survival of the bacterium in the infested soil was studied by uniformly mixing suspension of *X. c. pv. vesicatoria* (2×10^6 cfu / ml) with steam sterilized soil and unsterilized soil.

The infested soil was poured in plastic pots and stored at room temperature. 10g soil sample was drawn at an interval of 15 days and assayed for the bacterial population. The recovery of bacterial cells was detected by serial dilution of soil samples followed by dilution plating on Tween-80 medium.

3.7.4 Epiphytic survival of *X. c. pv. vesicatoria* on weeds and non-host crop plants.

The investigation were conducted to find out the possible role of weeds and non-host crop plants in the survival of *X. c. pv. vesicatoria* acting as source of inoculum.

Fourteen weed species and one non-host crop plant viz; *Phaseolus vulgaris*, as indicated in table-2 were collected from fallow fields which were previously affected with

Table 2. Weed species and non host plant tested for the epiphytic survival of
Xanthomonas campestris pv. *vesicatoria*

I. Weeds species

1. *Ageratum conyzoides* Linn.
2. *Bidens pilosa* L.
3. *Burhhoia diffusa* L.
4. *Commelina benghalensis* Linn.
5. *Cyanotis cristata* (L.) D. Don.
6. *Cynodon dactylon* Pers.
7. *Cyperus rotundus* Linn.
8. *Digitaria marginata* Link.
9. *Echinochloa stagnina* (Retz) Beauv.
10. *Euphorbia geniculata* Ort.
11. *Hemilia sanchifolia* L.
12. *Legascea mollis* Cav.
13. *Marselia quadrifolia* L.
14. *Tridax procumbens* Linn.

II. Non-host crop plants

1. *Phaseolus vulgaris* Linn.
-

bacterial spot of tomato and from fields adjacent to infected tomato plots after (15, 30, 45 and 60 days of harvesting of tomato. The leaves and tender stems of weed species were separated from the plants and the samples were assayed for the isolation of *X. c. pv. vesicatoria*.

Each plant sample consisting of 10 g (fresh weight) was suspended in 100 ml sterilized distilled water taken in 250 ml Erlenmayer flask and agitated for 10 min. on a reciprocal shaker. The wash water was diluted in a tenfold series and 100 µl aliquot was poured onto the surface of Tween-80 medium contained in sterilized petriplate and the suspension was spread with the help of sterilized spreader. The inoculated plates were incubated at 28°C for 72 hours. Observations for the appearance of typical colonies of *X. c. pv. vesicatoria* on Tween-80 medium is made by looking for colonies showing zones of white crystals around them, and the population of bacterial cells were recorded. Further, identity of the bacterium was confirmed by Outcherlony's agar double diffusion test.

3.8 STUDIES ON THE ROLE OF INSECTS IN TRANSMITTING *X. c. pv. vesicatoria*

The possible role of insects in transmitting *X. c. pv. vesicatoria* from disease plants to healthy plants was investigated.

Six insect spp. were collected, while they were feeding on the bacterial spot affected tomato plants in the affected field. The insects were collected by insect net and the active flying type of insects such as white flies were collected using aspirator.

The insects collected were categorized according to the type of mouth parts such as biting and Chewing type, piercing and sucking type as shown in Table 3.

The mouth parts of the insects viz., mandible, probasis, prothorax, maxial, and intestine were separated and disinfected in 70 per cent alcohol for 10 seconds and plated

Table 3. Insects collected from tomato affected with bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria*

Sl. No.	Common name	Scientific name	Type of mouth parts
1.	Chrysomelid beetle	<i>Aulacophora cincta</i> Fabr.	Biting and chewing type
2.	White fly	<i>Bemisia tabaci</i> Gennadius.	Piercing and sucking type
3.	Long horned grasshopper	<i>Eucoptaera praemorsa</i> Stal.	Biting and chewing type
4.	Coccinellid beetle	<i>Henosepilchna vigintioctapunctata</i> Fabr.	Biting and chewing type
5.	Coccinellid beetle (Predatory beetle)	<i>Menochilus</i> sp. Fabr.	Biting and chewing type
6.	Short horned grasshopper	<i>Phenoptera gracillus</i> Burmelster.	Biting and chewing type

on petriplate containing Tween - 80 medium. The plates were incubated at 28°C for 4 to 5 days and observed for the development of typical colonies of *X. c. pv. vesicatoria*. The presumptive colony developing on the Tween-80 media were purified and the identification of the bacteria was confirmed serologically by Outcherlony's agar double diffusion technique.

3.9 EFFECT OF BACTERIAL SPOT ON THE QUALITY OF TOMATO FRUITS.

The effect of the incidence of bacterial spot of tomato on the quality of fruit was assessed by estimating the total soluble solids, total sugars, reducing sugar and the ascorbic acid (Vitamin-C) content of the diseased fruits in comparison with the healthy fruits.

Tomato fruits belonging to cv. Pusa Ruby was selected for the purpose of estimating the quality parameter.

Ripened tomato fruits infected with bacterial spot (*X.c. pv. vesicatoria*) were collected from severely affected tomato fields, and also ripened healthy tomato fruits were collected from fields which is free from infection. Ten g of affected fruits were taken in six replication for the assay.

3.9.1 Estimation of total soluble solids (T.S.S.)

The total soluble solids of tomato was estimated as per method of Ranganna, (1986). The juice was extracted by mechanically crushing the fruit pulp in muslin cloth and used to measure T.S.S. The T.S.S. was determined by using Abbe refractometer (0-32°) and expressed as °Brix.

3.9.2 Estimation of Reducing sugars

Juice from affected and healthy tomato fruits was obtained as noted under 3.9.1. Ten ml of juice from the diseased and healthy fruits was taken and blended with distilled water to make up the volume to 250 ml after adding lead acetate and potassium oxalate 2 ml each which acted as a clarifying agent. The filtrate was collected and used for estimating reducing sugar by the method of Lane and Eyan as described in "Manual of analysis of fruit and vegetable productions". (Lane and Eynon, 1923) using 0.5% sugar solution as standard and the reducing sugars are expressed in percentage.

3.9.3 Estimation of total sugars

Twenty five ml of the aliquot prepared under 3.9.2 was acidified with 1:1 HCl and kept overnight for inversion at room temperature. The solution was then neutralised with 20 per cent sodium hydroxide solution using phenolphthalein indicator by adjusting the pH to 7.0. The volume was made up in a volumetric flask to 100 ml and resulting solution was used for the estimation of total sugars as in the case of reducing sugar and expressed in percentage.

3.9.4 Estimation of ascorbic acid (Vitamin-C):

The most important component of tomato fruits, is (Vitamin-C) i.e., Ascorbic acid. It was estimated in both diseased and healthy tomato fruits as per the method suggested by Anonymous, (1966) in "Manual of analysis of fruit and vegetables products" by Ranganna, (1986) using 2,6-Dichlorophenol Indophenol visual titration method.

Ten ml of tomato juice extracted from diseased and healthy fruits as indicated under 3.9.1 and was blended with four per cent oxalic acid solution. The volume was made up to 100 ml in a volumetric flask and filtered by using Whatman No. 4 filter paper. Ten ml of the filtrate was taken and titrated against 2,6 dichlorophenol Indophenol

dye. The titration was carried out till a light pink colour persisted for 15 second. The dye was prepared using 50 mg of the sodium salt of 2,6 dichlorophenol indophenol in approximately 150 ml of double distilled water containing 42 mg of sodium bicarbonate and the volume was made to 200 ml. Vitamin-C was expressed as milligram of ascorbic acid per 100 gm of sample.

3.10 STUDIES ON THE ENVIRONMENTAL FACTORS FAVOURING THE DEVELOPMENT OF BACTERIAL SPOT OF TOMATO

A field experiment was conducted to investigate the influence of environmental factors on the disease development and to correlate the disease incidence and severity with the weather conditions, by planting tomato seedlings at fortnightly intervals starting from first fortnight of July 1995 to first fortnight of October 1996 at the Regional Research Station, UAS, Bangalore.

The field trial was laid out in an area measuring 300 sq. meters (30 mt width x 10 mt length). Twenty five days old tomato seedlings cv. Pusa Ruby raised in nursery in the green house were transplanted in 10 meters long rows. The seedlings were planting with a spacing of 20 cm between plants and 30 cm row to row. All cultivation practices such as fertilizers N250, P250, K250 kg/ha. spacing, Inter cultivation and Irrigation were carried out as per the package of practices prescribed by the University of Agricultural Sciences, Bangalore. The crop was sprayed with neem kernel extract to check the menace of white flies transmitting tomato leaf curl virus.

Observations were recorded at fortnightly intervals on the per cent disease incidence and the severity of disease was calculated by counting the number of leaves infected, no. of leaf lets infected, and number of necrotic spots / cm². The disease incidence and severity were correlated with the weather condition such as max. and min. temperature, relative humidity, rainfall.

3.11 DETECTION OF *X. c. pv. vesicatoria* IN NATURALLY INFECTED TOMATO SEEDS BY SEROLOGICAL TECHNIQUE

3.11.1 Collection of seed samples

Seeds from tomato fruits showing typical symptoms of bacterial spot, as described under 3.1 due to infection by *X. c. pv. vesicatoria* were collected. The seeds were extracted from the infected fruits by mechanical method by crushing the fruits in water and removing the pulp. The suspension was passed through sieve under running tap water. The seeds were collected and dried on a blotting paper overnight at room temperature and stored in two layered butter paper bags at 5°C for future use.

3.11.2 Preparation of seed extract

Tomato seeds sub samples comprising of 100, 200 and 400 seeds were added to 20 ml sterilized distilled water taken in 100 ml Erlenmeyer flask separately. The flasks were incubated over night at 5°C. The seed extract was shaken after 4 hour of incubation by hand.

3.11.3 Preparation of Antigens

The culture of *X. c. pv. vesicatoria* isolated from tomato fruits cv. Pusa Ruby described earlier under 3.1 was grown on YDC agar plates for 72 hours at 28°C and used as antigens for the production of antiserum.

3.11.4 Production of antisera

The rabbit antiserum was prepared against *X. c. pv. vesicatoria* (tomato group) as described by Vrugink and Geasteranus mass (1975). The antiserum was stored at 5°C until used.

3.11.5 Application of Enzyme Linked Immuno Sorbent Assay (ELISA) in the detection and identification of *X. c. pv. vesicatoria* in naturally infected tomato seeds.

Purification of γ -globulin from antiserum developed against *X. c. pv. vesicatoria* and conjugation of γ -globulins with Horse radish peroxidase (HRPO) was carried out as described by Avrameas and Ternyck (1971), respectively. The protein concentration was determined by the Bradford method (1976) using γ -globulin (Sigma G-3500) adjusted at 280nm as standard.

The specificity of antiserum for *X. c. pv. vesicatoria* was tested using a number of pathovars of *Xanthomonas campestris* and other seed borne bacteria isolated from the seeds.

3.11.6 Testing the specificity of antiserum developed against *X. c. pv. vesicatoria*

The antiserum obtained as under 3.11.4 was determined for its specificity to *X. c. pv. vesicatoria* by testing the antiserum against well known seed borne bacterial pathogens viz., *X. c. pv. phaseoli*, *X. c. pv. campestris*; *X. o. pv. oryzae*; *X. o. pv. oryzzicola* *X. c. pv. carotae*, *X. c. pv. malvacearum*; *X. c. pv. citri*; *X. c. pv. dieffenbachiae*, *X. c. pv. moricola* and few *Pseudomonas spp.* such as *P. avenae*, *P. fuscovaginae*, *P. syringae pv. phaseolicola*, *P. s. pv. pisi*, *Erwina carotovora* sub. sp. *carotovora* and also against contaminating seed borne bacteria, isolated during the extraction and Isolation of *X. c. pv. vesicatoria* from the tomato seeds under 3.11.1. The bacteria were tested as antigens by Ouchterlony double diffusion method.

The titre of the antiserum was determined by tube agglutination (AG) (Kiralay *et al.*, 1970) and Ouchterlony double diffusion methods (Ouchterlony, 1958) against the strains of *X. c. pv. vesicatoria*.

The extraction titres for coating γ -globulin and HRPO enzyme conjugate was determined. The dilution for coating

γ -globulin was found to be 1:800, and the dilution of enzyme conjugated γ -globulin was 1:1000.

3.11.6.1 Protocol for ELISA

1. **Antigen layer** : 200 μ l seed extract was filled in each well in a microtitre plate (limbro plate - Flow laboratories USA) and incubated overnight at 37°C.
2. Add 200 μ l 0.1 % glutaraldehyde to each well of micro titre plate and incubate at 37°C for 30 min.
3. Rinse the wells thrice in phosphate buffer saline PBS (pH 7.2). at 3 min. interval.
4. Add 200 μ l normal goat serum in phosphate buffer saline and incubate for 2 hours at room temperature.
5. **Washing** : Wash the plates with PBS-T three times at 3 min. interval.
6. Add 200 μ l diluted Antiserum 1:800 in PBS-T with 0.1% bovine serum albumine and incubate for one hour at room temperature.
7. **Washing** : Remove the fluid and wash the plates twice with the washing solution (PBS-T) at two min. intervals.
8. **Addition of enzyme conjugated serum** :

The Horse radish peroxidase conjugated goat anti-rabbit immunoglobulin preparation was diluted 1:1000 times in dilution buffer and 200 μ l of the enzyme conjugate

was filled in each well. Incubate plates for 20 min. at room temperature.

9. Washing : Remove the fluid from the wells and wash 5 times with the washing solution (PBS-T) at 2 minutes intervals
10. Add 200 μ l of 1:20 diluted Tetra methyl benzadine (1:20 dil.) per well. incubate plates for 30 min. at room temperatures.
11. Add 100 μ l of 0.5 N H_2SO_4 to stop the reaction.
12. Controls : Positive control using *X. c. pv. vesicatoria* culture and negative control using PBS were maintained.
13. Absorbance of the plates was recorded at 450 nm in multi skan plus version 2.03 (U.S.A.)Elisa reader.

3.11.7 Sensitivity of enzyme linked immunosorbent assay in detecting the *X. c. pv. vesicatoria* in tomato seeds.

The experiment was designed by artificially mixing the naturally infected seeds extracted from the diseased fruit cv. Rupali with healthy seeds in different proportion so as to get 0.1, 0.2, 1.0, 10 and 100 per cent infection of diseased seeds in an artificially infested seed samples as shown in table 4. The healthy seeds were extracted from fruits collected from tomato field free from bacterial spot disease. In order to make sure that the seeds are not carrying any bacterium, a sample consisting of 400 seeds was drawn from healthy seed lot and the bacterium (if any) was extracted in water by soaking the seeds in 20 ml distilled water and incubating it for 12 hrs. at 5°C. The seed extract has plated on Tween-80 medium.

Table 4. Sensitivity of Enzyme linked Immuno sorbent Assay in detecting *Xanthomonas campestris* pv. *vesicatoria* in artificially infested seed samples.

Percent infection (%)	Diseased Tomato seeds	Healthy tomato seeds
100	100	0
10	10	90
1.0	1.0	99.0
0.2	1.0	499.0
0.1	1.0	999

The artificially infested seed samples containing different percentage of infected seeds soaked in 20 ml sterile distilled water for 12 hr. at 5°C and the seed suspension was assayed by drawing 100 µl of the seed extract at each level of infestation for ELISA and dilution plates. The procedure carried out for ELISA and dilution plating is as described under 3.11.6.1 and 3.11.8.4 described else where.

The extinction values of ELISA obtained for each level of infestation was compared with the population of *X. c. pv. vesicatoria* detected by dilution plating on Tween-80.

3.11.8 Efficiency of enzyme linked immunosorbent assay in detecting *X. c. pv. vesicatoria* in naturally infected tomato seeds.

The efficiency of enzyme linked Immuno sorbent assay (ELISA) in detecting *X. c. pv. vesicatoria* in naturally infected tomato seeds was evaluated by comparing with the population of the pathogen detected by dilution plating.

3.11.8.1 Seed samples

Tomato seed samples of cv. Rupali, Rajani, Naveen and Rashmi, collected from naturally infected tomato plants were divided into 36 sub-samples consisting of 100, 200 and 400 seeds in each sub samples.

3.11.8.2 Preparation of seed extract

Thirty six seed sub-samples belonging to Rupali, Rajani, Naveen and Rashmi respectively were soaked in 20 ml sterilized distilled water in 100 ml Erlenmeyer flasks separately, and incubated over night at 5°C.

3.11.8.3 Detection by ELISA

The detection of *X. c. pv. vesicatoria* in naturally infected tomato seed was carried out by ELISA as per the protocol described under 3.11.6.1

3.11.8.4 Detection by dilution plating

The bacterial cells released in the seed extract were isolated by dilution plating technique on Tween-80 medium.

One 100 µl of the seed extract for each sub sample and cultivar was poured on the surface of Tween-80 medium contained in sterilized petriplate and the seeds extract was spread on the surface of the agar medium with the help of sterilized spreader. The inoculated plates were incubated at 28°C for 72 hours and the colonies of *X. c. pv. vesicatoria* developing in each plate was counted.

In order to test the efficiency of ELISA, the ELISA extinction values were compared with the colony count obtained for each sub sample for each variety in dilution plating.

3.12. EFFECT OF BACTERICIDES / ANTIBIOTIC SPRAYS ON THE DEVELOPMENT OF BACTERIAL SPOT OF TOMATO CAUSED BY *X. c. pv. vesicatoria* UNDER FIELD CONDITIONS

Two field experiments were conducted during the Kharif 1995 (July planting) and 1996 (July Planting). Tomato seedlings cv. Pusa Ruby were raised in sterilized soil in the nursery in plastic bread boxes. Twenty five days old seedlings were transplanted in the plot measuring 3.6 sq meter with four replication, each replication comprising of 15 plants. The trial was laid out as per RCBD design during July, 1995 and 1996. The tomato strain belonging to race-1 of *X. c. pv. vesicatoria* isolated from GKVK Campus as noted under 3.1 was multiplied in nutrient broth taken in Erleyenmayer flask. The

Table:5 Bactericides / Antibiotics used as sprays to control bacterial spot of tomato caused by *xanthomonas campestris pv. vesicatoria* under field condition

ANTIBACTERICIDES ANTIBIOTICS	CONCENTRATION USED (PPM)
Paushamycin	500
Streptomycin Sulphate	500
Streptocycline	500
Copper Oxychloride	2000
Paushamycin+ Dithane M-45	500 + 2000
Streptomycin Sulphate + Copper Oxychloride	500 + 2000
Streptocycline + Dithane M-45	500 + 2000

inoculated flasks were incubated for 72 hours at 28°C. The concentration of bacterial cells in the suspensions was adjusted to 5×10^7 cfu/ml in spectrophotometer at 480nm (Spectron 20D-Milton and Roy, USA).

The tomato plants were sprayed with bacterial suspension after a week of transplanting in the field in order to induce the disease.

Three sprays were given with bactericides and antibiotics either alone or in combination as given in Table 5 on the 35, 55, and 75 days old plants. The observations on the incidence and severity of the disease was made by recording the number of plants infected and the number of leaves per plant infected, number of infected leaflets / leaf and number of bacterial spots per sq. cm. of the leaflet area. The incidence or severity of the disease was recorded a day before the spraying the plants and four days after the plants were sprayed in order to find out the effect of the bactericides / antibiotics on the development of the disease. The yield of fruits in each treatment was recorded at the end of the experiment.

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

Xanthomonas campestris pv. *vesicatoria* is an incitant of bacterial spot of tomato. The pathogen affects leaves as well as the fruits. The symptoms on the leaves appeared as irregular greasy spots, which became dark brown, frequently with yellow margins (Plate 1). The leaves turned yellow and drop off. On fruit the spot appeared as circular tan coloured upto 8-9 mm in dia, slightly sunken with a central scab and irregular lobed margins (Plate 2).

4.1 ISOLATION OF *X. c.* pv. *vesicatoria* FROM INFECTED TOMATO LEAVES AND FRUITS

Isolation made from the bacterial ooze from the infected leaves and fruits collected from different agroclimatic zones of Karnataka State, in sterile distilled water followed by dilution plate technique on nutrient agar yielded *Xanthomonas* colonies. The colonies were dull white, mucoid, glistening, slimy, convex odourless and round on nutrient agar.

4.2 Purification of bacterial culture

Well separated bacterial colonies (Plate 3) were further purified by streaking on the surface of YDC agar. The cultures were stored on nutrient agar slants at 5°C. and also in sterile distilled water by suspending few loopful of the cultures in 3ml sterile distilled water taken in small screw cap tubes. The strains isolated from tomato plants collected from 14 locations in different agroclimatic zones of Karnataka were designated as Bangalore-1 and Bangalore-2, Chitradurga, Davanagere, Hassan, Kolar, Mangalore, Mysore, Mandya, Shimoga, Tumkur, IIHR-1 and IIHR - 2 (Bangalore) strains.

4.3 IDENTIFICATION OF CAUSAL ORGANISMS

4.3.1 Morphological, physiological and biochemical characteristics

All the 14 strains of *X. c.* pv. *vesicatoria* presented distinguishing characters of *X. c.* pv. *vesicatoria* viz; dull white, coloured colonies on N.A. medium. They were rod shaped, obligately aerobic, Gram negative, oxidase negative, and utilized glucose,

Plate 1. Tomato leaf showing symptoms of bacterial spot due to *X. c. pv. vesicatoria*.

Plate 2. Tomato fruits showing symptoms of bacterial spots due to *X. c. pv. vesicatoria*

a) Infected tomato fruits

b) Healthy tomato fruits

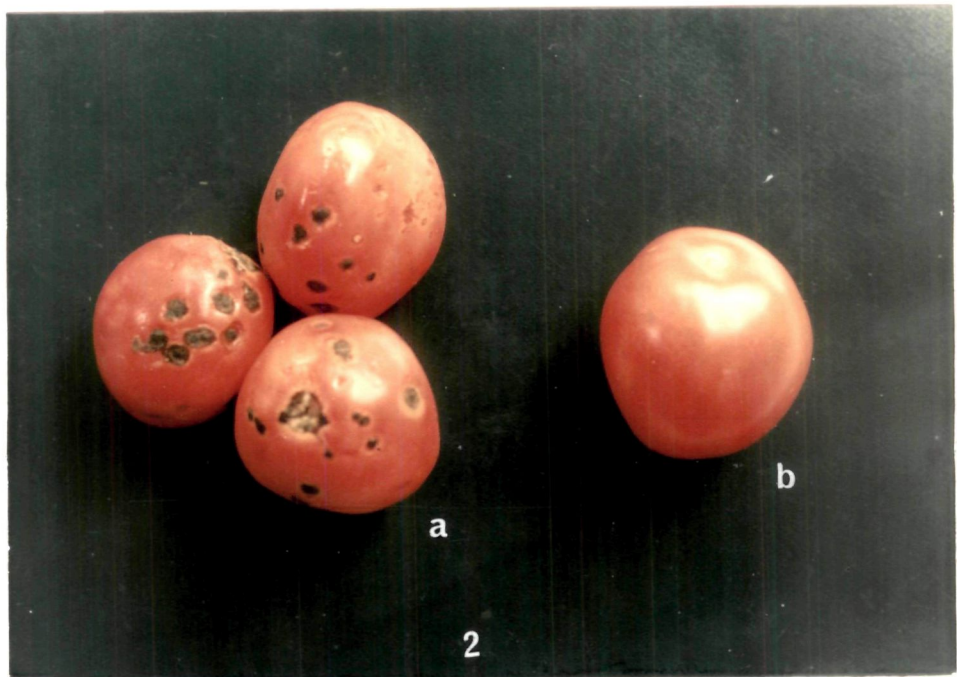
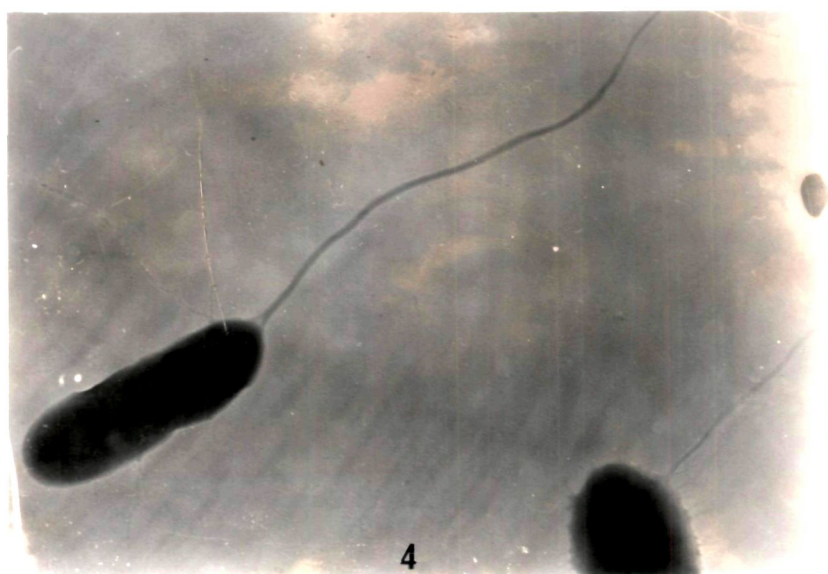
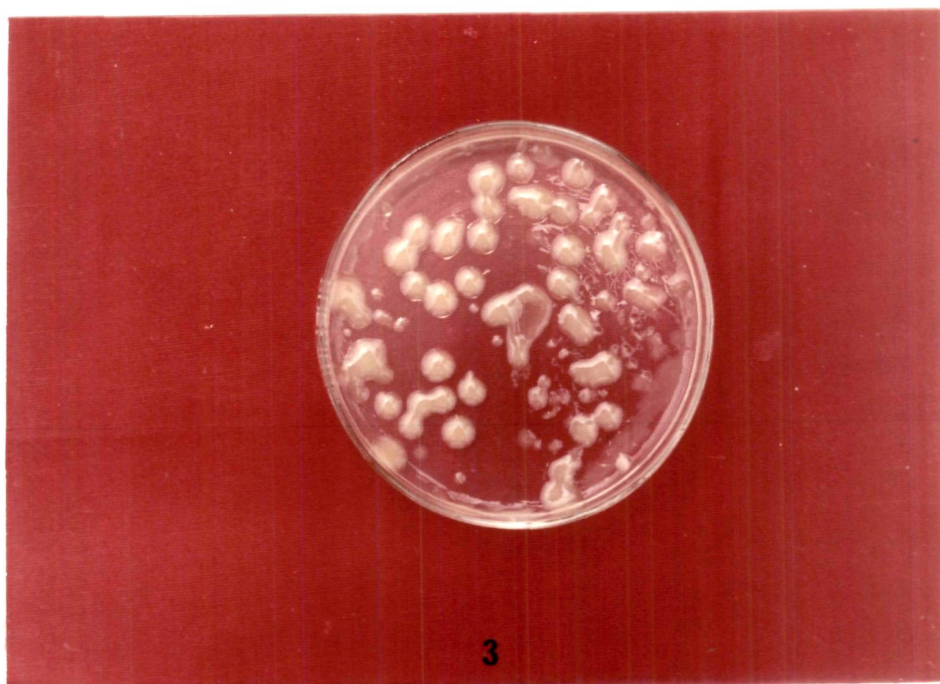


Plate 3. Light yellow colonies of *X. c. pv. vesicatoria* cultivation nutrient broth glucose yeast extract agar.

Plate 4. A single cell of *X. c. pv. vesicatoria* with monotrichous flagellum under Electron microscope (X 20,000).



arabinose, mannose, trehalose and cellobiose for acid production, monotricously flagellated (Plate 4) but none utilized asparagine as a sole source of carbon and nitrogen. They were positive for catalase reaction, hydrolysed geletin and casein and produced hydrogen sulphide and did not produce indole, in addition, the strains failed to reduce nitrates to nitrites (Table 6). Except for one strain viz; Shimoga stains which hydrolysed of starch, the other strains did not hydrolyse starch.

4.3.2 Hypersensitive reaction on tobacco

All the 16 strains of *X. c. pv. vesicatoria* injected into intercellular spaces of tobacco leaves (*Nicotiana tabacum*) var. Samsun produced characteristics water soaked lesions within 16-20 hours of inoculation and after 24 hours the light yellow area started collapsing and formed a desiccated light brown necrotic area (Plate. 5).

4.3.3 Pathogenecity on tomato and pepper plants

The 16 strains of *X. c. pv. vesicatoria*, including two strains from pepper artificially inoculated to 25 days old tomato and pepper seedlings, cv. Pusa Ruby and California wonder respectively, by spraying the bacterial cultures to preinjured leaves were found to be pathogenic to tomato and pepper plants, respectively. The plants started producing small greenish yellow to dark brown spots on the leaves. The yellow lesions becomes necrotic leading to blighting of infected leaves (Plate 6). The first symptoms of the disease was observed 15 days after inoculation of the plants. The tomato strains did not produce any symptoms on pepper plants and similar two pepper strains did not infect the tomato plants.

Re-isolations of the bacterium made from artificially inoculated plants yielded yellow coloured colonies on YDCA and the 14 strains were confirmed to be that of *X. c. pv. vesicatoria*.

4.4 STUDIES ON THE PREVELANCE OF RACES OF *X. c. pv. vesicatoria*.

Studies on the prevelance of races of *X. c. pv. vesicatoria* in Karnataka was carried out by isolating *Xanthomonas campestris. pv. vesicatoria* from diseased tomato specimens collected from different agro climatic zone of the state as described in the materials and methods. The 16 strains of *X. c. pv. vesicatoria* were inoculated

Plate 5. Tobacco (*Nicotiana tabacum* var. Samsun) leaf showing hypersensitive reaction due to infiltration of *X. c. pv. vesicatoria* isolated from infected tomato fruits.

Plate 6. Tomato leaf showing bacterial spot symptoms induced by *X. c. pv. vesicatoria* on artificial inoculation.



Table : 6 Physiological and biochemical characteristics of the 14 strains of *Xanthomonas campestris* pv. *vesicatoria* causing bacterial spot of tomato.

Characteristics	Strains										IIHR (Bangalore)		
	Bangalore GKVK 1	Hoskote 2	Chitra durga	Daven gere	Hassan	Kolar	Mudi gere	Mysore	Mandya	Mangalore Shimoga	Tumkur	1	2
I Morphology:													
Shape	Small rods	Small rods	medium rods	Small rods	Small rods	Small rods	Small rods	Small rods	medium rods	Small rods	Small rods	Small rods	Small rods
Occurrence	In single	In single	In single	In single	In single	In single	In single	In single	In single	In single	In single	In single	In single
Flagellation	Mono trichous	Mono trichous	Mono trichous	Mono trichous	Mono trichous	Mono trichous	Mono trichous	Mono trichous	Mono trichous	Mono trichous	Mono trichous	Mono trichous	Mono trichous
II Staining:													
Gram reaction	G - ve	G - ve	G - ve	G - ve	G - ve	G - ve	G - ve	G - ve	G - ve	G - ve	G - ve	G - ve	G - ve
capsule staining	+	+	+	+	+	+	+	+	+	+	+	+	+
spore stuning	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid fast	-	-	-	-	-	-	-	-	-	-	-	-	-
III Bio chemical characters:													
Utilization of glucose+ arabinose,mannose, trehose, cellobiose, for acid production	+	+	+	+	+	+	+	+	+	+	+	+	+
Utilization of Asparagine as sole source of carbon and nitrogen	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase reaction	+	+	+	+	+	+	+	+	+	+	+	+	+

(10^8 cfu/ml) to three week old tomato, genotypes or hybrids and pepper cultivar/the three near isogenic lines and the results are presented in Table 7. All the strains of *X. c. pv. vesicatoria* isolated from infected tomato plants were found to infect all the popular tomato hybrids viz., Bony Best, Rashmi, Roopali, Rajani, Naveen, Vyshali, NS-386 and NS-815.

It is interesting to now that none of the tomato strain were virulent on tomato genotype Hawaii -7998 and a hypersensitive reaction was produced within 24 hours in the inoculated leaves of this genotypes.

Further all the tomato strains infected Hawaii line 7981 except Shimoga strain which produced on hypersensitive reaction. However, none of the 14 strains isolated from tomato infected tomato genotype H7997, with the exception of Shimoga strain which again produced HR reaction on this genotype. None of the tomato strains infected the highly susceptible pepper cultivar California wonder and the three near isogenic line, viz., ECW 10R, ECW 20R, ECW- 30R carrying resistant genes BS1, BS2, BS3. respectively. Similarly none of the pepper strains obtained from IIHR and Himachal Pradesh (HP) infected any of the tomato cultivars/genotypes, however the two pepper strains infected the susceptible California wonder cultivar and produced typical bacterial spot symptoms within 15 days. Further, the two pepper strains showed some differential reactions on the three isogenic pepper lines. The pepper strain from Himachal Pradesh infected ECW-10R, while ECW-30R was infected with IIHR Bangalore strains. The ECW-20R remained completely free from infection by any of the two pepper strains.

4.5 PHYSIOLOGICAL CHARACTERIZATION OF THE STRAINS OF *X. c. pv. vesicatoria*

4.5.1 Amylolytic activity

Except Shimoga strains of *X. c. pv. vesicatoria* isolated from tomato, none of the strains hydrolysed starch indicating that the strains were negative to amylolytic activity; Even the Shimoga strains showed a weak amylolytic activity (Plate 7) as evidenced by the small and faint zone of hydrolysis in the starch amended medium after 5 days of inoculation (Table 8).

Table 7. Differential reaction of tomato and pepper genotypes / cultivars to 16 strains *Xanthomonas campestris* pv. *vesicatoria* isolated from tomato and pepper plants.

Genotypes/Cultivar	Tomato Isolates										Pepper isolates				
	Bangalore		Chitra	Davane- gere	Hassan	Kolar	Mysore	Mudigere	Shimoga	Tumkur	IIHR		Manga- Mandya lore	IIHR	HP
	1	2	durga								1	2			
TOMATO															
Hawaii-7998	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	HR	-	-
Hawaii-7981	+	+	+	+	+	+	+	+	HR	+	+	+	+	-	-
Hawaii-7997	-	-	-	-	-	-	-	-	HR	-	-	-	-	-	-
Bonybest	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Rashmi	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Roopali	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Rajani	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Naveen	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Vyshali	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
NS-386	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
NS-815	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
PEPPER															
ECW-10R	-	-	-	-	-	-	-	-	-	-	-	-	-	HR	+
ECW-20R	-	-	-	-	-	-	-	-	-	-	-	-	-	HR	HR
ECW-30R	-	-	-	-	-	-	-	-	-	-	-	-	-	+	HR
California Wonder	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+

HR = Hypersensitive reaction ; IIHR= Indian Institute of Horticultural Research Station; HP = Himachal Pradesh.

Plate 7. Plate showing hydrolysis of starch by the tomato strain of *X. c* pv. *vesicatoria* isolated from Shimoga.

Plate 8. Plate showing a single plasmid detected in the strains of *X. c* pv. *vesicatoria* isolated from different agro-climatic zone of the Karnataka.

- | | | |
|----------------|------------------------|----------------------|
| 1. Bangalore-1 | 6. Mudigere | 11. Mangalore |
| 2. Chitradurga | 7. Mysore | 12. Mandya |
| 3. Davangere | 8. Tumkur | 13. IIHR-Bangalore |
| 4. Hassan | 9. Shimoga | 14. Himachal Pradesh |
| 5. Kolar | 10. IIHR-1 (Bangalore) | |

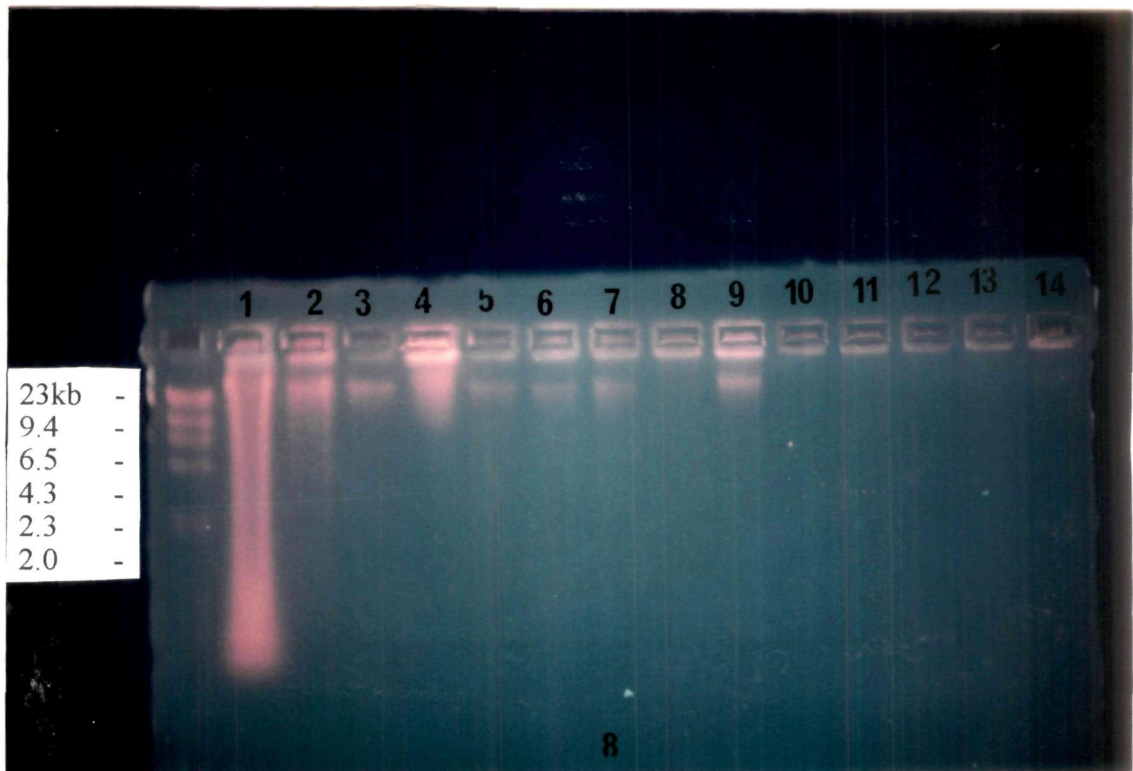
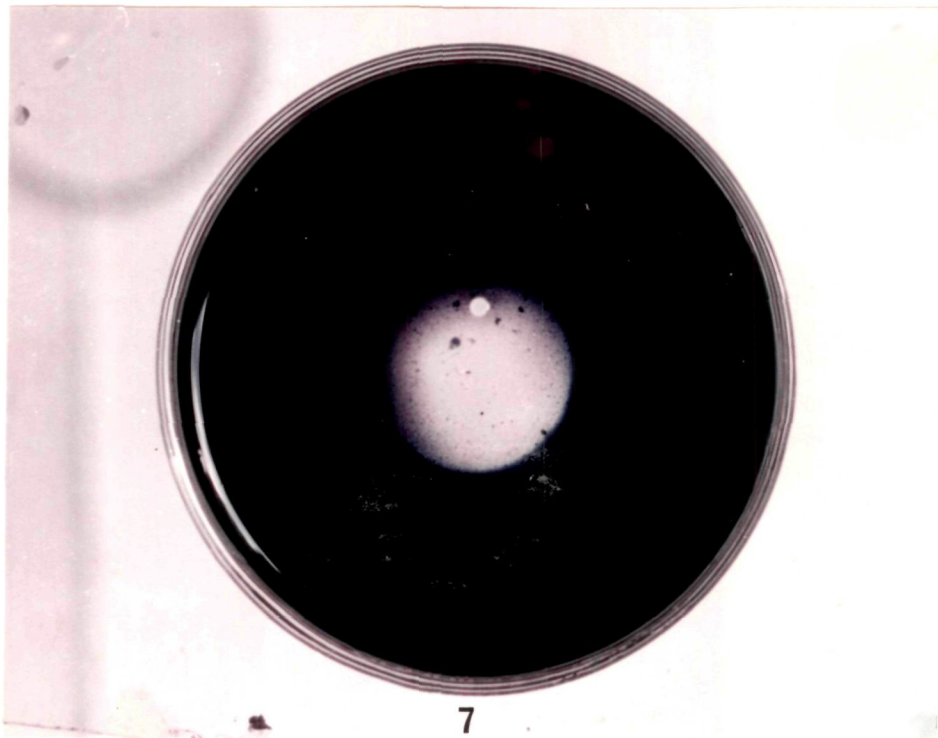


Table 8. Anylolytic and Pectolytic activity of the 16 strains of *Xanthomonas campestris* pv. *vesicatoria*

Location	Strain designation	Host	Amylotic activity	Pectolytic activity
Bangalore (GKVK)	Bangalore-1	Tomato	-	-
Bangalore (Hoskote)	Bangalore-2	Tomato	-	-
Chitradurga	Chitradurga	Tomato	-	-
Davangere	Davangere	Tomato	-	-
Hassan	Hassan	Tomato	-	-
Kolar	Kolar	Tomato	-	-
Mudigere	Mudigere	Tomato	-	-
Mysore	Mysore	Tomato	-	-
Shimoga	Shimoga	Tomato	+	+
Tumkur	Tumkur	Tomato	-	-
IIHR-Bangalore	IIHR-1 (Bangalore)	Tomato	-	-
IIHR-Bangalore	IIHR-2 (Bangalore)	Tomato	-	-
Mangalore	Mangalore	Tomato	-	-
Mandya	Mandya	Tomato	-	-
(Bangalore)	Bangalore	Pepper	-	-
(Himachal Pradesh)	HP	Pepper	-	-

+ = Positive reaction

- = Negative reaction

4.5.2 Pectolytic activity

Out of 16 strains of *X. c. pv. vesicatoria* belonging to tomato and pepper group tested for pectolytic activity, only the Shimoga strain exhibited weak pectolytic activity on crystal violet pectate medium. The strain produced shallow small pits after one week of inoculation (Table 8).

4.6 DETECTION OF PLASMIDS IN THE STRAINS OF *X. c. pv. vesicatoria*

Few colonies of *X. c. pv. vesicatoria* developed on the nutrient medium containing 6000ppm of streptomycin sulphate and in case of copper oxychloride a couple of colonies appeared on medium containing upto 0.8% copper oxychloride. The colonies of *X. c. pv. vesicatoria* which showed resistance to streptomycin sulphate at 6000ppm were picked up, sub-cultured and used for plasmid isolation. The data is presented in table 9.

The plasmid could be isolated by Lysis by boiling method of Holmes and Quigley (1981), while the other two methods i. e., Alkaline lysis method (Birnboim and Doly, 1979) modified by Ish-Horowitz and Burke, (1981) and rapid plasmid isolation method of Wheatcroft and Williams (1981), failed.

The results on the detection of plasmid in the streptomycin sulphate resistant strains of tomato obtained from Bangalore, Chitradurga, Davanagere, Hassan, Kolar, Mudigere, Mysore, Shimoga, Tumkur, Mangalore, Mandya and IIHR and 2 pepper strains obtained from IIHR and Himachal Pradesh of *X. c. pv. vesicatoria* are presented in Table 10.

The number of plasmids noticed in the different strains was only one. The tomato strains from Bangalore, Chitradurga, Davanagere, Hassan, Kolar, Mudigere, Mysore, Shimoga and the pepper strain from Himachal Pradesh possessed a single plasmid, while no plasmid was detected in tomato strains isolated from Mandya, Tumkur, IIHR, (One strain) Mangalore and the pepper strain from IIHR-1 (Bangalore). The plasmid size determined by electrophoretic mobility in agarose gels using lambda Hind III marker revealed that single plasmid detected in the 9 strains were approximately 30 kb in size (Plate 8).

Table 9. Development of resistance to antibiotic and bactericide by the 16 strains of *Xanthomonas campestris* pv. *vesicatoria* isolated from tomato and pepper plants.

Different strains	Streptomycin sulphate concentration (ppm)							Copper sulphate concentration (ppm)						
	1000	2000	4000	5000	6000	7000	8000	10000	4000	5000	7000	8000	9000	10000
Bangalore (GKVK)-1	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Bangalore (Hoskote)-2	-	-	-	-	-	-	-	-	+	+	+	+	-	-
Chitradurga	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Davangere	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Hassan	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Kolar	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Mudigere	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Mysore	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Shimoga	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Tumkur	+	+	+	+	+	-	-	-	+	+	+	+	-	-
IIHR-1	-	-	-	-	-	-	-	-	+	+	+	+	-	-
IIHR-2	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Mangalore	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Mandya	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Pepper IIHR-(Bangalore)	+	+	+	+	+	-	-	-	+	+	+	+	-	-
Pepper (HP)	+	+	+	+	+	-	-	-	+	+	+	+	-	-

+ = Resistance to streptomycin and copper sulphate.

- = Sensitivity to streptomycin and copper sulphate.

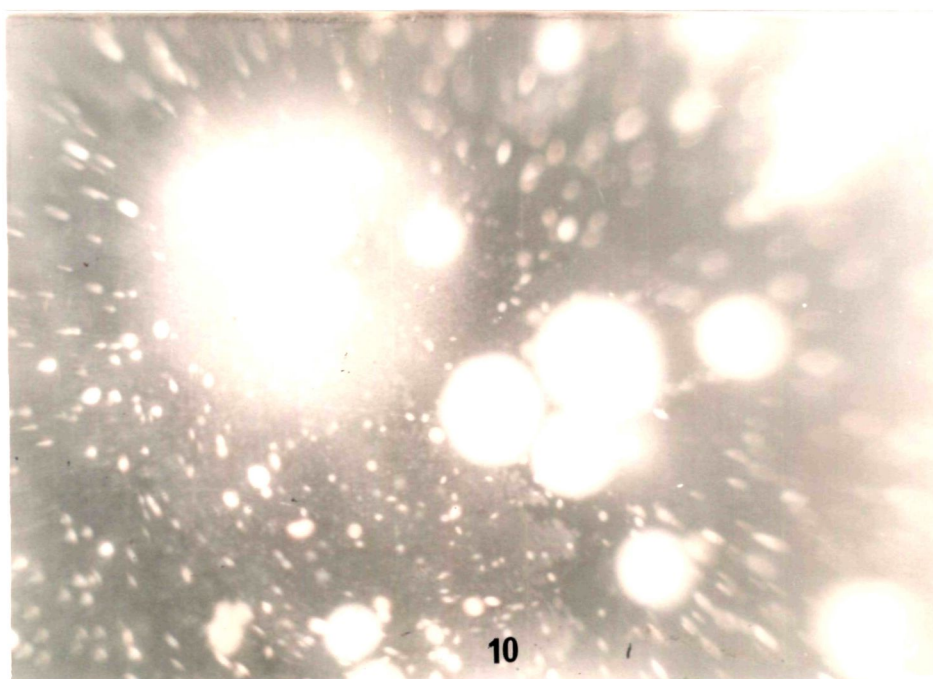
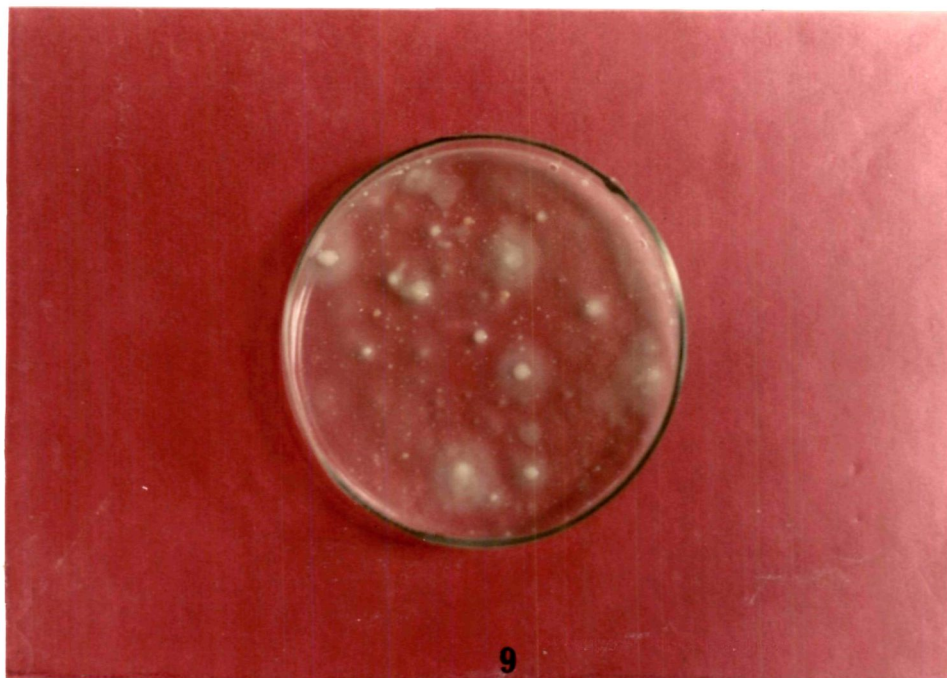
Table 10. Detection of plasmid in 14 strains of *Xanthomonas campestris* pv. *vesicatoria*.

Sl. No.	Strain	Detection of plasmid
<u>Tomato group</u>		
1	Bangalore	+
2	Chitradurga	+
3	Davanagere	+
4	Hassan	+
5	Kolar	+
6	Mudigere	+
7	Mysore	+
8	Tumkur	-
9	Shimoga	+
10	II HR -1	-
11	Mangalore	-
12	Mandya	-
<u>Pepper group</u>		
13	Bangalore (IIHR)	-
14	Himachal Pradesh	+

Plate 9. Detection of *X. c. pv. vesicatoria* from the seed of naturally infected tomato on Tween-80 medium.

Plate 10. Colonies characteristics of *X. c. pv. vesicatoria* on Tween-80 medium.

Note the whitish halo zone around the bacterial colonies.



4.7 STUDIES ON THE SURVIVAL OF *X. c. pv. vesicatoria*

4.7.1 Survival of *X. c. pv. vesicatoria* on tomato seeds

The bacterium were detected on the dry tomato seeds stored at 5°C for a period of 7 1/2 months, while it was detected upto 6 1/2 months on seeds stored at room temperature on Tween-80 medium. The bacterial population was gradually reduced from 25.67 cfu/ml from 0 day storage to 1.0 cfu / ml after 7 1/2 months in case of seed stored at 5°C. Similar trend was observed in seeds stored at room temperature in which 25.67 cfu/ml declined to 3.17 cfu/ml on the 6 1/2 months of sampling as seen from the data presented in Table 11, Figure 1 and Plate 9.

The colonies of *X. c. pv. vesicatoria* and Tween-80 medium were white, circular, raised, surrounded by a halogone comprising of whitish crystal (Plate 10).

4.7.2 Crop residues and infested soils.

The survival of *Xanthomonas campestris pv. vesicatoria* in the soil and crop residue buried at different depths in both sterilized and unsterilized soil was investigated.

The soil samples were assayed at an interval of 15 days for the population of the pathogen by dilution plating technique using Tween-80 medium.

It is seen from the data presented in table 12 that the infected crop residues burried at 15 cm depth in soil, the pathogen did not survive beyond 120 days in both the sterilized and unsterilised soils. The population of the pathogen on the 15th day of burrial of crop residue in soil was 51 cfu/g, which gradually declined to 5cfu/g of soil on the 120th day in sterilized soil and in unsterilized soil the pathogen population which was 41cfu/g on the 15th day, was reduced to 2.53cfu/g of soil on the 120th day.

The bacterial population survived beyond 135 days in the treatment in which the crop residue was burried at 7.5 cm. depth in both the sterilized and unsterilized soils. The population of the pathogen which was 95.5 safe you per gram of soil on the 15th day and gradually declined to 6.73cfu/g on the 135th day in sterilized soil and in unsterilized soil the pathogen population which was 76.67cfu/g on the 15th day was reduced to 4.73cfu/g on the 135th day. However, the bacterial pathogen could be recovered beyond

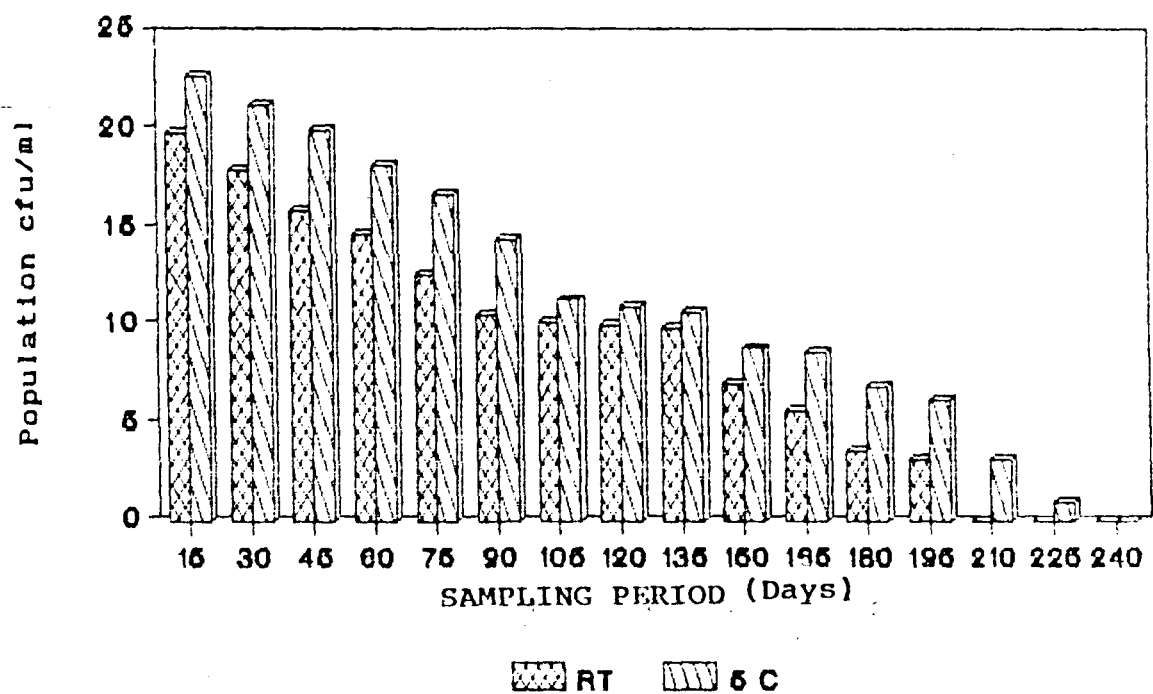


Fig1: Recovery of *Xanthomonas campestris* pv. *vesicatoria* from naturally infected tomato seed.

Table 11. Recovery of *Xanthomonas campestris* pv. *vesicatoria* in naturally infected tomato seeds under different storage conditions.

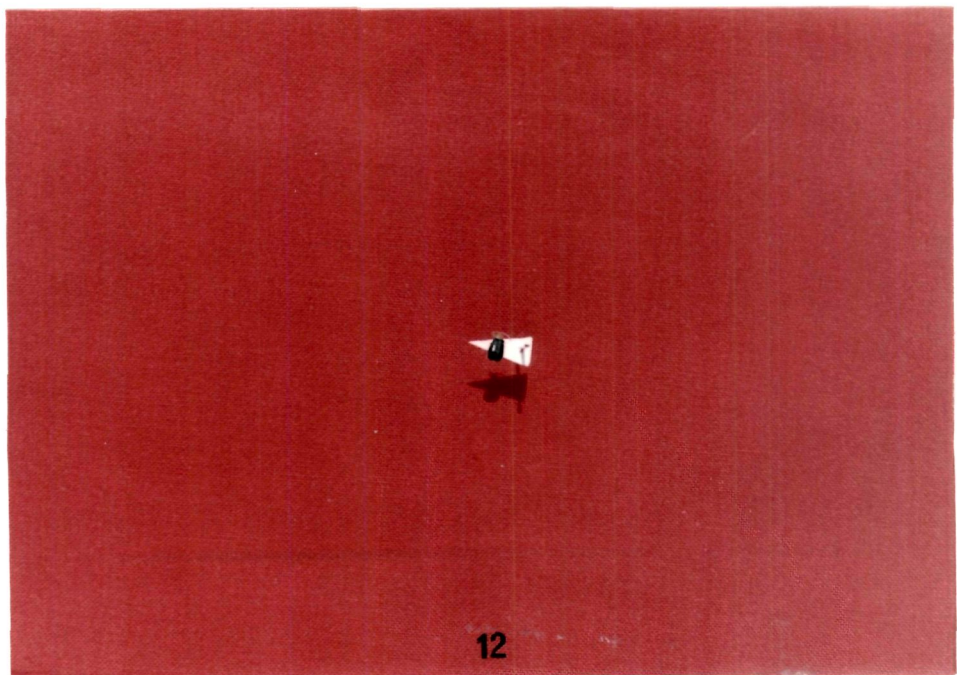
Duration of observation (Days)	Population <i>X. c.</i> pv. <i>vesicatoria</i> (cfu/ml seed extract)	
	RT	5°C
0	25.67	25.67
15	19.87	22.73
30	18.00	21.20
45	15.87	20.00
60	14.67	18.20
75	12.67	16.67
90	10.63	14.40
105	10.17	11.33
120	10.00	11.00
135	9.87	10.77
150	7.00	8.87
165	5.67	8.67
180	3.63	6.87
195	3.17	6.20
210	0.00	3.17
225	0.00	1.00
240	0.00	0.00

RT = Room temperature (25±3C°)

Plate 11. Colonies of *X. c. pv. vesicatoria* on Tween-80 medium isolated from the leaves of weed species.

1. *Cyperus rotundus*
2. *Cynodon dactylon*
3. *Marsellia quadrifolia*

Plate 12. Plate showing Chrysomelid beetle from which *X. c. pv. vesicatoria* was isolated.



180 days, in the treatment in which the crop residues was placed at surface of soil. The population of the pathogen on the 15 day was 1.05×10^2 cfu/g, gradually declined to 1.0 cfu / g on 180th day in sterilized soil and in unsterilized soil the pathogen population declined from 89.67cfu/g on 15th day to 2.87cfu/g of soil on the 165th day (Table 12).

However, the pathogen could be recovered upto 240 days in sterilized soil infested artificially, and the population of the pathogen on the 15th day was 1.10×10^2 cfu/g, gradually declined to 0.67cfu/g on the 240 day. In case of unsterilized soil the Pathogen population was 93.33cfu/g on the 15th day and gradually reduced to 0.33cfu/g by the 195th day.

4.7. 4 Epiphytic survival *X. c. pv. vesicatoria* on weeds and non-host crop plants

During the sampling period, fourteen weed species and one non-host bean plants *Phaseolus vulgaris* were assayed for the presence of *X. c. pv. vesicatoria* and the data presented in Table 13 and Plate 11. The bacterium was isolated for a period of 60 days from *Cyperus rotundus* and *Cynodon dactylon*, while it could survive only for 15 days on *Hemilia sanchifolia* and *Marselia quadrifolia* as evidence by the number of bacterial colonies isolated on Tween-80 medium. The bacterial population was gradually reduced from 40cfu/g leaf from 15th day sampling to 12cfu/g leaf on the 60th day in case of *Cyperus rotundus*. Similar trend was observed in case of *Cynodon dactylon* (36cfu/g leaf to 8cfu/g leaf) also during corresponding period of sampling.

The identity of the colonies was confirmed by studying morphological and staining characteristics of the bacteria, serologically by Outcherlouys agar double diffusion test and hyper sensitive reaction on tobacco leaves cv. Samsun.

4.8. STUDIES ON THE ROLE OF INSECTS IN TRANSMITTING *X.c. pv. vesicatoria*

Six insects species collected from the bacterial spot affected tomato plants in the fields were assayed for the presence of *X. c. pv. vesicatoria*. The bacterium was isolated from four insects species viz., *Aulacophora cincta*, *Eucoptera praemorsa*, *Henosepilachna vigintioctopunctata* and *Phenoroptera gracillus* (Table 14 and plate 12 to 15). It is interesting to note that the bacterium was isolated from the insects with biting and chewing type of mouth parts only while it was not isolated from white flies (*Bemisia tabaci*) which possessed piercing and sucking type of mouth parts.

Plate 13. Plate showing coccinellid beetle from which *X. c. pv. vesicatoria* was isolated.

Plate 14. Plate showing long horned grasshopper from which *X.c. pv. vesicatoria* was isolated.

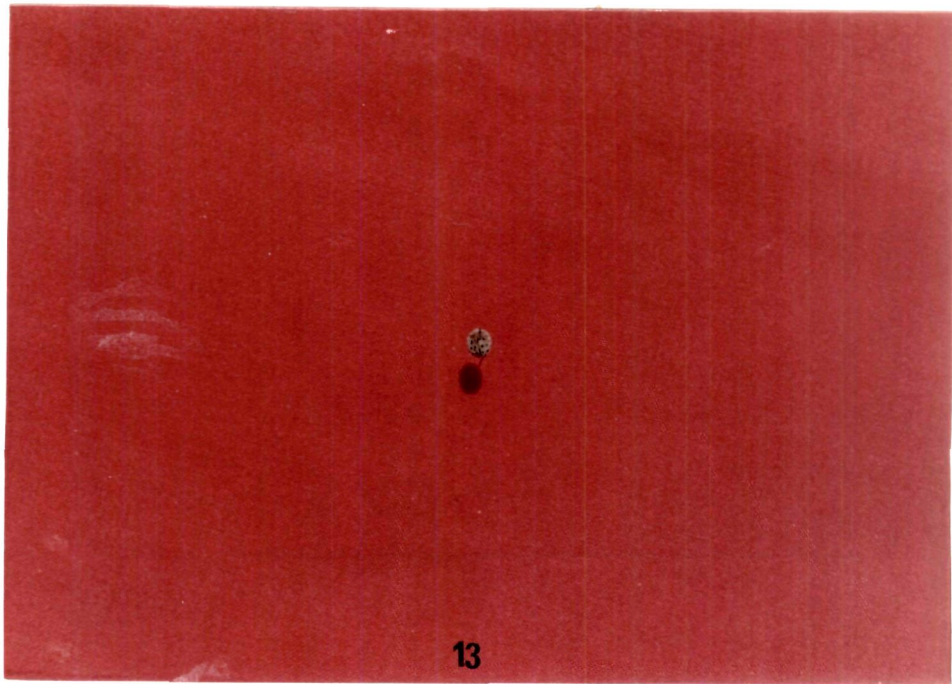


Table 12. Recovery of *Xanthomonas campestris* pv. *vesicatoria* from the infected crop residue buried at different depths or placed on surface of soil.

Treatment	Sampling period (1995-96)															
	(Population of pathogen in cfu/g of soil)															
No. of days	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240
1. Crop residue buried at 7.5cm																
a) Sterilised soil	95.50	83.50	82.33	80.00	75.67	68.67	54.67	27.00	6.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00
b) Unsterilised soil	76.67	73.00	69.83	69.17	60.50	54.00	32.67	16.83	4.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2. Crop residue buried at 15cm																
a) Sterilised soil	51.00	37.53	32.83	31.33	24.67	21.67	14.50	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
b) Unsterilised soil	41.10	29.00	27.67	26.17	20.33	15.33	9.17	2.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3. Crop residue on surface of soil																
a) Sterilised soil	1.05x10 ²	81.17	77.00	75.33	68.67	63.17	53.83	33.50	20.67	9.53	4.73	1.00	0.00	0.00	0.00	0.00
b) Unsterilised soil	89.67	74.17	71.33	68.67	60.67	52.67	40.17	30.00	19.17	6.67	2.87	0.00	0.00	0.00	0.00	0.00
4. Infested soil																
a) Sterilised soil	1.10x10 ²	95.33	85.00	84.00	81.67	76.33	68.33	62.00	41.50	29.00	15.87	8.33	4.37	2.93	1.67	0.67
b) Unsterilised soil	93.33	84.67	76.83	75.67	72.50	65.67	53.00	45.00	27.83	19.50	5.00	1.30	0.33	0.00	0.00	0.00

Plate 15. Plate showing short horned grass hopper from which *X. c. pv. vesicatoria* was isolated.

Plate 16. Plate showing the colonies of *X. c. pv. vesicatoria* isolated from the mouth parts of Chrysomelid beetle on Tween-80 medium.

Note the typical crystallized zone around the colonies of *X. c. pv. vesicatoria* on Tween-80 medium.

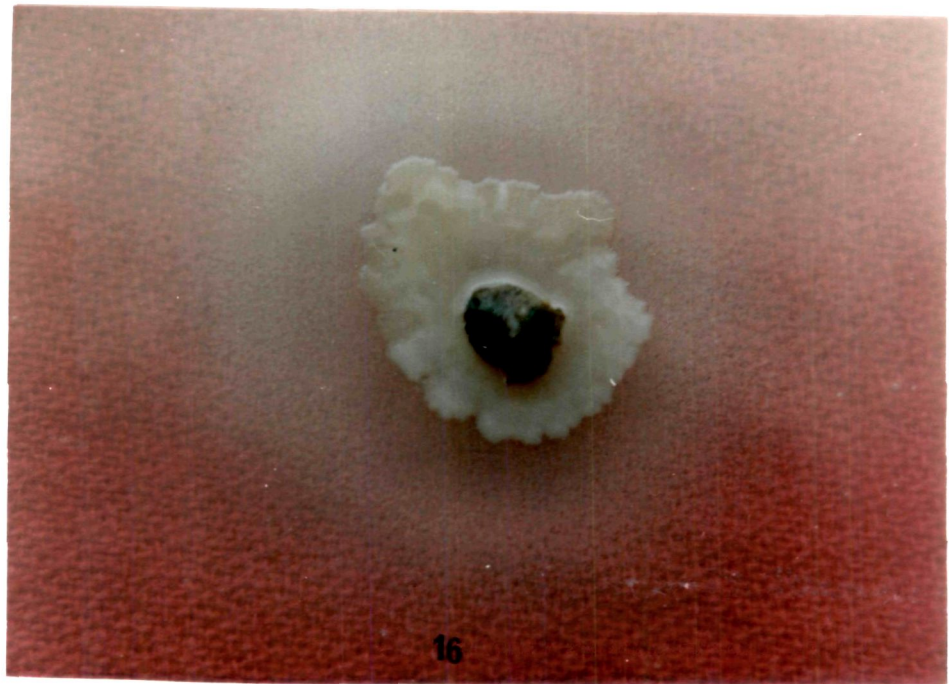
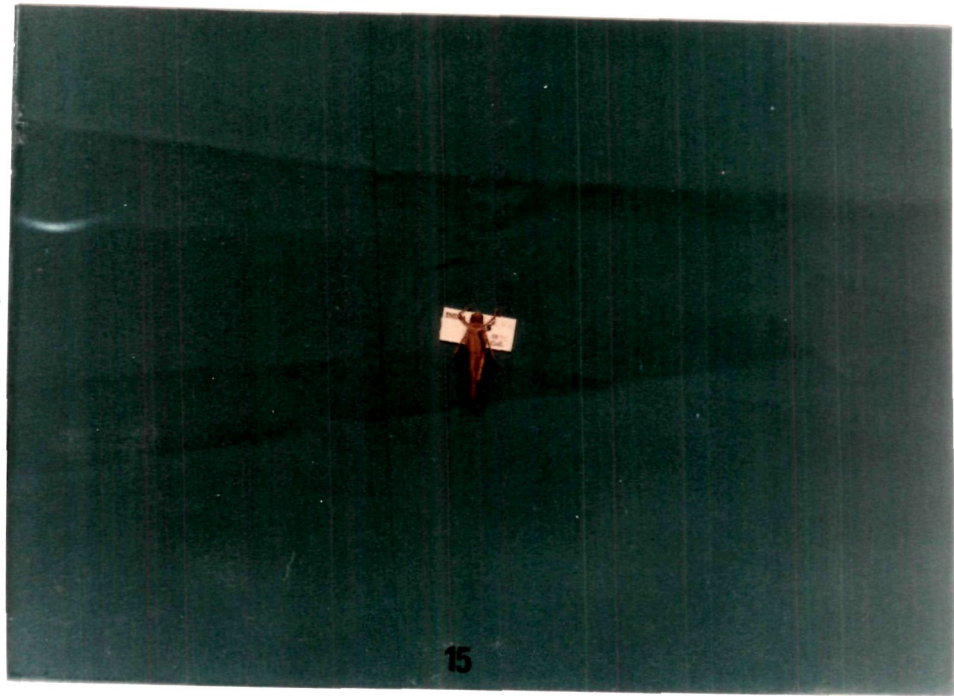


Table 13. Epiphytic Survival of *Xanthomonas campestris* pv. *vesicatoria* on weed species and non host crop plant.

Weed species	Sampling intervals (in days ^a) (Population in cfu/ml)				
	15	30	45	60	75
I Weeds					
<i>Ageratum conyzoides</i> Linn.	-	-	-	-	-
<i>Bidens pilosa</i> L.	-	-	-	-	-
<i>Burhhoea diffusa</i> L.	-	-	-	-	-
<i>Commelina benghalensis</i> Linn.	-	-	-	-	-
<i>Cyanotis cristata</i> (L.)D Don	-	-	-	-	-
<i>Cynodon dactylon</i> Pers.	36.00	18.00	14.00	8.00	0
<i>Cyperus rotundus</i> Linn.	40.00	22.67	18.67	12.00	0
<i>Digitaria marginata</i> Link.	-	-	-	-	-
<i>Echinochloa Stagnina</i> (Retz)Beauv	-	-	-	-	-
<i>Euphorbia geniculata</i> Ort.	-	-	-	-	-
<i>Hemilia sanchifolia</i> L.	3.00	-	-	-	-
<i>Legascea mollis</i> Cav.					
<i>Marselia quadrifolia</i> L.	4.67	-	-	-	-
<i>Tridax procumbens</i> Linn.	-	-	-	-	-
II Non-host crop plants					
<i>Phaseolus vulgaris</i> Linn.	-	-	-	-	-

a = Sampling and isolation done after harvest of tomato plants.

- = Pathogen was not detected.

Table 14. Isolation of *Xanthomonas campestris* pv. *vesicatoria* from the mouth parts of insects collected from bacterial spot affected tomato plants in the field.

Sl. No.	Common name	Name of insect	Mouth parts	Detection of XCV
1.	Chrysomelid beetle	<i>Aulacophora cincta</i> Fabr.	Biting and chewing type	+
2.	White fly	<i>Bemisia tabaci</i> Gennadius.	Piercing and sucking type	-
3.	Long horned grasshopper	<i>Eucoptaera praemorsa</i> Stal.	Biting and chewing type	+
4.	Coccinellid beetle	<i>Henosepilchna vigintioctapunctata</i> Fabr.	Biting and chewing type	+
5.	Coccinellid beetle (Predatory beetle)	<i>Menochilus</i> sp. Fabr.	Biting and chewing type	-
6.	Short horned grasshopper	<i>Phenoptera gracillus</i> Burmelster.	Biting and chewing type	+

XCV - *Xanthomonas campestris* pv. *vesicatoria*

Plate 17. Tobacco leaves var. Samsun showing the Hypersensitive reaction induced by *X. c.* pv. *vesicatoria* isolated from the mouth parts of insects (i) Coccinellid beetle (2) long horned grass hoppers.



The bacterial colonies on Tween-80 medium appeared as circular, convex white surrounded by a narrow hallow zone (Plate 16). Further, the identity of the bacterium was confirmed serologically by Outcherlouys agar double diffusion technique and by the hypersensitive reaction on tobacco cv. Samsun plants (Plate 17).

4.9 EFFECT OF BACTERIAL SPOT ON THE QUALITY OF TOMATO FRUIT

The data on the various quality parameter of tomato fruits, cv. Pusa Ruby, like total soluble solids (TSS), per cent reducing sugar, total sugar and ascarbic acid content of tomato in relation to infection of fruits by *X. c. pv. vesicatoria* are given in Table. 15

The TSS accounting to 5.56°B was recorded in healthy tomato compared to 5.39°B in the diseased fruits. There was no significant difference between the disease and healthy tomato fruits with respect to the per cent reducing sugar and total sugar. The per cent reducing sugar recorded in healthy tomato (1.53%) was allmost on par with that of the diseased tomato fruits (1.52%). Similarly the total sugar content in healthy tomato fruit was 2.17% compared to 2.15% in diseased fruits.

A slight differences in the ascorbic acid content of diseased and healthy tomato was observed. The ascorbic acid content of the healthy tomato fruits was 23.02mg/100g of fruits as compared to 22.78 mg/100g in case of diseased tomato fruits.

4.10 STUDIES ON THE ENVIRONMENTAL FACTORS FAVOURING THE DEVELOPMENT OF BACTERIAL SPOT OF TOMATO

The data on field trial conducted during the years 1995 and 1996, to study the correlation between the weather data such as Temperature, Relative humidity and Rainfall with the development of bacterial spot of tomato are presented in Table 16 and Fig. 2. The data are analysed by simple correlation.

It is seen from the Table 16 that disease incidence started increasing gradually from 2nd fortnight of July to 2nd Fortnight of Nov. 1995. Incidence of disease as measured by percentage of plants infected during this period ranged from 15 to 58 per cent and the maximum temperature was around 28 to 29°C and minimum temperature ranged between 16.4 to 21°C. The relative humidity was also fairly high during this period

Table 15. Effect of bacterial spot disease on the quality of tomato fruits (cv. Pusa Ruby).

Nature of fruits/ Quality	TSS P ^o Brix	Reducing sugar (%)	Total Sugar (%)	Ascorbic acid (mg/100gm)
Diseased tomato fruit	5.39	1.52	2.15	22.78
Healthy tomato fruit	5.56	1.53	2.17	23.02
F. test	NS	NS	NS	NS
SEm $\pm$	0.120	0.030	0.046	0.300
CD at 5%	0.2644	0.0622	0.0980	0.6589

(Average of 6 fruit and 3 replication)

Table 16. Development of Bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* in relation to weather conditions prevailing during 1995-96.

Standard days		Temperature °C		Relative humidity(%)		Rain fall (MM)	Percentage of Plants infected	Per cent leaf infected/ plant	Per cent leaf let infected /leaf	No. of spots /cm ² leaflets
		Max.	Min.	I	II					
16th July - 29th July	1995	28.90	20.50	91.0	62.5	48.05	15.00	30.50	14.50	04.23
30th July - 12th August	1995	28.70	20.25	93.0	62.0	33.00	17.00	38.00	22.83	05.38
13th Aug. - 26th Aug	1995	29.00	20.45	94.0	65.5	54.30	24.10	44.50	27.80	06.50
27th Aug. - 9th Sep.	1995	27.85	19.90	91.5	69.0	27.65	28.50	48.10	38.50	08.78
10th Sep. - 23rd Sep.	1995	28.60	19.90	93.5	60.5	22.95	35.00	50.00	40.16	09.50
24th Sep. - 7th Oct.	1995	29.40	20.15	94.0	62.0	48.35	42.50	55.50	45.18	10.50
8th Oct. - 21st Oct.	1995	28.20	19.65	92.5	66.0	32.30	52.10	68.00	48.16	11.60
22nd Oct. - 4th Nov.	1995	28.25	19.55	95.0	61.5	11.25	58.00	78.50	55.00	11.80
5th Nov. - 18th Nov.	1995	28.70	18.45	92.0	46.5	12.30	40.10	80.00	70.50	12.50
19th Nov. - 2nd Dec.	1995	28.55	16.40	93.0	49.0	12.30	22.00	55.00	65.00	12.80
3rd Dec. - 16th Dec.	1995	27.15	15.15	92.0	39.0	00.00	05.00	20.00	40.00	08.00
17th Dec. - 31st Dec.	1995	26.75	12.25	85.0	35.5	00.00	00.00	00.00	00.00	00.00
1st Jan. - 14th Jan.	1996	27.35	14.70	91.0	78.0	00.00	00.00	00.00	00.00	00.00
15th Jan. - 28th Jan.	1996	28.60	15.00	78.0	29.5	00.00	00.00	00.00	00.00	00.00
29th Jan. - 11th Feb.	1996	28.00	12.00	79.0	23.0	00.00	00.00	00.00	00.00	00.00
12th Feb. - 25th Feb.	1996	28.75	14.90	82.0	32.0	00.00	00.00	00.00	00.00	00.00
26th Feb. - 11th March	1996	32.00	16.25	74.0	34.5	00.00	00.00	00.00	00.00	00.00
12th March- 25th March	1996	32.90	14.75	70.0	32.0	00.00	00.00	00.00	00.00	00.00
26th March- 8th April	1996	34.55	19.35	78.5	31.0	00.00	00.00	00.00	00.00	00.00
9th April- 22nd April	1996	32.50	19.85	82.0	40.0	00.00	00.00	00.00	00.00	00.00
23rd April- 6th May	1996	32.65	20.75	77.0	52.5	00.00	00.00	00.00	00.00	00.00
7th May - 20th May	1996	35.00	20.25	86.5	34.5	00.00	00.00	00.00	00.00	00.00
21st May - 3rd June	1996	34.55	19.70	79.0	41.0	32.10	8.350	25.00	12.00	05.00
4th June - 17th June	1996	31.85	18.50	88.5	54.0	64.50	15.50	42.25	15.50	06.30
18th June - 1st July	1996	25.85	18.85	90.5	73.5	83.90	25.75	58.40	35.00	07.10
2nd July - 15th July	1996	30.10	18.90	88.0	51.5	08.10	29.30	60.50	48.20	08.80
16th July - 29th July	1996	27.60	18.60	88.0	68.5	07.75	35.38	65.80	52.50	09.50
30th July - 12th August	1996	28.20	18.20	88.0	69.5	03.20	40.00	68.50	65.00	10.50
13th Aug. - 26th Aug.	1996	27.50	17.85	89.5	67.0	36.90	35.35	75.50	72.00	12.00
27th Aug. - 9th Sep.	1996	27.00	17.05	91.0	70.5	74.80	48.50	80.25	80.00	13.50
10th Sep. - 23rd Sep.	1996	26.65	17.75	92.0	73.0	48.90	50.50	65.00	55.00	14.00
24th Sep. - 7th Oct.	1996	27.15	17.40	92.5	73.5	26.90	42.60	60.00	48.00	15.00
8th Oct. - 21st Oct.	1996	26.80	16.25	91.5	71.5	22.55	35.20	40.25	30.00	12.30

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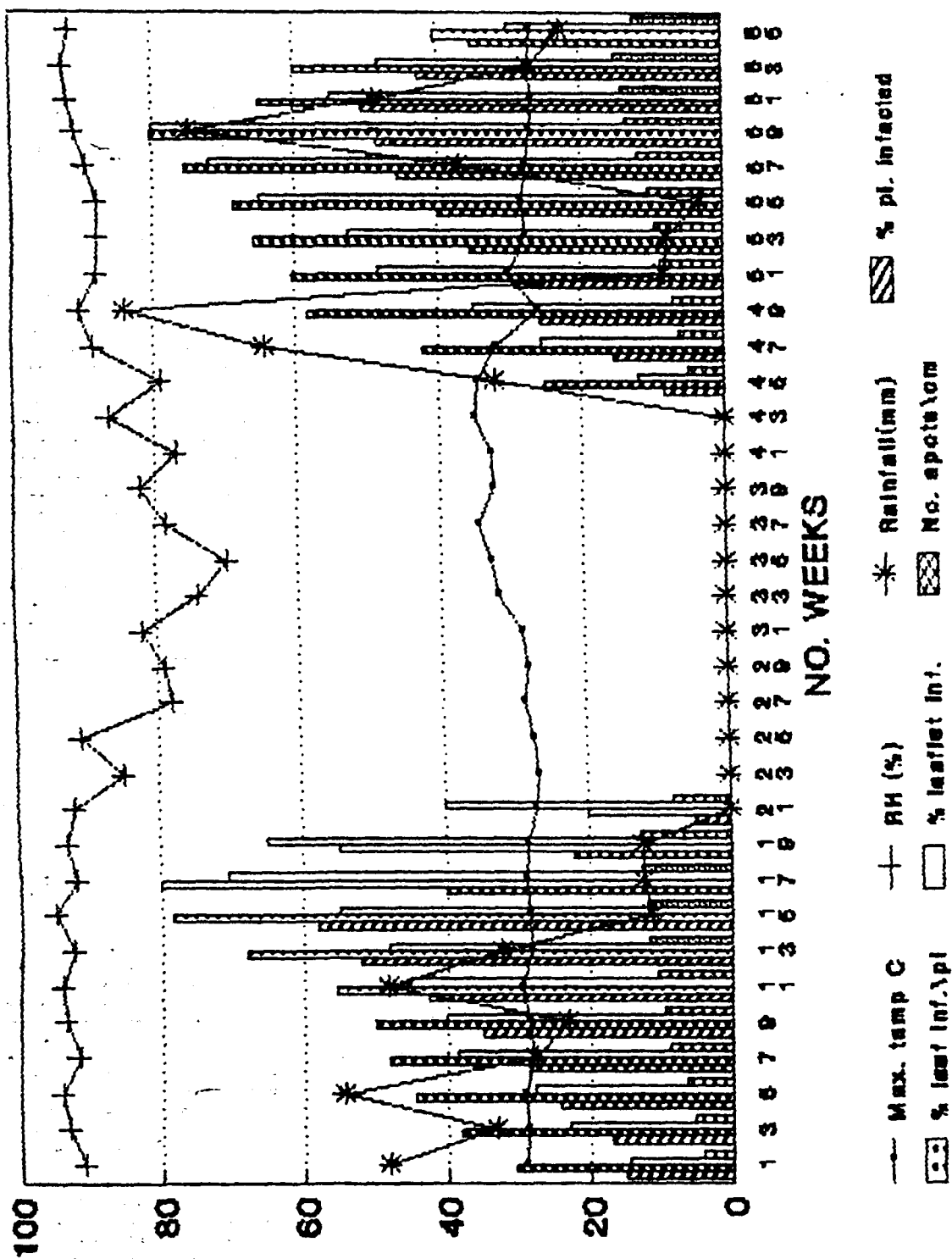


Fig. 2 Influence of weather conditions on the development of bacterial spot of tomato caused by *X. c. pv. vesicatoria* (1995-96).

(91-95 per cent.) Similarly good rainfall was ranging from 12.30 to 48.35mm was received during these months. The severity of disease gradually increased in terms of per cent leaf infected per plant, per cent leaf let infected per leaf and no. of spots per cm² area of leaflet. The number of bacterial spots per cm² area of leaf let ranged from 4.32 to 12.8.

However, the disease incidence suddenly declined to 5 per cent and severity of disease was also reduced to 20 per cent of leaf infected per plant in crop planted during the first fortnight of Dec. 1995 as compared to high severity of disease ranging from 30 to 80 per cent of leaves infected per plant during 2nd fortnight of July to 2nd fortnight of November. No disease incidence was recorded from 2nd fortnight of December to 3rd week of May 1996. Again the disease started appearing during 3rd week of May and gradually increased upto 3rd week of October 1996 and the incidence of the disease ranging from 8.35 to 35.20 per cent and severity of disease also was fairly high ranging 25 to 80.25 per cent leaves infected and number of bacterial spots per cm² recorded ranged from 5 to 15 and the maximum temperature during corresponding period ranged from 27 to 31.85°C and relative humidity was also fairly high in the range of 79 to 92 per cent and good rainfall also was recorded as seen from the data presented in Table 16 and Fig. 2.

Correlation coefficient analysis done to establish relationship between environmental factors and disease development during 1995-96 revealed that as the maximum temperature increases the disease incidence decreases, since no disease was observed during summer months, viz., from February to May 1996. during which the maximum temperature ranged from 35-32°C. Positive correlation could be established (Table 17) between Relative humidity and rainfall with the incidence, and severity of bacterial spot.

4.11 DETECTION OF *X. c. pv. vesicatoria* IN NATURALLY INFECTED TOMAOT SEEDS BY SEROLOGICAL TECHNIQUES.

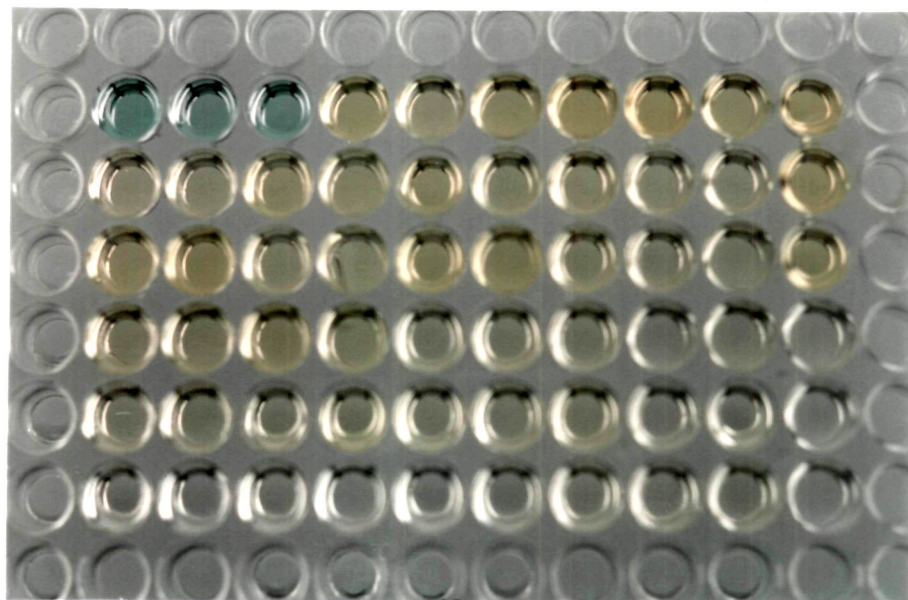
4.11.1 Application of enzyme linked immunosorbent assay for the identification and detection of *X. c. pv. vesicatoria*

Table 17. Correlation between disease incidence and environmental factors prevailing during 1995-96.

Weather factors	Correlation Co-efficient "r"			
	Percentage of plants infected	Per cent leaves infected / plant	Per cent leaflets infected / plant	No. of spots/cm ² leaf let
Maximum temperature	-0.5015	-0.3917	-0.4572	-0.5185
Minimum temperature	-0.1526	-0.0473	-0.4362	-0.5231
Relative humidity I	0.3733	0.2471	0.2425	0.3613
Relative humidity II	0.6334	0.4167	0.1685	0.3352
Rainfall	0.0366	0.0022	0.1982	0.1960

Plate 18. Identification of *X. c. pv. vesicatoria* by ELISA using HRPO enzyme conjugate and Tetramethyl benzidine substrate system, well on B.2-4 control with distilled water- Blue colour.

Note the decrease in the intensity of yellow colour with increase dilution of yellow colour with increase dilution of homologous suspension of *X. c. pv. vesicatoria*.



4. 11. 1. 1. Specificity of antiserum and identification of *X. c. pv. vesicatoria*

The protein concentration of γ -globulin was adjusted to 900 μ g/ml.

No cross reaction occurred with the any of pathovars of *X. campestris*, except with one strain of undiluted suspension of *X. c. pv. phaseoli*. However, the extinction value of *X. c. pv. phaseoli* was always lower as compared to *X. c. pv. vesicatoria* as seen from the data presented in Table 18.

The optimum dilution of coating γ -globulin was found to 1:800 and the optimum dilution of enzyme conjugate was 1:1000.

The ELISA carried out with the homologous antigen i.e. *X. c. pv. vesicatoria* race-1 gave extinction values of 0.320 for 10.5×10^4 cfu/ml cells and 0.225 for 54×10^5 cfu/ml at 450nm wavelength (Table 19).

Further, ELISA technique was standardized for the purpose of identifying *X. c. pv. vesicatoria* by using a new Enzyme-Substrate System viz., HRPO enzyme conjugate and Tetramethyl benziden substrate for the first time in place of Alkaline phosphatase and generally used in ELISA for detecting bacterial pathogens.

4.11.2 Detection of *X. c. pv. vesicatoria* in the naturally infected tomato seeds by enzyme linked immuno sorbent assay (ELISA)

The data on the detection *X. c. pv. vesicatoria* in naturally infected tomato seeds by ELISA are presented in Table 20 and Plate 18. Of the 36 seed sub samples belonging to four tomato cultivar viz., Rupali, Rajani, Naveen and Rashmi tested by ELISA. 30 seed samples were found to be positive for the presence of *X. c. pv. vesicatoria* in the seeds. Extinction values ranging from 0.227 to 0.237 were recorded in sub sample consisting of 400 seeds (cv. Rajani). Similarly, in another cv. Naveen the extinction values ranging from 0.193 to 0.209 for a sub sample consisting of 400 seeds. In sub sample consisting of 100 seeds in cv. Rupali, the extinction values ranged from 0.179 to 0.185. The extinction value for a similar sub sample in case of cv. Naveen ranged from 0.167 to 0.170. Higher extinction values were recorded for sub samples consisting higher number of seeds i.e. 400 as compared to extinction values obtained for sub samples consisting of 100 seeds. Values greater than or equal to 0.165 were considered

Table 18. Specificity of Antiserum developed against *Xanthomonas campestris* pv. *vesicatoria* antiserum.

Dilution of bacterial suspension	<i>X. c. pv. vesicatoria</i>	<i>X. c. pv. phaseoli</i>	<i>X. c. pv. vignicola</i>	<i>X. o. pv. oryzae</i>	<i>X. c. pv. phaseoli</i> (fusceans)	<i>X. c. pv. campestris</i>	<i>X. c. pv. citri</i>	<i>X. c. pv. mori</i>	<i>X. c. pv. malvacearum</i>
Undiluted	2.00	0.07	0.05	0.05	0.06	0.05	0.05	0.05	0.05
10 ¹	1.24	0.05	-	-	-	-	-	-	-
10 ²	0.95	-	-	-	-	-	-	-	-
10 ³	0.52	-	-	-	-	-	-	-	-
10 ⁴	0.28	-	-	-	-	-	-	-	-
10 ⁵	0.17	-	-	-	-	-	-	-	-
10 ⁶	0.09	-	-	-	-	-	-	-	-
10 ⁷	0.05	-	-	-	-	-	-	-	-

Note: Extinction value 3 were measured at 450 nm in titrate multiscan plus 2 photometer version 2-03 USA.

Table 19. Application of Enzyme Linked Immuno Sorbent Assay (ELISA) in identification and detection of *X. c. pv. vesicatoria*

Dilution of bacterial suspension	E _{values}	Bacterial population (cfu/100μl)
10 ⁴	0.243	7.5x10 ⁴ .
	0.258	7.8x10 ⁴ .
	0.320	10.5x10 ⁴ .
	0.320	10.2x10 ⁴ .
10 ⁵	0.225	5.4x10 ⁵ .
	0.217	5.2x10 ⁵ .
Control (water)	0.156-0.166	0

E_{values} measured at 450nm

Table 20. Detection of *Xanthomonas campestris* pv. *vesicatoria* in naturally infected tomato seeds by enzyme linked immunosorbent assay (ELISA)

Variety	No. of seeds	ELISA
		Extinction values
Rupall	100	0.185
		0.164
		0.179
	200	0.175
		0.196
		0.197
	400	0.206
		0.209
		0.220
Rajani	100	0.164
		0.165
		0.166
	200	0.223
		0.215
		0.206
	400	0.227
		0.235
		0.237
Naveen	100	0.167
		0.170
		0.156
	200	0.183
		0.189
		0.189
	400	0.193
		0.209
		0.205
Rashmi	100	0.160
		0.164
		0.156
	200	0.219
		0.248
		0.218
	400	0.228
		0.233
		0.227
Control		0.156-0.164

E values measured at 450nm in titrate multiscan plus 2 photometer UAS.

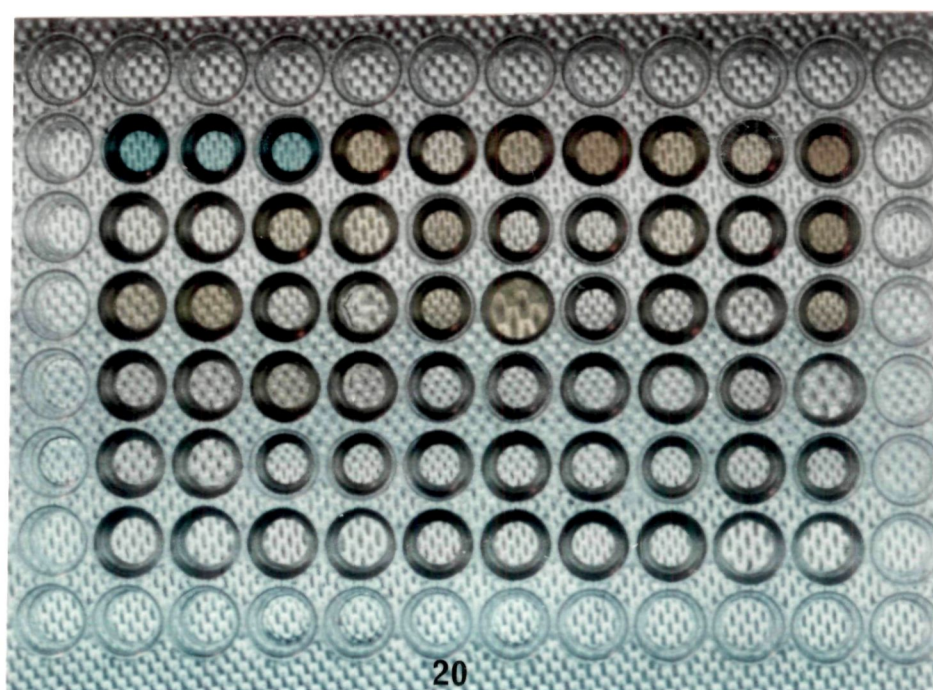
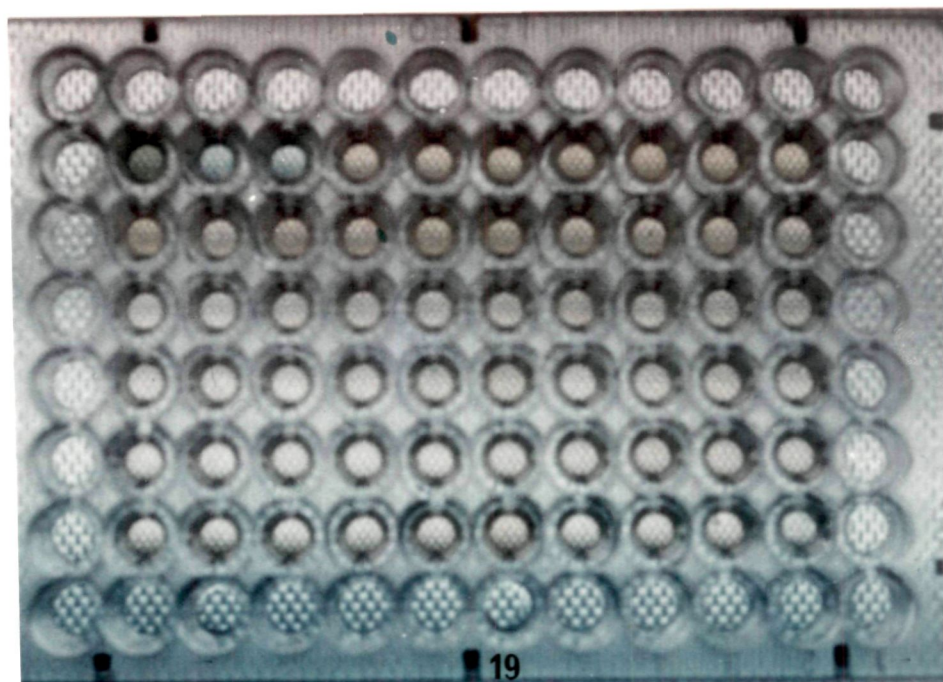
Plate 19. Sensitivity of ELISA in detecting *X. c. pv. vesicatoria* in artificially infected seed samples at different levels of infestation.

B2 to 4 negative control with distilled water. Note the blue colour.
B5 to B10 100% naturally infected tomato seeds. Note the deep yellow colour.

Well nos. C 2-10 -	10%	infected seed
Well nos. b. 4-10	1%	infected seed
Well nos. E-2-5	1%	infected seed
Well nos. F-2-10-	2%	infected seed
Well nos. G-2-10	0.1%	infected seed

Well nos. B-11, C-11, D-11, D-2 to 3] Positive control with homologous antigen i. e. *X. c. pv. vesicatoria*.
Note the deep yellow colour.

Plate 20. Detection of *X. c. pv. vesicatoria* in seeds obtained in naturally infected tomato fruits by ELISA. Development of yellow colour indicate positive reaction for the presence of *X. c. pv. vesicatoria* in the seed samples of 400, 200 and 100 seed belonging to different cultivars. The three well with blue colour (B2 to 4) are control with buffer only.



as positive in view of the fact than the extinction values recorded for control consisting of only buffer were in the range of 0.156 to 0.164.

4.11.3 Sensitivity of Ezyme linked Immunosorbent assay (ELISA) in detecting *X. c. pv. vesicatoria* in artificially infested tomato seed samples

The ELISA was found to be sensitivity in detecting *X. c. pv. vesicatoria* in artificially infested seed samples as seen from the data presented in Table 21 Plate 19.

In three sub seed samples comprising of 100 per cent naturally infected seeds, ELISA was able to detected the presence of the pathogen in the seed as the extinction values obtained were 0.185, 0.179 and 0.185 and the population of the pathogen detected by dilution plating in the corresponding samples were 51, 48 and 54cfu/100µl seed extract respectively. Similarly ELISA detected the presence of the pathogen at infestation level of 10 per cent and the extinction values obtained for three sub-samples were 0.173; 0.180 and 0.169 and the population of the pathogen detected in the corresponding seed extracts were 50, 60 and 40cfu/100µl. However, the extinction values obtained in sub-sample consisting of 1, 0.2 and 0.1 per cent diseased seed were in the range of control, also no population was detected in these seed samples on dilution plating on Tween-80 medium.

4.11.4 Efficiency of ELISA in detecting *X. c. pv. vesicatoria* in naturally infected tomato seeds

The efficiency of ELISA in detecting of *X. c. pv. vesicatoria* in naturally infected tomato seeds was evaluated by comparing with the population of *X. c. pv. vesicatoria* detected by dilution plating on Tween-80 semi-selective medium.

The extinction values obtained for the sub samples compared well with the population of the bacterium detected by dilution plating for most of the seed samples. A better comparison was obtained between ELISA and dilution plating, in sub samples containing 400 seeds than in sub-samples containing lower number of seeds. The data present in Table 22 and plate 20, revealed that sub-samples consisting of 400 seeds belonging to cv. Rupali, extinction values of 0.206, 0.209 and 0.220 compared well with the population of 2.93×10^2 , 2.90×10^2 and 2.90×10^2 cfu/100µl seed extract, respectively detected by dilution plating. While the extinction values for sub samples

Table 21. Sensitivity of Enzyme linked Immuno sorbent Assay in detecting *Xanthomonas campestris* pv. *vesicatoria* in artificially infested seed samples.

Percent infection (%)	Diseased Tomato seeds	Healthy tomato seeds	E-values	Population of pathogen /100µl seed extract
100	100	0	0.185 0.179 0.185	51 48 54
10	10	90	0.173 0.180 0.169	50 60 40
1.0	1.0	99.0	0.152 0.161 0.135	0 0 0
0.2	1.0	499.0	0.167 0.168 0.160	0 0 0
0.1	1.0	999	0.162 0.168 0.168	0 0 0
Control	-	-	0.152 - 0.164	-

Extinction values measured at 450nm in titrake multiscan plus 2 photometer version 2-03 USA.

Table 22. Efficiency of Enzyme linked Immunosorbent assay in detecting *Xanthomonas campestris* pv. *vesicatoria* in naturally infected tomato seeds.

Cultivars	No. of seeds	E values	Population of pathogen cfu/100µl seed extract	No. of seeds	E-values	Population of pathogen cfu/100µl seed extract	No. of seeds	E values	Population of pathogen cfu/100µl seed extract
Rupali	100	0.185	50	200	0.175	75	400	0.206	(2.93x10 ²)
		0.185	52		0.196	77		0.209	(2.90x10 ²)
		0.179	51		0.197	79		0.220	(2.90x10 ²)
Rajani	100	0.165	66	200	0.223	143	400	0.227	(3.18x10 ²)
		0.166	67		0.215	140		0.235	(3.11x10 ²)
		0.156	8.0		0.206	139		0.237	(3.11x10 ²)
Naveen	100	0.167	48	200	0.183	80	400	0.193	(3.18x10 ²)
		0.170	52		0.189	79		0.209	(3.19x10 ²)
		0.134	0.0		0.185	79		0.205	(3.19x10 ²)
Rashmi	100	0.165	32	200	0.219	32	400	0.233	(3.05x10 ²)
		0.167	36		0.248	62		0.227	(3.01x10 ²)
		0.144	0.0		0.156	5.0		0.165	10.0
Control		0.141-0.164							

Extension values measured at 450 nm in titrax multiscan plus 2 photometer version 2-03 USA.

containing 200 seeds in the same cultivar were 0.175, 0.196 and 0.197 and the population of the pathogen detected in the corresponding seed extracts by dilution plating were, 75,77 and 79cfu/100µl seed extracts. Similarly, in case of cultivar Naveen the extinction values for the two sub sample consisting of 100 seeds were 0.167 and 0.170 and the population of *X. c. pv. vesicatoria* detected in the corresponding seed extracts by dilution plating was 48 and 52cfu/100µl seed extract. Higher extinction values were obtained for seed extracts containing higher number of seeds and the population of the pathogen detected was also higher. In a sub-samples consisting of 200 seed, E value of 0.183 and 0.189 were recorded and the population of the pathogen detected was 80 and 79cfu/100µl respectively and higher extinction values of 0.209 and 0.205 were obtained in seed samples containing 400 seeds which compared well with higher number of cells detected by dilution plating on Tween-80 medium i.e. 3.19×10^2 and 3.18×10^2 cfu/100µl seed extract. However, with regards to the extinction values obtained and the population of the pathogen detected in tomato cv. Rajani, correlation could not be established particularly in sub sample consisting of 200 and 400 seeds.

4.12. EFFECT OF BACTERICIDES / ANTIBIOTICS SPRAYS ON THE DEVELOPMENT OF BACTERIAL SPOT CAUSED BY *X. c. pv. vesicatoria* UNDER FIELD CONDITIONS.

4.12.1 First field trial conducted during kharif 1995

A field trial was conducted during the kharif 1995, using antibiotics and bactericides to control bacterial spot of tomato. The result of the chemical control trial is presented in Table 23 and Fig. 3.

It was observed that spraying streptomycin sulphate alone at 500ppm or in combination with copper oxychloride at 2000 ppm was very effective in reducing the disease severity in the field in which the disease was induced artificially by spraying the bacterial suspension (5×10^7 cfu/ml). The disease severity was least in the plots sprayed with a combined spray of streptomycin sulphate and copper oxychloride as measured by the percentage of leaves infected (37.13%), per cent leaf let infected (26.75%) and the number of spots / cm² of leaf let (1.30).

Further the disease was effectively controlled in plots sprayed with streptomycin sulphate alone at 500ppm. The mean percentage of leaves infected. Leaf let infected/leaf

Table 23. Effect of bactericides /antibiotics sprays on the development of bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* (kharif 1995).

Treatment	Concen- tration (ppm)	Per cent leaves infected/plant					Per cent leaf let infected/leaf					No. of spots/cm ² leaf/let				
		Spray intervals					Spray intervals					Spray intervals				
		1st spray (days)	2nd spray (days)	3rd spray (days)	Mean		1st spray (days)	2nd spray (days)	3rd spray (days)	Mean		1st spray (days)	2nd spray (days)	3rd spray (days)	Mean	
		35	55	75			35	55	75			35	55	75		
Streptomycin sulfate	500	66.07	29.25	16.25	37.19		42.70	26.25	13.75	27.56		2.02	1.15	0.85	1.34	
Streptocycline	500	65.62	34.25	19.82	39.89		47.95	27.25	15.87	30.35		2.07	1.62	1.42	1.70	
Paushamycin	500	70.07	39.77	22.37	44.07		47.62	33.12	21.00	33.91		2.15	1.92	1.75	1.42	
Copper oxychloride	2000	71.55	44.97	27.22	47.91		48.87	38.62	22.50	36.66		2.10	1.96	1.91	1.99	
Streptomycin	500															
Copper oxychloride	2000	66.70	29.25	15.75	37.13		40.50	26.12	13.65	26.75		1.97	1.12	0.81	1.30	
Streptocycline	500															
Dithane M-45	2000	67.55	34.30	19.82	39.89		41.32	27.12	15.75	28.07		2.07	1.65	1.41	1.71	
Paushamycin	500															
Dithane M-45	2000	67.62	39.75	27.87	45.08		48.40	33.00	20.87	34.09		2.22	2.0	1.82	2.01	
Control		75.67	80.50	83.20	79.79		52.07	71.32	66.87	63.42		2.72	5.25	6.60	4.86	
		68.86	41.50	29.04			46.18	35.35	23.78			2.16	2.08	2.07		

Bactericides /Antibiotic

F- test	NS	*	*	NS	*	*	NS	*
SEm ±	3.22	1.43	1.03	3.38	1.10	1.09	0.18	0.15
CD at 5%	9.47	4.22	3.34	9.96	3.24	3.21	0.53	0.43

Table : 23b (Continuation)

Treatment	Concentration PPM	Percent leaf infected / plant						Per cent leaf infected / plant						No. of spots / cm ² of leaflets					
		I st		Spray intervals I nd		III rd		I st		Sprays intervals I nd		III rd		I st		Spray intervals I nd		III rd	
		Spray B	B	Spray B	B	Spray B	B	Spray B	B	Spray B	B	Spray B	B	Spray B	B	Spray B	B	Spray B	B
Streptomycin sulphate	500	65.97	45.75	22.20	42.45	32.12	18.50	1.95	1.80	1.05									
Streptocyclin	500	69.50	48.20	25.25	47.90	35.62	19.25	2.00	1.87	1.52									
Paushamycin	500	69.10	49.75	28.62	47.00	45.12	22.75	2.00	2.22	2.00									
Copper oxychloride	500	70.87	53.50	33.82	48.57	45.75	29.00	2.02	2.00	1.95									
Streptomycin + Copper oxychloride	500 + 2000	66.62	39.87	22.00	40.37	32.07	18.45	1.92	1.80	1.00									
Streptocyclin + Dithane M-45	500 + 2000	63.65	48.20	25.37	41.07	35.75	19.10	1.87	1.82	1.57									
Paustamycin + Dithane M-45	500 + 2000	67.45	49.55	28.50	48.22	45.37	22.87	2.10	2.17	1.95									
Control		75.65	69.25	81.25	48.50	65.12	71.50	2.02	3.45	5.65									
		68.14	50.51	33.38	45.51	42.11	27.68	1.98	2.14	2.09									
Chemicals / antibiotics																			
F-test	NS	*	*	*	NS	*	*	NS	*	*				NS	*	*	*	*	*
SEM $\pm$	3.33	1.25	1.25	1.25	3.38	2.14	1.21	0.18	0.17	0.14				0.55	0.51	0.51	0.51	0.51	0.51
CD at 5%	9.80	3.70	3.69	3.69	9.98	6.29	3.57	0.55	0.51	0.42				0.55	0.51	0.51	0.51	0.51	0.51

* B = Before spray

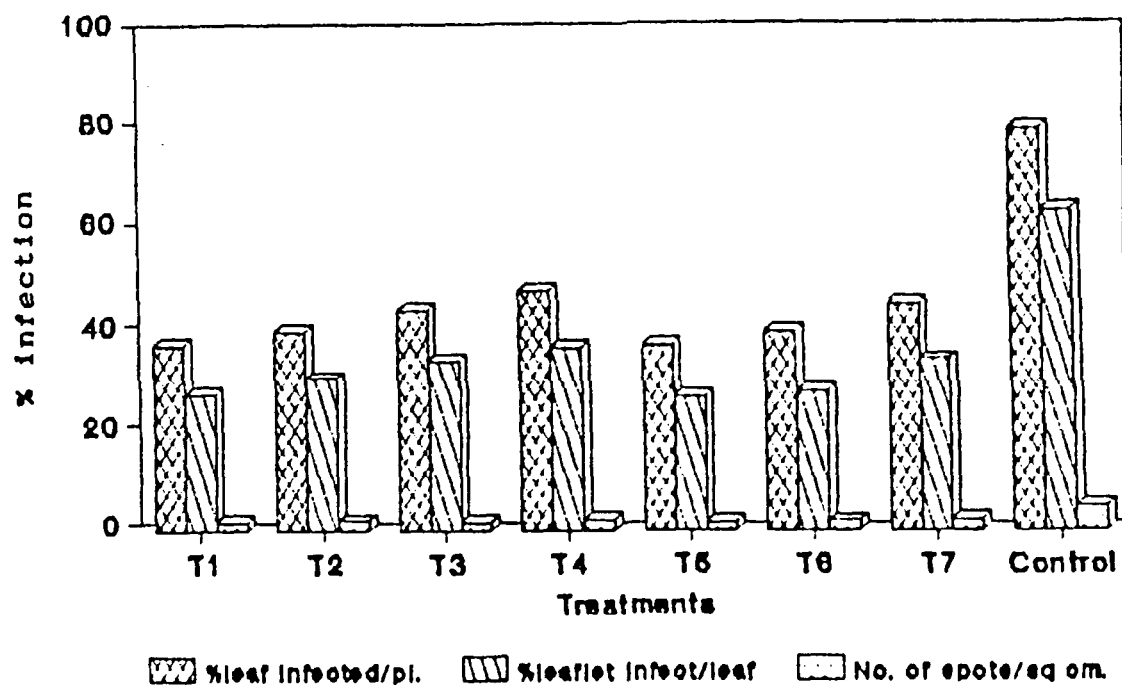


Fig3 Effect of bactericides/antibiotics on the development of bacterial spot of tomato (Kharif-1996)

- T₁ : Streptomycin sulfate
 T₂ : Streptocyclin
 T₃ : Paushamycin
 T₄ : Copper oxycloiride
 T₅ : Streptomycin sulfate + Copper oxycloiride
 T₆ : Streptocyclin + Dithane M-45
 T₇ : Paushamycin + Dithane M-45
 T₈ : Control

and number of spots / cm² leaf let were 37.19, 27.56 per cent and 1.34 respectively. The reduction in disease severity was statistically on par with the plots sprayed with the combination of streptomycin sulphate + copper oxychloride. The next best antibiotic, found to be effective was streptocycline at 500 ppm in which the 39.89 per cent of leaves was infected with 30.35 per cent of the leaf lets being affected producing over an average 1.70 spots / cm². The other antibiotics / bactericides were moderately effective. The unsprayed control plot recorded 80 per cent diseased leaves with 63.42 percentage of the leaf lets infected produce a very high number of spots i.e. 4.86 spots/cm².

4.12.2 Second field trial conducted during kharif 1996

In the field second trial conducted during kharif 1996 using the same antibiotics / bactericides which were applied 1st trial during 1995, the data on the efficacy of these chemicals in controlling the disease is given in Table 24 and Fig. 4. In this trial also streptomycin sulphate sprayed at 500ppm stood first in effectively controlling the disease, the percentage of leaves, leaf let infected and number of spots / cm² in streptomycin sulfate sprayed plots were 42.34, 28.93 and 1.27 respectively, while in the control plot the percentage of leaves, leaflets infected and number of spots /cm² were 77.52, 65.63 and 4.90 respectively.

From among the treatments comprising of combination sprays, the most effective treatment was combined sprays of streptomycin sulphate (500ppm) with copper oxychloride (2000ppm) in which 42.54 per cent of leaves were infected while 27.84 diseased leaf lets/leaf and 1.35 spots / cm² were observed. Similarly combined spray of streptocycline with Dithane-M45 also gave a good control of the disease,. However, all the above three treatments were statistically on par with each other in their effectiveness in controlling the disease.

4.12.3 YIELD LOSS DUE TO THE INCIDENCE OF BACTERIAL SPOT

In the field trial conducted during the kharif 1995, on the effect of chemical strays on the development of bacterial spot of tomato, the yield loss due to bacterial spot was also estimated and the data is presented in Table 25. It was observed that combined spray of streptomycin sulphate (500ppm) with copper oxychloride (2000ppm) or spraying streptomycin sulphate (500ppm) or streptocycline (500ppm) alone were effective in controlling the disease and increasing the yields of tomato fruits.

Table 24. Effect of bactericides/antibiotics sprays on the development of bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* (khanif 1996)

Treatments	Concen- tration ppm)	Per cent leaves infected/plant				Per cent leaf let infected/leaf				No. of spots/cm ² leaf let			
		Spray intervals				Spray intervals				Spray intervals			
		1st spray (days) 35	2nd spray (days) 55	3rd spray (days) 75	Mean	1st spray (days) 35	2nd spray (days) 55	3rd spray (days) 75	Mean	1st spray (days) 35	2nd spray (days) 55	3rd spray (days) 75	Mean
Streptomycin Sulfate	500	64.80	42.75	19.47	42.34	43.60	27.62	15.57	28.93	1.93	1.07	0.82	1.27
Streptocycline	500	66.57	44.75	20.12	43.81	48.12	28.75	19.25	32.04	1.95	1.37	1.42	1.58
Paushamycin	500	68.07	46.75	20.50	45.11	47.62	32.12	23.00	34.25	2.26	1.87	1.68	1.94
Copper oxychloride	2000	71.62	48.20	21.62	47.15	49.87	38.87	24.00	37.58	2.30	2.00	1.80	2.03
Streptomycin +	500												
Copper oxychloride	2000	68.37	40.30	18.95	42.54	41.50	26.82	15.22	27.84	2.32	0.98	0.75	1.35
Streptocycline +	500												
Dithane M-45	2000	68.50	43.45	19.37	43.77	42.37	28.00	18.87	29.75	2.12	1.30	1.27	1.56
Paushamycin +	500												
Dithane M-45	2000	69.75	46.50	20.50	45.58	49.07	33.75	22.37	35.06	2.43	1.80	1.65	1.96
Control		74.12	76.75	81.70	77.52	61.52	67.62	67.75	65.63	2.97	4.97	6.77	4.90
		68.97	48.43	27.78		47.95	35.44	25.75		2.28	1.92	2.02	

Bactericides /Antibiotic

F - test

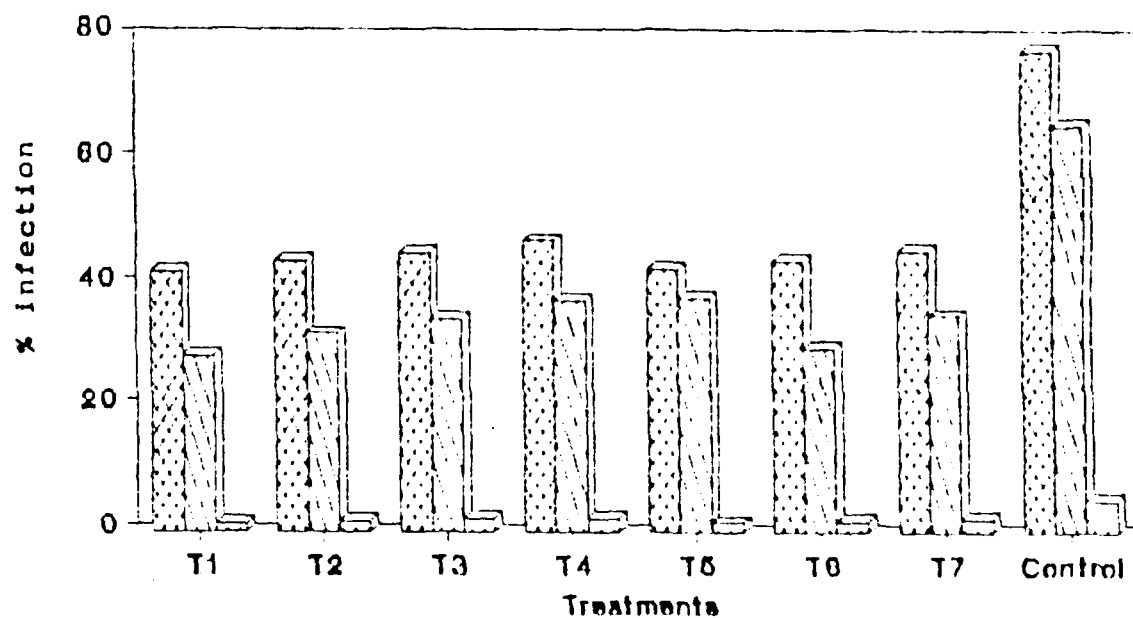
SEm ±

CD at 5%

NS	*	*	*	NS	*	*	*	NS	*	*
2.88	2.86	0.62	2.90	1.33	1.03	0.16	0.11	0.17	0.16	0.11
8.48	8.42	1.84	8.53	3.92	3.05	0.48	0.48	0.50	0.48	0.48

Table : 24b (Continuation)

Treatment	Concentration	Percent leaf infected / plant						No. of spots / cm ² leaflets					
		Spray intervals			Sprays intervals			Spray intervals			Spray intervals		
		Ist Spray B	IIInd Spray B	IIIrd Spray B	Ist Spray B	IIInd Spray B	III Spray B	Ist Spray B	IIInd Spray B	IIIrd Spray B	Ist Spray B	IIInd Spray B	IIIrd Spray B
Streptomycin sulphate Streptocyclin Paushamycin Copper oxychloride Streptomycin + Copper oxychloride Streptocyclin + Dithane M-45 Paushamycin + Dithane M-45 Control	PPM												
	500	64.75	55.62	32.87	43.47	33.12	20.75	1.98	1.75	1.05			
	500	66.37	60.12	36.50	48.00	33.87	21.37	1.91	1.87	1.30			
	500	67.82	63.12	37.05	47.25	45.37	27.25	2.20	2.40	1.80			
	500	71.37	65.25	37.70	49.12	46.00	28.62	2.18	2.20	1.90			
	(500 + 2000)	68.25	50.25	31.75	41.37	32.50	19.72	2.22	1.75	0.92			
	(500 + 2000)	67.50	56.75	36.37	42.25	33.75	20.87	2.03	1.90	1.25			
	(500 + 2000)	69.37	62.70	37.00	48.75	45.12	26.87	2.38	2.15	1.70			
		74.00	76.75	75.50	51.87	65.07	71.70	2.26	3.85	6.25			
		68.68	66.81	40.59	46.51	41.85	29.64	2.16	2.39	2.02			
Bactericides / antibiotics F-test SEm ± CD at 5%		NS	*	*	NS	*	*	NS	*	*			
		2.78	2.29	1.69	2.90	1.67	0.83	0.18	0.15	0.12			
		8.20	6.73	4.98	8.55	4.96	2.43	0.54	0.45	0.34			



% leaf infected/pl.
 % leaflet infected/leaf
 No. of spots/eq cm.

Fig4 Effect of bactericides/antibiotics on the development of bacterial spot of tomato (Kharif 1996).

- T₁ : Streptomycin sulfate
 T₂ : Streptocyclin
 T₃ : Paushamycin
 T₄ : Copper oxycloiride
 T₅ : Streptomycin sulfate + Copper oxycloiride
 T₆ : Streptocyclin + Dithane M-45
 T₇ : Paushamycin + Dithane M-45
 T₈ : Control

Table 25. Effect of bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* on the yield of tomato fruits (kharif 1995).

Treatments	Concen- tration (ppm)	Average No. of fruits/pl.	No. of infected fruits/pl.	Per cent diseased fruits	Yield/pl. (g)	Yield/plot (Kgs) control	Yield/ac (Tonnes)	% increase in yield over
			a	b				
Streptomycin sulphate	500	24.00	0.0 (0.71)	0.0 (0.0)	460.00	7.67	21.30	126.60
Streptocycline	500	21.33	0.0 (0.71)	0.0 (0.0)	455.00	7.58	21.15	125.00
Paushamycin	500	16.33	3.0 (1.86)	18.37 (25.38)	416.67	6.95	19.30	105.30
Copper oxychloride	2000	15.00	5.0 (2.34)	33.33 (35.26)	398.33	6.63	18.40	95.74
Streptomycin + Copper oxychloride	500	24.33	0.0 (0.71)	0.0 (0.0)	461.67	7.70	21.40	127.65
Copper oxychloride	2000							
Streptocycline + Dithane M-45	500	21.67	0.0 (0.71)	0.0 (0.0)	455.67	7.60	21.10	124.47
Dithane M-45	2000							
Paushamycin + Dithane M-45	500	16.00	1.0 (1.17)	6.25 (14.48)	417.33	6.96	19.35	105.83
Control	2000	9.00	7.3 (2.79)	81.10 (64.24)	203.33	3.39	9.40	
F- test	*	*	*	*	*	*	*	*
SEm ±	1.44	0.13		0.015	5.763	0.0143		0.0193
CD at 5%	4.35	0.39		0.041	17.482	0.0433		0.0585

a. Figures in parenthesis are square root transformed values.

b. Figures in parenthesis are angular transformed values.

Plants sprayed with streptomycin sulphate (500ppm) plus copper oxychloride (2000 ppm) gave 21.40 tonnes/acre an increase of 11.90 tonnes per acre in yield over control. While plants sprayed with streptomycin sulphate (500ppm) or streptocycline (500ppm) alone yielded 21.30 tonnes and 21.15 tonnes /acre of fruits respectively.

Copper oxychloride and paushamycin were less effective in controlling fruits infection though there was increase in yield of tomato fruits (18.40 and 19.30 tonnes/acre respectively). But, fruit infection was fairly high in case of copper oxychloride (33.33%) and paushamycin (18.37%) sprayed plants, fruit infection to extent while, fruit infection in control plants was found to be 81.10 per cent. In the 2nd field trial conducted during kharif 1996, on the effect of chemical control on the development of bacterial spot of tomato, a similar trend in fruit infection and increased in yield in sprayed plot was observed. The data on the yield loss is presented in Table 26. Plants sprayed with streptomycin sulphate plus copper oxychloride gave maximum yield of 18.14 tonnes/acre followed by streptomycin sulfate (500ppm) 18.10 tonnes/acre and streptocycline (500ppm) 17.97 tonnes/acre. Fruit infection was observed in paushamycin, copper oxychloride and paushamycin plus Dithana-M45 sprayed plants to an extent of 17.45, 27.27 and 13.18 per cent respectively. The incidence of fruit infection in control plants was 73.03 per cent and the yield recorded was only 8.72 tonnes per acre.

DISCUSSION

V DISCUSSION

Bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye. Syn. *Xanthomonas vesicatoria* (Ex. Doidge) Vauterin, Hoste, Kersters and Swings, 1995 has become a serious threat to tomato cultivation in Karnataka in recent years, especially under moist and warm conditions prevailing in the major tomato growing areas in the state. The disease is known to reduce not only the plant growth and yield but more importantly the quality of the fruit is also affected.

Xanthomonas campestris pv. *vesicatoria* has been classified into three groups viz., tomato group (XCVT). Pepper group (XCVP) and pepper-tomato (XCVPT) on the basis of its virulence on tomato and pepper and further each group comprises of races differentiate mainly on the basis of the reaction of the strains on tomato genotypes and pepper near isogenic lines. A gene for gene relationship in pepper bacterial spot pathogen system has been established by many workers.

Effective management of bacterial spot depends on the combination of practices such as use of pathogen free seeds and seedlings, sanitation, crop rotation, resistant cultivars and chemical control. Fixed copper compounds and antibiotics such as streptomycin is generally followed by the farmers both in the nursery and field conditions. Resistance in *Xanthomonas campestris* pv. *vesicatoria* for both these chemicals have been reported from abroad by several workers in recent years and as such control of bacterial spot with copper or streptomycin sprays have been erratic because of the variation in population of pathogen in its resistance to bactericides. This resistance to the bactericides/antibiotics are reported to be plasmid encoded in *Xanthomonas campestris* pv. *vesicatoria*. Since it is difficult to control bacterial spot with foliar application of chemicals, once it become established, other control measures are needed to reduce disease losses. Therefore, prevention of the problem through exclusion of the bacteria is one such possibility, the other possibility being, reducing the inoculum of the pathogen. In order to achieve this it is necessary to determine the source of inoculum many workers have shown that the bacterium can survive between crops in or on tomato seeds, or the bacterium over winter on crop refuse. The epiphytic survival of the pathogen has been reported either on volunteer plants of the same crop or on alternate cultivated plants acting as non hosts, or on weed plants. Practically no information is available with regards to the disease development in relation to the environmental conditions prevailing in the state of Karnataka.

Hence, the present investigation were undertaken on the prevalence of races in Karnataka state detection of the plasmid / plasmid profile among the strains obtained from different agroclimatic zones of the state, sources of the survival of the bacterium, influence of weather conditions on the disease development, the role of insects in the transmission of the pathogen, and chemical control of the disease and the results are discussed in the following paragraphs.

Isolation and Identification of the causal organism

Strains *Xanthomonas campestris* pv. *vesicatoria* isolated from infected tomato leaves and fruits showing typical symptoms of bacterial spot, collected from different agroclimatic zones of Karnataka viz., Bangalore (GKVK and Hosakote) Chitradurga, Davanagere, Hassan, Kolar, Mandya, Mangalore, Mudigere, Mysore, Shimoga, Tumkur and IIHR, Bangalore were identified as *Xanthomonas campestris* pv. *vesicatoria* on the basis of colony and morphological characters, staining reaction, physiological and biochemical characteristics, hypersensitive reaction on tobacco leaf var. Samsun and on the basis of pathogenicity test on tomato plants.

The bacterial colonies were round, smooth, convex, glistening and light yellow on YDC agar medium. The bacterial cells were small, rod shaped with monotrichously flagellated, Gram negative, as evidenced by Gram staining technique and reaction in 3 per cent KOH solution (Suslow *et al.*, 1962), non capsulated, non-spore forming and non acid fast. All the 14 strains behaved similarly with regards to different physiological and biochemical characteristics (Table 6) ; such as utilization of glucose, arabinose, mannose, trehalose, and cellobiose for acid production. None of the strains utilized asparagine as sole source of carbon and nitrogen, all the strains were positive to catalase reaction, hydrolysed gelatin and casein, and produce hydrogen sulphide. None of the strains produce indole, negative to urease, and methyl red reaction and did not reduce nitrate to nitrite (Gardner and Kendrick, 1923, Gitaitis *et al.* 1987, Beleva and Sotirova, 1975, Beleva *et al.* 1976, Schaad and Stall, 1988, Ward and OGarro, 1992).

The 16 strains of XCV including two strains isolated from pepper produced confluent necrosis in 24 hours, when infiltrated into tobacco leaves var. Samsun.

Further, the pathogenicity test revealed that, all the 14 strains isolated from tomato were virulent on tomato cv. Pusa Ruby only and the two strains of pepper received from

IIHR, Bangalore and Himachal Pradesh were pathogenic to pepper plants cv. California wonder, producing typical bacterial spot symptoms in 15 days. Thus the 14 strains of tomato and two strains of pepper were identified to be *X. c. pv. vesicatoria*. None of the strains of *X. c. pv. vesicatoria* isolated from tomato were virulent on pepper cv. California wonder. Two pepper strains behaved similarly and were avirulent to the tomato plants, but infected the pepper plants.

Prevalence of Races of *X. c. pv. vesicatoria*

The results of the inoculation studies of the strains of *X. c. pv. vesicatoria* isolated from tomato revealed the prevalence of *X. c. pv. vesicatoria* (XCVT) tomato group in Karnataka, since all the 14 strains isolated from tomato infected tomato cultivars only and were non pathogenic to pepper. Similarly, the two pepper strains infected only pepper plants cv. California wonder, but failed to infect tomato plants, indicating that the two strains from pepper belonged to *X. c. pv. vesicatoria* pepper (XCVP) group. Three groups of the bacterium namely the tomato group (XCVT), the pepper group (XCVP) and the pepper - tomato group (XCVPT) are usually associated with the disease according to Minsavage *et al.* (1990) and Reifshneider *et al.* (1985). The tomato group is virulent only on tomato plants, the pepper group is virulent only on pepper and both tomato and pepper plants are susceptible to the tomato - pepper group. Strains belonging to XCVT and XCVP groups are further divided into races on the basis of virulence on tomato genotype and pepper near isogenic lines (Bouzar *et al.* 1994; Kousik and Ritchie, 1995; Minsavage *et al.* 1990 ; Ritchie and Dittapongpitch, 1991 and and Sahin and Miller, 1995). The data presented in Table 7 on the inoculation studies indicated that the 14 tomato strains infected all the commercial hybrids of tomato, however the strains varied with regards to the infection on tomato differentials / genotypes viz., Hawaii-7998, Hawaii-7997, and Hawaii-7981.

The 14 tomato strains were avirulent on the tomato genotype Hawaii-7998 and produced hypersensitive reaction within 24 hours, while all the tomato group strains except Hawaii-7981 except the Shimoga strain, which produced hypersensitive reaction on this genotype. Further, none of the 14 strains infected tomato genotype Hawaii 7997, while Shimoga strains which produce hypersensitive reaction on this genotype also. Thus on the basis of race nomenclature suggested by Minsavage *et al.* (1990) and Bouzar *et al.* (1994), the 13 strains belonging to XCVT group isolated from different agroclimatic areas of Karnataka could be designated as belonging tomato race-1 (T1) in tomato group.

Since the strain from Shimoga behaved slightly differently by producing hypersensitive reaction even on Hawaii 7981 genotype and in view of the fact that this strain produce hypersensitive reaction on Hawaii 7998 also, which is known to posses multigenic resistance to *X. c. pv. vesicatoria*, on which all the strains of race-T1 are known to produce hypersensitive reaction, hence tentatively the tomato strain from Shimoga could be designated as race T3. However, further work on this strain is needed to classify it into a definite race, also in view of the fact that even with regards to amylolytic and pectolytic activity this strain differed from the other 13 tomato strain, which will be discussed later.

Race-1 and Race - 2 of *X. c. pv. vesicatoria* have been differentiated on tomato cultivars ; Walter and Hawaii 7998 (Ward and O'Garro, 1992). Walter has no resistance to strains of XCVT and Hawaii 7998 restricts race-1 but not race-2 of this group (Scott and Jones, 1986, Ward and O'Garro 1992). Jones *et al.* (1995) reported a third race of tomato (T3) as the strain produced a compatible reaction on tomato genotype Hawaii 7998, however this strain produced a rapid hypersensitive response in another tomato genotype Hawaii 7981, PI 126932, PI 128216.

As regard the two pepper strains, the strains produced a compatible reaction on pepper cultivar California wonder, indicating that the two strains belonged to pepper group (XCVP). The inoculation studies of the two strains on the three near isogenic lines of pepper viz., ECW-10R, ECW-20R and ECW-30R revealed that pepper strain from IIHR, Bangalore produced hyper sensitive reaction on ECW-10R and ECW-20R, however on ECW-30R the strain produced a compatible reaction indicating that the IIHR, Bangalore strain belonged to race-2 (XCVP-2), while the pepper strain from Himachal Pradesh produced a compatible reaction on ECW-10R, while inducing HR reaction on ECW-20R and ECW-30R, revealing that the HP pepper strain belonged to race-1 (XCVP-1). Single dominant gene in pepper (*Capsicum annuum* L.) have been reported to confer resistance to bacterial spot caused by *X. c. pv. vesicatoria*. These genes have been designated BS1, BS2 and BS3 and were first found in accessions, P1 163192, P₁ 260435 and P₁ 271322 respectively (Cook and Guvevara 1982 ; Cook and Guvevara 1984 ; Hibberd *et al.* 1987 and Hartman 1990). The genes counter hypersensitive reaction to certain strains of *X. c. pv. vesicatoria*. The HR associated with these genes could be recognisized after infection of lines with the concentrated inoculum (10^8 cfu/ml) in compatible strains (Hibberd *et al.*, 1987). Minsavage *et al.* (1990) developed a nomenclature system for races of *X. c. pv. vesicatoria*, they distinguished the groups of *X. c. pv. vesicatoria* strains based on the response of tomato cultivar Walter and a set of near-

isogenic lines of *Capsicum annuum*. The near isogenic lines derived from Early Calwonder (ECW), ECW-10R, ECW-20R and ECW-30R contain a resistance gene BS1, BS2 and BS3 respectively. The XCVT group was avirulent on all capsicum lines and virulent on Walter. The XCVP group was avirulent on specific pepper lines and also avirulent on Walter, and the XCVPT group was avirulent on specific capsicum lines but virulent on Walter. The pepper race-1 is pathogenic on breeding lines ECW-10R carrying BS1 resistance gene, strains of race-2 induce hyper sensitive reaction in plants with BS1. Genes BS3 and BS1 confer resistance to race-1 and race-2 respectively, whereas gene BS2 restricts races 1, 2 and 3 (Ward and O'Garro 1992). Ritchie and Dittapougpitch (1991) proposed a new race of pepper on the basis of the fact that a strain of *X. c. pv. vesicatoria* isolated from Early Calwonder elicited a HR response in the three near isogenic lines of early Calwonder, hence they designated this strain as race-0.

Kousik and Ritchie (1995) isolated pepper race-4 and race-5 from USA. They stated that three single dominant gene conferred resistance to these races. Gene BS1 provided resistant to race P0, P2 and P5, gene BS2 to race P0 and P3 and gene BS3 to race P0 and P4. Race P4 produced HR reaction on ECW-30R but not on ECW-20R or ECW-10R. Further they also designated race-P5 on the basis of the HR observed on ECW-10R and a compatible reaction on ECW-20R and ECW-30R.

Sahin and Miller (1995), reported the prevalence of race-6 (P6) from Ohio state in USA, as the strain did not cause a HR on any of the three pepper isogenic lines. Thus based on of race designation system of Bouzar *et al.* (1994), the two pepper strain studied in the present investigation, could be categorized conclusively into two races, IIHR Bangalore strain into race-P2 and the pepper strain from Himachal Pradesh to race- P1.

Physiological characterization of the strains of *X. c. pv. vesicatoria*

Strains of *X. c. pv. vesicatoria* have been clearly distinguished into 2 groups viz., A and B on the basis of their amylolytic activity (Stall, 1994 ; Canteros (1990) ; Beaulieu *et al.* (1991) and Jones *et al.* (1993), Bouzar *et al.* (1994a,b)) and Sahin and Miller 1996). Further it was reported that strains of *X. c. pv. vesicatoria* pathogenic on tomato were strongly amylolytic and those from pepper were not. (Burkholder and Li, 1941; Dye 1962 and Marigoni and Kimati, 1987). The result of our investigation revealed the presence of both amylolytic and pectolytic strains among the 14 strains belonging to XCVT group. One strain of tomato obtained from Shimoga hydrolysed starch after 5 days of incubation

and also produced small pits in the crystal violet pectate medium indicating its ability to hydrolyse pectate. While the two strains from pepper were negative to amylolytic and pectolytic activity. Both amylolytic and pectolytic and non amylolytic and non pectolytic strains of *X. c. pv. vesicatoria* from tomato and pepper have been widely recorded (Beaulieu *et al.* (1991) ; Bouzar (1994a,b) ; Jones *et al.* (1993) and Jones *et al.* (1995)).

Further Bouzar *et al.* (1994a,b) and Jones *et al.* (1995) reported that the non amylolytic strains belonged to T1-race while the amylolytic strains to T2 race. Our results clearly indicated that the 13 strains of XCVT group which belonged to race-T1, were non amylolytic while the strain from Shimoga belonging to race-T3 (which produced HR on Hawaii-7981) was both amylolytic and pectolytic.

Jones *et al.* (1995), also reported that tomato race T3 was both amylolytic and pectolytic in nature. Thus the findings of our investigations on amylolytic and pectolytic activity agree with the reports of Bouzar *et al.* (1994 a,b) and Jones *et al.* (1995).

The two pepper strains were negative to both amylolytic and pectolytic activity, thus confirming the reports of Sahin and Miller, (1994) and Bouzar *et al.* (1994b) who also reported variations in the amylolytic and pectolytic activity among the strains races of pepper.

Detection of plasmid in the strains of *X. c. pv. vesicatoria*

As a part of study to characterize the strains of *X. c. pv. vesicatoria* with respect to resistance to copper and streptomycin sulphate, the 14 strains of *X. c. pv. vesicatoria* (two tomato strains from Bangalore were deleted) were tested. Lysis by boiling method (Holmes and Quigly, 1991) was found to be efficient and the plasmid could be isolated by this method whereas the other two methods failed.

Although no definite association was observed between streptomycin resistance and the strains obtained from different geographical area of the state, a single plasmid was detected in 9 out of 14 tomato strains tested. No plasmid was detected in the strains belonging to both XCVT and XCVP group obtained from Tumkur, IIHR, (Bangalore), Mangalore, Mandya and in one pepper strain from IIHR Bangalore. The size of plasmid on the basis of movement on agarose gel with lambda Hind III as marker as found to be about 30 kb. However, from among 9 strains in which the plasmid was detected, the 3

strains obtained from Bangalore, Shimoga and pepper strain from Himachal Pradesh possessed plasmid slightly bigger than 30 Kb. On the basis of the classification of plasmids into 13 categories by Canteros *et al.* (1995) in *X. c. pv. vesicatoria*, it can be concluded that the plasmid detected among the nine strains of *X. c. pv. vesicatoria* in our present study belonged to class 'K' in which plasmid with a size ranging from 25-33 kb are included. Stall *et al.* (1986) isolated self transmissible plasmid which was related to copper resistant and were avirulent to ECW-10R pepper plants with an approximate size of 193kb. Hender *et al.* (1988) also related copper resistance with the presence of plasmid designated as PXV 10A measuring 190 kb.

In 1990, Minsavage *et al.* reported plasmid mediated resistant in *X. c. pv. vesicatoria*. The streptomycin resistance locus was found on plasmid of 68 kb. Dittapongpitch and Ritche (1993) were able to detect 4 mini plasmids in strains of *X. c. pv. vesicatoria* with sizes ranging from 1.7 to 2.28 kb. Canteros *et al.* (1995) studied the diversity of plasmid in 522 strains of *X. c. pv. vesicatoria*, the number and size of plasmid varied among the strains and the size ranged from 3-300 kb. Ten strains had no detectable plasmids and they observed 71 different plasmid profile among 522 strains of *X. c. pv. vesicatoria*.

Survival of *X. c. pv. vesicatoria* on seeds, crop residue and soil

Survival in seeds :

Survival of pathogenic bacteria on seed and propagative parts is wide spread and is the source of serious epidemics of disease of annual crops (Leben 1981). As few as five pathogen infested seeds among 10,000 bean seeds can result in substantial crop loss, provided conditions are favourable for development of disease and spread of the pathogen (Trigalet *et al.* 1978). Gardner and Kendrick (1921) reported that *X. vesicatoria* survived for 16 1/2 months on dry seeds, while Bashan *et al.* (1982) observed that *X. c. pv. vesicatoria* survived on infected dry seeds for 10 years. Our present study on the survival of the pathogen on seeds, revealed that the bacterium could survive for 6 1/2 months on dry seeds stored at room temperature, While it survived for longer period (7 1/2 months) on seeds stored at 5°C. However the population of the bacterium on the seeds gradually declined over a period of time. Thus it is evident that seeds serve as primary and important source of survival and inoculum for the pathogen. Shekhawath and Chakravarti (1978) reported that pepper strains of *X. c. pv. vesicatoria* survived for 14 and 10 months on

seeds stored at 8°C and 10-38°C respectively, while Jones *et al.* (1986) could not detect the presence of *X. c. pv. vesicatoria* on 293 tomato seed lot extracted by fermentation procedure from 53 commercial pepper seed lot, further they stated that infestation or infection by *X. c. pv. vesicatoria* in tomato seeds planted in Georgia and Florida appeared to be at extremely low level. Thus they concluded that seed may not play an important role in the epidemiology of the disease.

Survival on soil and crop residue

There is much evidence to show that plant pathogenic bacteria survive from season to season in soil (Leben 1981). But it is generally believed that bacterial pathogens of plant foliage are not well adapted to survive in soil. However, infected plant debris has been considered a source of inoculum for epiphytic plant pathogenic bacterium (Hirano and Upper 1983). In the present investigation, it was observed that the pathogen survived for 8 months in sterilized soils artificially infested with the bacterial suspension while it could survive for a lesser duration i.e. 6 1/2 months only in unsterilized soil. However, Peterson (1963) reported that *X. c. pv. vesicatoria* survived for a very short period of 2 weeks only in laboratory soils held at 25°C and accordingly he designated *X. c. pv. vesicatoria* as exochthonous, while Shekhawat and Chakarvarti (1978) stated that *X. c. pv. vesicatoria* could survive in autoclaved soil held at 8°C and 75 per cent moisture for 105 days. Our present investigation revealed that soil could act as important source of survival and inoculum for the pathogen and agree with the findings of Sato and Takahashi (1973). As regarding the difference in the survival of bacteria in sterilized and unsterilized soil, they observed that the duration of survival of *P. mori* in sterilized soil was influenced by size of soil particles i.e. the bacteria in a soil consisting of particles smaller than 3 mm in dia. survived for a longer period than on the particles smaller than 0.50 mm. Further, they stated that the number of bacteria decreased more rapidly in the non-sterilized soil than in the sterilized soil and this tendency was remarkable at 20°C or 30°C.

The bacterium could survive for a longer period (5 1/2 and 6 months) in crop residues placed at the surface of unsterilized and sterilized soil while it could survive for a short period of 4 1/2 months in crop residue buried at 7.5 cm depth in both the sterilized and unsterilized soil. The pathogen survived for only 4 months in crop residue buried at much deeper layers in the soils (15 cm depth). Thus it is evident that the bacterium is able to over winter in the crop residue and that the duration of survival was dependent on the rate of decomposition of the crop residue in soil, as evidenced by fact that the pathogen

survived for a short period of 4 months only in crop residue buried in deeper layer of soils (15 cm).

Shekhawat and Chakravarti (1978) observed that the *X. c. pv. vesicatoria* survived for 105 days and 15 day in autoclaved and unautoclaved soil respectively maintained at 8°C at 75 per cent moisture holding capacity and the survival was reduced at 30-35°C. Further they stated that the pathogen could not survive for longer period when crop residue is buried in soil.

The results of our investigator are in conformity with the findings of Takimota (1939) and Peterson (1963) who could detect the pathogen in diseased plant refuse and stated that the bacterium could over winter in plant debris and also with the report of Jones *et al.* (1986) who observed that the *X. c. pv. vesicatoria* could over winter for a period of 6 month and over summer for 1 1/2 to 3 months during the summer months. Foliar infecting plant pathogenic bacteria survived for short period either in soil or crop residue either placed on surface of soil or buried at different depths in soil for a short period (Khan, 1989 ; McCarter *et al.*, 1983 and Wimalajeewa and Nagearow, 1980).

Further the differences observed with regards to duration of survival in sterilize and unsterilized soils could be attributed to the intense microbial competition for food, rate of decomposition and colonization by microbes as compared to sterilized soil (Buddenhgen, 1965, Jones *et al.* 1986 and Khan 1989).

The ability of plant pathogenic bacteria to survive epiphytically on weeds or non-host plants, thereby serving as reservoir of primary inoculum has been very well established. The epiphytic survival of *X. c. pv. vesicatoria* on weeds was observed in the present studies. The bacterium was detected upto 60 days on two weed species namely *Cynodon dactylon* pers. and *Cyperus rotundus* Linn., however the population level detected was very low. The other two species which supported the epiphytic survival of *X. c. pv. vesicatoria* are *Hemilia sanchifolia* L. and *Marselia quadrifolia* L. which the bacterium survived for only 15 days. However, the pathogen could not be detected on non-host crop plant namely *Phaseolus vulgaris*. Thus our study revealed that *X. c. pv. vesicatoria* is able to lead a resident phase for a short period on weed species. However, the significance of the resident phase of bacterium on these weeds in relation to the epidemiology needs further investigation as we have not related the resident population

of the pathogen with the level of disease observed under field conditions in the subsequent tomato crop.

Several pathovars of *X. campestris* are known or suspected to have potential for epiphytic growth or survival (Hirano and Upper, 1983), Cafati and Saettler (1980) recorded upto 105 cfu of *X. c. pv. phaseoli* from the surface of healthy bean leaves in field studies in disease situation. Similarly Daniel and Boher, (1978) found high population of *X. c. pv. manihotis* on aerial parts of cassava in rainy season and low populations during the dry season in diseased fields, but bacteria were completely absent from healthy fields. In all the above cases epiphytic population may have been derived from bacteria produced in disease tissue indicating that Xanthomonads can survive as epiphytes for a short period of time but not necessarily multiply on healthy leaf surface. Leben (1963) however, demonstrated the multiplication of *X. c. pv. vesicatoria* on non diseased tomatoes grown from infested seeds and further stated that the pathogen persisted as epiphytes for long periods without producing symptoms. Timmer *et al.* (1987), observed that *X. c. pv. vesicatoria* could multiply on tomato leaves only under high relative humidity conditions, when the relative humidity is low the pathogen does not survive well in plants sprayed with *X. c. pv. vesicatoria*.

The result presented in Table 13 clearly revealed that the bacterium did not multiply on the surface of the leaves when assayed after 15 days of harvest of the tomato crop, on the other hand the population gradually declined and no bacterium was detected after 75 days of sampling indicating that the epiphytic survival of *X. c. pv. vesicatoria* on weeds species appeared to be unimportant in the epidemiology of bacterial spot of tomato. Jones *et al.* (1986) also made similar observation in their study, they could detect very low level of population of *X. c. pv. vesicatoria* on weeds. The pathogen detected on only 11 out of 203 weed samples from fields under current tomato production or adjacent to tomato volunteer plants suggesting that weeds were poor source of *X. c. pv. vesicatoria*. Jones *et al.* (1986) quoted the work of Mc Guire and Jones in which they detected *X. c. pv. vesicatoria* after two weeks only on *Solanum americanum*. Laub and Stall (1967) reported *Solanum nigrum* and *Physalis minima* were not the host of *X. c. pv. vesicatoria*, thus our result support the findings of Jones *et al.* (1986) and Schneider and Grogan, (1977), who stated that weed species play minor role in epidemiology of bacterial spot.

Role of insects in the transmission of *X. c. pv. vesicatoria*

The role of the insects in the epidemiology of bacterial diseases particularly caused by *Erwinia* has been very well established and insects are known to be associated in the dispersal the bacterial pathogens. (Beer 1979, Guevara *et al.*, 1995; De-wael *et al.*, 1992 ; Harrison *et al.*, 1977 ; Hossin and Mew 1989 ; Kloepper *et al.*, 1979 and Surkv and Yu, 1981). The bacterium was isolated from the mouth parts of Chrysoneid beetle (*Aulacophora cincta*), long horned grass hopper (*Euoptaera praemorsa*), short horned gross hopper (*Phenoptera gracillus*) and coccinellid beetle (*Henosepiluchna viginiotapunctata*) in the present study. However, the pathogen was not detected in the mouth parts of white fly (*Bemisia tabaci*) insects. Incidentally the bacterium was detected in insects with biting and chewing type of mouth parts only but not from insects posing peircing and sucking type of mouth parts (*B. tabaci*). Obviously it can be concluded that the bacterial pathogen is acquired as contaminant on the mouth parts insects biting and Chewing type. Stanley *et al.* (1988) also established relationship between insects activity, viz., noctuid (*Spodoptera eridania*) and agromyzid (*Liriomyza trifolii*) with the severity of bacterial spot of tomato caused by *X. c. pv. vesicatoria* in Florida state. In the present study, however, it was not possible to definitely establish the relationships between the insect population with incidence/ severity of bacterial spot, as we have not fed the insect which had acquired bacterium on the health tomato plants.

Effect of disease on the quality of the tomato fruits

The disease had a very slight effect on the quality of fruits especially on the ascorbic acid content. A slight reduction in the level of ascorbic acid was noted in diseased fruit compared to healthy fruits. However, with regards to other parameters like per cent total sugar, and reducing sugar the disease had no deleterious effect.

Relationship between environmental factors on the development of bacterial spot of tomato

Bacterial spot of tomato is known to occurs every year during the rainy season in Karnataka state. The weather conditions prevailing in the tomato growing areas such as fairly high relative humidity and abundant rainfall might have influence on the disease, therefore the effect of relative humidity, rainfall, temperature on the development of disease was investigated in the present studies.

The results presented in Table 16, and Fig. 2. revealed that the incidence and severity of disease was more in crop grown during the period in which maximum temperature ranged from 27-32°C and RH was between 80-95 per cent. The disease incidence was not noticed during the summer month i.e. Feb to May during which period the maximum temperature was fairly high i.e. 32-35°C and relative humidity was low, and further, no rainfall was received, indicating that the development of bacterial spot is not favoured by high temperature and dry conditions. The Environmental condition which favouring disease development were moderate temperature ranging from 27-32°C, high relative humidity in the 80 -95 per cent fairly good rain. and Rain fall. The results of our studies are supportive of findings of Gorlenko and Voronkevich (1950) who observed fairly high temperature combined with high Relative humidity appeared conducive for the disease development, the time just before and beginning of fruit maturity (June and July). Shekhawat and Chakravarti (1976) observed bacterial spot of chilli is maximum during July to September when there was high RH and temperature between 22-34°C. Daib *et al.* (1982) also noticed that the bacterial spot of pepper required high RH more than 85 per cent for one or two day and low RH was found to be unfavourable for disease development.

Detection of *X. c. pv. vesicatoria* in naturally infected tomato seeds by serological technique

Application of enzyme linked immuno sorbent assay for the identification and detection of *X. c. pv. vesicatoria*.

Enzyme linked immuno sorbent assay was modified by using -

- (i) Tetra methyl benzidine instead of 1, 2-phenylendiamine-dihydrochloride which is photosensitive
- (ii) by reducing the incubation period of test samples from 18-24 hrs. to 10-12 hrs and Enzyme conjugate from 2 to 4 hours to 20 min. thus saving considerable time and
- (iii) by using horse radish peroxidase enzyme conjugate which has been found to be more sensitive, (Khan, 1996, Vester gaard, 1986), as compared to the usual alkaline phasphotase.

Specificity

The coating γ -globulin and the enzyme conjugate γ -globuline preparations were specific to *X. c. pv. vesicatoria*. Though a very mild reaction was noticed with undiluted culture of *X. c. pv. phaseoli* it was ignored as the low extinction values were low as compared to the extinction values obtained for homologous antigen. Khan (1996) and Vrugink (1978) also ignored the cross reaction in ELISA in view of the low extinction value obtained in their studies on the detection of seed borne pathogenic bacteria.

The titres of the coating and enzyme conjugate γ -globulins were found to be 1:800 and 1:1000 respectively.

Xanthomonas campestris pv. vesicatoria was identified by applying enzyme linked immunosorbent assay using horse radish peroxidase and tetramethyl benzidine enzyme substrate system.

The technique was carried out using homologous antigen (XCV) suspension of tomato strain. The extinction values obtained with the samples of homologous antigen were 0.320 and 0.225 and the number of cells recorded by dilution plating on Tween-80 medium were 105 and 54cfu/100 μ l seed extract respectively in the corresponding samples. (Table 19). Thus good correlation could be established between ELISA and dilution plating in view of the fact that the higher extinction value were recorded for cell suspension carrying higher population of the pathogen.

Detection of *X. c. pv. vesicatoria* in naturally infected tomato seeds by ELISA

Xanthomonas campestris pv. vesicatoria was detected in 30 out of 36 seed samples belonging to different cultivars. This technique was found to be more promising and sensitive. Several workers, (Khan 1996, Vrugghink 1978; Weaver and Guthrie 1978 ; Barzic and Triglet 1982 ; Shepperd, 1983 and Alvarez and Lou, 1985) applied ELISA in the detection of plant pathogenic bacteria in seeds.

In the present study, extinction values ranging from 0.227 to 0.237 was recorded in cv. Rajani in sub samples consisting of 400 seeds. Similarly in cv. Naveen the extinction values recorded ranged from 0.193 to 0.209 for sub samples consisting of 400

seeds. Low extinction values were obtained in ELISA for seed samples consisting of 100 seeds carrying lower number of pathogen cells.

Sensitivity of ELISA in detecting Enzyme Linked Immuno sorbent assay in detecting *X. c. pv. vesicatoria* in artificially infested tomato seed samples

ELISA was found to be sensitivity in the detection of *X. c. pv. vesicatoria* in naturally infected tomato seeds. The fact that the extinction values obtained were within the range of negative control for infestation level of one per cent and below in a artificially infested seed samples clearly corroborated with the dilution plating technique in which no pathogen was detected in sample at these levels of infestation. However, ELISA detected the presence of pathogen at 10 per cent infestation level. Thus ELISA is less sensitive technique in detecting the presence of bacteria in naturally infected seed sample at lower level of infestation.

The results are in agreement with the findings of Barzic and Triglet (1982) ; Khan Van Vuurde *et al.* (1983) who reported that ELISA was less sensitive then IF in detecting presence of seed borne plant pathogenic bacteria. Van Vuurde *et al.* (1984a,b) reported the sensitivity of ELISA is about 10 to 100 times lower than indirect IF in detecting seed borne pathogenic bacteria.

Efficiency of Enzyme Linked Immuno sorbent assay in detecting *X. c. pv. vesicatoria* in naturally infected tomato seeds

The extinction values obtained for sub sample compared well with the population of the pathogen detected by dilution plating on Tween-80 medium for most of the seed samples. A better comparison was obtained between ELISA and dilution plating in sub sample containing higher number of seeds (Table 21). In cv. Rupali extinction values of 0.206, 0.209 and 0.220 compared well the population of 2.93×10^2 , 2.90×10^2 and 2.90×10^2 cfu/ml seed extract respectively, while the extinction values in the same cultivar in sub sample containing 200 seeds were 0.175, 0.196 and 0.197, and the pathogen population detected by dilution plating in corresponding seed extracts were 75, 77 and 79 cfu/ml. Similar trends was obtained in other cultivars also. It could be seen that high extinction values were obtained for seed samples containing higher population of *X. c. pv. vesicatoria* which compared well with the dilution plating technique. Thus the result of our investigation confirm reports made by other workers. ELISA was found to be efficient and

promising in detecting *P. s. pv. phaseolicola* (10^6 cfu/ml) in bean seeds (Weaver and Guthrie, 1978 and Van Vuurde and Bovenkemp, 1981), Wesley (1982) also reported that ELISA could be used in the detection of *Corynebacterium michiganense* in tomato seeds. Further, he reported that ELISA was very efficient and that single seed ELISA was effective in determining infection rates of one per cent or more. Whereas batch ELISA was able to detect at least 0.06 per cent infection rate.

Barzic and Trigalet (1982) demonstrated the use of ELISA in detection of seed borne *P. s. pv. phaseolicola*. Shepperd (1983) strongly advocated the use of ELISA and IF in detection bean blight. Khan (1996) found ELISA to be efficient technique in detection of *X. c. pv. vignicola* in naturally infected cowpea seeds.

Effect of bactericides/antibiotics sprays on the development of bacterial spot of tomato caused by *X. c. pv. vesicatoria* under field condition

The two field trials conducted during monsoon seasons of 1995 and 1996, using bactericides and antibiotics demonstrated that the three sprays given on 35, 55, and 75 days old plants with antibiotics alone viz., streptomycin sulphate (500ppm) or streptocyclin (500ppm) or a combined spray with of streptomycin sulphate (500ppm) and copper oxychloride (2000ppm) very effectively reduce the development of bacterial spot of tomato both on the leaves as well as the fruits.

The number of spots / cm^2 observed in the plants sprayed with streptomycin sulphate + copper oxychloride, streptomycin sulfate and streptocycline alone were 1.30, 1.34, 1.70 respectively as compared to 4.86 spots/ cm^2 of leaf let recorded in control plants in a field trial conducted during 1995. Similarly the per cent leaf let infected per leaf was also reduce by 50 per cent as compared to control.

The same trend was also observed in a 2nd field trial conducted during 1996 in which 1.35, 1.27 and 1.58 spots / cm^2 were observed in plants sprayed with a combination of streptomycin + copper oxychloride or streptomycin sulphate or streptocycline alone.

As regards the infection on fruits, no disease incidence was recorded in fruits sprayed with any of the antibiotics. However, 18.37 and 17.45 per cent of fruits were found to be infected in case of Paushamycin (500ppm) and copper oxychloride (2000ppm) alone sprayed plants during 1995. During 1996, 33.33 and 27.27 per cent diseased fruits

were recorded in plants sprayed with chemicals. While, plants sprayed with combination of Paushamycin + Dithane M-45 recorded 6.25 and 13.18 per cent diseased fruits during 1995 and 1996 respectively as compared to 72.03 and 81.5 per cent infected fruits in control.

The increase in yield in plants sprayed with a combination of Streptomycin sulphate + copper oxychloride during the two year (1995 and 1996) was 127.65 and 105 per cent respectively while streptomycine (126.60% and 107.56%) alone or streptocycline alone (125 and 106 per cent) recorded higher yield over control. Falico de Alcaroz (1973) effectively controlled bacterial spot of tomato and capsicum by streptomycin sulphate. Jones (1979) observed that the applications of streptomycin sulphate as spray under field conditions suppressed disease development. Kishun and Sohi (1982) reported that three application of Agrimycin-100 were effective in reducing the intensity of infection due to *X. c. pv. vesicatoria* in tomato. Conover and Gerhold (1981) reported that Basic copper sulphate plus maneb, basic copper sulphate plus mancozeb were effective against *X. c. pv. vesicatoria* on tomato. Jones and Jones (1986) noted that fresh application of copper hydroxide plus mancozeb twice at weekly interval reduced disease severity. Maringoni *et al.* (1986) reported that in field trial, application of maneb plus Zineb and copper oxychloride at 7 days interval reduced the bacterial spot disease incidence.

Verma *et al.* (1980) reported that Fytolon (copper oxychloride) sprayed to rice plant reduced the intensity of bacterial leaf blight and bacterial streak of rice. Shekaraiah (1981) reported moderate control of bacterial blight of cowpea by spraying blitox at 2000ppm at 10 day interval. Gitaitis *et al.* (1986) reported that sprays of copper and copper plus maneb mixture controlled leaf spot and stem canker symptoms and significantly increased cowpea yield.

Rajpurohit and Lodha (1982), stated that combined sprays of Agrimycin - 100 + blitox - 50 (copper oxychloride) reduced the intensity of black arm of cotton caused by *X. c. pv. malvaceum* by 26.57 per cent. Thapliyal and Dubey (1986) reported that combination of Agrimycin -100 plus blitox-50 recorded improved control of *X. c. pv. phaseoli* on soybean and highest yields were obtained.

Hernandez and Medina (1991) advocated seed treatment of tomato with sodium hypochlorite and chlorohydric acid at two and three per cent for 20 min to effectively controlled the disease. Mandge *et al.* (1992) from India reported that *X. c. pv. vesicatoria* was effectively controlled with 3 sprays of Agrimycin + copper oxychloride + corboryl.

Jones *et al.* (1993), observed suppression of population of *X. c. pv. vesicatoria* in the leaves sprayed with cupric hydroxide + mancozeb, while mancozeb alone or copper ammonium carbonate had no effect on the population build up of the pathogen.

Koushik *et al.* (1994), reported that bacterial spot of pepper could be effectively controlled by weekly combined spray of copper + mancozeb.

SUMMARY

VI SUMMARY

Bacterial spot of tomato caused by *Xanthamonas capestris* pv. *vesicatoria* has become a serious threat for tomato cultivation in the recent years in Karnataka State. The bacterium isolated from infected leaves and fruits showing typical symptoms of bacterial collected from different agro-climatic zone of the Karnataka state was identified as *X. c.* pv. *vesicatoria* on the basis of morphological, physiological, biochemical, hyper sensitive reaction on tobacco cv. Samsun and pathogenicity test.

The 14 strains of *X. c.* pv. *vesicatoria* isolated from tomato were found to infect all the popular tomato cultivars tested viz., Bony best, Rashmi, Roopali, Rajani, Naveen, Vyshali, NS-286 and NS-815. However, none of the strains were virulent on tomato genotype viz., Hawaii-7998 and one strain caused hyper sensitive reaction on Hawaii-7997. Further, all the 14 strains isolated from tomato infected tomato genotype Hawaii-7981, except Shimoga strain which induced Hyper sensitive reaction. None of the tomato strains of *X. c.* pv. *vesicatoria* infected the highly susceptible pepper cultivar California wonder and the three near isogenic line ECW-10R, ECW-20R and ECW-30R carrying resistant genes BS1, BS2 and BS3. The two strain isolated from pepper infected pepper cv. California wonder, but fail to infect any of the tomato cultivar. Further, the pepper strain from Himachal Pradesh produced a compatible reaction on ECW-10R while the strain from Bangalore caused infection in ECW-30R. On the basis of pathogenicity on tomato plants, the 14 strains isolated from tomato are categorized into tomato group. Further, on the basis of reaction of the 14 strain on the tomato genotypes, the 13 strains could be designated as race-1 (T1) while the Shimoga strain is designated as race T3, prevailing in Karnataka state while 2 strains isolated from pepper are categorized into pepper group in view of that there infectivity on pepper cultivar and on the basis of their reaction on near isogenic lines of pepper, the pepper strain from Himachal Pradesh is designated as race-1 and Bangalore strain into race-2 of pepper group.

Except for one tomato strain isolated from Shimoga none of the strain of *X. c.* pv. *vesicatoria* belonging either to tomato group or pepper group hydrolysed starch nor exhibited pectolytic activity.

There was variation in the presence of plasmids isolated from strains of *X. c. pv. vesicatoria*. A single plasmid with a size approximately 30kb was detected 9/14 strains.

Xanthamonas campestris. pv. vesicatoria could survive upto 6 ½ and 7 ½ months on tomato seeds stored at 25°C and 5°C, respectively.

The pathogen survived on crop residue placed at the surface of soil upto 6 ½ months, while it could survive for 4 to 5 months in the crop residue buried at a depth of 7.5cm or mixed uniformly in the soil. The bacteria survived 6 ½ and 8 months in unsterilized and sterilized infested soils, respectively.

The bacterium survived epiphytically for a period of 60 days on *Cyperus rotundus* and *Cynodon dactylon* and for 15 days *Hemilia sanchifolia* and *Marselia quadrifolia* indicating that the pathogen has a short resident phase.

The pathogen was isolated from the mouth parts of 4 insects mainly *Aulocophora cincta*, *Ecoptaera praemorsa*, *Henosepilachna vigintioctopunctata* and *Phenoroctera gracillius* and it was not isolated from *Bemisia tabaci* and *Menochilus spp.*

The disease had slight effect on the quality of fruit especially with regards to the ascorbic acid content, a slightly reduction in ascorbic acid content of was recorded in disease fruits (22.78mg/100g) as compared to healthy fruit (23.02mg/100g).

The incidence and severity of disease was more in crops grown during the period in which the maximum temperature ranged from 27-32°C and relative humidity was in the range of 80-95 per cent with fairly good rainfall. The disease was noticed during the summer months (i.e., Feb - May) during which maximum temperature was fairly highly (32-35°C).

Enzyme linked immunosorbent assay applied with slight modification using HRPO-tetra methyl benzidine substrate system with reduced duration of incubations. The technique was found to be effective in detect in *X. c. pv. vesicatoria* in naturally infected

tomato seeds. The efficiency of ELISA compared well with that of dilution plating. High extinction were obtained (0.235-0.237) for seed sample carrying higher population of pathogen (318cfu/ml) as detected by dilution plating. However, ELISA was found to be less sensitive in detecting the pathogen in the seed. It could detect infestation level of 10 per cent but not lower level.

Studies conducted on the control of Bacterial spot of tomato by using bactericides/antibiotics either alone or in combination revealed that streptomycin sulphate (500ppm) plus copper oxychloride (2000ppm) or streptomycin sulphate at 500ppm or streptocycline (500ppm) alone were effective in suppressing the development of disease.

Plants sprayed with streptomycin sulphate or streptocycline alone are in combination with copper oxychloride reduced the disease incidence on fruits and highest yield per hectare was obtained in streptomycin sulphate plus copper oxychloride (21.4 and 18.14 tons/acre) sprayed plot followed by streptomycin (21.30 and 18.10 tons/acre) or streptocyclene (21.15 and 17.97 tons/acre) alone at the 500ppm, where as the yield in control plot was only 9.40 and 8.72tons/acre accounting for 127.65 and 108 per cent increase in yield over control during the year of trails i.e., 1995 and 1996 respectively.

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VII REFERENCES

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* Original are not seen

APPENDIX

APPENDIX-1

Buffers and Reagents used :

A. Enzyme linked immunosorbent assay (ELISA)

(i) Coating Buffer (pH. 9.6)

Na₂CO₃ 1.59 g (15 mm)
NaHCO₃ 2.93 g (35 mm)
NaN₃ 0.20 g
Stored at 4°C for max. 14 days.

(ii) Washing solution (pH 7.2)

Phosphate buffer saline Tween (PBS Tween)

(PH 7.2)

NaCl 80.0 g
KH₂PO₄ 2.0 g
Na₂HP₄.2H₂O 29.0 g
Kcl 2.0 g
NaN₃ 2.0 g
Tween -20 5.0 ml
Distilled water adj. 1000 ml.

(iii) Dilution buffer (pH 7.2)

Albumin - Bovin 10.0 g (Sigma at. 8082)
PBS-Tween adj 1000 ml

(iv) Conjugate - globulin : Goat anti-rabbit IgG

(v) Enzyme : Horseradish peroxidase

(vi) Enzyme substrate : Tetra methyl benzidine (TMB.)

Solution I

50 mM glucose
25 mM tris cl (PH 8.0)
10 mM EDTA (pH 8.0)

Solution II

0.2 N NaOH (freshly diluted from a 10 N stock)
1% Sodium dodecyl sulphate (SDS)

Solution III

5 M potassium acetate 60 ml
Glacial acetic acid ... 11.5 ml
Water 28.5 ml

Solution A: TE (Tris EDTA) buffer containing 5% Dow corning antifoam and Xylene cyanol (0.1mg/ml).

Solution B : 1 M Sod. hydroxide containing 2% SDS.

Phenol : Chloroform reagent : Equal volume of phenol equilibrated with Tris pH 8.0 and chloroform : isoamyl alcohol (24: IV/V).

100 ml STET solution :

0.1 M Nacl
10 mM Tris cl (pH 8.0)
1 mM EDTA (pH 8.0)
5% Triton X-100 or 10% SDS.

45% Neutral lead acetate solution : Dissolve 225 g of neutral lead acetate in water and dilute to 500 ml.

22 % potassium oxalate solution : Dissolve 110 g potassium oxalate ($K_2C_2O_4 \cdot H_2O$) in water and dilute to 500 ml.

2. Composition of various non-selective, semi-selective media.

(i)	Nutrient agar (NA)	in grams g/l
	Peptone (Dif Co)	5 g
	Beef extract	3 g
	Agar (Difco)	18 (pH 7.2 ± 0.2)

(ii) Yeast extract - dextrose $CaCO_3$ agar (YDA) (After Schaad, 1980)

Yeast extract	10.0
Dextrose (glucose)	20.0
Calcium carbonate (Light powder)	20.0
Agar	18.0

* Autoclaved separately.

Calcium carbonate was finely ground to avoid precipitation quickly. The autoclaved medium was cooled to $50^\circ C$ in a water bath and $CaCO_3$ suspended by Swirling before pouring the plates.

(iii)	Tween -80 media (Mc Guire and Jones, 1984)	g/l
	Peptone	10.00
	Potassium bromide	10.00
	Calcium chloride	00.25
	Agar	15.00
	Tween-80	10.00 ml
	Cycloheximide	50.00 mg
	Cephalexin	35.00 mg
	5-Fluorouracil	12.00 mg
	Tobramycin	00.40 mg

- Cycloheximide, Cephalexin, 5-Fluorouracil and Tobramycin was filter sterilized and *added after autoclaving*.

Lugol's iodine solution (1% Iodine, 2% potassium iodide).

LB-broth. Lory and broth

EDTA : Ethylene diamine tetra-acetic acid (Disodium salt)