EPIDEMIOLOGY AND PATHOGENESIS OF BACTERIAL BLIGHT OF GUAR CAUSED BY *Xanthomonas campestris* pv. *cyamopsidis* (PATEL, DHANDE & KULKARNI) DYE

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JAYANTI PRASAD JAIN

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Dr. B.P. Chakravarti
Ph.D. (Minn.), Assoc.IARI, FPSI
Associate Professor & Head
Department of Plant Pathology

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CERTIFICATE

THIS IS TO CERTIFY THAT THIS THESIS ENTITLED "EPIDEMIOLOGY AND PATHOGENESIS OF BACTERIAL BLIGHT OF GUAR CAUSED BY Xanthomonas campestris pv. cyamopsidis (PATEL, DHANDE AND KULKARNI) DYE" SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE SUBJECT OF PLANT PATHOLOGY OF THE UNIVERSITY OF UDAIPUR IS A BONAFIDE RESEARCH WORK CARRIED OUT BY Mr. JAYANTI PRASAD JAIN UNDER MY SUPERVISION AND THAT NO PART OF THIS THESIS HAS BEEN SUBMITTED FOR ANY OTHER DEGREE. THE ASSISTANCE AND HELP RECEIVED DURING THE COURSE OF INVESTIGATION HAVE BEEN FULLY ACKNOWLEDGED.

B.P. Chakravarti
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(B.P. CHAKRAVARTI)

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J. P. Jain

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INTRODUCTION

Guar or clusterbean, Cyamopsis tetragonoloba (Linn.) Taub. (Syn. = Cyamopsis psoralioides DC), belongs to family Leguminosae. According to Vavilov (1950) guar, although never seen in wild state is supposed to be native to India while Gilletts (1958) considers Tropical Africa to be its probable centre of origin.

The crop is extensively grown in arid and semi-arid regions. In India, chief guar growing areas lie in the states of Rajasthan, Gujarat, Haryana, Punjab and Uttar Pradesh. The total acreage in India under this crop is 2,409.4 thousand hectares with a total production of 566.7 thousand tonnes (Anonymous, 1981). In Rajasthan it covers 1,965.4 thousand hectares with a total production of 314.6 thousand tonnes (Anonymous, 1981). Among all the states, Rajasthan ranks first in acreage as well as in production. Guar is well adapted to conditions prevailing in Rajasthan and is grown as vegetable, green manure, feed and fodder crop. Of late, the crop has been credited with having potentialities of producing an excelled gum for industry.

Guar is generally taken as mixed crop with sorghum (Sorghum vulgare Pers.), pearl millet (Pennisetum typhoides Stapf. and Hubb.) and other crops. It is a crop of 2½ - 3 months duration, taken more commonly as rainy season crop. Summer crop is sown in February-March and harvested in May.

[illegible], gum [illegible] from

Guar seed is highly nutritive and is often fed as a concentrate to all livestock. Raw pods are a source of nutritive vegetables and contain 82.5% moisture, 3.7% protein, 0.2% fat (ether extract), 2.3% fibre, 9.9% carbohydrates, 1.4% mineral matter, 0.13% calcium, 0.05% phosphorus, 58 mg/100 g iron, 330 IU/100g carotene (calculated as vitamin A) and 49 mg/100g vitamin C.

Guar flour due to its colloidal nature, is used as a stabilizer and thickner in food products like bakery mixtures, salad dressings and ice creams. Guar flour is a commercial source of gum which is reported to be the most economical and effective viscosity building agent, and it is due to this property that it is finding increasing use in various applications. Guar gum is used in textile industry in certain finishing operations, wrap sizes and printing posters and in paper industry it aids in improving sheet formation. In mining industry, guar gum has found its use as flocculant and filterant in the refinement of mineral ores. It is commercially used in explosives in forming a plastic seal which cutt-off entry of water in cartridges and, thus they are rendered moisture proof. Guar fruits are laxative and are useful in biliousness and night blindness.

Besides fungal and viral diseases, guar suffers from bacterial blight disease caused by Xanthomonas campestris pv. cyamopsidis which is responsible for appreciable loss especially during rainy season (Srivastava and Rao, 1963b). It appears

almost every year and at times may cause considerable damage, depending upon the variety and environmental conditions. Gupta (1978) and Lodha and Gupta (1981) have reported 50 - 60 per cent yield losses under artificial inoculation.

Despite its destructive nature, enough information on guar bacterial blight is not available. Investigations were carried out with respect to occurrence and severity, extent of loss, epidemiology, method of detection of bacterium in seed, altered host physiology and varietal reaction, results of which are presented herein.

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REVIEW OF LITERATURE

Cyamopsis tetragonoloba (Linn) Taub. locally known as guar is an annual legume of Indian origin. It is attacked by more than 30 fungal, bacterial and viral pathogens. Among them, bacterial blight caused by Xanthomonas campestris pv. cyamopsidis (Patel et al.)Dye has assumed importance recently as the disease often becomes destructive under favourable conditions. The disease was first reported from Patna (Bihar) and Khopoli (Bombay) by Patel et al. (1953) and the bacterium was named as X. cyamopsidis Patel, Dhande and Kulkarni. They observed only olive coloured spots on leaf lamina, therefore, the disease was named as bacterial leaf spot. Later, Patel and Patel (1958) described a severe leaf blight disease on the same host from Anand, Bombay and indicated that the symptoms of the disease resembled those caused by X. campestris. They attributed it to a different species which was described and named as X. cyamophagus. Srivastava and Rao (1963) recorded severe outbreak of bacterial blight of guar during the monsoon of 1961 at the farm area of Indian Agricultural Research Institute, New Delhi. They studied the symptoms in detail and observed that leaf blight and leaf spot symptoms were due to hydathodal and stomatal infection respectively and concluded that the same causal organism was responsible for the entire symptom complex of the disease. The pathogen was found to produce symptoms on all plant parts except flowers and fruits. It was further

suggested that the pathogen X. cyamopsidis should stand valid on the basis of priority and the other species, X. cyamophagus which was described later, should be invalidated.

Dye (1962) made a comparative study of 209 phytopathogenic xanthomonads comprising 57 recognised species using 30 different tests and suggested that many species within the genus Xanthomonas could well be regarded as single species comprising special forms adapted to particular hosts. However, he stressed the necessity of further investigations to determine the most susceptible host, the host range, and the mechanisms of pathogenicity of xanthomonads. Dye and Lelliott (1974) recognised only 5 species of Xanthomonas and as such X. cyamopsidis was considered to be sub-species of X. campestris as the former was generally indistinguishable from the latter except by their host range and requested to the Judicial Commission of the International Committee on Systematic Bacteriology to accept this approach in naming phytopathogenic bacteria. Young et al. (1978) suggested the use of term 'pathovar' at the infrasubspecific level on the basis of their host range. Accordingly, the name of guar bacterial blight pathogen was proposed as X. campestris pv. cyamopsidis (Patel et al) Comb. novo. Recognising the pathovar system, International Society of Plant Pathology, Committee on Taxonomy of Phytopathogenic Bacteria named the bacterium as X. campestris pv. cyamopsidis (Patel, Dhande and Kulkarni) Dye, 1978.

Srivastava and Rao (1963a) and Orellana et al. (1965) have described the symptoms in detail and reported that symptoms of bacterial blight disease appeared on all aerial plant parts except flowers. On leaves, symptoms appear as round oily spots which later coalesce and result into dark brown to black necrotic spots and often caused partial or total blighting. Under field conditions, large, light coloured, angular lesions bounded by veins were most common symptoms. Infection develops systemically and advances through the petiole into the stem producing black longitudinal streaks. Wilt of apical leaves, curvature of the stem and finally splitting and breaking of the stem. Srivastava and Rao (1963b) also observed heavy spotting on pods and reported that the pathogen was internally seed borne. Blackening of the stem or formation of black lesions which completely girdle the stem at soil level and yellowing of cotyledons were found to occur on young seedlings as a result of seed borne bacterial infection (Orellana et al., 1965). However, Srivastava and Rao (1963b) reported that seedlings from diseased seeds developed cotyledonary lesions soon after emergence.

Biochemical and cultural characteristics of bacterium have been reported by several workers (Patel et al., 1953; Srivastava and Rao, 1963a; Orellana et al., 1965). The colonies are yellow and gummy on nutrient agar but colourless on potato dextrose agar media. Bacterium is rod shaped with rounded ends, borne singly or in short chains, 0.8 x 1.7 μm in size, gram

negative, capsulated, uniflagellate, and non-spore forming. The organism grew well between 27-35°C with optima between 28-30°C, produced acid from sucrose, glucose, xylose, galactose and mannose, nitrates reduced, indol not produced, H_2S produced, starch hydrolysed, gelatin liquefied and litmus milk made alkaline.

Orellana and Kinman (1970) reported that susceptibility of certain field resistant guar varieties to bacterial blight in Texas was due to presence of virulent race of Xanthomonas campestris pv. cyamopsidis. Based on reactions to 6 different varieties of guar, which they called as differentials, two races of guar bacterium viz. race 0 and race 1 were recognised. Later, Orellana and Weber (1971) based on serological techniques considered races 0 and 1 of X. campestris pv. cyamopsidis as serotypes rather than distinct races.

The importance of intensive and general utility surveys has been emphasized by Miller (1946), Chester (1959), Vallega and Chiarappa (1964) and Church (1971). In the survey conducted during 1968 and 1969 at 11 locations in 9 different states (Patel et al., 1971) found 10 bacterial disease including bacterial blight of guar. Singh and Edward (1975) recorded occurrence of 17 bacterial diseases in Allahabad but they did not find bacterial blight on guar.

Severe losses on account of bacterial blight pathogens have been reported, both quantitatively and qualitatively in different crop plants. Information on overall losses are not

available, although, some workers have given loss figures for some specific diseases. Gupta (1978) has reported yield losses as high as 58 per cent in Nav Bahar variety under artificial inoculation conditions in Udaipur, Rajasthan. Lodha and Gupta (1981) observed 50 per cent loss in grain yield in variety E.C. 248 at 72 per cent blight intensity under arid land conditions of Jodhpur (Rajasthan). They further observed that the effect of various disease intensities on plant height, number of pods per plant and seed yield were statistically significant. The yield losses may be further aggrevated in areas with more rainfall and favourable temperature.

According to Srivastava and Rao (1963b, 1970) bacterial blight is wide-spread in guar growing areas of India and the disease is most destructive to rainy season crop. They reported that rainfall, high humidity and suitable temperature were essential for effective dissemination and spread of the pathogen and found that the summer grown crop under irrigated condition was more or less free from disease. Shekhawat and Chakravarti (1976) reported that high humidity coupled with temperature ranging between 22-34°C were most favourable for bacterial leaf spot of chillies (*Xanthomonas vesicatoria*). Shekhawat and Patel (1977) while studying on bacterial blight of cowpea (*X. vignicola*) and leaf spot of gram (*X. phaseoli*) observed that both diseases became severe during monsoon with RH 50-95 per cent and temperature 24-32°C.

The ability of the pathogen to infect and establish itself on many hosts other than the suscept leads to the danger of epiphytotic and is also important in relation to the recurrence of the disease. However, in this respect xanthomonads differ because of their host specificity, although, the host ranges of various species and pathovars within the species have never been completely determined. There is some support for the opinion that xanthomonas may not be strictly host specific (White, 1930; Dye, 1958; Sabet, 1959; Starr, 1959; Klement and Lovrekovich, 1961; Starr and Dye, 1965; Rangaswami and Sanne Gowda, 1963; Shekhawat, 1975; Vakili, 1977; Kishun and Sohi, 1979; Beniwal and Parashar, 1979). According to Patel (1972) X. campestris pv. cyamopsidis also infects french bean but information on type of symptoms and nature of damage has not been given.

Klement and Goodman (1967) have shown that plant pathogenic bacteria multiply, produce high populations and cause typical symptoms when inoculated into their natural host plants, but failed to continue to multiply and cause only a typical necrosis when inoculated into non-host or incompatible plants.

Bacteria enter leaves and stems mainly through stomata, lenticels and wounds. Among artificial methods of inoculation, spraying bacterial suspension under pressure onto a leaf surface without causing visible water soaking of the

leaves probably approximates natural field infection. Matthee and Dains (1968) reported that the symptoms of the disease on pepper caused by Xanthomonas vesicatoria and X. pruni on peach were easily produced, if bacteria were spray inoculated. Vakili (1967) demonstrated that sand blasting of tomato leaves which caused epidermal abrasion and breakage of leaf hairs, and subsequent spraying of such leaves with a suspension of X. vesicatoria resulted in enhanced lesion number as compared to inoculation by infiltration under pressure. The latter method gave only 0-14 spots per plant while the former resulted in thousands of lesions. Incidence was similarly increased by breaking the leaf hairs with hand. Abrasion with 600 grit carborundum prior to inoculation was found better over hair brush method for producing a systemic toxemia, local chlorosis, water soaking and necrosis. Carborundum method has also been found suitable and convenient for X. vesicatoria on tomato (Shekhawat, 1975). Daft and Leben (1970) reported that only when leaf damaging winds during a rain occurred, soybean blight due to Pseudomonas glycinea developed.

Klement (1963) reported infection infiltration as the best method for rapid test of pathogenicity with phytopathogenic pseudomonads. However, this method has been found unsatisfactory with thin leaves of cereal plants by Hagborg (1970). He prepared an improved apparatus for injecting solutions and suspensions into the thin leaves of plants which

overcame this difficulty encountered by Klement's method. The syringe method was excellent according to Starr and Dye (1965) but they found it tedious and preferred pricking with multineedle for inoculation of various phytopathogenic bacterial species into bean pods. Rangarajan and Chakravarti (1970) however, found injection of bacterial suspension by hypodermic syringe into the stalk of maize a suitable method for the development of stalk rot by Erwinia carotovora. Basu (1966) was also of similar opinion that pre-inoculation host injury to tomato plants produced more disease by X. vesicatoria, Corynebacterium michigananse and P. tomato. Hartman and Kelmen (1973), however, reported that no infiltration or wounding of the tissues was needed in case of maize stalk rot caused by E. carotovora when they used some detergent with the inoculum. They suggested whorl inoculation method with 0.7 per cent of Tween 40 and inoculation by this method gave consistent results.

The process of reproduction leads to multiplication of bacteria and this is variable, depending upon the host. The extent of multiplication of bacteria in host is much influenced by the host reaction; development of symptoms depends on the number of cells present within the host tissues at a time. Diachum and Rroutman (1954) correlated the reaction of different hosts and rate of multiplication of Pseudomonas tabaci. They found that the bacterial population was maximum in leaves of susceptible Ky 56 Burley tobacco and minimum in the resistant

Burley 1819-19 while the population in their hybrid TL-100 was intermediate. Similar observations were recorded by Chamberlain (1962) who found that Xanthomonas phaseoli var. sojense multiplied in the leaves of resistant variety CNS soybean, but at a slower rate than in leaves of susceptible variety Lincoln. Chand and Walker (1964) correlated the tolerances of older leaves of cucumber to P. lachrymans which was 20 to 40 times lesser than in the younger leaves. Stall and Cook (1966) working with X. vesicatoria found that initial population of the bacterium was affected significantly by the host reaction and which in turn affected the symptom development in two chilli cultures viz. Yolo improved B (susceptible) and XV 17-78 (resistant). They inoculated the plants with two different suspensions containing 10^5 and 10^8 cells per ml of X. vesicatoria and observed that differences in lesion development were related to bacterial concentration. The lesser concentration of inoculum did not multiply significantly to induce necrosis or desiccation in resistant leaf tissues and the bacterial concentration in these tissues was much lesser than that in susceptible tissues. Leben et al. (1968) studied the population levels of P. glycinea in relation to symptom development in soybean and reported that (i) high populations of bacterium prevailed during the early stages of symptom development i.e. between 7-15 days after inoculation, (ii) a low inoculum concentration resulted in lower population maxima and delayed symptom expression, and (iii) the hypersensitive

isolate-variety combination produced lower population than water soaking combinations. Kawamato and Lorbeer (1972) observed an increase in population of P. cepacia to a level of 4.8×10^9 cells/leaf disc in young leaves from initial level of 10^4 cells/leaf disc. However, in mature leaves the bacterial population fluctuated widely, but did not exceed 4×10^5 cells/leaf disc, and leaves failed to develop symptoms. Further, they observed that the initial symptoms of water soaking and wilting was expressed when the peak population was attained and the population thereafter declined and none was reisolated once leaves became dry and brittle. HSu and Dickey (1972) while studying comparative growth of X. phaseoli and X. vesicatoria and development of symptoms respectively in bean and tomato leaves reported that growth pattern of both the bacteria were similar in their natural hosts, beans and tomato respectively but not in non-hosts. The population of the bacterium in natural hosts initially increased rapidly in the leaves, then gradually decreased and tissue necrosis appeared when the populations reached the maxima. However, the population of X. phaseoli decreased rapidly in tomato leaves, whereas the population of X. vesicatoria increased in bean leaves and both bacteria induced visible necrosis in leaves of their host, although, the former caused a rapid development of necrosis than later. Shekhawat (1975) reported that multiplication of X. vesicatoria was slower in chilli variety NP 46A (tolerant) as compared to IC 13257 (susceptible) in the

initial stages but after 11 days the population was almost same in both the varieties reaching 1.4×10^9 cells/leaf disc and 2.7×10^9 cells/leaf disc, respectively. Ranga Reddy (1981) observed maximum multiplication of X. campestris pv. oryzae in stem and leaves between 12 and 15 days after inoculation of bacterium through roots. The plants wilted when the bacterial population reached the level of 10^4 or 10^5 cells/ 2 cm leaf sample and even apparently normal leaf contained as many as 8×10^3 cells/2 cm leaf sample. Verma et al. (1981) found that bacterium X. campestris pv. malvacearum could produce symptoms on cotton leaves only at a population of $1\text{-}5 \times 10^{10}$/ gram fresh weight of leaves.

Scharen (1959) reported that typical symptoms appeared in resistant species when inoculated with very heavy inoculum concentration of X. phaseoli isolated from bean and with less number of cells per ml, a large bacterial population persisted in the resistant tissues without producing any symptoms. Basu (1966) has reported that minimal level of X. vesicatoria , C. michigananse and P. tomato to cause infection on tomato differed. X. vesicatoria could cause infection at minimal level of 1×10^3 cells/ml while this level was 1×10^6 for C. michigananse and P. tomato. Forster and Echandi (1973) and Hopkins and Schenck (1972) while working with C. michigananse on tomato and P. lachrymans on watermelon respectively reported similar results. Kawamoto and Lorbeer (1972) found

that initial symptom expression in young onion leaf blades infected with different concentration of P. cepacia was delayed from 1 to 5 days as inoculum concentration was decreased from 10^8 cells/ml to 2.8×10^3 cells/ml. Concentration less than 10^3 cells/ml failed to induce symptoms. Similar findings have been reported by Shekhawat (1975) in X. vesicatoria on tomato who found 6×10^3 cells/ml as minimal concentration to cause infection when inoculated with carborundum, however, this concentration was 6×10^5 when bacteria were spray inoculated. Beniwal and Parashar (1979) observed that with c. 3.5×10^3 cells/ml of X. phaseoli symptoms developed on Vigna radiata when abrasion was used. Most of these reports indicated that minimum concentration of 10^3 cells/ml or above was required to cause infection in bacterial plant pathogens.

Age of the host plant conditions its physiology and, consequently, its reaction to a pathogen. Last (1959) observed that in similar leaves of different ages, Xanthomonas malvacearum spread least in the oldest cotton leaves and initial invasion was more rapid in young leaves of young plants than in young leaves of old plants. Similar results were also reported by Salgado and Balmer (1975) and Silva and Cia (1975). For X. malvacearum on cotton, Nayudu and Walker (1964) and Kore and Dahiwal (1980) for X. vesicatoria on tomato, Chand and Walker (1964) for P. lachrymans on cucumber and Patel and Walker (1963) for P. phaseolina and X. phaseoli on beans.

On the contrary, Rangaswami (1962) reported that older chilli leaves were more diseased than younger leaves from bacterial leaf spot caused by X. vesicatoria. Shekhawat and Chakravarti (1976) and Kore and Dahiwal (1980) have reported that tomato plants of 43-50 days age group were more susceptible under artificial inoculation conditions to X. vesicatoria as compared to younger or older plants.

Host nutrition does affect the severity of bacterial plant diseases, but it is only one of the several factors that pre-disposes the plants to infection. Allington and Johnson (1942) showed that the liability of water soaking of potassium deficient tobacco plants was accompanied by susceptibility to Pseudomonas tabaci infection. Shear and Wingard (1944) concluded that increase in the severity of the disease in potassium deficient seedlings is due to an accelerated rate of bacterial multiplication resulting from increase in nitrate nitrogen in the conductive tissues which in turn was consequent on the shortage of potassium. On the contrary, bacterial wilt of cucumber caused by Erwinia tracheiphila was found to be promoted by a low nitrogen and high potassium supply (Anonymous, 1951). Nayudu and Walker (1960) reported that bacterial spot of tomato was markedly reduced by high nitrogen, low and high phosphorus or high potassium. Bacterial spot of peach was enhanced by high nitrogen and retarded by high phosphorus (Bachelder et al., 1956), high nitrogen, low phosphorus or potassium favoured

bacterial blight of limabean (Thaung and Walker, 1957), and high nitrogen or low potassium favoured angular leaf spot of cucumber (VanGundy and Walker, 1957). The diseases halo blight and common blight of bean caused by Xanthomonas phaseolicola and X. phaseoli, respectively have also been reported to be influenced by host nutrition (Patel and Walker, 1963). In both the cases, there was less disease when plants were supplied with either low or very high dosages of N, P and K; high dosages of P and K induced more common blight. Lipke (1968) studied the effect of nitrogen and potassium nutrition on the pathogenicity of X. malvacearum in cotton and reported that the pathogenicity was reduced by -N, -K and -N, +K treatments in nutrient culture experiments. Similar results for X. malvacearum on cotton have also been reported by Salgado and Balmer (1975) who observed fewer symptoms in plants in nutrient solutions lacking N and P. Shekhawat (1975) reported that tomato plants when supplied with low nitrogen or potash and high phosphorus had more disease, maximum being where K was entirely lacking. It was also observed that at lower and higher concentrations of Hoagland's nutrient solution the disease was more as compared when supplied with normal solution. Rao and Devdath (1977) reported that in bacterial leaf streak of rice, increased nitrogen tended to increase lesion length, whereas increased potassium decreased it; phosphorus at all levels had no effect on lesion length. It was also observed that on plants which were not supplied with nitrogen, the incubation period was prolonged.

Seeds play a vital role in associating pathogenic bacteria which may prove hazardous for the seed or the new plant growing from it. Seed borne bacterial plant pathogens produce seed rots, seedling rots, diseases at various stages of crop growth and influence the crop stand and ultimately the yield. Seed is also known to be a potent source of survival and dissemination of bacteria (Orton, 1931; Cormack, 1961; Hunter and Brinkerhoff, 1964; Crosse, 1968; Rangarajan and Chakravarti, 1970; Anil Kumar and Chakravarti, 1971; Shekhawat and Chakravarti, 1976, 1979). The guar blight bacterium has been reported to be internally seed borne (Srivastava and Rao, 1963b; Orellana et al., 1965). Srivastava and Rao (1963b) isolated the bacterium from apparently diseased and shrivelled seeds of several varieties when plated on nutrient agar after surface sterilization. The infection of the seeds ranged from 3 to 95 per cent in various varieties. However, they did not describe the symptoms on the diseased seeds on the basis of which apparently diseased seeds were selected for plating. They also reported that seedlings from seeds collected from summer grown crop were free from infection, although, it was not clear that how long the bacterium could survive in seeds collected from rainy season crop. Based on field and nursery observations on the development of bacterial blight disease on guar. Orellana et al. (1965) concluded that the bacterium X. campestris pv. cyamopsidis was definately seed borne in nature.

It is not always easy to detect seed borne bacterial plant pathogens from seeds. Often the percentage of infected seeds in sample may be so low that detection become extremely difficult. Attempts have been made by various workers for seed detection of bacterial pathogens and different methods have been used by them (Katznelson and Sutton, 1951; Shackeleton, 1962; Guthrice et al., 1965; Parker and Dean, 1968; Srinivasan et al., 1973; Srinivasan, 1977; Shekhawat and Chakravarti, 1979; Gupta and Chakravarti, 1981; Randhava and Singh, 1981). One of the simplest techniques, i.e. visual separation of bluish white fluorescence on bean seeds infected with Pseudomonas phaseolicola was used by Parker and Dean (1968) and Taylor (1970). Bacteriophage method in detection of Xanthomonas phaseoli and P. phaseolicola on beans (Katznelson and Sutton, 1951) and X. campestris pv. malvacearum on cotton (Randhava and Singh, 1981) and serological techniques to detect P. phaseolicola on beans (Guthrice et al., 1965) have also been used but have not been advocated because of procedural complexities (Wallen et al., 1963).

The most acceptable test for phytopathogenic bacteria may be plating or growing of infected seeds on suitable substrates under optimum conditions for the development of typical colonies or disease symptoms on seedlings. Direct plating of seeds on agar media have been used in most studies (Wallen et al., 1963; Srivastava and Rao, 1963b; Chakravarti and

Rangarajan, 1967; Hoitink et al., 1968). Wallen et al. (1963) detected X. phaseoli var. fuscans from bean seeds by plating them on 1.5 per cent Difco nutrient agar after surface sterilization with 2% chlorine solution for 10 minutes. Further differentiation of the pathogen X. phaseoli var. fuscans from X. phaseoli was made by transferring the culture on proteinaceous agar media on which the former pathogen produced a brown pigment. Srivastava and Rao (1963b) successfully isolated X. campestris pv. cyamopsidis from guar seeds by plating them on nutrient agar after surface sterilization. Production of fluorescin colonies by P. syringae onto Difco Pseudomonas agar-F was found to be reliable for detecting the pathogen from bean seeds (Hoitink et al., 1968). Chakravarti and Rangarajan (1967) isolated a virulent strain of X. oryzae from rice seeds by plating seeds on agar medium. However, this method has its own limitation because of development of fast growing saprophytes.

Selective media have been developed in some cases to isolate plant pathogenic bacteria from seed and more commonly to confirm identification of the pathogen (Kado and Huskett, 1970; Anil Kumar and Chakravarti, 1971).

Shackeleton (1962) developed a method for detection of Xanthomonas campestris in which Brassica seeds were germinated on the moist absorbant paper in enclosed but not air tight plastic boxes and incubated at 22°C. Progressive

collapse, blackening and death of seedlings with in 18 days after sowing was indicative of the black rot pathogen which was further confirmed by injecting macerated extract of collapsed seedlings into Brassica indicator plants with sterile needle. If X. campestris is present characteristic leaf symptoms appear within a few days after inoculation.

Parashar and Leben (1972) used cotyledon method of seed detection for Pseudomonas glycinea. The bacterium was detected by pre-disposing germinated soybean seeds and seedlings to water saturated environment which favoured the formation of typical water soaked lesions on cotyledons. The method has provided evidence that the green house can be used to produce pathogen free seed from infected seed lot. Shekhawat and Jain (1978) reported that the bacterium X. campestris in cabbage and cauliflower seeds could easily be detected on the seedlings on water agar. Presence of a yellowish exudate on infected seeds, collapsed condition of seedlings or presence of V-shaped black rot lesions on cotyledon gave a clear indication of the infection caused by X. campestris, which was further confirmed by pathogenicity tests. Srinivasan et al. (1973) developed and standardised a method for detection of Xanthomonas campestris in cauliflower seeds. Seeds soaked in 200ppm solution of Aureofungin were plated on 1.5% Bacto agar and incubated at 20°C. The normal healthy seedlings grew rapidly while in diseased seeds germination was delayed and emerging

hypocotyl and cotyledons were yellowish and pulpy. Such seedlings were found to collapse on agar surface. In infected seedlings V-shaped marginal lesions were observed on emerging hypocotyls which showed bacterial ooze on examination. The association of bacterium was further confirmed by proving Koch's postulates. Shekhawat and Chakravarti (1979) advocated the use of cotyledon method to detect X. vesicatoria in chilli seeds. The seeds after 15 days of sowing in sterilized soil were exposed for 3-5 days to 95-100% RH and 24-30°C temperature, thereafter, the plants were kept in cage house. The infection on cotyledons developing from infected seeds appeared 30-40 days after planting in the form of small ash coloured spots. The bacterium was isolated on triphenyl tetrazolium chloride medium and Koch's postulates were proved by inoculating chilli seedlings. Gupta and Chakravarti (1981) reported that the infection in cowpea seeds can be detected by growing seeds in sterilized soil for 10-11 days. The cowpea seeds inoculated with bacterium when grown in sterilized soil yielded 100 per cent infected seedlings.

For successful pathogenesis, the parasite must obtain at site of its localization on the host, the kind and amount of various nutrients for its multiplication. The ecological concept to explain biochemical nature of parasitism has been proposed by various investigators as balance hypothesis of parasitism (Lewis, 1953) and the nutritional inhibition

hypothesis (Garber, 1956, 1961). When a pathogen successfully colonizes a host cell the metabolism of the cell is altered to the advantage of the pathogen. After infection certain biochemical changes, because of altered metabolic pathways operating in host-pathogen system, have been noticed in case of several bacterial diseases (VanGundy and Walker, 1957; Buddenhagen and Kelman, 1964; Goodman et al., 1967; Nayudu, 1969; Sinclair et al., 1970; Sridhar et al., 1979; Krishna Rao and Nayudu, 1979).

Kuprevicz (1947) observed 32 to 64 per cent chlorophyll reduction in potato variety Early Rose as a result of infection of Xanthomonas vesicatoria. Similar results were reported by Shekhawat and Chakravarti (1977) in chilli leaves infected with X. vesicatoria and by Padmanabhan et al. (1974) in citrus leaves infected with X. citri. Sridhar et al. (1979) observed that infection of detached rice leaves by X. translucens f. sp. oryzicola floated on water resulted in the loss of chlorophyll pigment compared to healthy tissues.

The evidence at hand which describes alterations in transpiration in plant tissues affected by bacterial pathogens is comparatively meagre. Grieve (1941) found that in spite of a considerable reduction in the healthy leaf area during the early stage of wilting, evidences were found of a high rate of transpiration in plants infected with Pseudomonas solanacearum.

On the contrary Buddenhagen and Kelman (1964) reported decreased transpiration in the bacterial wilt caused by P. solanacearum.

Plants affected by bacteria usually show an increase in the respiratory rate and it was attributed to (i) uncoupling of oxidative phosphorylation whereby O_2 is reduced to water by electron transport system, (ii) ATP produced by the plant is used up in metabolism stimulated by the pathogen, and (iii) utilization of oxygen in oxidative systems (Strobel and Mathre, 1970). Increased respiration as a result of infection with bacterial pathogen have been reported in tumor tissue from beet plants infected with Agrobacterium tumefaciens (Neish and Hibbert, 1943-44), potato variety Early Rose infected with Xanthomonas vesicatoria (Kuprevicz, 1947), bacterial wilt caused by Pseudomonas solanacearum (Buddenhagen and Kelman, 1964), ahd halo blight of bean caused by P. phaseolicola (Deverall, 1964). Hasan and Sabet (1960) observed that respiration first decreased and reached maximum in 5-7 days in Domain Sakel cotton plants inoculated with X. malvacearum. however, after appearance of pin head spots, respiration began to increase and was more than the healthy one.

Padmanabhan et al. (1974) studied the mineral composition of canker, halo and green region of citrus leaves infected by Xanthomonas citri. It was observed that potassium and calcium were less and sodium was more in the canker and halo regions of diseased leaves, although, in case of potassium the

decrease in halo region was not appreciable. Sidhan and Parashar (1981) estimated mineral elements in 24 guar varieties/ lines showing various degrees of disease intensity to Xanthomonas campestris pv. cyamopsidis and reported the calcium concentration decreased in diseased leaves. They also reported a highly significant positive correlation between sodium and disease intensity and highly significant negative correlation between potassium, sodium and disease intensity.

A general increase or decrease in total, reducing and non-reducing sugars has been recorded for a number of bacterial diseases. Chanturiya (1959) reported that in mulberry leaves infected by Pseudomonas mori both total sugar and reducing sugar increased. Keen et al. (1967) observed increasing accumulation of reducing sugars till the 4th day of inoculation of cucumber plant by P. lachrymans. However, Mohan et al. (1978) found a decrease in the amount of reducing and non-reducing sugars in Co. Pusa 4, V 38 and CM 11 varieties of cowpea following infection with Xanthomonas vignicola. Sridhar et al. (1979) observed no change in reducing sugar level in healthy leaves and those inoculated by X. translucens f. sp. oryzicola. In both the cases, although reducing sugar increased initially during the first day of incubation in leaves floated on water, the values declined from second day onwards. Changes in the amount of carbohydrates in rice leaves infected by X. oryzae were also reported by Misawa and Miyazaki (1972), Moses et al. (1975), and Krishna Rao

and Nayudu (1979). Moses et al. (1975) reported that decrease in reducing and nonreducing sugars in rice leaves developed after bacterial blight infection was associated with the intensity of disease. Krishna Rao and Nayudu (1979) studied the changes in the organic constituent of rice leaves upto 18 days after inoculation with X. oryzae and reported that blight infection resulted in an increase of reducing sugars, but a decrease in non-reducing sugars. The total sugar content in diseased leaves 4 and 8 days after inoculation decreased whereas an increase was recorded in earlier and later stages after inoculation. Padmanabhan et al. (1974) observed a decrease in total sugar contents in citrus leaves infected with X. citri.

Nitrogen metabolism of the host has been known to be affected as a result of infection with pathogens though the degree of its effect is governed by the nature of host-parasite interaction. A review by Braun (1962) has acknowledged general agreement on the fact that there were marked differences in the nitrogenous constituents between normal resting cells and actively dividing tumor cells, incited to division by the crown gall bacterium, Agrobacterium tumefaciens. Neish and Hibbert (1943) have reported that tumor tissues had three times the protein of normal tissue and the difference in water soluble protein was 6:1 in favour of tumor tissues. Klein (1952) reported that greatest increase in protein and soluble nitrogen became apparent in tumor tissue late in its development 27-35 days after inocu-

lation. He postulated that nitrogen of the whole plant was being mobilised into the tumor and result in nitrogen-deficient appearance of the tumor plant. Lee (1952) recorded the highest percentage of total nitrogen and crude protein in the bacteria free crown gall tissues.

Patel and Walker (1963) conducted a full analysis of alterations in the amino acid and amide pool, as a result of pathogenesis by halo blight bacterium, Pseudomonas phaseoli cola in bean. They observed that free amino acid and amides accumulated in large quantities in infected bean tissues as compared to healthy tissues. Misawa and Miyazaki (1972) and Krishna Rao and Nayudu (1979) recorded a decrease in water soluble, water insoluble and total nitrogen in the blight infected rice leaves and attributed this decrease because of inhibition of synthesis of various nitrogenous constituents as a result of infection besides some utilization by the bacterium. Decrease in protein content has also been reported by Chanturiya (1959) in mulberry leaves infected with P. mori and by Mohan et al. (1978a)in case of chilli leaves inoculated with X. vesicatoria.

Changes in phenolic substances of almost all the inoculated plants have been reported (Moustafa and Whittenbury, 1970; Sridhar et al., 1973; Reddy and Sridhar, 1975; Jalali et al., 1976; Mohan et al., 1978; Krishna Rao and Nayudu, 1979; and Sindhan and Parashar, 1981). However, the direct partici-

pation of an altered or activated phenol metabolism has been demonstrated less frequently as a factor of resistance in diseases caused by bacteria. Moustafa and Whittenbury (1970) observed that pathogenic species of Pseudomonas morsprunorum, P. syringae , P. tabaci and P. phaseolicola possess properties of counteracting phenolic compounds in host plants, whereas non-pathogenic species were inhibited by these compounds. They found that enzymatic activity related to mobilization of phenols and their conversion to quinone increased in bean plants infected by pathogenic isolates and thereby was responsible for decreased amount of phenol in diseased leaves. Such decrease in total phenols have also been reported by Jalali et al. (1976) in cotton plants infected with X. malvacearum and by Krishna Rao and Nayudu (1979) in rice leaves infected by X. oryzae. However, Mohan et al. (1978) reported that following infection with X. vignicola, the accumulation of total phenol in cowpea varieties Co. Pusa 4, V 38 and CM-11 increased. Sidhan and Parashar (1981) estimated total phenol in 24 guar varieties/lines showing various degrees of bacterial blight infection and reported that the resistant varieties had higher concentration of total phenols as compared to susceptible ones.

Ascorbic acid, which occurs as L-ascorbic acid and its oxidised form dehydroascorbic acid almost in all plants. The evidences regarding the alteration in ascorbic acid amount in plant tissues affected by bacterial pathogens are rare.

Its possible role in disease resistance in rice has been emphasized by Sridhar and Mahadevan (1979). Chanturiya(1959) observed that in mulberry leaves, ascorbic acid content was reduced by Pseudomonas mori.

Cellulose, a linear polymer of D-glucose units with B-1, 4 linkages, is the major constituent of plant cell wall. Cellulases secreted by bacteria are important in pathogenesis as they are known to hydrolyse cellulase and thereby cell walls collapse. Cellulose is attacked by cellulose (Cx) enzyme which hydrolyses the long glucose chains to cellobiose and perhaps other short chain polymers of glucose (Goodman et al., 1967). These are subsequently degraded to glucose by beta glucosidases. Husain and Kelman (1958) compared virulent and avirulent strains of Pseudomonas solanacearum from tomato and found that cellulolytic activity was 13 times more in culture filtrates of the virulent strains as compared to avirulent strains. This was also confirmed by Kelman and Cowling (1965). Goto and Okabe (1958, 1959) tested 13 species of Pseudomonas, 7 of Xanthomonas, 2 of Erwinia and one each of Corynebacterium and Agrobacterium for cellulolytic enzymes and reported that P. setariae, P. solanacearum, X. campestris, X. citri, X. nigromaculens, X. oryzae, X. pisi, X. pruni, X. resicatoria, E. carotovora, E. milletiae and C. sepedonicum produced cellulolytic enzymes. Guar bacterial blight pathogen X. campestris pv. cyamopsidis was reported to produce cellutolytic enzyme both in in vitro and

in vivo by Porwal and Chakravarti (1971). Hayward (1977) reported that *X. albilineans* and 3 nomen species of *X. campestris* group had beta-glucosidase activity whereas Kelman and Cowling (1965) reported that *P. solanacearum* did not possess beta-glucosidase activity. Kuprevicz (1947) did not observe any essential differences in the activity of protease in healthy and diseased tissues infected with *Xanthomonas vesicatoria* in potato. Friedman (1962) and Keen *et al.* (1967) reported proteolytic activity of *Erwinia carotovora* and *Pseudomonas lachrymans* respectively. Friedman (1962) postulated that greater virulence of *E. carotovora* could be attributed to increased proteolytic activity as a result of accelerated hydrolysis of host protein.

The polyphenol oxidase catalyses oxidation of phenols to antimicrobially active quinones and semiquinones (Farkas and Kiraly, 1962). The efficacy of the oxidised phenols seems also to be dependent upon their being able to remain in the oxidised stages in the region of parasitization. According to Maine (1960) and Maine and Kelman (1961) the polyphenol oxidase activity was more in tobacco stem tissues inoculated with *Pseudomonas solanacearum* and they correlated the enzyme activity to resistance to *P. solanacearum*. Sequeira and Kelman (1962) and Sequeira (1964) confirmed these results. Farkas and Lovrekovich (1965), however, found that polyphenol oxidase activity decreased in tobacco plants infected with *P. tabaci*.

Addy and Goodman (1972) correlated polyphenol oxidase activity with the intensity of foliar browning by Erwinia amylovora in apple leaves. It was observed that when virulent and avirulent strains of E. amylovora were inoculated on apple leaves the polyphenol oxidase activity with the virulent strain was as much as twice that associated with the control leaves and between the strains virulent showed more activity than avirulent.

Catalase i.e. hydrogen peroxide oxido-reductase catalyses decomposition of hydrogen peroxide or oxidation of various hydrogen donors. The importance of the enzyme in metabolism is still uncertain but decomposition of hydrogen peroxide is important as accumulation of hydorgen peroxide is toxic to cells. Increased activity of catalase has been recorded in few host parasite combinations only. Mohanty (1951) studied in vitro production of catalase by 22 strains of Corynebacterium fasciens obtained from different host plants and reported that all strains showed Copious formation of catalase in culture media. Kuprevicz (1947) reported a decrease in the catalase activity in potato plants infected with Xanthomonas vesicatoria whereas Chanturiya (1959) found that catalase activity was increased in mulberry leaves infected with Pseudomonas mori. Increased catalase activity has also been reported in bacterial wilt of potato caused by P. solanacearum (Buddenhagen and Kelman, 1964). Studying interaction of peroxidases and catalases between Phaseolus vulgaris and P. phaseolicola, Rudolf and Stahman (1964)

observed that in a comparison of several isolates of P. phaseolicola, the virulent isolates had higher catalase activity than the less virulent ones. It was proposed that the influence of catalase on host metabolism would be an inhibition of host peroxidases involved in the defense reactions of the host. They further suggested that the net result of the interaction between these two enzymes in either a susceptible bean plant or one infected by a virulent form of P. phaseolicola seems to be a redox potential which favours maintaining phenolic compounds in their reduced microbially less active form, rather than as antimicrobially active quinones.

The peroxidases, commonly known as POD, catalyses the dehydrogenation of a large number of compounds such as phenols and aromatic amines (hydroquinones and hydroquinoid amines) and especially, benzene derivative. The enzyme is known to be produced in several host-parasite combinations and is reported to be involved in pathogenesis by reacting with phenolic toxicants (Mahadevan, 1979). Matirhevska (1958) estimated peroxidase activity in 3 different varieties of tomato inoculated with Xanthomonas vesicatoria. He observed that in 3 tomato varieties inoculated with bacterium, intensive optical absorption 200 per cent higher than in the control, occurred in Marglobe while in Break O' day the increase was 123 per cent. In both the tomato varieties an increase of peroxidase activity was noticed for 6-7 days, whereas in variety-Skorospelka the period

was much shorter. The increase of the enzyme in the 3 varieties was not equal. Chanturiya (1959) and Lipetz and Galston (1959) also reported increased peroxidase activity in mulberry leaves infected with Pseudomonas mori and in crown gall tissue cultures of Parthenocissus tricuopidata infected with Agrobacterium tumefaciens, respectively. Peroxidase activity was studied by Rudolph and Stahmann (1964) in connection with their possible role in the virulence of P. phaseolicola and the susceptibility of the bean plant Phaseolus vulgaris to the pathogen. It was observed that peroxidase activity remain unchanged or decreased in susceptible bean leaves with a virulent strain of P. phaseolicola and increased following infection by a less virulent one. Analogously, peroxidase activity in a resistant bean variety showed a greater increase following infection with virulent strain than did a susceptible variety and concluded that peroxidase activity favoured resistance to P. phaseolicola. Lovrekovich et al. (1968) also observed that tobacco leaves having more peroxidase were resistant to infection with P. tabaci. They found that older leaves had more peroxidase activity than younger ones and correlated the age of tobacco leaves and susceptibility with the bacterium to the peroxidase activity. Urs and Dunlevy (1974) found that peroxidase from horse raddish (Armoracia rusticana) was bactericidal to the bacterium X. phaseoli var. sojensis in presence of potassium iodide and water at 50 µg/ml.

Breeding resistant varieties, although is a costlier method of plant disease control, yet it is one of the best methods to avoid losses caused by bacterial diseases in plants. Attempts have been made by various workers to find out resistant lines against bacterial blight of guar. Patel (1972) reported that none of the varieties of guar was resistant to Xanthomonas campestris pv. cyamopsidis. Chahal et al. (1979) screened 331 guar lines collected from Punjab and Haryana against bacterial blight pathogen and reported that 4 varieties of guar viz. G.85, G. 102, G. 126 and G. 255 were resistant and 15 moderately resistant; remaining 312 lines were either susceptible or highly susceptible. Sharma and Dubey (1981) screened 65 varieties of guar under conditions of natural epiphytotics and found 29 varieties as resistant, 12 as moderately resistant and the rest as susceptible or highly susceptible. They also observed that glabrous vegetable types were more susceptible than hairy grain types. Srivastava and Rao (1963b) reported that bacterial blight was more destructive in pubescent varieties and glabrous varieties and some of of pubescent branched types showed considerable tolerance, although they did not mention the names of different varieties.

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MATERIALS AND METHODS

Occurrence and severity of the disease:

Guar fields from different areas of Rajasthan viz. Udaipur, Jaipur, Sawai Madhopur, Alwar, Bharatpur, Ajmer, Bhilwara, Chittor and Jodhpur were surveyed during August-October, 1980 and 1981. From each field, 100 plants at random were selected and the records were made on the incidence and severity of the disease.

Isolation and pathogenicity tests:

Xanthomonas campestris pv. *cyamopsidis* , the causal organism of guar blight, was isolated from the diseased leaves collected from various places in guar growing areas of Udaipur. Small pieces from infected portion were surface disinfected with 0.1 per cent mercuric chloride solution for 1½ - 2 minutes and washed thoroughly in two changes of sterile distilled water. Bits were transferred on to each glass slide in few drops of sterile distilled water kept in sterile petri plate. The pieces were then cut into smaller pieces with razor blade and left for few minutes for bacterial oozing. The ooze was then streaked on the surface of nutrient dextrose agar medium in petri plates and incubated at 25°C for 48 hours. The yellow single colonies developing on the medium were picked up and restreaked on to the same medium. This process was repeated until all colonies developing on the medium were of similar cultural appearance.

The pathogenicity of resulting cultures were tested on Pusa Nav Bahar variety of guar. Inoculations were done on 20-30 days old plants by spraying bacterial suspension in water and by giving abrasions with fine carborundum powder (300 mesh). The pathogen was reisolated after appearance of symptoms and compared with the original culture. The culture was maintained at 4-10°C in the refrigerator on nutrient dextrose agar or yeast extract glucose chalk agar media for subsequent studies.

Preparation of inoculum:

Forty five to 50 hours growth of bacterium on nutrient dextrose agar or yeast extract glucose chalk agar slants was harvested in 10 ml sterile distilled water and immediately used for inoculations on plants. Number of cells/ml was calculated by using serial dilution technique. For mass inoculation, the culture was raised by pouring melted but inoculated medium in petri plates.

Glassware and chemicals:

All glassware was of Corning make and used after cleaning with Vim detergent powder or chromic acid followed by washing in tap water and/or distilled water. For physiological and biochemical studies, glassware were soaked overnight in chromic acid solution followed by washing in tap water, rinsed in distilled water and finally rinsed with double glass distilled water. All chemical used in precision work were of 'GR',

'Analar', 'Proanalysis' and/or high purity grade except for routine media preparation in which ordinary grade chemicals were used. In biochemical analysis work, double glass distilled water was used throughout.

Media:

The composition of different media employed was as follows:

i. Nutrient dextrose agar -

Beef extract	-	3.0 g
Peptone	-	5.0 g
Dextrose	-	10.0 g
Agar	-	20.0 g

Distilled water to make up - 1000.0 ml

pH adjusted to 7.0

ii. Yeast extract glucose chalk agar -

Yeast extract	-	10.0 g
Glucose	-	20.0 g
Calcium carbonate	-	20.0 g
Agar	-	20.0 g

Distilled water to make up - 1000 ml

iii. Triphenyl tetrazolium chloride agar (Kelman, 1954)-

Peptone	-	10.0 g
Casein hydrolysate	-	1.0 g
Glucose	-	0.5 g

Agar - 17.0 g

Distilled water to make up - 1000 ml

pH adjusted to 7.0

1% triphenyl tetrazolium chloride (1 g in 100 ml) solution was prepared separately and autoclaved at 10 p.s.i. for 10 minutes and added at the rate of 0.5 ml/100 ml medium.

iv. Modified Peterson's medium (Peterson, 1963; Tuite, 1969) -

No. 1. Glucose - 5.0 g

Peptone - 5.0 g

DL-methionine - 0.1 g

Actidione - 0.04 g

Agar - 17.0 g

Distilled water to make up - 1000 ml

pH adjusted to 7.0

Sterilize at 15 lbs p.s.i. for 15 minutes.

No.2 (Oxine)-

Solution A:- 0.22 g Ferric-8-hydroxyquinoline to be dissolved in 4 ml ethanol and diluted to 20 ml by sterile water.

Solution B:- 0.48 g Ferric sulphate in 10 ml water, heat to dissolve.

Mix solution A and B. Take 1 ml and add 49 ml sterile water. No sterilization necessary.

No.3. 0.3 g Sodium lauryl sulphate in 100 ml water. Sterilize at 10 lbs p.s.i. for 10 minutes. No.1 of the medium was dispensed in 250 ml flasks and autoclaved. Three ml of No.2 (oxine) and 1 ml of No.3 solutions were then added aseptically in individual flasks of No.1 and poured in sterilized petri plates.

v. Starr's xanthomonas synthetic medium (Starr, 1946)-

Glucose	-	5.0 g (to be added after separate autoclaving)
Ammonium chloride	-	1.0 g
Potassium dihydrogen phosphate	-	2.0 g
Boric acid	-	5.0 mg
Calcium carbonate	-	100.0 mg
Calcium sulphate(hydrated)	-	10 mg
Ferrous sulphate	-	100.0 mg
Molybdic acid	-	10.0 mg
Potassium iodide	-	10.0 mg
Manganese sulphate (hydrated)	-	10 mg
Zinc sulphate (7 hydrated)	-	50.0 mg
Distilled water to make up	-	1000 ml
pH adjusted to 6.8		

vi. Potato dextrose agar-

Peeled potatoes	-	250 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water to make up	-	1000 ml
pH adjusted to 7.0		

vii. Water agar -

Agar	-	20 g
Distilled water to make up	-	1000 ml.

Buffers:

i. Sodium acetate-acetic acid buffer -

Sol. A. 0.2M Acetic acid (11.55 ml/litre)

Sol. B 0.2M Sodium acetate (16.4 g of $C_2H_3O_2Na$ or 27.2 g of $C_2H_3O_2$ Na. $3H_2O$/litre).

Mix . 10.5 ml of sol. A and 39.5 ml of sol. B and diluted to 100 ml for pH 5.2 buffer.

ii. Phosphate buffer (Gomori, 1955)

	Dibasic sodium phosphate (Na_2HPO_4. $7H_2O$)	Monobasic sodium phosphate (NaH_2PO_4. $2H_2O$)	pH
Stock solution	0.2M(35.61g/litre)	0.2M(31.21g/litre)	
Mix	12.3	87.7	6.0
	37.5	62.5	6.6
	49.0	51.0	6.8
	61.0	39.0	7.0

Diluted to 200 ml with water.

Disease rating key:

Based on the pattern of disease development and symptoms produced, disease rating key was devised in which plants were graded into 6 arbitrary classes (0-5) to calculate the infection index.

0 = No infection

1 = Only few black oily, minute spots scattered on the leaf lamina. Stem and petiole without infection.

2 = Necrotic brown spots, more in number, covering about $\frac{1}{4}$ leaf surface. Infection on petiole and stem rare.

3 = Spots coalesced and cause blightening of leaf lamina covering ½ leaf surface. Spots on leaf petiole and stem may or may not be present.

4 = Whole plant infected with blighting of leaf lamina covering ¾ leaf surface, infection on leaf petiole and stem present, invariably chlorosis of leaves occurs.

5 = Plants severely infected, crumpling drying and defoliation of foliage, black longitudinal streaks on stem, wilting of apical leaves and curvature of the stem. Infection on pod may be present.

Infection index (McKinney, 1923; Chester, 1959; Wheeler, 1969):

$$\text{Infection index} = \frac{\text{Sum of numerical rating}}{\text{No. of plants assessed}} \times \frac{100}{\text{Max. disease rating}}$$

Estimation of loss:

Experiments to determine the relationship between disease severity and yield loss due to bacterial blight were conducted in field. Guar variety Pusa Nav Bahar was sown in 20 plots each plot measuring 4x2.5 m. To have plots with different infection indices, 8 plots were spray inoculated(c.10^6cells/ml) 40 days after sowing and 8 others 60 days after sowing. Number of inoculations were also varied i.e. one inoculation in the evening, and two inoculations with 24 hrs interval. Four plots were kept free from disease by fortnightely sprays of streptocycline (250 ppm). To control powdery mildew, the plants were sprayed with Bayleton (0.1%) as and when required. Observations on disease severity on individual plants were recorded 20 days before harvest and the data were pooled to calculate the

infection index for individual plots. Seed yield from each plot (c. 100 plants) was recorded after harvest and the per cent loss in seed yield or 'injury coefficient' was calculated by using Klemm's (1940) expression (Klemm, 1940):

$$Q = \frac{a-b}{a} \times 100$$

Where:

a = Mean yield per plot from healthy plants
b = Mean yield per plot from diseased plants
and Q = Percentage loss in yield.

Besides this, 20 plants with each disease category (0-5 scale) were tagged 20 days before harvest. The yield data were taken after harvest and the per cent loss in crop yield (seed weight) under each intensity grade was calculated.

Host range:

Plants belonging to different families were raised in 25 cm pots in cage house. Inoculations were made on one month old plants with bacterium (c. 10^6 cells/ml). Ten plants of each host were inoculated by gently pressing the surfaces of the leaves with inoculum mixed with carborundum powder and observations were recorded upto one month. Some plant species growing in the college area were inoculated *in situ*.

Inoculation techniques:

In order to develop a convenient but efficient method of inoculation, the following methods were tried:

i) Atomizing the inoculum with hand atomizer (Klement, 1968): Bacterial suspension was sprayed on one month old guar

plants with a hand atomizer and left as such in natural conditions. The plants were divided into 3 sets. The first set was inoculated once and second and third sets were inoculated twice and thrice, respectively at 10-15 hrs interval.

ii) Spray inoculation under pressure (Klement, 1968): Plants were spray inoculated under pressure with atomizer connected to a vacuum-cum-pressure pump from a distance of about 30 cm.

iii) Spray inoculation after pricking with multineedle (Andrus, 1948; Starr and Dye, 1965): Leaves were injured with the help of a multineedle prepared by fixing 9 fine entomological pins (No. 20) in a cork. Pointed end of needle was kept 1 mm projected and the opposite surface of the cork was dipped in melted sealing wax to fix the needles. The multineedle so prepared, was then applied on the surface of leaves of young plants.

iv) Abrasion with carborundum powder (Leben et al., 1968): A piece of sterile cotton was dipped in bacterial suspension and then touched with carborundum powder (300 mesh). This was gently pressed on to the leaf surface.

v). Injection infiltration method (Klement, 1963): Inoculations were made by injecting 0.1 ml of bacterial suspension in the leaf mesophyll by hypodermic syringe (needle No. 24).

Multiplication of bacterium in host:

Multiplication of X. campestris pv. cyamopsidis was studied in 2 varieties of guar viz. Pusa Nav Bahar and RGC 237, which were found to be highly susceptible and less susceptible, respectively. Third leaf from top of each plant was used for inoculation. Abrasion was made with fine carborundum powder adhered to flat end of a glass rod (0.3 mm diameter) near the mid rib at 4 places. A drop of inoculum (appox. 0.05 ml) c. 2.4×10^7 cells/ml was applied on the injured area with the same rod. Samples from each variety were collected first at 24 hours interval upto 4 days after inoculation and then at 2 days intervals upto 16 days. From each sample 2 leaf discs of 5 mm diameter were removed, washed with sterile distilled water, crushed in 2 ml of sterile water in a sterilized pestle and mortar and suspended in 10 fold dilution series in sterile water. One tenth millilitre of proper dilutions of the suspensions were spread on to TTCA medium in triplicate with glass hockey stick and incubated at 30°C. Observations on number of viable cells recovered per leaf disc were recorded.

Bacterial blight development after artificial inoculation throughout the year:

Bacterial blight development throughout the year for two years (July, 1980-June, 1982) as affected by environmental factors was studied by inoculating guar variety Pusa Nav Bahar at intervals of 7 days using carborundum abrasion technique

(c. 10^6 cells/ml). Plants were raised in 15 cm pots, inoculated when one month old and kept in pot house. Observations on disease severity was recorded 21 days after inoculation.

Bacterial blight development in field:

Field experiments were laid out to study the progress of the disease during Kharif and summer seasons of 1980-81 and 1981-82. The crop was raised in 4 plots, mensions of each plot being 4 x 3 m. When one month old, it was spray inoculated (c. 10^6 cells/ml) on 21st July and 16th March, respectively on Kharif and summer crops. Observations on infection indices were taken after every week. The data on rainfall, relative humidity and temperature were obtained from Meteorological laboratory, C.T.A.E., Udaipur.

Inoculum concentration and disease development:

Plants of guar variety Pusa Nav Bahar were raised in 15 cm pots in cage house and each pot had 3 plants. One month old plants were inoculated by spraying cell suspension and by rubbing cell suspension with carborundum powder. Seven different concentrations of bacterial suspension i.e. 7.3×10^1-7.3×10^7 were used. Twenty plants by spraying and 50 leaves by carborundum abrasion method were inoculated by each dilution. Observations on number of plants/leaves infected, incubation period and infection index 21 days after inoculation were recorded.

Bacterial concentration at the time of appearance of initial water soaking was estimated by inoculating one month old plants of variety Pusa Nav Bahar. Leaves were inoculated by carborundum abrasion technique with bacterial suspension (c. 7.3×10^4). Immediately after the development of water soaking, the area was removed by a circular cork borer, washed with sterile water and number of viable cells recovered per water soaked area was determined. The population was estimated in 20 such water soaked areas.

Age of the host:

Guar plants of Pusa Nav Bahar were raised by sowing at one week interval in 25 cm earthen pots. Each pot had 3 plants. At the time of inoculation, position of leaf on a plant was numbered downward from the topmost leaf. All the plants were inoculated (c. 10^6 cells/ml) by carborundum abrasion technique. Observation on bacterial blight intensity was recorded 15 days after inoculation on each leaf along with its leaf position as per scale given below:

Numerical value	Approx. area involved (%)	Description
0	0	No sign of disease.
1	1-10	Spots few, water soaked translucent, just begining to turn brown, covering upto 10% leaf area.
2	11-35	Spots enlarged, brown with small chlorotic haloes, covering upto 35% leaf area.

3	36-75	Spots, coalesced, brown necrotic, conspicuous chlorosis occurs, leaf blighted covering upto ¾th leaf area.
4	above 75	More than 3/4th leaf area covered, leaf severely blighted, extensively chlorotic and may defoliate.

The disease index was calculated as given earlier. In another experiment, seeds of guar variety Pusa Nav Bahar were sown in 25 cm earthen pots in cage house at 7 days intervals to get plants of 14 to 56 days old. Each pot had 2 plants and there were 10 plants in each treatment. Inoculations were done by spraying bacterial suspension three times with 12 hours intervals. Observations on infection index were recorded 21 days after first inoculation. The experiment was repeated.

Host nutrition:

To study the effect of host nutrition on bacterial blight development, quartz sand culture technique (Gallegly and Walker, 1949) was used. Fifteen centimeter plastic pots with plastic cap against the drain hold, after thorough washing with double glass distilled water, were filled with acid washed quartz sand. Seeds of variety Pusa Nav Bahar were sown and watered with glass distilled water for seed germination. The plants were kept in open during day time and in night covered with polythene sheet. During day hours too, the plants were protected from rain, as and when required, by covering them with polythene sheet. After germination of seeds,

thining of the seedlings was done after first leaf unfolding stage keeping 5 seedlings into each pot. Each treatment was replicated 10 times.

The basal nutrient solution was modified to give solutions as per requirements (Hoagland and Arnon, 1950; Meyer et al., 1963; Hewitt, 1966). For preparing various nutrient solutionsthe following stock solutions were prepared:

A. Stock solution	Concentration	Amount in g/litre
$Ca(NO_3)_2 . 4H_2O$	1 M	236.14
$MgCl_2 . 6H_2O$	1 M	203.31
$MgNO_3 . 6H_2O$	1 M	256.40
KCl	1 M	74.56
KNO_3	1 M	101.11
KH_2PO_4	1 M	136.08
$NaH_2PO_4 . H_2O$	1 M	156.02
Na_2SO_4	1 M	142.00
$MgSO_4 . 7H_2O$	1 M	246.48
$CaCl_2$	1 M	110.99
NH_4NO_3	1 M	80.05

B. Iron tartarate solution:

$FeCl_3$ - 5 g

Tartaric acid - 5 g

Double glass distilled water - 1 litre.

C. Micrometabolic solution:

H_3BO_3	2.50 g
$MnCl_2 . 4H_2O$	1.50 g
$ZnSO_4 . 7 H_2O$	0.21 g
$CuCl_2 . 2H_2O$	0.05 g
MoO_3	0.05 g

Double glass distilled water - 1 litre.

In order to prepare the nutrient solutions under different treatments, molar solutions of different chemicals were added in requisite amount as shown in Table 1.

The nutrient treatments were applied on alternate days at the rate of 100 ml per pot. The plants were spray inoculated 20 days after sowing and the infection index was recorded 20 days after inoculation.

Detection of bacterium in seed:

Guar seeds of Pusa Nav Bahar collected from the field affected with bacterial blight pathogen was used. The seeds after harvest were stored in a refrigerator. Seeds were tested in the laboratory for the presence of X. campestris pv. cyamopsidis by following techniques.

1. Agar plate technique (Muskett and Malone, 1941; Srinivasan et al., 1973).
2. Test tube agar method (Khare et al., 1977)
3. Growing on test (Eggebrecht, 1949).

Table 1: Volume of molar solutions used in preparation of different nutrient solutions*

Treatment	Millilitre of molar solutions added											Iron tartarate solution (ml)	Micro metaboli solution (ml)
	KH_2PO_4	KCl	$CaCl_2$	$MgSO_4$	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	NaH_2PO_4	Na_2SO_4	$Mg(NO_3)_2$	$MgCl_2$		
0 N	1	5	5	2								1	1
½ N	1		5	2	5	1.25						1	1
2 N	1		5	2	5	7.50						1	1
0 P		0.63		1.26	3.33		3.33					1	1
½ P	0.31	0.31		1.26	3.33		3.33					1	1
2 P	1.26	2.66		1.26		1.66	3.33					1	1
0 K				1.26		1.66	3.33	0.63				1	1
½ K	0.63	1.26		1.26		1.66	3.33					1	1
2K	0.63	4.00		1.26	3.33		3.33					1	1
Minus Ca	2				2				2	3		1	1
Minus Mg	2				2		3		2			1	1
Minus S	2				2		3				2	1	1
Minus Fe	2			2	2		3					-	1
0.25 Hoagland	0.25			0.5	1.25		1.25					0.25	0.25
0.5 H	0.50			1.0	2.5		2.5					0.5	0.5
1.0 H	1.00			2.0	5.0		5.0					1.0	1.0
2.0 H	2.0			4.0	10		10					2.0	2.0

* Volume raised to one litre by double glass distilled water.

Agar plate technique: Seeds were plated on nutrient dextrose agar, triphenyl tetrazolium chloride agar, modified Peterson's medium and Starr xanthomonas agar. Each treatment consisted of 400 seeds, 10 seeds per plate and these were incubated at 30°C under natural conditions.

In the first experiment, seeds washed with sterile distilled water and those surface sterilized with 1 per cent sodium hypochlorite for 5 minutes were plated on all the above media. Observations were recorded upto 10 days after plating. The yellow colonies which appeared and suspected to be of X. campestris pv. cyamopsidis were streaked on nutrient dextrose agar and the resulting cultures were inoculated on the guar plants to prove pathogenicity.

In second experiment, the seeds after surface sterilization were soaked for 4-5 hours in sterile distilled water. Each seed was then placed in a sterile petridish, cut into two pieces by a sharp scalpel and plated in serial order at equidistant on nutrient dextrose agar media. Each plate accommodated 10 cut pieces of 5 seeds. All the plates were incubated and observations were recorded as above.

In the 3rd experiment, seeds after soaking in 200 ppm solution of aureofungin for 30 minutes with and without pretreatment with sodium hypochlorite were plated on nutrient agar and 2% water agar. The petri plates were incubated under

dark after wrapping them in black paper. After 10 days of incubation each seed was examined for yellow Xanthomonas colony. Also, the collar region and cotyledons of each seedling were examined for any bacterial ooze. In positive cases the bacterium was isolated and inoculated on young guar plants to prove pathogenicity.

In the 4th experiment, seeds treated with 0.1 per cent Vitavax for 10 minutes followed by with and without pre-treatment with sodium hypochlorite were also plated on nutrient dextrose agar medium and observations recorded as above.

Test tube agar method: Guar seeds after soaking in 0.1 per cent Vitavax for 30 minutes were divided into two lots. Seeds of one lot were transferred to nutrient agar slants (culture tube size 25x200 mm) at the rate of one seed per tube. Seeds of second lot were further pretreated with 1% sodium hypochlorite solution and transferred to nutrient agar and 2% water agar slants. The tubes were incubated under dark and observations were recorded upto 15 days after incubation on the (i) development of typical colony on the medium (ii) symptoms on collar region or cotyledons.

A similar experiment was also done by using aureofungin in place of Vitavax in which seeds were soaked in 200 ppm solution of aureofungin for 6 hrs followed by with and without pretreatment with sodium hypochlorite and plated on nutrient

dextrose agar and 2% water agar. Observations were recorded on mentioned above.

Growing on test: Plastic pots of 15 cm size after rinsing their surfaces with rectified spirit were filled with acid washed quartz sand. Guar seeds of Pusa Nav Bahar variety collected from blight affected field were sown and watered daily for first 4 days and later on alternate days with sterile water. A total of 400 seeds were taken and in each pot 10 seeds were sown. Collar region and cotyledons of toppled seedlings were examined under microscope for bacterial ooze from time to time. From suspected seedings, isolations were made and pathogenicity was proved by inoculating young guar plants. Observations were recorded upto one month after sowing.

In the second experiment, seeds collected from pods artificially inoculated after making abrasion with carborundum powder were used. Simultaneously seeds collected from healthy guar plants, and those artificially inoculated by soaking in bacterial suspension (c. 6.2×10^7 cells/ml) for 4 hrs were also used. Four hundred seeds in each treatment were planted in quartz sand and observations were recorded upto 30 days after sowing. The experiment was repeated.

Sampling for physiological changes in plants after infection:

For estimating different organic and inorganic consti-

tuents in plants infected by bacterial blight, leaf tissues surrounding the diseased portion were used to avoid bacterial population as far as possible. Leaves of almost same age taken from one month old diseased and healthy plants were used for analysis.

Estimation of chlorophyll and carotenoids:

Chlorophyll and carotenoids were estimated both qualitatively and quantitatively.

Quantitative determination: Chlorophyll and carotenoids were extracted and qualitatively measured by using chromatographic method developed by Mishra and Jha (1967). Two grams of freshly collected leaves of healthy and diseased plants were homogenized in 2 ml of 90 per cent ethanol in a pestle and mortar. Five ml of ethanol was again added to the pulp and filtered through muslin cloth to get clear extract. Strips of 25x5 cm size were cut from Whatman No. 1 chromatographic paper. Half millilitre of the extract from both healthy and diseased stocks were applied on the strips in the form of streaks along the width of the paper 5 cm from below and allowed to dry. The chromatogrames were run in petroleum ether and acetone (9:1 V/V) solvent system in a glass jar. Chromatograms were dried under ceiling fan and observation on different bands and the colour intensity representing chlorophyll a, chlorophyll b and carotenoids were taken.

Quantitative determination: The chlorophyll and carotenoids were extracted and quantitatively measured by the method of Arnon (1956), and Bruinma (1963) respectively with slight modification. Leaf samples (100 mg each) from 1½ month old diseased (about 50% severity) and healthy plants were collected and thoroughly ground in a pestle and mortar with small amount of glass beads in a dark room. To this paste, 5 ml of 85% acetone (chilled) was then added and the supernatent was decanted into a flask. The extraction was repeated 3-4 times and the total volume raised to 50 ml by 85% acetone. Ten ml aliquot from each was centrifuged at 4000 rpm for 15 minutes at 4°C and clear supernatent was transferred to clean tubes and optical density was measured at 663, 645 and 450 mm with Systronics Spectro Colorimeter. Acetone (85%) was used to adjust absorbance at zero. Chlorophyll a, chlorophyll b and carotenoids were calculated by the following formulae.

$$\text{Chlorophyll a (mg/g tissues)} = \frac{12.7\ A_{663} - 2.69 \times A_{645}}{1000 \times W} \times V$$

$$\text{Chlorophyll b (mg/g tissues)} = \frac{22.9\ A_{645} - 4.69\ A_{663}}{1000 \times W} \times V$$

$$\text{Chlorophyll a+b(mg/g tissues)} = \frac{20.2\ A_{645} + 8.02\ A_{663}}{1000 \times W} \times V$$

$$\text{Carotenoids (mg/g tissues)} = \frac{10\ A_{450} \times V}{2500 \times W}$$

Where A_{645}, A_{663} and A_{450} represent absorbancies of the extract at 645, 663 and 440 nm respectively, V -volume of total extract and W - weight of tissues extracted in gram.

Transpiration:

Shift in transpiration as a result of infection was measured by finding out loss in weight of water (Vijay Kumar and Rao, 1979). Healthy and diseased leaves with different infection indices from 3rd trifoliate from top were selected and removed along with petiole. The cut end of the petiole was dipped in water filled in a plastic vial (10x2.5 cm size) while leaf lamina was kept projected out side. To prevent evaporation from surface, few drops of liquid paraffin were poured on the surface of the water. The leaf along with tube filled with water was weighed at intervals and the loss in weight due to transpiration calculated. Transpiration rate was expressed as loss of water in mg/sq. cm leaf area per hour and as loss of water in mg per gram leaf per hour. The diseased and healthy leaf area of each leaf was measured by tracing them on graph papers and counting the squares. There were 10 leaves in each treatment and the experiment was repeated twice.

Respiration:

Respiratory changes in guar plants infected with X. campestris pv. cyamopsidis was measured in Warburg's respirometer (supplied by M/s K.K. Supplier, New Delhi). Manometer

along with flask was calibrated using mercury before actual measurement. The total weight of the mercury in flask and upto the reference point (250 mm) was recorded. This was divided by density of mercury (13.5217 at 30°C) to obtain the total volume for a given flask and manometer. Flask constant (k) for each manometer and flask assembly was calculated by the following formula (Umbrett et al., 1964)

$$\text{Flask constant } (k) = \frac{Vg \ \frac{273}{T} + Vf \ L}{Po}$$

Where,

Vg = Volume of gas phase in flask including connecting tube down to the reference point.

Vf = Volume of fluid in vessel

Po = Standard pressure which is 760 mm Hg or 10000mm of Kreb's fluid.

T = Temperature of bath in absolute degrees (273 + temperature in °C)

L = Solubility in reaction liquid of gas involved (values for L obtained from table given by Umbrett et al.(1964) which is 0.0261 for Hg at 30°C)

Disease and healthy tissues (1 g) of same age taken from one month old plants were sliced into thin pieces by a sharp blade. These were then washed in running tap water, rinsed several times in distilled water, blotted with filter paper and transferred to the main compartment of a clean dry Warburg's flask equipped with a central well. One ml of phosphate buffer (pH 6.8) was added to the tissues. In the central well, 0.2 ml of 20 per cent KOH was transferred, after greasing the upper rim of the well. A small piece of filter paper folded in the

form of an accordion was placed on the central well to increase the surface area. The flask was attached to the manometer filled with Kreb's manometer fluid of following composition

Sodium bromide	- 44 g
Triton X-100	- 0.3 g
Acid Fuchsin	- 0.3 g
Water	- 1000 ml

Density of fluid at 28°C = 1.03057

The manometer was fixed on the apparatus and flask placed in the constant water bath. The whole system was shaken at 200 rpm for about 5 minutes and all the connections were tightned. The system was equilibrated for 10 minutes after which the manometer was adjusted with stopcock open on the closed side of it to the reference mark (250 mm). The stopcock was now closed and the readings were taken at 30 minutes interval. Liquid level in the closed arm was always adjusted to the reference mark by manipulating the screw clamp in the rubber reservoir. To correct the changes in pressure of room and temperature of water bath, thermobarometer control was maintained simultaneously. A Warburg's manometer with a flask containing 2 ml of phosphate buffer served as thermobarometer control. Any rise in the thermobarometer reading, which shows a decrease in the pressure, was added to the readings of the reaction flasks and vice versa. The amount of gas exchanged (μl O_2 uptake) was calculated by multiplying alteration in reading on open arm of manometer (h) with flask constant (k).

$\mu l\ O_2$ uptake = h x k

There were 3 replications for each treatment and the experiment was repeated twice.

Changes in sodium, potassium and calcium:

The amount of sodium, potassium and calcium in diseased and healthy leaves was determined by flame photometer (Systronics digital flame photometer type 125) as per method of Burried-Marti and Ramirej Munoz (1957). Analar grade chemicals and double glass distilled water was used throughout. The stock solutions and working standards of sodium, potassium and calcium were prepared as follows:

1. Sodium - Thousand parts per million solution was prepared by dissolving 2.5416 g sodium chloride in a litre of water.

2. Potassium - One thousand parts per million solution was prepared by dissolving 1.9070 g of KCl in a litre of water.

3. Calcium - One thousand parts per million solution was prepared by dissolving 2.497 g of calcium carbonate in 300 ml water + 10 ml concentrated hydrochloric acid and diluted to 1000 ml with water.

From these stock solutions, working standars of 10, 25, 50, 70 and 100 ppm concentrations were prepared in 500 ml volumetric flasks.

Diseased and healthy leaves (approx. 5 g each), in triplicate, were collected and dried till constant weight at 60°C. Five hundred mg of oven dried material was charred at 220°C in an oven for 48 hours. The material was cooled and ground in pestle and mortar and digested with 5 ml of conc. hydrochloric acid. This was evaporated to dryness on sand bath over a hot plate. The process of digestion was repeated again and the dried material was dissolved in 50 ml of dilute hydrochloric acid (4 ml HCl + 96 ml water) for sodium and in 100 ml for potassium and calcium and filtered through Whatman No. 42 filter paper. This solution was used as such for estimation.

In order to prepare standard curve, the most highly concentrated solution (100 ppm conc.) was atomized and the reading was calibrated to 100 in digital display unit of flame photometer. The setting operation was repeated several times in quick succession by checking zero position in display unit by feeding distilled water. The readings corresponding to the further dilution i.e. 70, 50, 25 and 10 ppm were then taken and standard curve was drawn on a graph paper. The unknown solutions were then atomised and the readings were recorded and compared with standard curve prepared to get the amount of element. The experiment was repeated twice and the data were averaged.

Estimation of sugars:

Preparation of extract - Diseased and healthy leaves were collected and dried till constant weight at 60°C. The oven dried material (400 mg) was extracted in 70 per cent ethanol, 50 per cent ethanol and finally in distilled water. These 3 fractions were pooled together, clarified by centrifugation and the total volume raised to 50 ml with 70% ethanol. To remove chlorophyll and other pigments, the extract was fractioned 3 times with equal volume of carbon tetrachloride. The lower layer was discarded and the chlorophyll free extract was collected and used. There were 3 replication in each case and the estimation was repeated.

Total sugars - The amount of total soluble sugars was estimated by anthrone reagent (Dubois et al., 1951). Anthrone reagent was freshly prepared by dissolving 200 mg anthrone dye in 100 ml concentrated sulphuric acid. 0.1 ml of the ethanol extract was taken in a test tube, dried on water bath and suspended in 2 ml water. To each tube 4 ml anthrone reagent was added by the side of the test tube. To prevent evaporation, aluminium foil was placed on the open end and the tube was placed in boiling water for 10 minutes. Thereafter, it was cooled in running tap water and the absorbance of the blue green solution was measured at 625 nm. Reagent blank containing 2 ml water and 4 ml of anthrone reagent was used to adjust absorbance at zero. The amount of sugar present in the extract

was calculated by standard curve prepared from different conc. of glucose (1-5 mg/100 ml).

Reducing sugars - Reducing sugars were estimated following dinitrosalicylic acid method (Millar, 1972). Dinitrosalicylic acid reagent (DNS) was prepared as follows:

Sol. A - To 75 ml of 4.5% NaOH; 220 ml of 1% dinitrosalicylic acid and 63.75 g of rochelle salt (Sodium potassium tartarate) were added.

Sol.B - To 2.5 g crystalline phenol; 5.5 ml of 10% NaOH and water to raise volume to 25 ml were added.

To 17.5 ml of solution B, 1.725 g of sodium bisulphite was added and mixed well with solution A. The reagent was stored in stoppered bottle at 4°C when not in use.

To 1 ml of ethanol extract in a tube, 3 ml of DNS reagent was added. The tube after shaking was put in boiling water for 5 minutes, thereafter cooled in ice water, diluted to 10 ml and the absorbance was measured at 575 nm. Blank consisted of 1 ml water + 3 ml DNS reagent finally diluted to 10 ml. The amount of reducing sugars in the sample was calculated by a standard curve prepared from different concentration of glucose. For this, 50 mg glucose was dissolved in 100 ml water and from this 0.2, 0.4, 0.6, 0.8 and 1 ml aliquots were drawn in tubes and the volume raised to 1 ml in each tube. The colour was developed and absorbance recorded as above.

Non-reducing sugars - The amount of non-reducing sugars was calculated by substracting the amount of reducing sugars from total sugars.

Estimation of nitrogen and protein:

Nitrogen and protein in healthy and diseased leaves were estimated as per method of Snell and Snell (1955). Hundred mg oven dried material was digested with 2 ml conc. sulphuric acid and pinch of digestion mixture in micro-Kjeldahl flask. Blank containing only digestion mixture was also digested simultaneously. When digestion completed, 2 ml of 30 per cent hydrogen peroxide was added and heated at 80°C till solution become clear, colourless and dense fumes started coming out. The solution was diluted to 100 ml with distilled water in a volumetric flask and kept overnight. To 1 ml of this solution in 50 ml volumetric flask, 2 ml of 2.5 N sodium hydroxide and 1 ml of 10% sodium silicate was added and the volume was raised upto the mark with distilled water, and thereafter 1.6 ml of Nesseler's reagent was added. The transmittance of the solution was read in Klett Summerson Colorimeter using green filter. Distilled water was used to adjust the transmittance at 100. To get actual reading, blank reading was substrated from sample readings. The amount of nitrogen in sample was calculated by a standard curve. In order to prepare standard curve, 4.714 g of ammonium sulphate was dissolved in a litre of double glass distilled water (1 μg N/ml). From this

0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 ml solution were taken in 50 ml volumetric flasks, chemical solutions added and per cent transmission read as mentioned above. The data were plotted on a graph, percent transmission on Y-axis and nitrogen concentration on X-axis.

The amount of protein was calculated by multiplying the nitrogen content in the sample with 6.25.

Estimation of total phenols:

Changes in total phenol as affected by bacterial blight infection was measured as per method of Bray and Thorpe (1954) using Foliin Ciocalteu reagent.

To prepare Foliin Ciocalteu reagent, 100 g sodium tungustate and 25 g sodium molybdate were dissolved in 700 ml distilled water. To this, 50 ml of 80% orthophosphoric acid and 100 ml of concentrated hydrochloric acid was added and the mixture was boiled under reflux for 10 hours. Then it was cooled and 150 g lithium sulphate dissolved in 50 ml water and 4-5 drops of bromine were added. The mixture was boiled for about 15 minutes to remove excess of bromine, cooled, volume raised to one litre and filtered through Whatman No. 42 filter paper. This golden yellow coloured reagent was stored in brown bottle at 4°C when not in use. The reagent was used after diluting it with 2 volumes of water.

Ethanol extracts prepared earlier for estimation of sugars were used. One fifth ml extract was taken in a tube and evaporated to dryness on a water bath and 1 ml distilled water added. To this was added 1 ml of Foliin ciocalteu reagent followed by 2 ml of 20% aq. sodium carbonate. The contents of the tube were mixed and the tube was put in boiling water for 1 minute. Thereafter, the contents were cooled, diluted to 25 ml and the absorbance of reaction mixture was measured at 650 nm. Blank containing all the reagents minus plant extract was used to adjust the absorbance at zero. The amount of total phenol in sample was calculated from a standard curve prepared from catechol.

For standard curve, stock solution of catechol was prepared by dissolving 10 mg catechol in small volume of ethanol and volume raised to 100 ml. From this 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml aliquots were taken in tubes and the volume was raised to 1 ml in each case by adding distilled water. These were processed in the same way and the per cent transmission plotted against concentration of catechol.

Estimation of orthodihydric (O.D.) phenols:

Ethanol extracts prepared for estimation of sugars were used for O.D. phenol estimation. O.D. phenols in healthy and diseased leaves were estimated by Arnow's method (Arnow, 1937) using Arnow's reagent. The reagent was prepared by dissolving 10 g sodium nitrite and 10 g sodium molybdate in .

100 ml distilled water. The reagent was kept in brown bottle at 4°C.

To 1 ml of ethanolic extract, 2 ml of 0.05N hydrochloric acid, 1 ml of Arnow's reagent and 2 ml of 1N sodium hydroxide was added and the volume was raised to 10 ml with distilled water. The intensity of the pink colour developed was read in a spectrophotometer at 515 nm against reagent blank without extract. O.D. phenols present in the sample was calculated from a standard curve prepared with known concentrations of catechol.

For standard curve, 100 mg catechol was dissolved in 2 ml of 70% ethanol and the volume was raised to 100 ml. From this, 0.1, 0.2, 0.4, 0.5, and 0.6 aliquots were removed, diluted to 1 ml, processed as above and per cent transmission was plotted on a graph against concentration of catechol.

Estimation of ascorbic acid:

Ascorbic acid in healthy and diseased guar leaves was estimated by visual titration method (Roe, 1954) using 2,6-dichlorophenol indophenol dye.

Ascorbic acid from plant tissues was extracted by grinding 5 g tissues in 20 ml of 0.4% oxalic acid. The extract was filtered through 2 layers of muslin cloth and centrifuged at 5000 rpm for 20 minutes at 4°C. The final volume of the clear liquid was raised to 25 ml.

Indophenol reagent was prepared by dissolving 50 mg of 2,6-dichlorophenol indophenol dye in 150 ml of hot glass distilled water. To this 42 mg of sodium bicarbonate was added, cooled and final volume was raised to 200 ml. The reagent was stored at 2°C and used within a week's time.

Ascorbic acid standard solution was prepared by dissolving 50 mg of ascorbic acid in 250 ml of 0.4% oxalic acid (0.2 mg of ascorbic acid/ml). Five ml of this standard ascorbic acid solution was titrated against the indophenol dye till the solution became pink and persisted for atleast 15 seconds. To estimate ascorbic acid amount in unknown, 5 ml of oxalic acid extract was titrated against standard indophenol dye solution and the ascorbic acid contents were determined by the following formula:

$$\text{mg ascorbic acid/100 g tissue} = \frac{I \times S \times D}{A \times W} \times 100$$

Where,

I = ml indophenol dye used in titration

S = mg of ascorbic acid reacting with 1 ml indophenol dye solution

D = Volume of the extract in ml

A = Aliquot titrated in ml

W = Weight of the sample in gram.

Production of cellulase:

Production of enzyme cellulase was measured by estimating the amount of reducing sugars released from cellulosic substrate as per method of Gascoigne et al. (1960). Twenty

five gram of healthy and diseased leaves were blended in waring blender in chilled sodium acetate acetic acid buffer (pH 5.2). The extract was filtered through 2 layers of muslin cloth. The final volume was made to 40 ml by adding buffer. The extract was centrifuged at 10,000 rpm for 30 minutes at 4°C. The supernatant was decanted, few drops of toluene added in the extract and stored at 4°C.

Dinitrosalicylic acid (DNS) reagent was prepared by dissolving 880 ml of 10% DNS and 255 g of sodium potassium tartarate into 300 ml of 4.5 per cent sodium hydroxide (Solution A). Separately 10 g crystalline phenol was dissolved in 22 ml of 10 per cent sodium hydroxide and the volume was raised to 100 ml by distilled water (Solution B). To 69 ml of solution B, 6.9 g of sodium bisulphite was added and mixed with solution A. The reagent was stored at 4°C.

Carboxy methyl cellulose was used as substrate and prepared by blending 0.5 g carboxy methyl cellulose in 100 ml of sodium acetate acetic acid buffer (pH 5.2) for 8-10 minutes and filtered. Reaction mixture consisted of 4 ml carboxymethyl cellulose, 1 ml sodium acetate acetic acid buffer (pH 5.2) and 2 ml enzyme extract. Fifteen minutes after, 1 ml of enzyme substrate mixture was transferred to 25 ml tube and 3 ml of DNS reagent was added. The tube was placed in boiling water for 5 minutes, thereafter cooled in ice water and diluted to 25 ml with water. Per cent transmission was measured at 575 nm

against a blank in which, in place of extract water was used. Enzyme activity was expressed as the amount of glucose released per millilitre of the enzyme extract per unit time which was calculated from standard curve prepared from various concentration of glucose (125-1000 ppm).

Production of B-glucosidase:

B-glucosidase activity in healthy and bacterial blight affected guar leaves was measured by estimating a glucose formed with Foliin Ciocalteu reagent (Olah and Sher-wood, 1973).

Plant extract used was same as in cellulase estimation. To 10 ml of 0.01M salicin prepared in sodium acetate acetic acid buffer (pH 5.2), 3 ml of enzyme extract and 1 ml of 4 per cent sodium fluoride was added. This was kept in water bath at 30 ± 1°C. One millilitre aliquots were withdrawn at intervals of 5 minutes and to these were added 1 ml Foliin ciocalteu reagent, 2 ml of 20 per cent sodium carbonate, shaken vigorously, placed in water bath for one minute, cooled and volume raised to 10 ml. Transmission was measured in a spectrocolorimeter at 650 nm. Similarly, reaction mixture with boiled enzyme extract and sample withdrawn at zero time were maintained as control. The amount of aglucone released in terms of catechol was measured by comparing the data with standard curve prepared from known concentrations of catechol.

Estimation of protease:

Protease activity was measured by measuring the quantity of amino acids liberated from a protein substrate with ninhydrin reagent (Davis and Smith, 1955). Enzyme extract prepared for cellulase estimation was used. To 10 ml of one per cent casein, prepared by dissolving in minimal quantity of 0.1N sodium hydroxide and the volume raised by phosphate buffer (pH 7.0), 5 ml of 0.1M phosphate buffer (pH 7.0) and 5 ml of enzyme extract were added. The reaction mixture was mixed and incubated at 30°C in a water bath. Thereafter, aliquots of 1 ml were withdrawn at 0, 30, 60, 90 and 120 minutes interval and 2 ml of ninhydrin reagent (5 g ninhydrin dissolved in 125 ml methyl cellosolve was mixed with 125 ml phosphate buffer of pH 7.0 containing 0.2 g of strontium chloride). The open end of the tube was closed with aluminium foil, placed in boiling water bath for 20 minutes, cooled in water, diluted to 10 ml with 50 per cent propanol and colour intensity measured in a spectrocolorimeter at 570 nm. Boiled enzyme extract and reaction mixture removed at zero time served as controls. The amount of amino acid released in mg/ml enzyme extract/hour was determined from a standard curve prepared from different concentrations of glutamic acid (25-200 ppm).

Estimation of polyphenol oxidase:

Polyphenol oxidase in healthy and diseased leaves was estimated by measuring oxidation of catechol in spectro colori-

meter (Farkas and Kiraly, 1962). Two grams of leaves collected from healthy and diseased plants were ground in 2.5 ml of chilled phosphate buffer (pH 6.6). The extract was squeezed through muslin cloth and centrifuged at 10,000 rpm at 4°C. The clear supernatant was decanted and used for enzyme estimation.

To 2 ml of enzyme extract, 3 ml of phosphate buffer (pH 6.0) was added and the tube was placed in a spectrophotometer set at 495 nm and absorbance adjusted to zero. Thereafter, the tube was removed and 1 ml of catechol (0.01M in phosphate buffer, pH 6.0) mixed into it and placed again in spectrophotometer. Changes in absorbance were recorded upto 20 minute and represented as unit changes in absorbance per minute per ml enzyme extract.

Estimation of Ascorbic acid oxidase:

Ascorbic acid oxidase was measured by determining the residual ascorbic acid in the reaction mixture by UV Spectrophotometer (Oberbacher and Vines, 1963). The enzyme extract from healthy and diseased guar leaves was prepared as for polyphenol oxidase except that in this case 2.5 ml of buffer per gram of leaf was taken.

To 0.1 ml of tissue extract in a cuvette, 1 ml of phosphate buffer (pH 6.0), 1.8 ml of distilled water and 0.1 ml of L-ascorbic acid (0.005M) were added. Cuvette containing all without ascorbic acid was used to adjust the absorbance at

zero. The average change in absorbance was measured and presented as unit change in absorbance per minute per ml enzyme extract.

Estimation of catalase:

Catalase activity was measured by estimating the residual hydrogen peroxide in the reaction mixture by permangnate titration method of Von Euler and Josephson (1927). The reagents for this were prepared as follows:

Hydrogen peroxide (1N) - 5.67 ml of 30 per cent hydrogen peroxide dissolved in glass distilled water and final volume raised to 100 ml.

Hydrogen peroxide (0.01N) - 10 ml of normal hydrogen peroxide mixed with 67 ml of phosphate buffer (pH 7.0) and volume raised to one litre.

Sulphuric acid (2N) - 5.6 ml of sulphuric acid (36N) diluted to 100 ml with distilled water.

Potassium permangnate solution (0.05N)- 1.6 g of potassium permangnate dissolved in 100 ml water. This was diluted in 1:99 proportion with distilled water.

Ferrion indicator - 1.485 g of orthophenanthorin monohydrate was dissolved in 100 ml of 0.025M ferrous sulphate (0.695 g of $FeSO_4.7H_2O$ in 100 ml distilled water). Colour changes from orange red to pale yellow and the indicator may be stored in brown bottle in a refrigerator.

One gram of well washed fresh leaves was ground in 10 ml of 0.05M phosphate buffer (pH 7.0) with the help of a chilled pestle and mortar, filtered through muslin cloth and centrifuged at 7000 rpm for 15 minutes at 4°C. To 5 ml of 0.01N hydrogen peroxide pipetted in 50 ml conical flask, 0.2 ml of enzyme extract was added and incubated exactly for 5 minutes. The reaction was stopped by adding 5 ml of 2N sulphuric acid. Simultaneously, a control was maintained in which the enzyme activity was stopped by adding sulphuric acid prior to the addition of enzyme extract. Blank and incubated aliquots were titrated against 0.005N potassium permangnate solution to find out residual hydrogen peroxide. Colour change from red orange to light blue was the end point. The enzyme activity was expressed as μ mol. units of hydrogen peroxide per minute per gram of fresh weight which was calculated by taking into consideration that 1 ml of 0.005N $KMnO_4$ = 0.5 μ mol H_2O_2.

Estimation of peroxidase:

Peroxidase was assayed by measuring the purpurogallin, a coloured derivative, formed during the reaction of enzyme with pyrogallol in presence of hydrogen peroxide (Addy and Goodman, 1972).

Three millilitre of 0.05M pyrogallol solution prepared in 0.1M phosphate buffer (pH 6.0) and 0.1 ml of enzyme extract were taken in a cuvette and the absorbance was adjusted to zero at 420 nm in UV-spectrophotometer. Thereafter, 0.5 ml of one

per cent hydrogen peroxide was added to the cuvette, mixed and immediately replaced in the spectrocolorimeter changes in absorbance at 30 seconds interval for 3 minutes was measured and the average change in absorbance per minute between 60 and 150 second was recorded and compared.

Germplasm screening:

Seeds of different guar varieties/selections were obtained from Economic Botanist (Guar), Agricultural Research Station, Durgapura. Seeds of Pusa Nav Bahar variety were obtained from National Seeds Corporation. These varieties were screened during kharif 1980 and 1981 in field under artificial inoculations. Seeds of each entry were sown in 5 m long row with 15-20 cm spacing from plant to plant and 40 cm from row to row and was replicated four times. One month old plants were spray inoculated by an air compression sprayer. First inoculation was followed by two more inoculations at 24 hrs interval. Disease ratings on each plant within a variety were recorded 20 days after first inoculation and the infection index was calculated.

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EXPERIMENTAL RESULTS

I. Occurrence and severity of the disease:

Observations on bacterial blight prevalence and infection index in different guar growing areas of Rajasthan during kharif and summer seasons of 1980-81 and 1981-82 were made. From each field, 100 plants at random were selected and the data from different fields were averaged to represent the prevalence and infection index for a particular place. In 1980-81, observations were recorded during kharif in 130 fields from 34 places among 8 districts of Rajasthan and during summer in 28 fields from 8 places in 4 districts of Rajasthan. In 1981-82, during kharif 104 fields from 26 places in 5 districts and during summer 47 fields from 15 places in 3 districts of Rajasthan were surveyed.

The disease was prevalent under natural conditions in all the 9 districts where observations were recorded. The prevalence was as high as 35 per cent in Durgapura (Jaipur) in kharif 1981. The disease was mostly prevalent in Jaipur, Alwar, Bharatpur, Ajmer, Chittor, Bhilwara and Udaipur, although, the infection indices varied from place to place and field to field in a district. Bacterial blight was almost absent in several villages of Udaipur district viz. Bhupalpura, Thur, Iswal, Nandeshma, Loyra and Bamanwas. It was also absent in Asalpur Jobner (Jaipur).

In 1980 kharif the maximum infection index of bacterial blight (16.3%) was recorded in Barodameo (Alwar) followed by infection index of 14.2 in Fagi (Jaipur). In 1981 kharif, the infection index was maximum (15.4) in Shahpura (Jaipur). Bacterial blight infection index in Udaipur district varied from 0-9.1 with an average of around 4-5 in both the years (Table 2).

Table 2. Prevalence and severity of bacterial blight of guar in Rajasthan during kharif 1980 and 1981

S.No.	Place	Year	Prevalence (%)	Infection index
1	2	3	4	5
1.	Horticulture Farm, Udaipur	1980	5	2.3
2.	Vallabhnagar, Udaipur	"	16	6.3
3.	Rundeda, Udaipur	"	15	6.1
4.	Gumanpura, Udaipur	"	12	7.3
5.	Bhupalpura, Udaipur	"	0	0.0
6.	Bhatewar, Udaipur	"	10	4.2
7.	Khedi, Udaipur	"	9	4.5
8.	Thur, Udaipur	"	0	0.0
9.	Iswal, Udaipur	"	0	0.0
10.	Khamnor, Udaipur	"	13	5.9
11.	Kheroda, Udaipur	"	8	3.6
12.	Kharsan, Udaipur	"	9	4.3
13.	Jhadole, Udaipur	"	10	5.2
14.	Gogunda, Udaipur	"	21	9.1
15.	Nandeshma, Udaipur	"	0	0.0

Contd...

1	2	3	4	5
16.	Fatehnagar, Udaipur	1980	12	5.8
17.	Durgapura, Jaipur	"	20	11.3
18.	Bassi, Jaipur	"	19	10.5
19.	Jatwada, Jaipur	"	22	10.7
20.	Fagi, Jaipur	"	30	14.2
21.	Gangapur, Sawai Madhopur	"	12	6.1
22.	Agri. College, Jobner (Jaipur)	"	8	3.5
23.	Asalpur Jobner (Jaipur)	"	0	0.0
24.	Barodameo, Alwar	"	28	16.3
25.	Rajgarh, Alwar	"	21	12.9
26.	Tijara, Alwar	"	23	15.0
27.	Arniapant, Chittor	"	12	5.8
28.	Cultivator's field, Chittor	"	17	7.6
29.	Mandalgarh, Bhilwara	"	13	6.5
30.	Banera, Bhilwara	"	11	5.2
31.	Tabiji Farm, Ajmer	"	15	8.6
32.	Cultivator's field, Ajmer	"	22	12.8
33	Near Mandore Farm, Jodhpur	"	15	7.3
34.	Cultivator's field, Jodhpur	"	16	8.5
35.	Chikalwas, Udaipur	1981	12	5.8
36.	Bhuwana, Udaipur	"	9	5.3
37.	Pula, Udaipur	"	15	6.5
38.	Loyra, Udaipur	"	0	0.0
39.	Cultivator's field, Bharatpur	"	22	11.3

Contd....

1	2	3	4	5
40.	Nagar, Bharatpur	1981	12	5.8
41.	Kanpur, Udaipur	"	8	2.8
42.	Pula, Udaipur	"	17	6.2
43.	Badgaon, Udaipur	"	5	2.0
44.	Vidya Bhawan, Udaipur	"	8	4.1
45.	Mahla, Jaipur	"	9	5.0
46.	Bagru, Jaipur	"	15	8.0
47.	Agri. College, Jobner (Jaipur)	"	7	4.3
48.	Mavli, Udaipur	"	10	5.3
49.	Barapal, Udaipur	"	9	3.3
50.	Bamanwas, Udaipur	"	4	Trace
51.	Durgapura, Jaipur	"	35	15.1
52.	Nangal, Jaipur	"	19	10.8
53.	Mahla, Jaipur	"	26	13.3
54.	Tijara, Alwar	"	24	14.3
55.	Shahpura, Alwar	"	25	15.4
56.	Jaisamund, Alwar	"	22	12.4
57.	Agric. College, Udaipur	"	7	2.9
58.	Deeg , Bharatpur	"	18	9.2
59.	Cultivator's field, Jodhpur	"	17	7.8
60.	Cultivator's field, Jodhpur	"	12	6.3

In summer season crop of 1981 and 1982 the disease was not recorded in most of the areas but in few fields the disease was observed in traces. The disease was observed in few fields

at Malakheda (Alwar), Rajgarh (Alwar), Tabiji (Ajmer), Bassi (Jaipur) and Shahpura (Jaipur) and the prevalence was less than 7-8 per cent.

In general, it was observed that the disease was less in those areas where crop was taken occasionally and in small isolated fields. Also, the disease was more severe on vegetable type guar than the crop raised for fodder or grain purposes. Field observations also indicated that bacterial blight damage was more in crop taken during kharif and negligible in summer season crops.

II. Symptoms:

Bacterial blight appeared on all above ground parts viz. leaves, leaf petioles, stem and pods. Infection on cotyledons and flowers was not observed in field and under artificial inoculation. However, in few cases cotyledons of inoculated guar seedlings became yellow, but no spotting or blighting was seen.

On leaves - On leaves, artificially inoculated by bacterium, the symptoms first appeared as minute, round oily spots on the lower surface of leaf lamina which measured 0.5 to 1 mm in diameter (Fig. 3 a). These spots were produced in interveinal area and were transparent when viewed through light. On upper surface of leaf lamina, the spots were olive coloured (Fig. 3 b,c). With age, the spots increased in size, became brown black, coalesced forming irregular spots surrounded by small

chlorotic zone (Fig. 1,2). The leaves also showed cuneate lesions which extended from leaf margin inwards and in such leaves veins became discoloured and resulted into blighting or withering of leaf lamina (Fig. 4, 5). Leaf drop was common in leaves artificially inoculated by rubbing with carborundum even without blighting. Such leaves became yellow soon after the appearence of pin-heads throughout on the leaf surface and defoliated within 2-3 days. The infection from blighted leaves develop systemically and often advances through the petioles into the stem. On petioles, large black lesions developed at the base of leaf lamina and/or at the point of attachment of petiole with stem, which enlarged and might completely girdle the petiole (Fig. 6). Such leaves became chlorotic, drooped and ultimately defoliated. In nature, the girdling of petiole was found to cause leaf shedding even though the spots on the leaf lamina may not be numerous.

Under field conditions, irregular light coloured, V-shaped spots that extended inward from the leaf margin were produced. The angular leaf stage was generally observed only after rains. The spots gradually increased in size and the central portion became pale brown and necrotic accompained by a yellow water soaked border. When such leaves were viewed through light, minute dark brown black pinheads were seen occasionally Under high humid weather these spots rapidly spread to the entire leaf lamina giving it a totally blighted appearance. Besides V-shaped spots produced as a result of hydathode

Fig. 1. Bacterial blight infected guar plant.

Fig. 2. Dark brown lesions on leaf.

Fig. 3. Initial symptoms produced on artificially inoculated guar leaves. a. Water soaked spots on lower surface, b. Olive coloured water soaked spots on upper surface, and c. Brown black spots on upper surface of the leaf.

Fig. 4. V-shaped lesion on guar leaf from bacterial infection.

Figs. 5-7. 5. Guar plant with completely blighted leaves with infection on petiole and stem, 6. Dark brown lesion on petiole at the base of the leaf lamina and 7. Linear brownish black longitudinal lesions on infected stem.

Figs. 8-11. 8. Curvature and breaking of stem in infected guar plant, 9. Severely blighted plant in field, 10. Symptoms of bacterial blight on pods, and 11. Collar infection on guar seedlings developing from infected seeds.

infection, dark brown to black, circular necrotic spots were also observed on leaves. With advance in age, these spots coalesced and caused partial or total blighting. Symptom on petioles was also observed.

On stem - The infection developed on stem in the form of small, minute, linear, brownish black spot which soon produced black longitudinal streaks (Fig. 7) and when such stems were split open longitudinally, the inner surface showed blackening along the entire length of the stem. In such plants apical leaves were wilted, stem curved and finally splitting and breaking of the stem were observed (Fig. 8). In due course of time the plant may show complete blighting (Fig. 9).

On pods - On pods initially small, round black spots were observed which soon enlarged and became irregular in shape (Fig. 10). Seeds, collected from infected parts of pods, were smaller, light in weight, shrunken and brownish black in colour.

On seedlings - In seedlings developing from infected seeds, the symptom first appeared as large black spot on collar region which extended upward and downward and completely girdled the stem portion at soil level (Fig. 11). Such seedlings turn yellow, droop and die prematurely. Blackening of stem and yellowing of cotyledons was also observed but latter symptom was observed in few cases only. Black streaks on stem throughout lead to wilting type symptoms on apical portion of the seedling.

Field and pot house observations definitely indicated that the seed-borne infection always leads to seedling mortality and plants inoculated and infected at seedling stage generally do not survive and bear fruits.

III. Isolation and pathogenicity tests:

Xanthomonas campestris pv. cyamopsidis, the guar bacterial blight pathogen, was isolated from diseased leaves. Isolations were made from diseased plant parts by streak plate method on nutrient agar medium after surface disinfection in 0.1 per cent mercuric chloride solution followed by 2-3 washings with sterile distilled water. Single yellow, circular and semi-transparent colonies were picked under stereoscopic microscope, purified and maintained on yeast extract glucose chalk agar slants for further work. On nutrient agar medium colonies were mustard yellow, gummy, smooth with lobate margins.

Pathogenicity tests were conducted on 20-30 days old guar plants of Pusa Nav Bahar raised in sterilized soil. Plants were inoculated by carborundum abrasion technique and by spraying bacterial suspension. After 3-5 days, round, oily pin heads developed on the lower surface of the leaves inoculated by carborundum abrasion technique (Fig. 12). The spots coalesced forming irregular spots surrounded by thin chlorotic zone and became brown black in colour. Symptoms on leaves inoculated by spraying bacterial suspension appeared after 8-10 days.

Fig. 12. Lower surface of guar leaf 6 days after inoculation by carborundum abrasion technique.

IV. Estimation of loss:

Plots with varied infection indices were created by inoculating plants 40 and 60 days after sowing with variable number of inoculations. Some plots were kept uninoculated and protected by spraying with streptocycline (250 ppm) at 15 days interval. The plants were also protected from powdery mildew by spraying Bayleton 25 WP (0.1 per cent). Observations on disease severity for individual plant were recorded 20 days before harvest. The data were pooled to calculate the infection index for each plot (100 plants/plot). Seed yield from each plot was recorded after harvest and percentage yield loss was calculated (Table 3). Also, the 20 plants of each arbitrary class (0-5 scale) were marked and seed yield/plant recorded (Table 4).

Table 3. Percentage loss in seed yield of guar variety Pusa Nav Bahar due to bacterial blight in field in kharif 1980-81 and 1981-82

S.No.	Infection index	Seed yield (g)/ plot (10 sq.m.)	Loss in seed yield (%)
1.	No disease	1100.0	-
2.	10.6	1041.6	5.31
3.	26.2	935.3	14.97
4.	47.5	733.2	33.35
5.	73.8	568.0	66.55

FIG. 13. LOSS IN YIELD OF GUAR SEEDS DUE TO BACTERIAL BLIGHT IN FIELD DURING KHARIF 1980-81 AND 1981-82

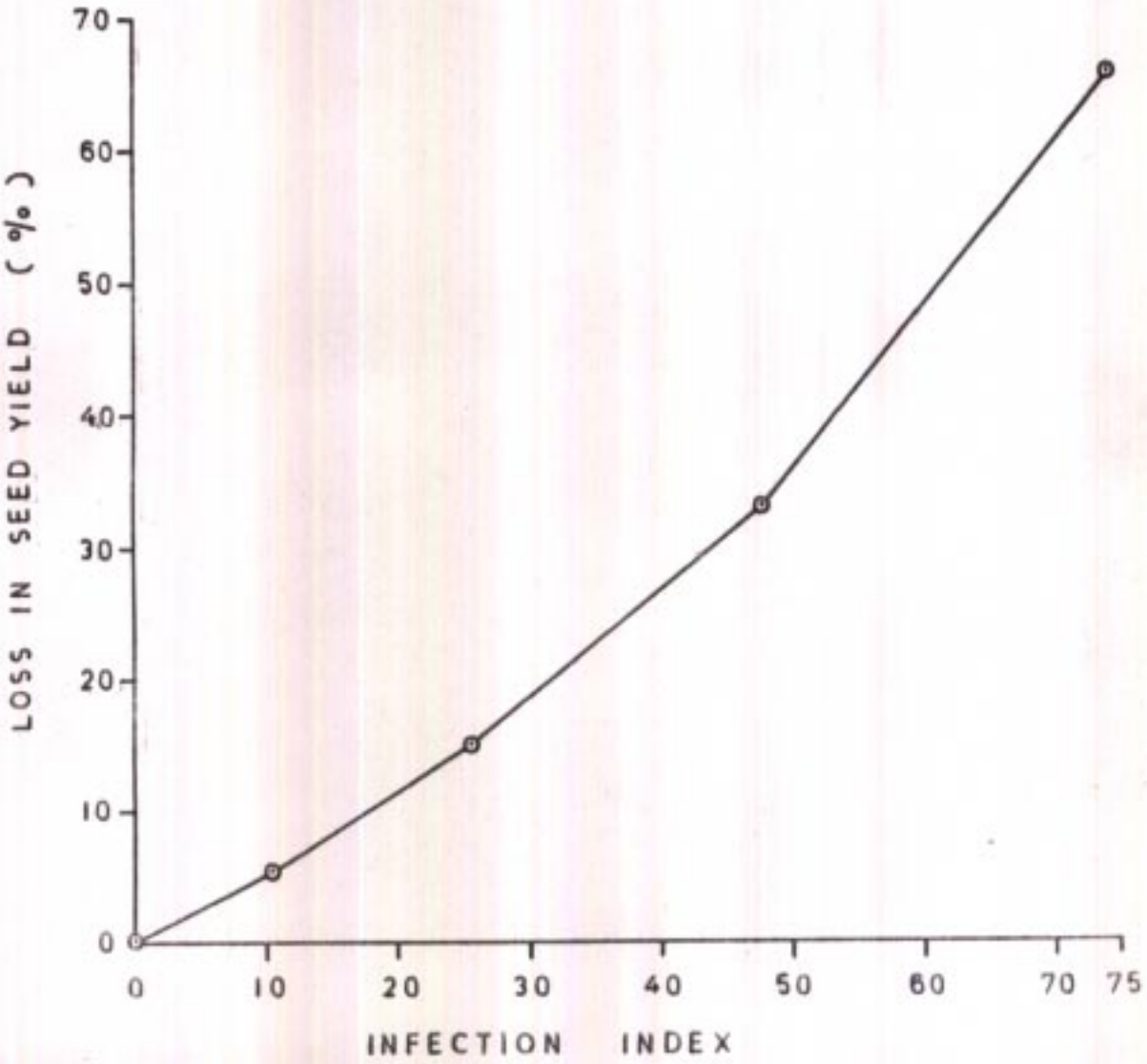


Table 4 : Loss in seed yield of guar variety Pusa Nav Bahar at different disease ratings

S.No.	Class rating	Seed yield in g/plant Range	Average	Loss in seed yield (%)
1.	0	10.32-12.81	11.58	-
2.	1	9.58-10.82	10.38	10.4
3.	2	8.04-9.68	8.87	23.4
4.	3	6.01-7.93	6.84	40.9
5.	4	3.87-6.02	4.87	58.0
6.	5	2.56-3.90	3.15	73.0

It is apparent from the results in table 3 that the loss in yield due to bacterial blight varied from 5.3 to 66.5 per cent depending upon the severity(Fig.13). The losses increased with increase in infection index, although the rate of increase in yield loss was higher at higher infection indices. It was minimum (5.3%) in plots with infection index of 10.6. The maximum loss in yield was as high as 66.5 per cent at infection index of 73.8. The amount of loss may even be more if the disease comes early and subsequently favoured by environmental factors. Besides loss in yield, the quality of seeds produced on infected plants was also adversely affected. Seeds harvested from bacterial blight affected plots had variable proportions of brown to black, under size seeds mixed with natural creamy white seeds.

The loss estimations for crop in the field were made separately for each disease class rating by taking into consideration the mean seed yield per plant. The data presented in table 3 clearly show that the seed yield decreased with increase in disease severity, ranging from 10.4 per cent loss in plants with class rating 1 to 73 per cent in plants with class rating 5. Severely infected plants only yielded 3.15 g of seeds as against 11.58 from healthy plants.

V. Host range:

Plants belonging to different families were inoculated with bacterium (c.10^6 cells/ml) by carborundum abrasion method and kept under observation for one month. The experiment was repeated again in similar way.

Out of 34 plant species, the bacterium could infect french bean (Phaseolus vulgaris) and field bean (Dolichos lablab) successfully in addition to its main host (guar). On french bean symptoms were first observed 8-10 days after inoculation, symptoms appeared in the form of brown nearly circular spots with extensive yellow halo (Fig. 14). These spots enlarged and leaves became pale yellow within few days and later drooped. On field bean, the symptoms appeared after 12-15 days only on few leaves. Leaves started showing yellowing and on one or two leaves light brown coloured patches developed on the leaf lamina (Fig. 15). Browning of veins was also noticed in some cases. When diseased leaf bits from both the hosts, after

thorough washing in sterile water, were cut with a sharp blade, copious ooze was seen which indicated that bacterium had multiplied in these hosts. From both the hosts, the bacterium were reisolated and inoculated on young guar seedlings which confirmed host susceptibility.

The bacterium induced resistant type reaction on groundnut and cotton. On these hosts, few small brown, necrotic lesions developed on lower leaves which were restricted in size (Fig. 16, 17). The lesions were more on groundnut leaves but in cotton only few lower leaves in 3 out of 10 plants showed such type of symptoms. Remaining 29 plant species proved to be non-hosts for guar blight bacterium (Table 5).

Table 5 : Host range of *X. campestris* pv. *cyamopsidis* under artificial inoculation by carborundum abrasion method on plants grown in pots (21.9-31.8°C)

S.No.	Host	Reaction*	Incubation period(days)
1	2	3	4
1.	Tomato (*Lycopersicon esculentum* Mill.)	-	-
2.	Chilli (*Capsicum annuum* L.)	-	-
3.	Chawlai (*Amaranthus viridis* L.)	-	-
4.	Bada Gokhru (*Xanthium strumarium* L.)	-	-
5.	Castor (*Ricinus communis* L.)	-	-
6.	Sorghum (*Sorghum bicolor* (L.) Moench.	-	-
7.	Maize (*Zea mays* L.)	-	-
8.	Groundnut (*Arachis hypogaea* L.)	R	8-10
9.	*Quisqualis indica*	-	-

Contd..

1	2	3	4
10.	Vinca rosea L.	-	-
11.	Lawsonia inernis L.	-	-
12.	Clerodendron inerme Gaertn.	-	-
13.	Zinnia elegans Jacq.	-	-
14.	Cotton (Gossypium arboreum L.)	R	7-8
15.	Green gram (Phaseolus aureus Roxb.)	-	-
16.	Black gram (Phaseolus mungo var. radiatus L.)	-	-
17.	Pea (Pisum sativum L.)	-	-
18.	Field bean (Dolichos lablab L.)	+	12-15
19.	Cowpea (Vigna sinensis Endl.)	-	-
20.	French bean (Phaseolus vulgaris Jacq.)	+	8-10
21.	Guar (Cyamopsis tetragonaloba (L.) Taub.)	+	5-6
22.	Arhar (Cajanus cajan (L.) Millsps.)	-	-
23.	Cauliflower (Brassica oleracea var. botrytis L.)	-	-
24.	Dhatura (Datura fastuasa L.)	-	-
25.	Tecoma stans Juss.	-	-
26.	Moth (Vigna aconitifolia (Jacq.) Marechal	-	-
27.	Amaltas (Cassia fistula L.)	-	-
28.	Albizzia (Albizzia lebbeck Benth.)	-	-
29.	Chenopodium album L.	-	-
30.	Neem (Azadirachta indica Juss.)	-	-
31.	Lucerne (Medicago sativa L.)	-	-
32.	Sesame (Sesamum indicum L.)	-	-
33.	Sunflower (Helianthus annuus L.)	-	-
34.	Lady's finger (Abelmoschus esculentus(L) Moench)	-	-

* Reaction : - No symptoms, + visible symptoms,
R-resistant reaction.

VI. Inoculation techniques:

In order to develop a convenient but efficient method of inoculation for the development of bacterial blight the following methods were tried:

1. Spray inoculation
   a. Single spray
   b. Two consecutive sprays
   c. Three consecutive sprays
2. Spray inoculation under pressure
3. Inoculation by rubbing cell suspension with carborundum powder (300 mesh)
4. Spray inoculation after pricking with multineedle
5. Inoculation by infection infiltration.

Guar plants raised in 15 cm earthen pots in cage house were used. Ten plants of one month age were inoculated by bacterial suspension (c.5.8x10^6) prepared from 36 hours old culture in sterile distilled water. In methods where injury was given, 50 leaves were inoculated by each method. The plants after inoculation were kept under cage house. The experiment was conducted during August, 1981 when humidity and temperature around 22-30°C prevailed. Observations were recorded for 21 days. The experiment was repeated and similar results were obtained, the averages of which are presented in table 6.

Out of 7 different inoculation methods tried, maximum infection index (65.00) was observed on plants whose leaves were inoculated by rubbing cell suspension with fine carborundum powder. Inoculation by injection infiltration and by three

Table 6: Methods of inoculation of *X. campestris* pv. *cyamopsidis* (c.5.8x10^6) in relation to disease development on Pusa Nav Bahar in pot house*

S.No.	Inoculation method	No.of plants or leaves		Incubation period (days)	Infection index
		Inoculated	Infected		
1.	Spray inoculation:				
	A. Single spray	10	8	8-12	38
	B. Two consecutive sprays	10	9	6-9	51
	C. Three consecutive sprays	10	9.5	5-8	64
2.	Spray inoculation under pressure	10	8	6-9	44
3.	Inoculation by rubbing cell suspension with fine carborundum powder	50	48	3-6	65
4.	Spray inoculation after pricking with multineedle	50	50	3-5	57
5.	Inoculation by injection infiltration	50	50	4-6	63

* Data are average of 2 separate experiments.

Table 7: Number of viable cells/leaf disc recovered at intervals after inoculation with c.2.4x10^7 cells/ml of *X.campestris* pv. *cyamopsidis* in varieties Pusa Nav Bahar and RGC 237

S.No.	Days after inoculation	No. of viable cells recovered/leaf disc*	
		Pusa Nav Bahar	RGC 237
1.	1	0.9x10^5	6.2x10^5
2.	2	7.2x10^5	2.9x10^5
3.	3	2.6x10^6	7.9x10^5
4.	4	8.7x10^6	3.8x10^6
5.	6	1.6x10^8	3.7x10^7
6.	8	6.7x10^8	1.8x10^8
7.	10	9.8x10^8	6.6x10^8
8.	12	1.3x10^9	6.5x10^8
9.	14	3.6x10^8	8.2x10^7
10.	16	9.1x10^5	5.3x10^5

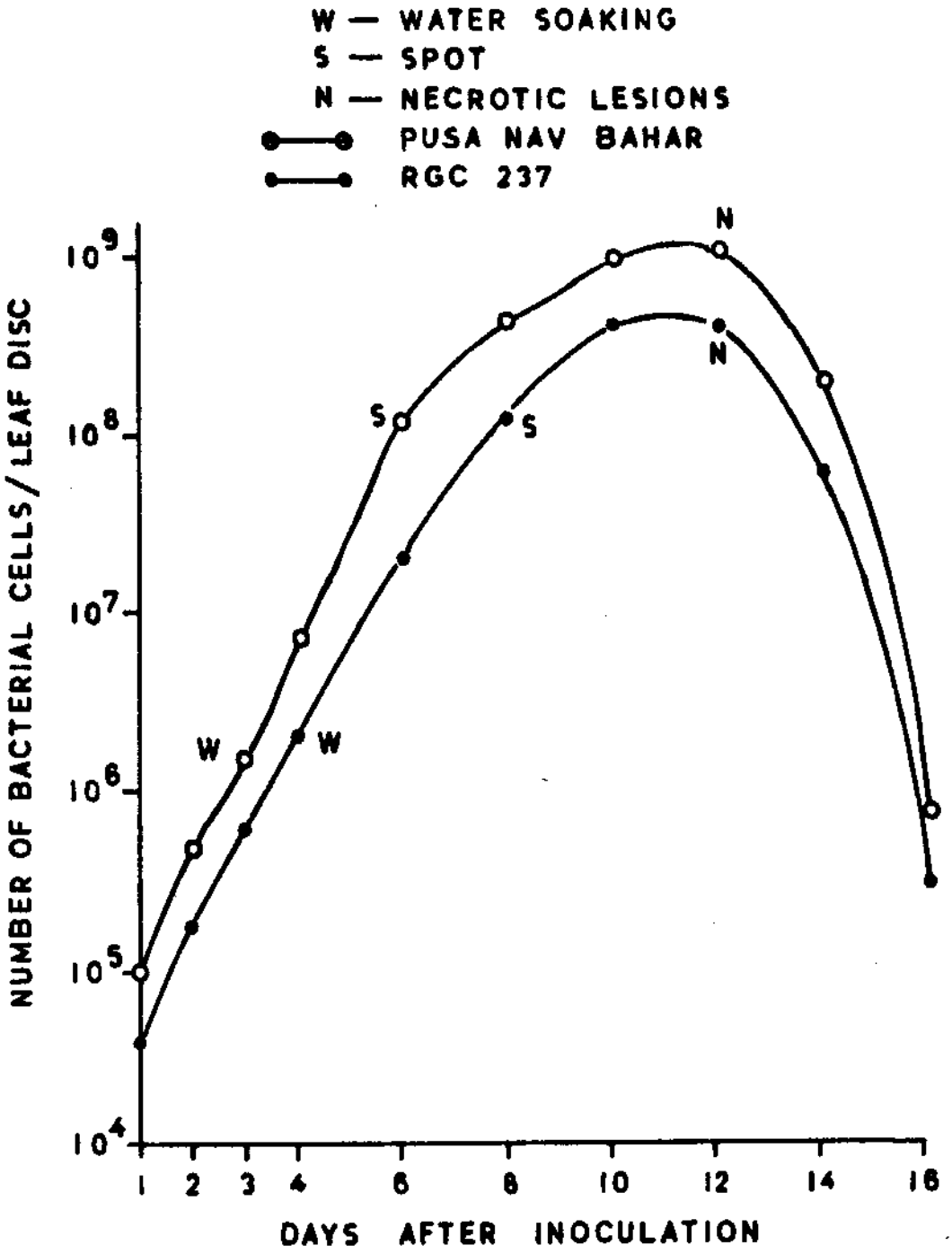
Each reading represents the average of 10 discs.

consecutive spray also gave equally good results and infection indices for these were 63 and 64 respectively. Spray inoculation under pressure was superior as compared to single spray inoculation done with atomizer, although, the difference in infection indices was not much. The infection index in former was 44 whereas in the latter it was 38. Injection infiltration method, though successful but it was rather inconvenient to use. In general, both carborundum abrasion technique and spray inoculation techniques were found successful. However, the latter may be more convenient under field conditions where inoculations on large population are to be carried out. It was also obserbed that incubation period was minimum in methods where injury was given. In these methods symptoms started appearing 3-4 days after inoculation. Incubation period was same when plants were either spray inoculated under pressure or spray inoculated twice at an interval of 10-15 hours. With increase in number of sprays of bacterial suspension the incubation period decreased. The incubation period was maximum 8-12 days in plants which were spray inoculated only once.

VII. Multiplication of bacterium in host:

Multiplication of X. campestris pv. cyamopsidis was studied in two varieties of guar viz. Pusa Nav Bahar (highly susceptible)and RGC 237 (less susceptible). Observations on number of viable cells recorded per leaf disc upto 16 days are presented in table 7 and Fig. 18 .

FIG. 18. NUMBER OF BACTERIAL CELLS / LEAF DISC RECOVERED AT DIFFERENT INTERVALS AFTER INOCULATION WITH BACTERIUM (C 10^7 CELLS / ml)



Results of multiplication of bacterium in two varieties of guar indicated that the bacterium multiplied in both the varieties of guar, however, the multiplication was more rapid in Pusa Nav Bahar than that in RGC 237. The first evidence of disease with both varieties inoculated with bacterium was chlorosis in the inoculated area in 2-3 days after inoculation. This was followed on third day in Pusa Nav Bahar and fourth day in RGC 237 by a discernible water-soaking on the under surface of the inoculated area and the population during this period was c.2.6×10^6 cells/leaf disc and c.3.8×10^6 cells/leaf disc respectively. Leaf spots were observed in variety Pusa Nav Bahar on 6th day when population was c.1.6×10^6 cells/leaf disc while in variety RGC 237 on 8th day with population level of c.1.8×10^8 cells/leaf disc. The maximum population in variety Pusa Nav Bahar reached c.1.3×10^9 cells/leaf disc in 12 days while in variety RGC 237 it was c.6.6×10^8 cells/leaf disc. It was observed that necrosis in inoculated leaves developed in both the varieties at the time when the bacterial population was near maxima (Fig. 18). Thereafter, the bacterial population declined in both the varieties.

VIII. Development of disease:

1. Bacterial blight development after artificial inoculation throughout the year -

In the field bacterial blight was observed mostly in rainy season crop and was rarely found in summer crop. Therefore, disease development throughout the year for two years (July 80

the relative humidity was maintained around 80 per cent and

-June 82) as affected by environmental factors was studied by inoculating guar variety Pusa Nav Bahar at 7 days interval using carborundum abrasion technique (c.10^6 cells/ml). One month old plants were inoculated each time and the disease severity was recorded 21 days after inoculation. The data are presented in table 8 and Figs. 19 and 20.

It was concluded that the bacterial blight could be produced artificially throughout the year, although, the infection index varied greatly and was dependent upon prevailing environmental conditions. In 1980-81, the disease was in traces or mild on plants inoculated during mid December to first week of June because of low relative humidity coupled with fluctuating temperature and no rainfall. During this period disease developed on guar plants inoculated on 3rd, 10th and 24th March, 1981 only mildly because of some precipitation after inoculation dates. Maximum disease was found on plants inoculated on July 29, 1980; high humidity, proper temperature and rainfall almost throughout the period provided conditions conducive to the development of the disease. July to end of September was most favourable for bacterial blight development as during this period the relative humidity was maintained around 80 per cent and temperature ranged between 20-34°C, During mid December to mid February, although, the high humidity prevailed because of winter rains but the disease did not develop and progress appreciably as the temperature was much low (4.3-27.8°C).

FIG. 19. BACTERIAL BLIGHT DEVELOPMENT ON PUSA NAV BAHAR, IN CAGE HOUSE, AFTER WEEKLY INOCULATIONS (JULY, 1980 - JUNE 1981)

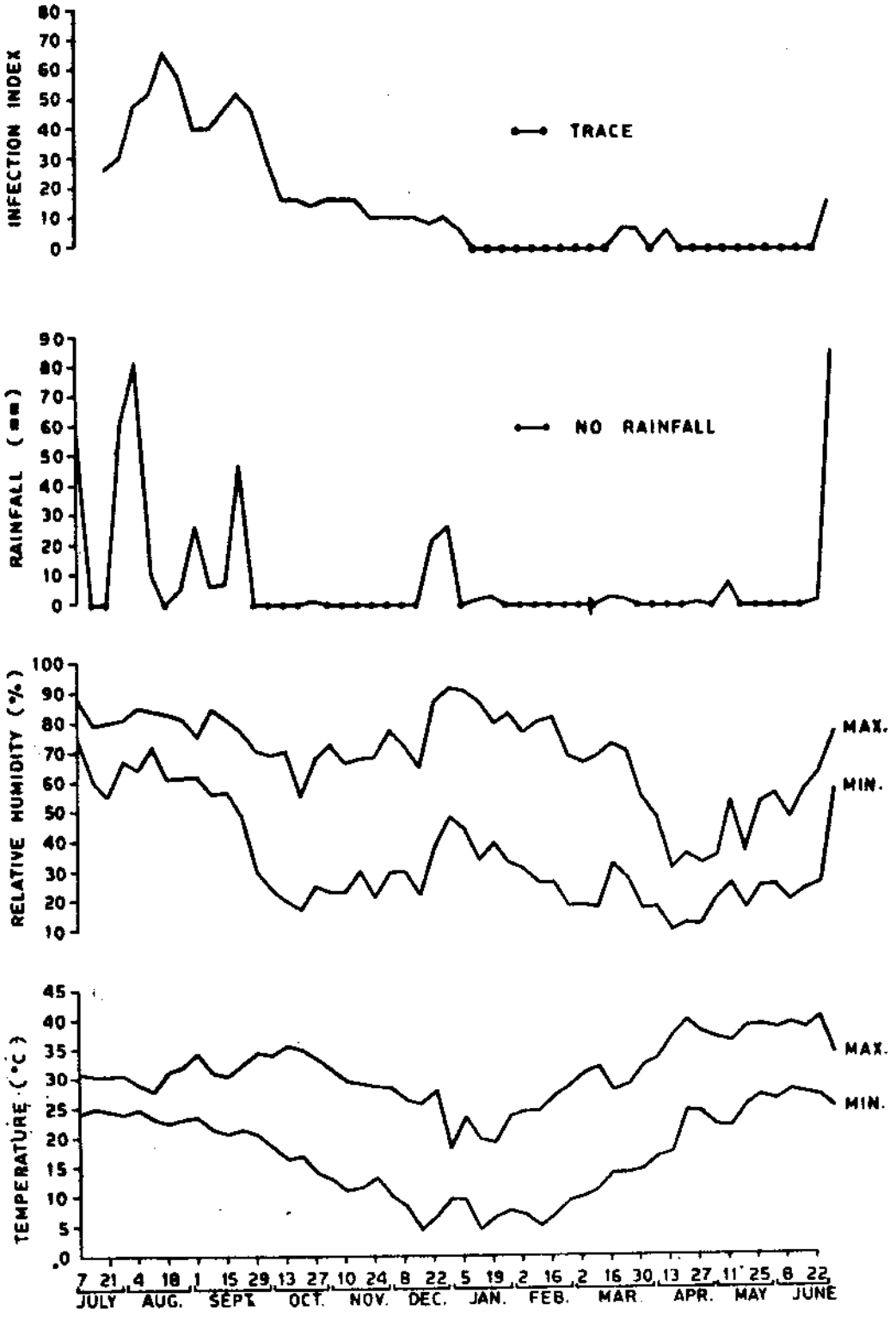
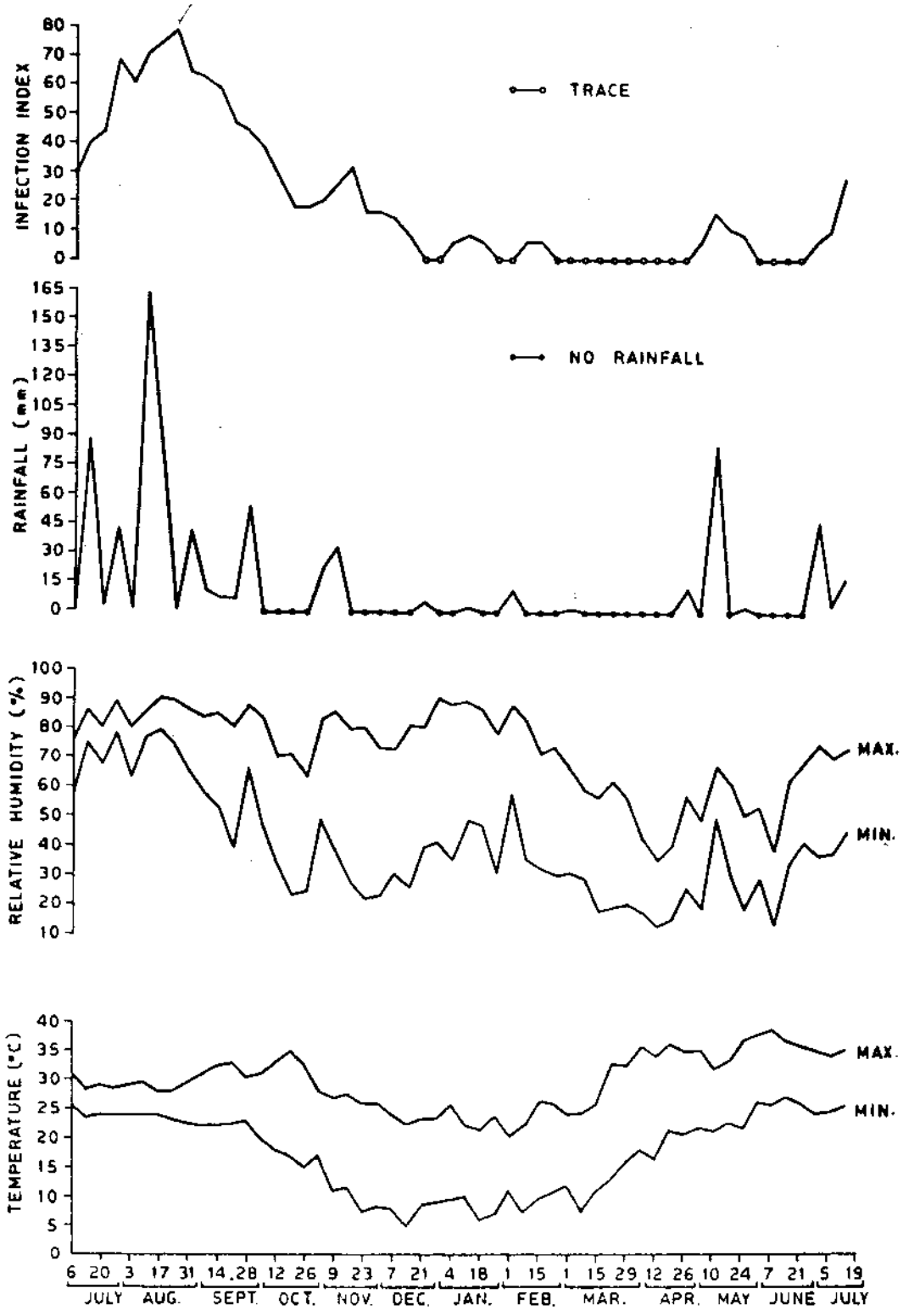


FIG. 20. BACTERIAL BLIGHT DEVELOPMENT ON PUSA NAV BAHAR, IN CAGE HOUSE, AFTER WEEKLY INOCULATIONS (JULY, 1981 - JUNE, 1982)



In 1981-82, the disease was more severe during July to September as compared to 1980-81 and this was because of intermittent rains, high humidity and favourable temperature. Moreover, the difference in maximum and minimum relative humidities and temperature was narrower during this period and this might have helped in the development of disease. In July, 1981, there were showers daily from 8.7.81 to 13.7.81 and thereafter from 22th July to 28th July which stabilized temperature between 22-30°C and relative humidity 75 per cent and above throughout the period. These conditions favoured the disease development and, therefore, plants inoculated on 7th July, 1981 had high infection index. Similarly, the infection index was high on plants inoculated on 14th, 21st and 28th July, 1981. The maximum infection index of 78 was recorded on plants inoculated on 4th August. This was because plants after inoculation got favourable environment throughout and during this period there was least fluctuations in the temperature and relative humidity. Disease declined in plants inoculated after August 4th and this trend continued upto mid December. The period between February to mid April was almost unfavourable for the disease development and, therefore, the disease development in traces on plants inoculated during this period. Subsequently, although there were rains during 7th to 10th May, 1982 but the disease did not progress sufficiently because of sudden drop in the relative humidity thereafter and hence the plants inoculated on 20th April, 1982 had infection index of 16.0 only.

Table 8 : Bacterial blight development on Pusa Nav Bahar, in cage house, after weekly inoculations

Year 1980-81		Year 1981-82	
Date of inoculation	Infection index	Date of inoculation	Infection index
1	2	3	4
July 1, 1980	25	June 30, 1981	44
July 8, 1980	30	July 7, 1981	68
July 15, 1980	48	July 14, 1981	60
July 22, 1980	52	July 21, 1981	70
July 29, 1980	66	July 28, 1981	74
Aug. 5, 1980	58	Aug. 4, 1981	78
Aug. 12, 1980	40	Aug. 11, 1981	64
Aug. 19, 1980	40	Aug. 18, 1981	62
Aug. 26, 1980	46	Aug. 25, 1981	58
Sept. 2, 1980	52	Sept. 1, 1981	46
Sept. 9, 1980	46	Sept. 8, 1981	44
Sept. 16, 1980	30	Sept. 15, 1981	38
Sept. 23, 1980	16	Sept. 22, 1981	28
Sept. 30, 1980	16	Sept. 29, 1981	18
Oct. 7, 1980	14	Oct. 6, 1981	18
Oct. 14, 1980	16	Oct. 13, 1981	20
Oct. 21, 1980	16	Oct. 20, 1981	26
Oct. 28, 1980	16	Oct. 27, 1981	32
Nov. 4, 1980	10	Nov. 3, 1981	16
Nov. 11, 1980	10	Nov. 10, 1981	16
Nov. 18, 1980	10	Nov. 17, 1981	14
Nov. 25, 1980	10	Nov. 24, 1981	8
Dec. 2, 1980	8	Dec. 1, 1981	T
Dec. 9, 1980	10	Dec. 8, 1981	T

Contd..

1	2	3	4
Dec. 16, 1980	T	Dec. 15, 1981	6
Dec. 23, 1980	T	Dec. 22, 1981	8
Dec. 30, 1980	T	Dec. 29, 1981	6
Jan. 6, 1981	T	Jan. 5, 1982	T
Jan. 13, 1981	T	Jan. 12, 1982	T
Jan. 20, 1981	T	Jan. 19, 1982	6
Jan. 27, 1981	T	Jan. 26, 1982	6
Feb. 3, 1981	T	Feb. 2, 1982	T
Feb. 10, 1981	T	Feb. 9, 1982	T
Feb. 17, 1981	T	Feb. 16, 1982	T
Feb. 24, 1981	T	Feb. 23, 1982	T
March 3, 1981	6	March 2, 1982	T
March 10, 1981	6	March 9, 1982	T
March 17, 1981	T	March 16, 1982	T
March 24, 1981	6	March 23, 1982	T
March 31, 1981	T	March 30, 1982	T
April 7, 1981	T	April 6, 1982	T
April 14, 1981	T	April 13, 1982	6
April 21, 1981	T	April 20, 1982	16
April 28, 1981	T	April 27, 1982	10
May 5, 1981	T	May 4, 1982	8
May 12, 1981	T	May 11, 1982	T
May 19, 1981	T	May 18, 1982	T
May 26, 1981	T	May 25, 1982	T
June 2, 1981	T	June 1, 1982	T
June 9, 1981	16	June 8, 1982	6
June 16, 1981	28	June 15, 1982	10
June 23, 1981	40	June 22, 1982	28

*-Based on observations of 10 plants.
T-traces

2. Bacterial blight development in field -

The relationship of various environmental factors to the disease development was studied both in kharif and summer for two consecutive years. The infection index based on 0-5 scale was taken at one week interval and are presented in Figs. 21 and 22.

During 1980 kharif, the disease started appearing 9-10 days after inoculation and progressed continuously till 18th August. This can be attributed to precipitation occurred 4 days after inoculation which increased the atmospheric humidity to a considerable extent and the temperature was 25-30°C. The progress in disease development continued till 22nd September, 1980 owing to further precipitation, although, the progress of the disease was slow. Later the progress of the disease was almost checked with the decrease in relative humidity, fluctuating temperature and onset of winter season. The bacterial blight infection index during this crop season reached 46.0 within 63 days of crop growth.

In summer season crop of 1981, the disease appeared 15 days after inoculation and progressed very slowly because of prevailing high temperature, low relative humidity and negligible precipitation. Only a marginal increase (6.0) in infection index was recorded over a period of 35 days after appearance of disease in the field. In latter two weeks the infection index reached was 13 from initial 6. This progress of the disease

FIG. 21. BACTERIAL BLIGHT DEVELOPMENT ON PUSA NAV BAHAR, IN FIELD, DURING KHARIF AND SUMMER, 1980-81

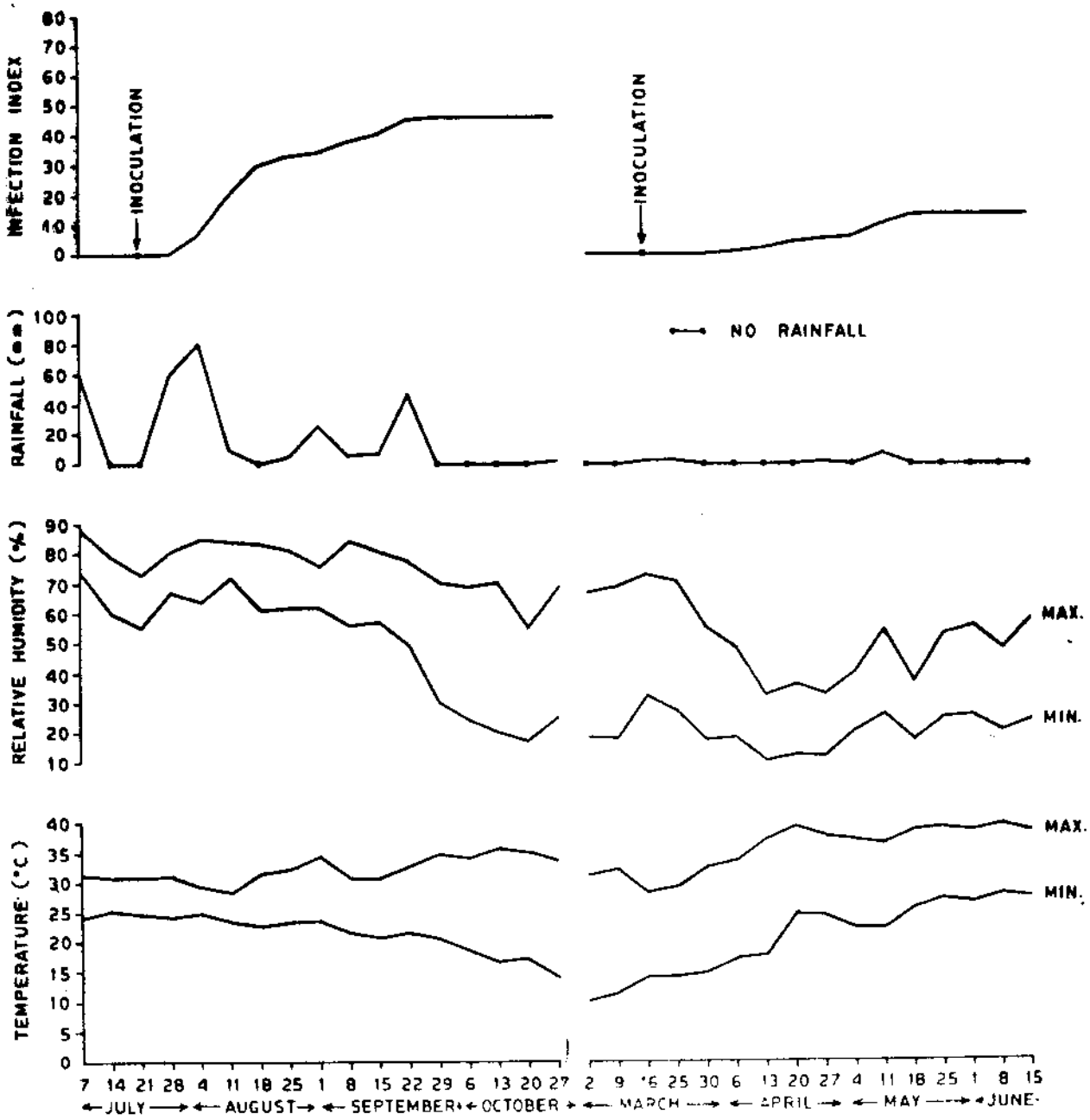
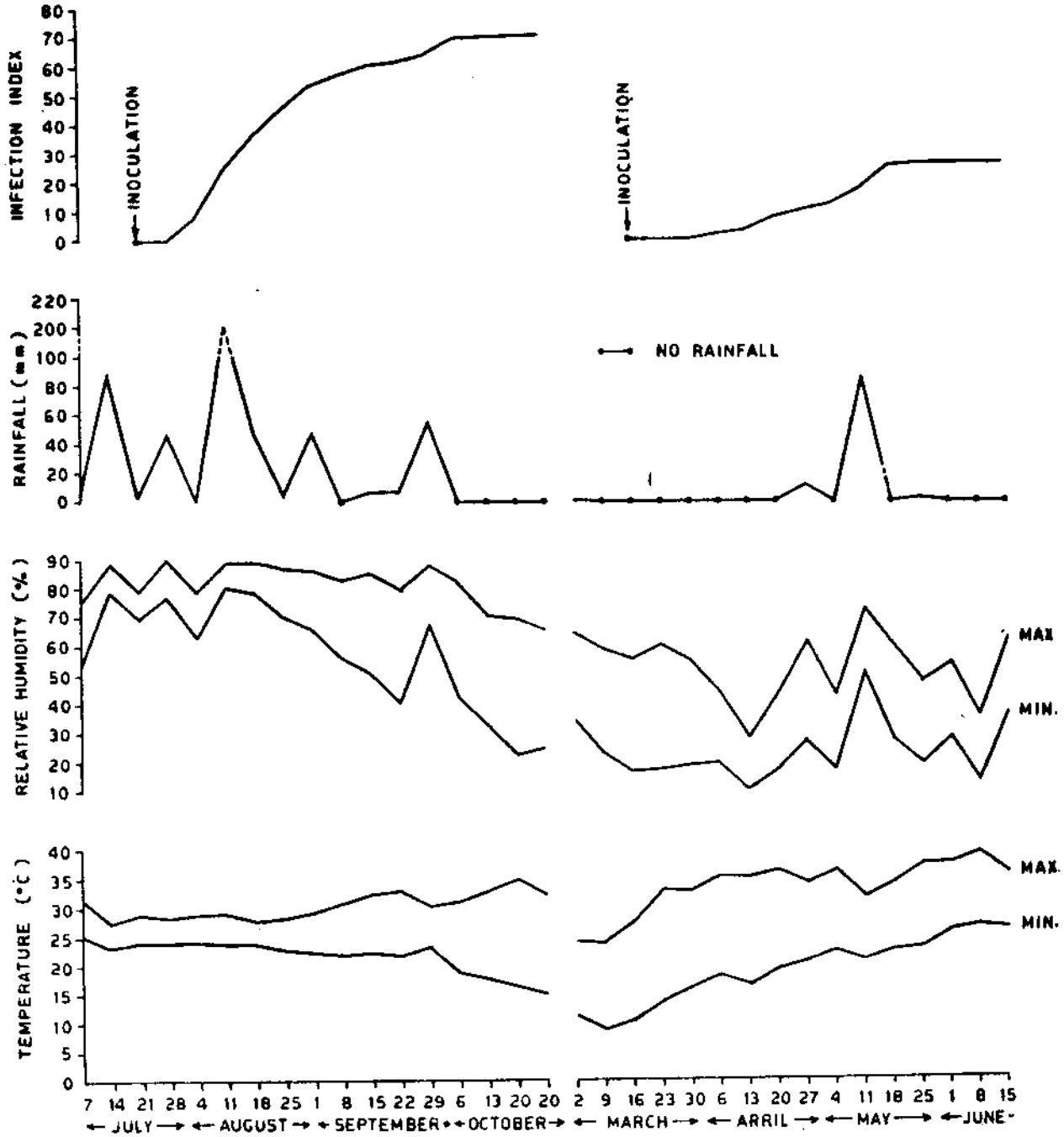


FIG. 22. BACTERIAL BLIGHT DEVELOPMENT ON PUSA NAV BAHAR, IN FIELD, DURING KHARIF AND SUMMER, 1981-82



can be attributed to slight precipitation on 7th and 9th May, 1982. After this the disease development was completely checked. The bacterial blight infection index during summer crop season of 1981 reached 14 within 2 months of crop growth.

During 1981 kharif, the disease started appearing 8-9 days after inoculation and reached to appreciable severity by 11th August due to heavy precipitation on August 6-7, 1981 which increased relative humidity to more than 85 per cent and maintained temperature in between 23-29°C. The progress in disease development continued till 1st September and thereafter the disease progressed with slow rate till 6th October. Later the progress of the disease was almost checked due to sudden drop in relative humidity and fluctuating temperature. Kharif 1981 season proved to be more favourable for bacterial blight development as compared to kharif 1980. The bacterial blight infection index during this crop season was 71.0 in 70 days after appearance of symptoms resulting into average increase of 1.01 per cent infection index per day as against 0.75 per cent increase in infection index per day during kharif 1980.

In March-June, 1982 crop, the disease appeared in the third week and progressed very slowly upto 4th May. Thereafter, due to good precipitation on 7th May and heavy shower on 9th May the atmospheric humidity increased and the temperature was brought down and as a result the disease progressed and reached to appreciable severity by 18th May. The further progress of

the disease was checked with the drop in relative humidity, increase in temperature and practically no rainfall. The bacterial blight infection index during this crop season reached 25 in 49 days after appearance of symptoms.

From the above observations it was concluded that bacterial blight is favoured by atmospheric temperature of 22-34°C coupled with relative humidity of 80 per cent and above and intermittent precipitation. In field the disease appeared even under unfavourable conditions but the disease progressed appreciably only if favourable weather conditions prevailed. It was also observed that rainy season crop suffered most in both the years as compared to summer crop, although, the disease was more during 1981-82 in both the seasons.

3. Inoculum concentration -

One month old plants of guar variety Pusa Nav Bahar were inoculated by different dilutions of bacterial suspension containing c.7.3×10^1 to 7.3×10^7 cells/ml following carborundum abrasion and spray inoculation techniques. Observations on number of plants/leaves infected, incubation period and infection indices 21 days after inoculation were recorded and are presented in table 9.

When the inoculum concentration of X. campestris pv. cyamopsidis under spray inoculation technique was decreased from 10^7 cells/ml to 10^4 cells/ml, the time interval between

inoculation and initial appearance of symptom expression increased. With the decrease in inoculum concentration infection index also decreased. The plants inoculated with inoculum concentration of $c.10^4$ cells/ml produced symptoms in 4 out of 20 plants and at higher concentration, viz. $c.10^7$ cells/ml and $c.10^6$ cells/ml, there was no difference in incubation period.

When leaves were inoculated by carborundum abrasion technique, the time interval between inoculation and initial symptom expression increased from 3 days to 5 days with decrease in inoculum concentration from 10^5 to 10^3. There was no difference in incubation period when leaves were inoculated by bacterial suspension containing 10^5 to 10^7 cells/ml. Also, there was not much difference in infection indices; it was 68 at a concentration of 10^7 cells/ml as against 60 at 10^5 cells/ml. Leaves inoculated with 10^3 cells/ml, although, produced symptoms but only in 28 out of 50 leaves inoculated and the infection index was minimum.

It is apparent from the results presented in table 9 that a minimum inoculum concentration of $7.3x10^3$ cells/ml, if inoculated by carborundum abrasion technique, and a concentration of $7.3x10^4$ cells/ml, if inoculated by spraying cell suspention, are needed for development of bacterial blight in guar variety Pusa Nav Bahar.

Table 9: Inoculum concentration of X. campestris pv. cyamopsidis in relation to disease development on Pusa Nav Bahar in pot house

S.No.	Inoculum concentration (Ca.)	Spray inoculation*				Rubbing cell suspension with carborundum*			
		No. of plants inoculated	No. of plants infected	Incubation period (days)	Infection index	No. of leaves inoculated	No. of leaves infected	Incubation period (days)	Infection index
1.	$7.3x10^7$	20	19	8	48	50	50	3	68
2.	$7.3x10^6$	20	18	8	45	50	50	3	66
3.	$7.3x10^5$	20	13	10	32	50	50	3	60
4.	$7.3x10^4$	20	4	12	4	50	47	5	42
5.	$7.3x10^3$	20	0	-	0	50	28	6	19
6.	$7.3x10^2$	20	0	-	0	50	0	-	0
7.	$7.3x10^1$	20	0	-	0	50	0	-	0

* Average of two experiments

In another experiment, plants of variety Nav Bahar were inoculated by carborundum abrasion technique and the bacterial population at the time of appearance of initial water soaking was estimated. It was observed that initial water soaking on inoculated leaves was produced only when a minimum population level of c.1.85x10^6 to 2.89x10^6 cells/ml with an average of c.2.34x10^6 cells/ml was attained.

4. Age of the host -

Seeds of guar variety Pusa Nav Bahar were sown in 25 cm earthen pots in cage house at 7 days interval to get the plants of different leaf stage, and 14 to 56 days old. In former case, the leaves were inoculated by carborundum abrasion technique whereas the plants were spray inoculated in latter case. Observations were recorded 15 and 21 days after inoculations, respectively. The experiments were repeated and average data are presented in tables 10 and 11.

Table 10 presents data on development of bacterial blight on leaves of guar plants with different leaf position. The stage of growth at inoculation was expressed in terms of the number of leaves expanded on the plant. Leaves were numbered beginning with top-most unfolded leaf of 2.5 cm size towards lower leaves. Observations indicated that in plants at 4 leaf stage or above there was progressive decrease in disease from 4th leaf position onwards. Second and third leaves from top were most diseased. The disease did not develop on lowest leaf after the plant had reached the 7th leaf stage.

Table 10: Relation of leaf-position to the bacterial blight development on guar variety Pusa Nav Bahar in cage house when inoculated by carborundum method (21.9-31.8°C)

Leaf position	Infection index of leaves inoculated at different leaf stages*						
	3	4	5	6	7	9	11
1	80	82	86	85	79	84	83
2	92	90	100	100	100	96	93
3	80	94	100	98	100	100	100
4		70	78	73	78	76	71
5			56	53	50	51	58
6				28	25	30	31
7					0	5	8
8						0	0
9						0	0
10							0
11							0

* Average of two experiments

Table 11: Relation of age of guar plant to the bacterial blight development in cage house under spray inoculation (21.9-31.8°C)

S.No.	Age of the plants (days)	Infection index*
1.	14	67.5
2.	21	65.0
3.	28	70.0
4.	35	62.5
5.	42	43.5
6.	49	38.0
7.	56	24.5

* Average of 2 experiments with total 20 plants in each treatment.

In guar plants of different age groups, the disease was more on plants of age groups between 14-35 days while infection index was minimum on 56 days old plants. Infection index decreased with increase in age of the plants beyond 35 days (Table 11).

5. Host nutrition -

Host nutrition does affect the bacterial diseases and their development (Van Gundy and Walker, 1957; Thaung and Walker, 1957; Nayudu and Walker, 1960; Taylor and Dobson, 1960; and Lipke, 1968). Recognising the importance of host nutrition similar studies were made with respect to guar bacterial blight using quartz sand culture technique. Three experiments (i) effect of different levels of nitrogen, phosphorus and potash, (ii) effect of calcium, magnesium, iron and sulphur, and (iii) effect of different concentration of a balanced nutrient solution on disease development were carried out. All experiments were repeated and average results of two experiments are given in tables 12, 13, and 14, respectively.

(i) Effect of different levels of nitrogen, phosphorus and potassium :- The basal nutrient was modified to give solutions as per requirements and used as given in materials and methods. The following treatments were used:

Basal nutrient solution without nitrogen (0N)

Basal nutrient solution with half nitrogen (1/2 N)

Basal nutrient solution with double nitrogen (2 N)

Basal nutrient solution without phosphorus (0P)
Basal nutrient solution with half phosphorus (1/2 P)
Basal nutrient solution with double phosphorus (2 P)
Basal nutrient solution without potassium (0K)
Basal nutrient solution with half potassium (1/2 K)
Basal nutrient solution with double potassium (2 K).
Basal nutrient solution with balanced N, P and K.

Observations recorded in table 12 indicated that different levels of N, P and K supplied to the guar plant affected the bacterial blight development differently. Where potassium was varied, infection index was highest (61.00) in the absence of potassium and declined with increase in potassium. The decline in infection index was more pronounced when potassium level was increased from 0K to 1/2 K as compared to increase in potassium level from 1/2 K to 2K. The infection indices were 38.15 and 41.60 at high (2K) and low (1/2 K) levels, respectively. Where nitrogen was completely lacking the infection index was 24.76 and with increase in nitrogen level to 1/2 N the infection index increased to 48.00. But a high level of nitrogen (2N) the disease was suppressed and it was 41.18 per cent. When P was varied, the infection indices did not differ much at 0P to 2P levels and varied in between 41 to 44. It indicated that the variation in the supply of phosphorus had no effect on the bacterial blight development. In normal Hoagland's solution the infection index was 50.43. The growth

of the plants was also affected with the manipulation in N, P and K contents. The growth was vigorous at high N level but at O N level the plants were poor in growth and chlorotic with phosphorus and potassium at higher levels, the plants were dark green in colour and at OK level the plants were slightly stunted with lower leaves yellowish green and other leaves rough in texture.

(ii) Effect of calcium, magnesium, sulphur and iron :- Effect of calcium, magnesium , sulphur and iron on the development of bacterial blight was studied in guar variety Pusa Nav Bahar. Throughout the studies high purity grade chemicals and double glass distilled water was used.

Results presented in table 13 indicated that the presence or absence of calcium, magnesium, sulphur and iron in the host nutrition affected the disease development, however, the trend was not similar in all cases. Absence of sulphur in nutrient solution markedly reduced the infection index from 50.20 to 39.16. The disease was also reduced by absence of magnesium and iron in the nutrient solution, however, the reduction in these cases was only marginal. On the contrary when calcium was absent in the nutrient solution the infection index marginally increased to 55.00.

(iii) Effect of different concentration of balanced nutrient solution :- The basal nutrient solution was modified into low

Table 12: Effect of different levels of nitrogen (N), phosphorus (P) and potash (K) on the development of bacterial blight in guar variety Pusa Nav Bahar in quartz sand culture*

S.No.	Treatments	No. of plants inoculated	No. of plants infected	Incubation in days	Disease rating (0-5 scale)	Infection index
1.	0 N	50	45	9	1.24	24.76
2.	0.5 N	50	47	7	2.70	48.00
3.	2.0 N	50	50	7	2.06	41.18
4.	0 P	50	50	7	2.20	44.00
5.	0.5 P	50	50	8	2.05	41.00
6.	2.0 P	50	50	8	2.16	43.20
7.	0 K	50	48	6	3.05	61.00
8.	0.5 K	50	49	7	2.08	41.60
9.	2.0 K	50	50	9	1.90	38.15
10.	1.0 (Hoagland)	50	50	7	2.50	50.43

* Average of 2 experiments.

Table 13 : Effect of calcium, magnesium, sulphur and iron on the development of bacterial blight in guar variety Pusa Nav Bahar in quartz sand culture*

S.No.	Treatments	No. of plants inoculated	No. of plants infected	Incubation in days	Disease rating (0-5 scale)	Infection index
1.	Minus Ca	50	49	6	2.75	55.00
2.	Minus Mg	50	50	7	2.33	46.66
3.	Minus S	50	48	8	1.95	39.16
4.	Minus Fe	50	50	7	2.30	46.00
5.	Normal Hoagland	50	50	7	2.51	50.20

* Average of 2 experiments.

Table 14: Effect of 4 different concentration of Hoagland's nutrient solution on bacterial blight development in guar variety Pusa Nav Bahar in quartz sand culture*

S.No.	Treatments	No. of plants inoculated	No. of plants infected	Incubation in days	Disease rating (0-5 scale)	Infection index
1.	0.25 H	50	50	6	3.22	64.44
2.	0.50 H	50	50	6	2.85	57.50
3.	1.00 H	50	50	7	2.52	50.40
4.	2.00 H	50	50	7	2.45	49.00

* Average of two experiments.

(0.25 H), medium (0.5 H), normal (1.0 H) and high (2.0 H) concentration keeping the normal concentration of all minor elements. Observations recorded on infection index are presented in table 14.

In plants supplied with different concentrations of nutrient solution, disease was reduced by higher concentrations of nutrient solution. The infection index was 66.44 at 0.25 H whereas it was 49.00 at 2.0 H. As the concentration of nutrient solution was increased from 1.0 H to 2.0 H, there was negligible decrease in infection index which indicated that concentrations higher than 1.0 H had no effect on bacterial blight development.

IX. Detection of bacterium in seed:

Agar plate technique, test tube agar method and growing in quartz sand in pots were tried to detect X. campestris pv. cyamopsidis in seeds of guar variety Pusa Nav Bahar.

Agar plate technique :- Seeds after different treatments were plated on nutrient dextrose agar (NDA), triphenyl tetrazolium chloride agar (TTCA), modified Peterson's agar (MPA), Starr's Xanthomonas agar (SXA) and 2% water agar (WA) media. Each treatment consisted of 400 seeds and 10 seeds were plated in each plate. The seeds were plated after giving the following treatments:

(i) Seeds washed with sterile water.

(ii) Seeds pretreated with 1% sodium hypochlorite solution for 5 minutes without washing.

(iii) Pretreated seed cut into two parts with sterile scalpel and plated (Fig. 23).

(iv) Seeds soaked in 200 ppm Aureofungin solution for 30 minutes.

(v) Seeds soaked in Aureofungin solution followed by pretreatment with sodium hypochlorite.

(vi) Seeds soaked in 0.1% Vitavax for 10 minutes.

(vii) Seeds soaked in vitavax followed by pretreatment with sodium hypochlorite.

Guar seeds, after pretreatment with sodium hypochlorite when plated on NDA, TTCA, MPA, SXA and WA, yielded bacterial colony only on NDA and TTCA media (Table 15). The bacterium

Table 15: Detection of X. campestris pv. cyamopsidis in guar seeds by agar plate method

S.No.	Treatments	Per cent infected seeds detected on*				
		NDA	TTCA	PMA	SXA	WA
1.	Seeds washed with sterile water	-	-	-	-	NT
2.	Pre-treated seeds	4	2	-	-	NT
3.	Pre-treated cut seed pieces	3	3	-	-	NT
4.	Seeds soaked in 200 ppm Aureofungin					
	A. Without pre-treatment	3	NT	NT	NT	-
	B. With pre-treatment	2	NT	NT	NT	0.5
5.	Seeds soaked in 0.1% vitavax					
	A. Without pre-treatment	2	NT	NT	NT	-
	B. With pre-treatment	1	NT	NT	NT	-

* = Observations based on 400 seeds
NT = Not tried.
- = Seed infection not observed.

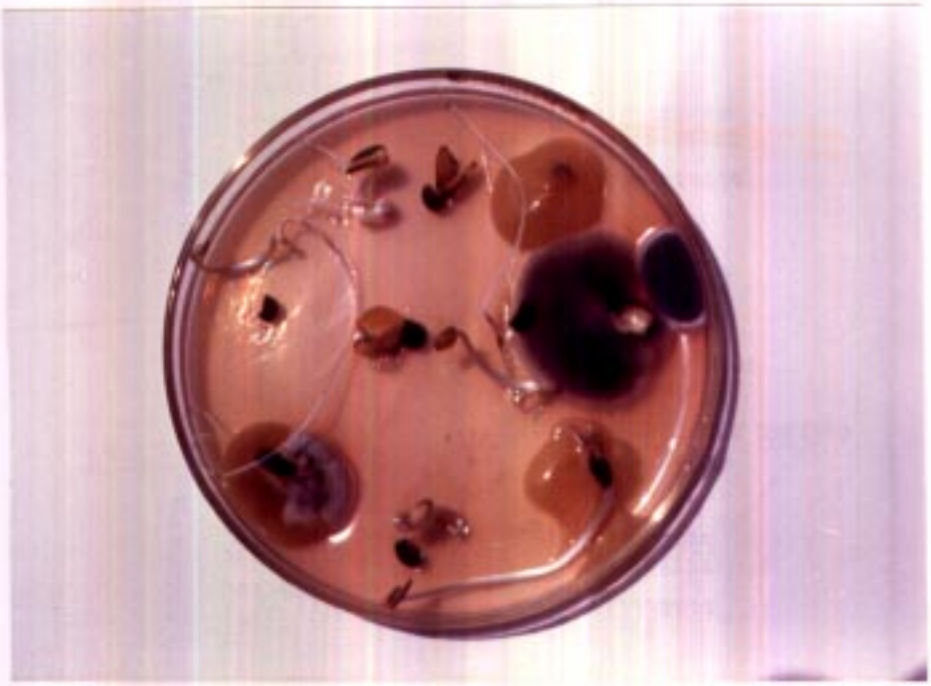


Fig. 23. Yellow slimy bacterial colonies developing from guar seeds pieces plated on NDA.

could not be detected in seeds plated on MPA, SXA and WA media. Among NDA and TTCA, plating of seeds on NDA medium gave better results as the bacterium could be detected from 3-4 per cent seeds whereas on TTCA medium the bacterium was isolated from 2-3 per cent seeds. The difficulty was experienced in isolating bacterium from seeds by plating them on agar media because of over growth of fungi on plated seeds and media.

To avoid fungal contaminants, the seeds were plated on NDA and WA media after pretreatment with antifungal antibiotic Aureofungin and fungicide Vitavax. By plating the seeds treated with Aureofungin and Vitavax on NDA seed infection could be detected in 3 and 2 per cent seeds, respectively (Table 15)

Test tube agar method:- Guar seeds, soaked in Aureofungin (200 ppm for 30 min) and Vitavax (1000 ppm for 10 min) separately followed by with and without pretreatment with 1% sodium hypochlorite, were sown in test tube @ one seed per tube on NDA and WA media. Observations were recorded upto 15 days after sowing (Table 16).

When seeds soaked in Aureofungin were sown in tubes on NDA medium, the bacterial colony developed on 3 per cent seeds. In Vitavax treated seeds plated on NDA with and without pretreatment with sodium hypochlorite, seed infection was detected only in 1% seeds. In water agar 1% seedlings were infected.

Table 16: Detection of X. campestris pv. cyamopsidis in guar seeds of variety Pusa Nav Bahar by test tube agar method

S.No.	Treatments	Per cent infected seeds/ seedlings detected on*	
		NDA	2% water agar
1.	Seed soaked in 0.1% Vitavax		
	A. Without pre-treatment	1	0
	B. With pre-treatment	1	0
2.	Seeds soaked in 200 ppm Aureofungin		
	A. Without pre-treatment	3	0
	B. With pre-treatment	1	1

* = Observations based on 400 seeds.

Growing in quartz sand:- Under more natural conditions the infected seeds and seedlings may develop symptoms comparable with those developed under field conditions. Therefore, the seeds were sown in quartz sand to compare this method with agar plate method. The following types of seeds were used:

(i) Seeds collected from pods artificially inoculated with carborunudm abrasion technique.

(ii) Seeds collected from blight affected plants which were used in agar plate method also.

(iii) Seeds soaked in bacterial suspension for 4 hours.

(iv) Seeds collected from apparently healthy plants.

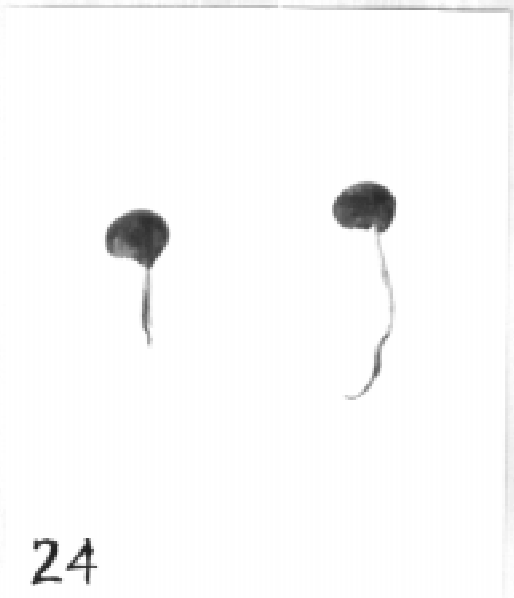
A total of 400 seeds were taken and in each pot 10 seeds were sown. Collar region and cotyledons of drooped

seedlings were examined under microscope for bacterial ooze and from suspected seedlings, bacterium was isolated and pathogenicity proved on young guar seedlings.

Guar seedlings started showing symptoms on collar region 11 days after planting, however, in artificially inoculated seeds the symptoms were first observed 12 days after sowing. The collar region in affected seedlings became brown to black and this browning extended upward and downward girdling the stem completely at soil level (Fig. 24 and 25). In few cases there was yellowing of cotyledons in seedlings developing from infected seeds. Presence of bacterium in collar and cotyledonary tissues was confirmed by ooze test (Fig. 26 and 27) and also by pathogenicity on young guar seedlings.

When seeds collected from artificially infected pods were sown, the maximum number of infected seedlings were detected in between 15 to 25 days after sowing. This period for detection of seedling infection in case of artificially inoculated seeds was in between 19 to 23 days after sowing.

Growing in quartz sand was found to be a relaible technique for detection of X. campestris pv. cyamopsidis in guar seeds as compared to other methods of seed detection used. Seeds soaked in bacterial suspension when planted in quartz sand yielded 31.5 per cent infected seedlings. In this treatment, all the seedlings had collar infection whereas only 0.75%



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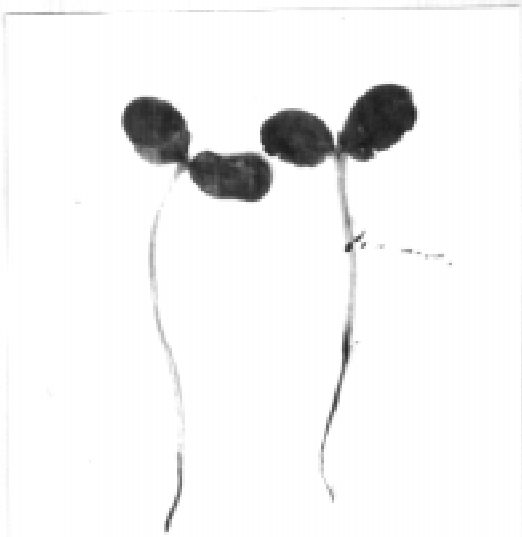

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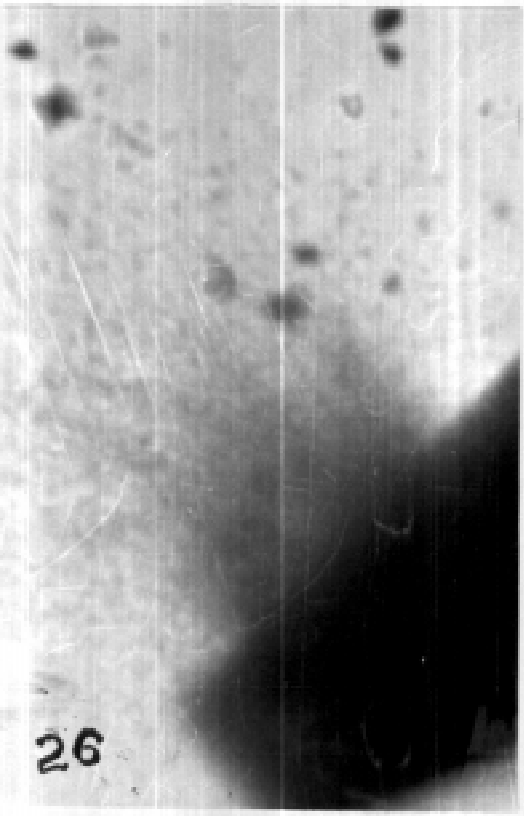

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Figs. 24-27. Seed detection of bacterium. 24. Seeds showing black infected area on germinated seeds, 25. Collar infection on seedlings developing from infected seeds, 26. Bacterial ooze from cotyledon showing yellowing in infected seedling, and 27. Ooze from infected collar region.

seedlings had infection of cotyledons (Table 17). Cotyledonary infection was highest (1.25%) in seedlings developing from seeds collected from artificially inoculated pods but the collar infection in this treatment was 18.5%.

Table 17: Detection of *X. campestris* pv. *cyamopsidis* in guar seeds of Pusa Nav Bahar by growing in quartz sand

S.No.	Treatments	Germination (%)	Seedlings showing infection(%)*		
			Collar region	Cotyledons	Total
1.	Seeds collected from artificially infected pods	80	18.5	1.25	18.5
2.	Seeds collected from blight affected plants	88	8.0	0.25	8.0
3.	Seeds soaked in bacterial suspension	87	31.5	0.75	31.5
4.	Seeds collected from apparently healthy plants	95	2.0	Nil	2.0

* Observations based on 400 seeds sown.

Collar infection on 8% while cotyledons of 0.25% seedlings were infected when seeds collected from blight affected plants were used. Cotyledon infection was not recorded in seedlings from apparently healthy seeds, although, collar region of 2% seedlings were infected.

Among agar plate, test tube agar and growing in quartz sand methods tried, maximum percentage of seed detection of the pathogen was found by growing seeds in quartz sand. By comparing the per cent seed detection of the bacteria it can be summarized as follows: Growing in quartz sand > Agar plate method > test tube agar method.

X. Physiological changes in plants after infection:

1. Chlorophyll and carotenoids -

Chlorophyll and carotenoids content of guar leaves infected with X. campestris pv. cyamopsidis were estimated qualitatively by chromatographic method and quantitatively by spectrocolorimeter.

Ethanol extracts from healthy and diseased leaves were strealod on Whatman No. 1 chromatographic paper. The chromatograms were run in petroleum ether and acetone (9:1) for 15 min. thereafter dried under fan. The pigments separated into 3 distinct bands. In chromatograms developed from healthy and diseased leaf extracts, although, the colour of the bands in latter were fainter as compared to those from healthy leaf extracts, the upper most band was yellowish orange representing carotene and xanthophylls, middle one bluish green of chlorophyll a and lower one yellowish green representing chlorophyll b. This study indicated that the bacterial blight influenced the chloroplast and carotenoid constitution of the host and their amount was reduced in diseased leaf tissues.

Quantitative changes in chlorophyll and carotenoids contents in guar leaves due to X. campestris pv. cyamopsidis were estimated by spectrocolorimeter using acetone extracts. The experiment was repeated 4 times and the average results are given in table 18.

Table 18: Chlorophyll and carotenoid contents in healthy and diseased leaves of guar variety Pusa Nav Bahar

S.No.	Content	Amount in mg/g fresh weight*		Per cent decrease
		Healthy	Diseased	
1.	Chlorophyll a	0.9512	0.6781	28.7
2.	Chlorophyll b	0.5642	0.3140	44.3
3.	Total chlorophyll	1.5155	0.9923	34.5
4.	Carotenoids	0.75	0.64	14.7

* = Average of 4 experiments.

In bacterial blight affected leaves, chlorophyll a decreased 28.7 per cent. Decrease in chlorophyll b content in diseased leaves was more as compared to chlorophyll a and per cent decrease in total chlorophyll was intermediate (34.5). The carotenoid content in healthy leaves was 0.75 mg/g fresh weight whereas it was 0.64 mg/g fresh weight in diseased leaves representing an decrease of 14.7 per cent (Table 18).

2. Transpiration -

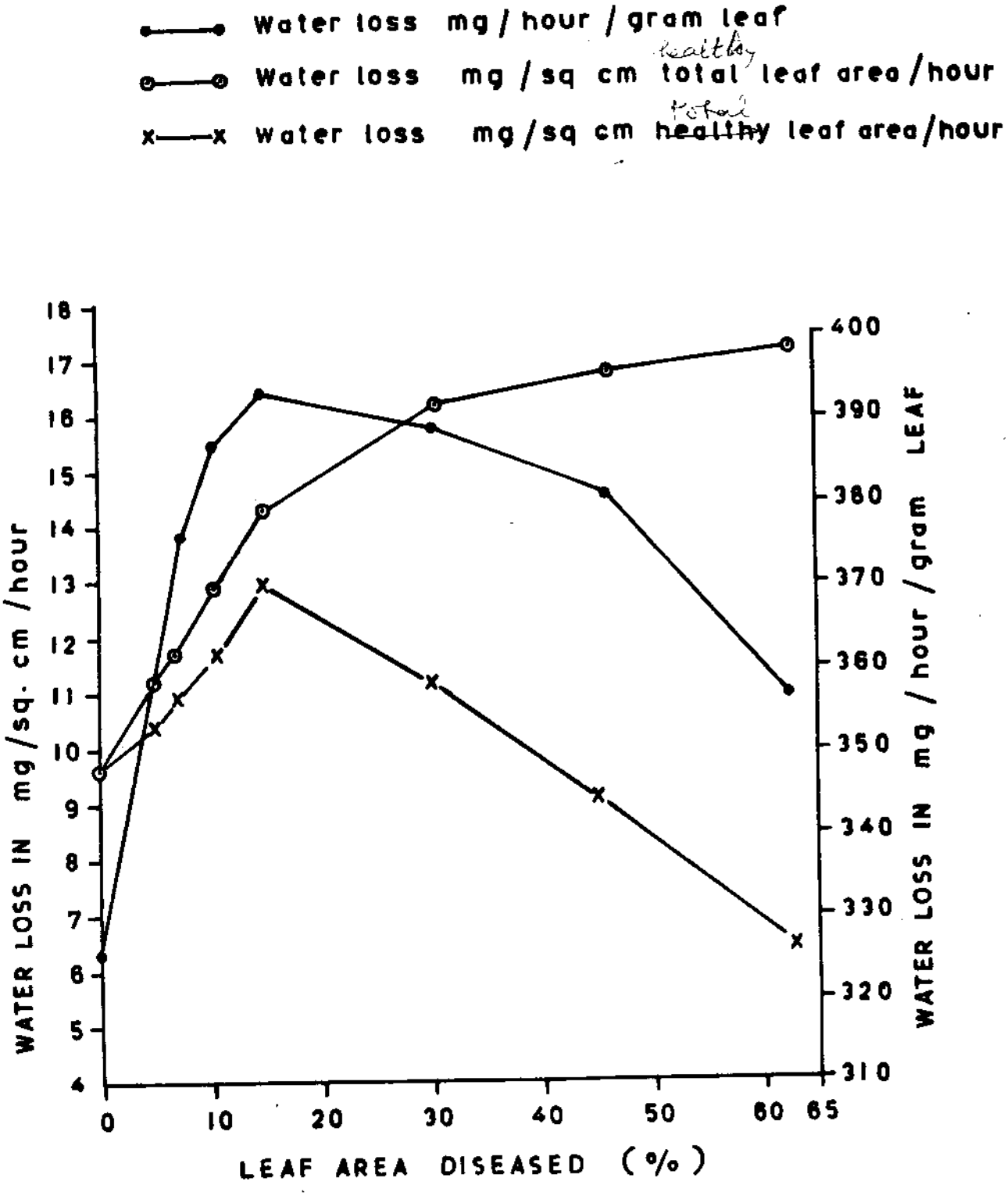
Experiments were carried out to compare transpiration rate in diseased and healthy guar leaves. Healthy, and diseased guar leaves with different infection indices were collected and cut end of petiole of each leaf was dipped in water filled in a plastic vial as described under materials and methods. The loss in weight of water from the tube in unit time, weight of the leaf without petiole and the healthy and diseased area

Table 19: Changes in transpiration rate of guar leaves infected to different degrees by *X. campestris* pv. *cyamopsidis*

S.No.	Leaf	Leaf area diseased (%)	Loss of water in mg/sq.cm healthy leaf area/hour*	Per cent increase in water loss	Loss of water in mg/hour/ gram leaf	Loss of water in mg/sq.cm total leaf area/hour
1.	Healthy	-	9.62	-	325.65	9.62
2.	Diseased	4.80	11.19	+ 16.32	357.90	10.43
3.	"	7.14	11.75	+ 22.14	375.72	10.91
4.	"	10.54	12.97	+ 34.82	386.81	11.71
5.	"	14.86	14.29	+ 48.54	392.97	13.02
6.	"	30.56	16.17	+ 68.09	388.54	11.23
7.	"	46.19	16.84	+ 75.05	380.83	9.06
8.	"	62.58	17.20	+ 78.79	356.51	6.44

* Average of 10 leaves in each treatment.

FIG. 28. CHANGES IN TRANSPIRATION RATE OF GUAR LEAVES INFECTED BY X. CAMPESTRIS pv. CYAMOPSIDIS



on each leaf were measured. The data obtained for 10 leaves having nearly similar per cent diseased leaf area were grouped together, averaged and presented in table 19 and Fig. 28.

It was observed that in diseased leaves, the transpiration rate was higher as compared to healthy leaves when calculated on the basis of healthy leaf area (Table 21). The increase in transpiration rate continued with the increase in diseased leaf area and the loss in water was maximum 17.2 mg/sq.cm. healthy leaf area/hour when 62.58 per cent of the leaf area was diseased (Fig. 28). This indicated that, although, the total healthy leaf area decreased with increasing infection, yet the rate of transpiration calculated on the basis of remaining healthy leaf area increased. When water loss was calculated on total leaf area basis, the transpiration rate initially increased with increase in diseased area and when the per cent diseased area exceeded beyond 14.86, the transpiration rate declined (Fig 28). Similarly, when transpiration rate was expressed as water loss in mg/hour/gram leaf, the transpiration rate continued to increase with increase in percent diseased leaf area upto 14.86, and thereafter the transpiration rate declined.

3. Respiration -

Respiratory changes in guar plants of variety Pusa Nav Bahar as a result of infection with X. campestris pv. cyamopsidis was measured in Warburg's respirometer. Each

Table 20: Respiration rate of healthy and bacterial blight affected leaves of guar variety Pusa Nav Bahar as measured by Warburg's respirometer

Plant material	Flask manometer assembly	Flask constant (K)	Actual change in barometer reading (mm/hour)*	µl. O_2 uptake	Average µl. O_2 uptake/hr/g fresh weight	Per cent increase in respiration rate
Healthy	1	2.924	40	116.96		
	2	2.563	38	97.39	108.36	
	3	2.260	49	110.74		
Diseased	4	2.648	78	206.54		
	5	2.423	81	196.26	207.37	+ 91.37
	6	2.238	98	219.32		

* Values are mean of 3 readings taken in each flask manometer assembly.

treatment was replicated 3 times and from each flask/manometer assembly 3 readings were taken. The experiment was repeated and average results of which are presented in table 20.

Bacterial infection resulted increase in respiration rate upto 91 per cent. In healthy leaves, oxygen uptake was 108.36 µl/hour/g fresh weight as against 207.36 µl/hour/g fresh weight in diseased leaf tissues (Table 20).

4. Sodium, calcium and potassium -

Changes in sodium calcium and potassium in guar variety Pusa Nav Bahar infected with X. campestris pv. cyamopsidis were estimated using Flame Photometer. All the analysis were carried out in triplicate and the experiment was repeated, averages of which are presented in Table 21 (Appendices III-IV).

Table 21: Changes in sodium, calcium and potassium as measured by Flame Photometer in Pusa Nav Bahar leaves infected X. campestris pv. cyamopsidis

S.No.	Element	Amount in mg/g on dry weight basis*		Per cent deviation**
		Healthy	Diseased	
1.	Sodium	2.1	1.5	-28.57
2.	Calcium	13.2	14.4	+ 9.09
3.	Potassium	16.6	19.2	+15.66

* = Average of two experiments.
** = (+) denotes an increase and (-) decrease as compared to healthy leaves.

In bacterial blight affected guar leaves, sodium content decreased upto 28.57 per cent while calcium and potassium content increased upto 9.09 and 15.66 per cent, respectively. The amount of 3 elements varied both in diseased and healthy leaves (Table 21). Healthy leaves contained 16.6 and 19.2 mg/g dry leaf of calcium and potassium, respectively. The amount of sodium was only 2.1 mg/g dry leaf in healthy leaves. The results indicated that both calcium and potassium accumulated in infected leaves, although, calcium accumulated in more quantity as compared to potassium.

5. Sugars -

For estimating reducing and total sugars, 400 mg of dried and ground leaf material was extracted in ethanol and water. The final volume was raised to 50 ml with 70 per cent ethanol reducing and total sugar content were estimated (Appendix-V) as per method given earlier. Non-reducing sugar content was calculated by substrating reducing sugar content from total sugar. There were 3 replication in each treatment and the experiment was repeated, average data of which are presented in table 22.

The reducing sugar content of diseased leaves was only slightely higher than that of the healthy one. This amount represented 30.77 per cent of total sugar in healthy leaves as against 37.59 per cent in diseased leaves. On the contrary, non-reducing sugar amount in diseased leaves was 25.11 per cent

Table 22: Effect of bacterial blight on the reducing, non-reducing and total sugar contents of guar leaves

S.No.	Content	Amount in mg/g dry weight*		Per cent increase or decrease	Per cent of total sugar	
		Healthy	Diseased		Healthy	Diseased
1.	Reducing sugar	12.500	12.687	+ 1.49	30.77	37.59
2.	Non-reducing sugar	28.125	21.063	-25.11	69.23	62.40
3.	Total sugar	40.625	33.750	-16.92	-	-

* Values are mean of three replications.
Non-reducing : reducing sugar ratios
Healthy = 1:0.44
Diseased =1:0.60

less than that of healthy. The amount of total sugar also decreased in diseased leaves. It was 40.625 mg/g in healthy leaves as against 33.750 mg/g in dry weight in diseased leaves. The non-reducing: reducing sugar ratio, which was more wide (1:0.44) in healthy leaves, was narrowed in diseased leaves.

6. Total nitrogen and protein -

Total nitrogen and protein content in guar leaves as affected by bacterial blight was estimated by micro-kjeldahl method (Appendix VI). The amount of protein was calculated by multiplying the nitrogen content in the sample with 6.25. The data on total nitrogen and protein content are presented in table 23.

Table 23: Changes in total nitrogen and protein in guar leaves (Pusa Nav Bahar) infected with X. campestris pv. cyamopsidis

S.No.	Constituent	Quantity in mg/100 mg dry*		Per cent deviation[1]
		Healthy	Diseased	
1.	Total nitrogen	1.94	1.28	-66
2.	Total protein (Nx6.25)	12.125	8.00	-66

[1](+) denotes an increase and (-) decrease as compared to healthy leaves.

* Average of two separate experiment with 3 replication in each treatment.

The data in table 23 indicated that the total nitrogen and protein content in diseased leaves decreased appreciably (66%) as compared to healthy one. The healthy dried leaves had 1.94 and 12.125 mg/100mg of total nitrogen and protein, respectively as against 1.28 and 8.00 mg/100 mg in healthy leaves.

7. Phenols and orthodihydroxyphenols -

Total phenols and orthodihydroxyphenols were estimated in healthy and diseased leaves of guar variety Pusa Nav Bahar (Appendix VII). All the analyses were carried out in triplicates . The average data of two experiments are presented in table 24.

Table 24: Total phenol and orthodihydroxyphenolic contents of healthy and diseased leaves of guar variety Pusa Nav Bahar

S.No.	Contents	Amount in mg/g dry weight*		Per cent increase (+) or decrease (-)
		Healthy	Diseased	
1.	Total phenols	6.00	5.62	- 6.33
2.	Orthodihydroxyphenols	1.25	1.36	+ 8.80

* Average of two experiments with 3 replications in each treatment.

8. Ascorbic acid -

Ascorbic acid content in diseased and healthy guar leaves were estimated by visual titration method using 2,6-dichlorophenol indophenol dye. The amount of ascorbic acid present in tissues was expressed as mg/100 g fresh weight (Table 25).

Table 25: Alternations in ascorbic acid content of guar leaves infected with X. campestris pv. cyamopsidis

S.No.	Material	Ascorbic acid (mg/100 g tissue)*	Per cent decrease over healthy
1.	Healthy leaves	46.5	-
2.	Diseased leaves	29.6	36.3

* Average of two experiments.

The ascorbic acid contents in diseased and healthy guar leaves were 29.6 mg/100 g fresh weight and 46.5 mg/100 g

fresh weight, respectively. A decrease in ascorbic acid content by 36.3 per cent was recorded in diseased leaves as compared to healthy leaves.

9. Production of cellulase -

The enzyme cellulase, as elaborated by infection with bacterium in guar leaves, was measured by estimating amount of reducing sugars released from carboxy methyl cellulase (Appendix VIII). The enzyme extract was expressed as the amount of glucose released per ml of the enzyme extract per unit time. The average data of two experiments are presented in table 26.

Table 26: Cellulase activity of diseased and healthy leaf extracts expressed as mg of glucose released/ml of enzyme extract/hour

S.No.	Material	Amount of glucose released in mg/ml/hour*	Increase in diseased leaves (%)
1.	Healthy leaves	0.575	-
2.	Diseased leaves	1.700	195.6

* Average of two experiments.

The enzyme cellulase activity was appreciably high in diseased leaf tissues as compared to healthy one. The enzyme extract from healthy leaves released 0.575 mg of reducing sugars per ml per hour, whereas, the enzyme extract from diseased tissues released 1.7 mg of reducing sugars per ml per hour, and this amount was 195 per cent higher than in uninfected leaves.

10. Production of beta-glucosidase -

Beta-glucosidase activity in healthy and bacterial blight affected leaves was measured by estimating the amount of aglucone released in terms of catechol. Observations indicated that beta-glucosidase was absent in healthy and diseased guar leaves. In both cases, no change in absorbancies was noticed even upto 35 minutes after the reaction set in.

11. Estimation of protease activity -

Protease activity in healthy and diseased leaf extracts was assayed by measuring the amount of amino acids liberated from casein with ninhydrin reagent (Appendix-IX). The data are presented in table 27.

Table 27: Protease activity in diseased and healthy leaf extracts expressed as mg of amino acid released/ml of enzyme extract/hour

S.No.	Material	Amount of amino acid released in mg/ml/ hour*	Per cent increase over healthy
1.	Healthy leaves	0.235	-
2.	Diseased leaves	0.298	26.8

* Average of two experiments.

An increase of 26.8% in protease activity was recorded in diseased leaves as compared to healthy leaves. The enzyme extract prepared from healthy leaves released 0.235 mg of amino acids/ml extract/hour as against 0.298 mg/ml extract/hour in extracts prepared from diseased leaves.

12. Estimation of polyphenol oxidase -

Polyphenol oxidase in healthy and bacterial blight affected leaves was estimated by measuring oxidation of catechol in spectrocolorimeter. Changes in absorbance were recorded upto 20 minutes and the data were expressed as unit change in absorbance 0.001/min /ml of enzyme extract.

It was observed that amount of polyphenol oxidase activity was less in extracts prepared from both healthy and diseased leaf tissues, although the activity in infected leaves was 29.4 per cent more than in healthy leaves (Table 28). The unit change in absorbance of 0.001/min /ml was 12 and 17 units in enzymes extracts of healthy and diseased leaves, respectively.

Table 28: Polyphenol oxidase activity in diseased and healthy leaf extract expressed as unit change (1 unit=change in absorbance of 0.001/min /ml enzyme extract)

S.No.	Material	Unit change in absorbance of 0.001/min / ml. enzyme extract*	Per cent increase over healthy
1.	Healthy leaves	12	-
2.	Diseased leaves	17	29.4

* Average of two experiments.

13. Estimation of ascorbic acid oxidase -

Ascorbic acid oxidase was measured by determining the residual ascorbic acid in reaction mixture by UV-Spectrocolorimeter. Data on enzyme activity, expressed as unit change in absorbance/min/ml enzyme extract, are presented in table 29.

Table 29: Ascorbic acid oxidase activity in diseased and healthy leaf extracts expressed as unit change in absorbance (1 unit = change in absorbance of 0.01/ min/ml enzyme extract)

S.No.	Material	Unit change in absorbance*	Per cent increase over healthy
1.	Healthy leaves	88.75	-
2.	Diseased leaves	108.75	22.5

* Average of two experiments.

The ascorbic acid oxidase activity was altered in diseased leaves due to bacterial blight infection. Ascorbic acid oxidase activity in healthy leaf extract was 88.75 units as against 108.25 units in enzyme extract prepared from diseased leaves, and the activity was 22.5 per cent higher in latter case.

14. Estimation of catalase -

Catalase activity in healthy and diseased plant tissue extracts was measured by permangnate titration method. Data on enzyme activity expressed as units/g fresh weight/min are presented in table 30.

Catalase activity in extract prepared from diseased leaf tissues was more by 17.3 per cent. The activity was 1.821 units/g fresh weight/min in healthy leaves as against 2.136 units/g gresh weight/min in diseased leaves.

Table 30: Catalase activity (units/g fresh weight/ min) in healthy and diseased leaves of guar variety Pusa Nav Bahar

S.No.	Material	Catalase activity (unit/g fresh weight)*	Per cent increase over healthy
1.	Healthy leaves	1.821	-
2.	Diseased leaves	2.136	17.3

* Average of 2 experiments with 3 replications in each treatment.

15. Estimation of peroxidase -

Enzyme peroxidase was assayed by measuring purpuragallin formed during the reaction of enzyme with pyrogallol in presence of hydrogen peroxide. Data on peroxidase activity, expressed as unit change in absorbance of 0.001/min/ml enzyme extract, are presented in table 31.

Table 31: Peroxidase activity in healthy and bacterial blight affected guar plant (activity expressed as unit change in absorbance of 0.001/min/ml enzyme extract)

S.No.	Material	Unit change in absorbance of 0.001/min/ml of enzyme extract	Per cent increase over healthy
1.	Healthy leaves	54	-
2.	Diseased leaves	63	16.6

* Average of two experiments.

Peroxidase activity was latered in diseased leaves due to bacterial blight infection, althouth, the increase was only 16.6 per cent. The enzyme activity in healthy leaf extract was 54 units (1 unit = change in absorbance of 0.001/ min/ml extract) as against 63 units in diseased leaves.

XI. Screening of germplasm in field:

Seeds of 55 guar varieties/selections obtained from Economic Botanist (Guar), Agricultural Research Station, Durgapura were used in present trials. The varieties were screened against bacterial blight during kharif 1980 and 1981 under artificial inoculations. Disease ratings were recorded by arbitrary scale (0-5) 20 days after inoculation when the plants were 50 days old and infection indices were calculated. The average data of two year trials are given in table 34.

Table 34: Reaction of guar germplasm to *X. campestris* pv. *cyamopsidis* in field during kharif 1980 & 1981*

S.No.	Variety/ selection	Source	Disease rating (0-5 scale)	Infection index	Reaction[1]
1	2	3	4	5	6
1.	RGC 587	Churu (Raj.)	2.17	43.33	T
2.	601	"	3.00	60.00	HS
3.	637	"	3.42	68.40	HS
4.	610	"	2.77	55.38	S
5.	647	"	2.25	45.00	T

Contd...

1	2	3	4	5	6
6.	RGC 107	Churu (Raj.)	2.83	56.66	S
7.	137	"	2.77	55.55	S
8.	136	"	2.50	50.00	S
9.	205	"	2.38	47.70	T
10.	679	"	3.10	62.00	HS
11.	702	"	2.73	54.54	S
12.	577	"	2.72	54.50	S
13.	581	"	2.91	58.18	S
14.	248	"	3.22	64.40	HS
15.	710	"	2.66	53.33	S
16.	237	"	2.00	40.00	T
17.	711	"	2.63	52.60	S
18.	718	"	3.37	67.48	HS
19.	239	"	2.07	41.43	T
20.	105	"	2.30	46.00	T
21.	242	"	2.63	52.62	S
22.	635	"	3.31	66.20	HS
23.	687	"	3.13	62.61	HS
24.	Durgajaya (B 19-1-55)	Badmer	3.00	60.00	HS
25.	RGC 916	Sojat	3.14	62.82	HS
26.	754	Gangapur(Bikaner)	3.48	69.60	HS
27.	755	Sirchi	2.87	57.50	S
28.	733	Mutha (Nagore)	3.11	62.22	HS
29.	757	Bhatla(Barmer)	3.08	61.60	HS

Contd...

1	2	3	4	5	6
30.	RGC 759	Urdsai (Churu)	2.86	57.14	S
31.	758	"	3.00	60.00	HS
32.	752	"	3.00	60.00	HS
33.	732	"	3.30	66.10	HS
34.	760	"	2.92	58.42	S
35.	730	"	3.42	68.00	HS
36.	761	"	2.25	45.00	T
37.	471	"	3.00	60.00	HS
38.	745	"	3.00	60.00	HS
39.	914	Selection (NBxD. safed)	2.86	57.33	S
40.	748	Mectha(Nagore)	3.00	60.00	HS
41.	756	Gungashia (Barmer)	2.56	51.20	S
42.	749	Tonk	3.32	66.40	HS
43.	735	Bachran (Barmer)	3.30	66.00	HS
44.	746	Laxmangarh (Alwar)	3.18	63.64	HS
45.	751	Khadd (Barmer)	3.00	61.68	HS
46.	741	Jalawar (Jodhpur)	3.26	65.33	HS
47.	737	Bidwana	2.75	54.00	S
48.	728	Bishangarh (Jalore)	3.05	61.17	HS
49.	740	Mutha (Nagore)	2.77	55.38	S
50.	736	JunJunu (Bharatpur)	2.69	53.84	S
51.	Sel. 1*	Selection(PNBxRGC 401)	3.20	64.00	HS
52.	SPS 119	Selection (PNBxD. safed)	3.50	70.00	HS
53.	SPS 63	Selection (PNBxRGC 401)	3.57	71.42	HS
54.	Pusa Nav Bahar	I.A.R.I.	3.60	72.00	HS
55.	Pusa Sada Bahar	I.A.R.I.	3.58	71.60	HS

* Data are average of two years' observations.
1 - Infection index 40-49 = T, 50-59 = S, and 60 or above = HS

Out of 55 entries tested in 1980 and 1981, 7 entries were found tolerant. These entries had infection indices of less than 50 (Table 34). Minimum infection index of 40 was recorded on RGC 237 followed by RGC 239 (41.43). Both these selections are from Churu district of Rajasthan. Eighteen entries were susceptible having infection indices in between 50 and 59 and remaining 30 entries were highly susceptible having infection index of 60 or more. Maximum infection index of 72 was recorded on variety Pusa Nav Bahar followed by 71.6 in Pusa Sada Bahar and 71.42 in SPS-83.

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DISCUSSION

Guar is an important crop of Rajasthan and used as vegetable, feed and fodder crop, and its flour is used as a commercial source for excelled gum. The crop suffers from bacterial blight caused by Xanthomonas campestris pv. cyamopsidis. It is cultivated in rainy and summer seasons but the disease was found mainly in rainy season in the months of July-September during 1980 and 1981.

The bacterial blight appeared on all aerial parts except flower, producing varying symptoms such as round, irregular and cuneate leaf spots, blighting, drooping and defoliation of leaves, spliting and breaking of stem, wilting and curvature of apical portion, girdling of collar region and spots on petioles. In nature and under artificial inoculations, lesions on cotyledons were not observed, although, it has been reported by Srivastava and Rao (1963 b). The symptoms on collar region on seedlings, as a result of primary infection from infected seeds, and spots on petioles were additional symptoms of the disease not observed by earlier workers (Patel et al., 1953; Patel and Patel, 1958; Srivastava and Rao, 1963 b; Orellana et al., 1965).

Some workers have given loss estimates for bacterial blight and in present investigation, results based on 2 years field trials, it was found that loss in seed yield varied from

5.31 to 66.35 per cent in variety Pusa Nav Bahar. However, loss in seed yield would also depend on age of the plant during infection, besides environmental and other factors.

Guar bacterial blight pathogen has been reported to be host specific, but in the present work, besides guar, it was found to infect french bean (Phaseolus vulgaris Jacq.) and field bean (Dolichos lablab L.). Considering the narrow host range, Young et al. (1978) named this bacterium as Xanthomonas campestris pv. cyamopsidis, however, its infective abilities on two hosts of leguminosae indicates that the present nomenclature of guar blight bacterium may not stand valid in future and comparative studies of this bacterium with other organisms with regard to host range will probably result in the reduction of several pathovars to synonomy, if present system of nomenclature is not changed. Trinomial nomenclature instead of binomial naming of the pathogen perhaps may need rethinking.

In guar plants inoculated at one week intervals throughout July, 1980 to June, 1982, it was found that in spite of artificial inoculations, the disease was severe only during July to September each year, when there were intermittent rains coupled with high humidity and temperature range of 22-34°C. Not only this, the progress of the disease in field during the crop growth period in rainy and summer seasons was much faster in rainy season than in summers. This perhaps can explain the negligible disease under natural conditions during summers as that much inoculum may not be available as under artificial inoculations.

Bacteria enter leaves and stems mainly through natural openings and wounds. Among artificial methods of inoculation, spraying bacterial suspension onto a leaf surface without causing visible water soaking on the leaves probably approximates natural field infection. In the present studies, 3 consecutive sprays of bacterial suspension were found as one of the successful, convenient and reliable methods of inoculation in the field. Similar results were reported by Matthee and Daines (1968) in case of X. vesicatoria on tomato. Not only methods of inoculation, but bacterial population available to cause infection also influenced the symptom expression. It was found that for successful infection under spray inoculation method, the suspension must contain atleast c.7.3×10^4 bacterial cells/ml. It is, however, not known about the minimum concentration of inoculum which may be necessary for initiation and development of disease in nature. Several workers have worked out the minimum inoculum level required for pathogenesis in different host-pathogen combinations (Basu, 1966; Kawamota and Lorbeer, 1972; Forster and Echandi, 1973; Shekhawat and Chakravarti, 1978; Beniwal and Parashar, 1979).

The extent of multiplication of guar blight bacterium was found to be influenced by the host reaction. In Pusa Nav Bahar, the rate of multiplication was faster than in RGC 237. This slower rate of multiplication of bacterium in RGC 237 is, perhaps, one of the causes for its less susceptible nature in

field. Similar observations have been reported by Chamberlain (1962) in X. phaseoli var. sojense on soybean, Stall and Cook (1966) in X. vesicatoria on tomato and by others. The fact that older leaves are less susceptible to infection points to the importance of a change in the host metabolism with age. In plants with 4-leaf stage or above, progressive decrease in disease was observed from 4th leaf position onwards. Second and third leaves from top were found highly diseased and the disease did not develop on lower leaves after plant had attained 7th leaf stage. This does not over rule the possibilities of developing resistance in leaves with age because of alterations in the host physiology. This may be the reason that plants of 15-35 days age groups had more disease in present case. During this age period, all the leaves present on a plant were infected. These results agree with Nayudu and Walker (1960) and Shekhawat and Chakravarti (1976) in case of tomato leaf spot caused by X. vesicatoria.

Besides age of the plants, the disease was found to be influenced by amount and kind of nutrition supplied to the plants. In general, low nitrogen, high potassium, absence of calcium or lower concentration of Hoagland's solution favoured more disease while plants supplied with high potassium or no nitrogen were found less diseased. Variable amount of phosphorus supplied to plants had no effect on disease development. In present case, with increase in nitrogen level from 1/2 N to

1 N, the infection index increased and thereafter, the increase in nitrogen supply adversely affected the disease development. These results agree with those reported by Nayudu and Walker (1960) in tomato leaf spot caused by X. vesicatoria and by Lipke (1968) for X. malvacearum on cotton. It appears probable that the high osmotic values of the nutrient substrates and/or the presence of any one ion in high concentration affect optimal absorption of the essential elements and that the consequent imbalance in the host metabolism affects both plant growth and the pathogenicity of the bacterial blight organism.

Seed is known to be a potent source of survival and dissemination of bacteria and guar blight bacterium is no exception to this. Detection of bacterium in seeds is a problem encountered with respect to many bacterial plant pathogens. Ordinary method of plating seeds on agar media is not always successful as the pathogen may not be able to grow out into the medium from the seed because of other fast growing saprophytes and often the percentage of infected seeds may be so low that detection becomes extremely difficult. This inability to isolate the pathogen from seeds may not indicate that the seed is pathogen free. Attempts were made to isolate the bacterium from seeds after plating them on various media and the pathogen could be isolated from 4% of the seed lot; seeds from the same lot when sown in quartz sand yielded 8% infected seedlings and the infection could be well detected on collar region. Superiority

of this technique in detection of bacterium in seeds was further confirmed by planting seeds collected from artificially inoculated pods and those healthy but inoculated by bacterial suspension in which infection on collar region could be well detected in 18.5% and 31.5% seeds, respectively. Srivastava and Rao (1963 b) reported that seedlings from infected seeds developed cotyledonary lesions soon after emergence but in present case lesions on cotyledons were not observed. Yellowing of cotyledons, which on isolation yielded bacterium, was observed only in 1.25% seedlings. Seedlings developing from infected seeds show collar infection and such seedlings droop and topple down later. Therefore, the role of seed borne inoculum in the epidemiology of disease needs further experimentation. The possible explanation may be that the disease might spread from the inoculum which reaches the cotyledons and remain available for some time. The bacterium present in collar region of infected seedlings may remain in field and may serve as source of inoculum. The inoculum may be made available from infected seedlings which themselves do not get infection in foliage but survive in field unnoticed for quite some time. The inoculum from such seedlings may spread to nearby plants by irrigation water or by spattering of rains. This view gets support from Orellana et al. (1965) who observed infection spreading along a single row on the side of a primary infection site.

Chlorophyll content in plants are reduced by plant pathogens by directly affecting the photosynthesis. However,

corresponding decrease in carotenoids suggests that the chlorophyll loss may be due to metabolic disruption rather that direct destruction. It is also possible that due to proteolysis, the chloroplast may degenerate (Meyer et al., 1960) and the production of proteolytic enzymes in present case by guar blight bacterium was observed. Moreover, pathogens which induce water loss would affect photosynthesis as a result of the restriction of gas exchange through the stomata. In present studies, increased transpiration rate might have been partly responsible for reduction in chlorophyll contents in infected tissues by affecting rate of photosynthesis.

Bacterial infection resulted in increased respiration in guar leaves and this may be due to abolition of pasteur effect (Turner, 1951), which seems to be a characteristic response of plant tissues invaded by bacteria and fungi. As a consequence, respiratory efficiency is decreased and this may be associated with the observed increased rate of O_2 uptake.

The killing of cells in the leaves <u>per se</u> by the pathogen would result in increased metabolic activity and in turn loss of cell permeability which would release minerals in the surroundings (Bateman, 1964; Yarwood and Jacobson, 1955). This may explain the accumulation of calcium and potassium in infected leaves. Reduction in sodium may be due to its transport to un-affected healthy region. Accumulation of calcium in infected leaves also get support from studies conducted on

effect of host nutrition on disease development in which a negative correlation was found.

On a dry weight basis, the reducing sugar content was not different in the healthy and infected tissues while non-reducing sugars and toatl sugar contents decreased. Decrease in total sugar content reflects a possible utilization by the bacterium and transportation of sugar from other parts of the plant towards infected area. The decrease in the amount of nitrogen in the infected tissues suggests possible inhibition of synthesis of various nitrogenous constituents as a result of infection besides some utilization by the bacterium. The total phenolic content of inoculated leaves was less than that present in the healthy tissues. The reduction in sugars at whose expense phenols are synthesized might have resulted in low phenolic contents of the diseased leaves. It is also probable that the reduction in total phenolics in diseased tissues might be due to the increased polyphenol oxidase activity in diseased host.

Biosynthesis of ascorbic acid in plants appears to be markedly influenced by the chlorophyll content of the leaves (Isherwood and Mapson, 1962) and also by the enzyme ascorbic acid oxidase. The loss of chlorophyll and increased ascorbic acid oxidase activity in the leaf tissues infected by bacterium might have resulted in the reduction of ascorbic acid. Both catalase and peroxidase are oxidative enzymes and their activity was found to increase slightly in infected tissues. This suggests that the terminal respiratory pathway is accelerated.

The possible control of diseases through host resistance is an important biological principle that is well established. The basic problem in the technique of breeding for disease resistance is that of providing a disease environment so that resistant plant may be distinguished from the susceptible one. Spraying bacterial suspension thrice onto the host was found to be a suitable technique of inoculation for screening guar varieties against bacterial blight. In different varieties screened, difference in susceptibility to X. campestris pv. cyamopsidis was observed.

Out of 55 lines, there were 7 lines which showed tolerance against the disease. Information regarding existance of distinct physiologic races is not available and further work is necessary to find out if the varieties which were found to be tolerant would remain so under different environmental conditions and in presence of different physiologic races, if and when found.

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SUMMARY

1. Bacterial blight of guar (*Cyamopsis tetragonoloba* (L.) Taub.) caused by *Xanthomonas campestris* pv. *cyamopsidis* (Patel, Dhande and Kulkarni) Dye was found to be prevalent in kharif seasons of 1980 and 1981 in different parts of Rajasthan. The incidence and infection index of the disease varied from 0 to 35% and 0 to 16.3, respectively. In general, the disease was not found to occur in summer season crop but in few fields the disease was present in traces. Symptoms were found on all above ground parts including leaf, leaf petiole, stem and pods but not on flowers. In seedlings developing from infected seeds symptoms were observed on collor region.

2. Loss in seed yield varied from 5.3 to 66.5% depending upon the amount of disease. At infection index of 10.6 loss in yield was 5.3%. Plants with maximum disease severity (0-5 scale) gave 73% less seed yield.

3. On artificial inoculation by carborundum abrasion technique, the bacterium produced symptoms on french bean (*Phaseolus vulgaris* Jacq.) and field bean (*Dolichos lablab* L.). The bacterium induced resistant type of reaction on groundnut (*Arachis hypogaea* L.) and cotton (*Gossypium arboreum* L.) in the form of brown necrotic lesions which were very small and remained restricted in size.

4. Five inoculation methods viz. (i) spray inoculation (single spray, 2 consecutive sprays and 3 consecutive sprays), (ii) spray inoculation under pressure with a rotary pressure-cum-vacuum pump, (iii) inoculation by rubbing cell suspension with carborundum powder (300 mesh), (iv) spray inoculation after pricking with multineedle, and (v) injection infiltration were tried to develop an efficient and convenient method of disease production. The disease could be produced by all the methods but, in general, carborundum abrasion technique for limited inoculations and inoculation by 3 consecutive sprays for field inoculation on large scale were found suitable and convenient.

5. The bacterium multiplied in highly susceptible (Pusa Nav Bahar) and in less susceptible (RGC 237) varieties of guar but the rate of multiplication was slower in variety RGC 237. The maximum bacterial population reached 1.3×10^9 cells/leaf disc in 12 days and 6.6×10^8 cells/leaf disc in 10 days after inoculation in highly susceptible and less susceptible varieties, respectively. In both the varieties, the spots became necrotic at the time when the bacterial population was near maxima. Thereafter the bacterial population declined.

6. The variety Pusa Nav Bahar was inoculated at 7 days interval throughout the year from July 1980 to June 1982. The symptoms could be produced artificially throughout the year but the disease development was dependent upon environmental

conditions. The maximum disease development was during July to September every year when there was high humidity and temperature range of 20-34.5°C. Disease developed only in traces during December to first week of June, in general, but infection index was somewhat higher during this period following rainy days. High humidity, rainfall and a temperature range of 20-34°C were conducive to disease development.

7. Progress of the disease was studied both in kharif and summer for 2 consecutive years. In field on artificial inoculation, the disease appeared even under unfavourable conditions but the disease progressed appreciably only under favourable conditions. The rainy season crop suffered most in both the years than summer crop.

8. For development of bacterial blight on guar a minimum inoculum concentration of c.7.3×10^3 cells/ml was required when plants were inoculated by carborundum abrasion technique while in spray inoculation it was c.7.3×10^4 cells/ml. No symptoms were produced below these levels when inoculated by both these methods. In general, when there was more number of bacterial cells/ml, the incubation period was slightly reduced and infection index was higher.

9. Initial soaking on inoculated leaves was produced only when a minimum population level of c.1.85×10^6 was attained.

10. Maximum infection was found on 2nd and 3rd leaves of plant from top. In plants at 4 leaf stage or more there was progressive decrease in disease from 4th leaf position onwards and the disease did not develop on lowest leaf after the plant had reached the 7th leaf stage. Bacterium was found to infect upto 50 days old plants but maximum infection was on 14-35 days old plants.

11. There was more disease in plants supplied with low level of nitrogen (upto 1N) and high level of nitrogen (2N) reduced the infection index. The infection indices were 48.00, 50.43 and 41.18 at 1/2 N, 1N and 2N levels, respectively and 24.76 when nitrogen was lacking. Phosphorus had no marked effect on disease development. Infection index was highest (61.00) when potassium was not supplied and disease declined with increase in potassium. Absence of calcium in the nutrient solution marginally increased the infection index from 50 to 55 and absence of sulphur markedly reduced the infection index from 50 to 39. Disease was comparatively more at low level of Hoagland's solutions and lowest at 2H level.

12. A method was developed to detect the pathogen in seed at seedling stage. To detect the bacterium in seed several methods were tried, viz. (i) agar plate method, (ii) test tube agar method, and growing in quartz sand in pots. In agar plate and test tube agar methods, 3-4% seed infection was detected whereas by growing seed in quartz sand in pots, in the same

seed lot 8% seed infection was detected. Seedlings infected with bacterium started developing symptoms on collar region 11 days after sowing. With this method, in seeds collected from artificially infected pods and those inoculated artifically by soaking in bacterial suspension, 18.5% and 31.5% seed infection could be detected respectively. This method of detection of bacterium in seed was found better and reliable over other methods.

13. In qualitative and quantitative estimation of chlorophyll and cartenoids, the amounts of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were reduced in infected leaves by 28.7%, 44.3%, 34.5% and 14.7% respectively.

14. Transpiration rate was found to increase with increase in diseased leaf area when calculated on the basis of loss of water in mg/sq.cm healthy leaf area/hour. When transpiration rate was calculated on total leaf area or on leaf weight basis, the transpiration rate initially increased with increase in diseased leaf area upto 14.86% and thereafter, the transpiration rate declined. Bacterial infection resulted increase in respiration rate upto 91%. In healthy and infected leaves oxygen uptake were 108.36 µl/hour/g fresh weight and 207.37 µl/hour/g fresh weight, respectively.

15. Sodium, non-reducing sugars, total sugars, total nitrogen and protein decreased in infected leaves whereas potassium

increased appreciably and there was slight increase in calcium and reducing sugars.

16. The amount of total phenols decreased by 6.35% in infected leves whereas an increased of 8.8% was recorded in orthodihydroxyphenols. Ascorbic acid contents also decreased appreciably (36.3%) in diseased leaves.

17. The enzymes cellulase, protease, polyphenol oxidase, ascorbic acid oxidase, catalase and peroxidase were elaborated in leaves infected with bacterium. In cellulase 195.6 per cent increase was observed whereas in case of other enzymes the activity increased in between 16.6% to 29.4%. No change was recorded in β-glucosidase activity.

18. None of the varieties was found to be resistant to X. campestris pv. cyamopsidis. Out of 55 entries, 7 entries viz. RGC 587, RGC 647, RGC 205, RGC 237, RGC 239, RGC 105 and RGC 471 were found to be tolerant. Remaining 38 entries were either susceptible or highly susceptible.

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* Original not seen.

APPENDIX - I

Weekly temperature, relative humidity and total rainfall during July 1, 1980 to July 12, 1982

Period	Average temperature(°C)		Average relative humidity (%)		Total rainfall (mm)
	Min.	Max.	Min.	Max.	
1	2	3	4	5	6
July 1-7, 1980	24.23	30.90	74.00	88.14	60.6
July 8-14, 1980	24.91	30.53	60.29	78.86	-
July 15-21, 1980	24.59	30.66	55.14	72.71	-
July 22-28, 1980	24.04	30.77	66.86	81.14	60.8
July 29-Aug. 4, 1980	24.76	29.04	63.86	84.71	81.0
Aug. 5-11, 1980	23.29	27.97	72.14	83.71	9.8
Aug. 12-18, 1980	22.46	31.31	60.71	83.14	-
Aug. 19-25, 1980	23.26	32.14	61.71	81.14	4.8
Aug. 26-Sept.1,1980	23.53	34.21	61.86	75.71	26.0
Sept. 2-8, 1980	21.54	31.11	56.14	84.29	6.2
Sept. 9-15, 1980	20.67	30.59	56.71	80.71	7.0
Sept. 16-22, 1980	21.49	32.49	49.00	77.29	47.0
Sept. 23-29, 1980	20.60	34.51	30.29	70.43	-
Sept.30-Oct.6, 1980	18.69	34.04	24.26	69.14	-
Oct. 7-13, 1980	16.53	35.50	20.00	70.14	-
Oct. 14-20, 1980	17.11	34.86	17.14	55.29	-
Oct. 21-27, 1980	13.90	33.59	24.86	67.71	1.2
Oct. 28-Nov. 3,1980	13.11	31.59	23.29	72.50	-
Nov. 4-10, 1980	11.04	29.74	23.00	66.43	-
Nov. 11-17, 1980	12.10	29.36	30.14	67.86	-

Contd...

1	2	3	4		5
Nov. 18-24, 1980	13.66	28.77	20.71	68.29	-
Nov. 25-Dec. 1, 1980	9.84	28.37	29.57	77.29	-
Dec. 2-8, 1980	8.54	26.41	30.00	72.43	-
Dec. 9-15, 1980	4.59	25.71	22.43	65.00	-
Dec. 16-22, 1980	6.70	27.80	38.29	87.29	21.2
Dec. 23-29, 1980	9.74	18.23	48.29	91.57	25.8
Dec. 30-Jan. 5, 1981	9.59	23.66	43.71	90.86	0.0
Jan. 6-12, 1981	4.30	19.77	34.14	87.29	1.4
Jan. 13-19, 1981	6.63	19.27	39.86	80.14	2.4
Jan. 20-26, 1981	7.50	23.77	32.86	83.00	-
Jan. 27-Feb. 2, 1981	7.16	24.60	30.71	76.71	-
Feb. 3-9, 1981	5.96	24.47	26.43	80.14	-
Feb. 10-16, 1981	6.93	21.11	26.57	82.00	-
Feb. 17-23, 1981	9.27	28.67	18.43	69.29	-
Feb. 24-Mar. 2, 1981	9.86	31.00	18.57	66.86	-
March 3-9, 1981	11.31	31.95	17.86	69.14	-
March 10-16, 1981	14.00	28.12	32.43	72.66	2.4
March 17-23, 1981	13.98	29.20	27.43	70.57	2.1
March 24-30, 1981	14.67	32.64	17.43	54.71	-
Mar. 31-Apr. 6, 1981	16.81	33.27	18.29	47.86	-
April 7-13, 1981	17.48	37.24	10.57	32.57	-
April 14-20, 1981	24.54	38.88	12.43	35.71	-
April 21-27, 1981	24.29	37.69	11.71	33.14	1.0
April 28-May 4,1981	21.91	36.79	20.57	40.13	-

Contd...

1	2	3	4	5	6
May 5-11, 1981	22.06	36.30	26.14	54.29	7.3
May 12-18, 1981	25.44	38.71	17.57	37.14	-
May 19-25, 1981	27.27	39.01	24.71	52.71	-
May 26-June 1, 1981	26.43	38.56	25.37	55.86	-
June 2-8, 1981	28.09	39.41	20.29	48.14	-
June 9-15, 1981	27.39	38.44	24.14	58.14	-
June 16-22, 1981	27.07	40.39	26.00	63.71	1.4
June 23-29, 1981	24.84	33.90	57.57	76.85	84.7
June 30-July 6,1981	25.36	31.53	55.71	75.00	1.0
July 7-13, 1981	23.59	28.37	75.14	85.72	89.5
July 14-20, 1981	24.13	29.13	68.29	80.57	3.1
July 21-27, 1981	24.19	28.65	78.29	89.43	44.6
July 28-Aug. 3, 1981	24.11	29.06	64.14	80.57	1.8
Aug. 4-10, 1981	24.19	29.44	77.14	86.14	162.2
Aug. 11-17, 1981	24.09	28.11	79.29	89.86	82.9
Aug. 18-24, 1981	23.13	28.20	74.14	89.29	1.6
Aug. 25-31, 1981	22.61	29.44	65.00	86.29	41.3
Sept. 1-7, 1981	22.21	30.94	57.86	83.86	10.8
Sept. 8-14, 1981	22.30	32.43	52.71	85.00	6.8
Sept. 15-21, 1981	22.36	32.90	40.14	81.00	6.7
Sept. 22-28, 1981	23.21	30.61	66.14	88.29	54.9
Sept. 29-Oct.5, 1981	20.11	31.12	47.57	83.57	-
Oct. 6-12, 1981	18.19	33.04	33.57	70.43	-
Oct. 13-19, 1981	16.90	35.00	22.86	71.00	-

Contd..

1	2	3	4	5	6
Oct. 20-26, 1981	14.81	32.46	24.43	64.00	-
Oct. 27-Nov. 2,1981	16.93	27.94	49.43	83.57	21.6
Nov. 3-9, 1981	10.99	26.99	38.00	86.00	32.8
Nov. 10-16, 1981	11.44	27.47	27.86	79.86	-
Nov. 17-23, 1981	7.40	25.86	21.71	80.43	-
Nov. 24-30, 1981	8.29	25.89	22.86	73.43	-
Dec. 1-7, 1981	8.10	23.71	30.71	73.00	-
Dec. 8-14, 1981	5.19	22.36	25.86	81.00	-
Dec. 15-21, 1981	8.39	23.33	39.57	80.57	4.4
Dec. 22-28, 1981	8.91	23.69	41.43	90.57	-
Dec. 29-Jan. 4, 1982	9.46	26.03	35.57	88.43	-
Jan. 5-11, 1982	9.89	22.37	49.14	89.57	1.4
Jan. 12-18, 1982	7.23	21.66	47.57	86.71	-
Jan. 19-25, 1982	6.91	24.11	30.86	79.29	-
Jan. 26-Feb. 1, 1982	11.21	20.50	58.43	88.43	11.2
Feb. 2-8, 1982	7.34	22.56	35.57	82.71	-
Feb. 9-15, 1982	9.47	26.36	32.14	72.14	-
Feb. 16-22, 1982	10.37	25.79	29.86	73.71	-
Feb. 23-Mar. 1,1982	12.01	24.21	31.14	67.29	1.0
March 2-8, 1982	7.60	24.33	28.71	59.57	-
March 9-15, 1982	10.89	25.73	17.71	56.86	-
March 16-22, 1982	13.14	32.76	18.71	62.57	-
March 23-29, 1982	16.04	32.69	20.14	55.71	-
March 30-Apri.5,1982	18.23	35.86	17.67	42.29	-

Contd...

1	2	3	4	5	6
April 6-12, 1982	16.39	34.36	13.14	35.00	-
April 13-19, 1982	21.47	36.37	15.14	40.14	-
April 20-26, 1982	20.69	34.87	26.14	57.57	12.4
April 27-May 3,1982	22.11	35.28	19.57	49.14	-
May 4-10, 1982	21.50	32.04	50.00	68.14	85.8
May 11-17, 1982	23.07	33.69	30.14	62.14	-
May 18-24, 1982	21.89	36.96	19.29	50.57	2.5
May 25-31, 1982	26.61	37.91	30.00	53.57	-
June 1-7, 1982	26.07	38.86	13.71	39.14	-
June 8-14, 1982	27.29	36.77	34.29	63.00	-
June 15-21, 1982	26.34	35.86	42.14	68.86	-
June 22-28, 1982	24.67	35.26	36.71	75.29	46.4
June 29-July 5,1982	24.81	34.61	37.71	71.14	3.0
July 6-12, 1982	26.16	35.50	45.43	73.71	16.8

APPENDIX-II

Weekly temperature, relative humidity and total rainfall during July 1, 1981 to October 27, 1981 and Feb. 24, 1982 to June 15, 1982

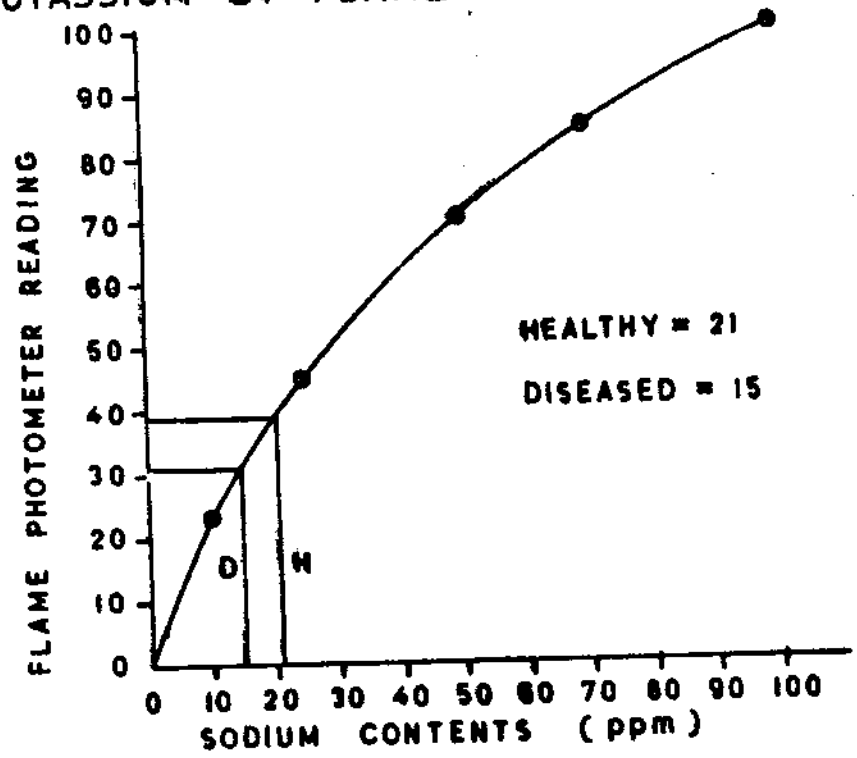
Period	Average temperature(°C)		Average relative humidity (%)		Total rainfall (mm)
	Min.	Max.	Min.	Max.	
1	2	3	4	5	6
July 1-7, 1981	25.61	32.20	52.86	75.00	1.0
July 8-14, 1981	23.40	27.69	79.00	88.43	89.5
July 15-21, 1981	24.26	29.00	69.86	79.57	3.1
July 22-28, 1981	23.98	28.61	77.43	90.29	46.4
July 29-Aug.4,1981	24.26	29.07	62.86	79.14	0.4
Aug. 5-11, 1981	23.86	29.30	80.71	89.00	200.6
Aug. 12-18, 1981	24.11	28.03	78.71	89.57	45.5
Aug. 19-25, 1981	23.13	28.56	70.29	87.29	4.8
Aug. 26-Sept.1, 1981	22.63	29.47	66.00	86.43	47.7
Sept. 2-8, 1981	22.16	31.20	56.29	83.00	-
Sept. 9-15, 1981	22.53	32.67	51.14	85.43	6.8
Sept. 16-22, 1981	22.13	32.83	40.86	79.86	6.7
Sept. 23-29, 1981	23.53	30.40	67.71	88.14	55.9
Sept. 30-Oct.6,1981	19.21	31.34	43.00	82.57	-
Oct. 7-13,1981	17.99	33.17	33.00	70.71	-
Oct. 14-20, 1981	16.46	34.86	23.00	70.00	-
Oct. 21-27, 1981	15.10	32.34	25.29	66.29	-

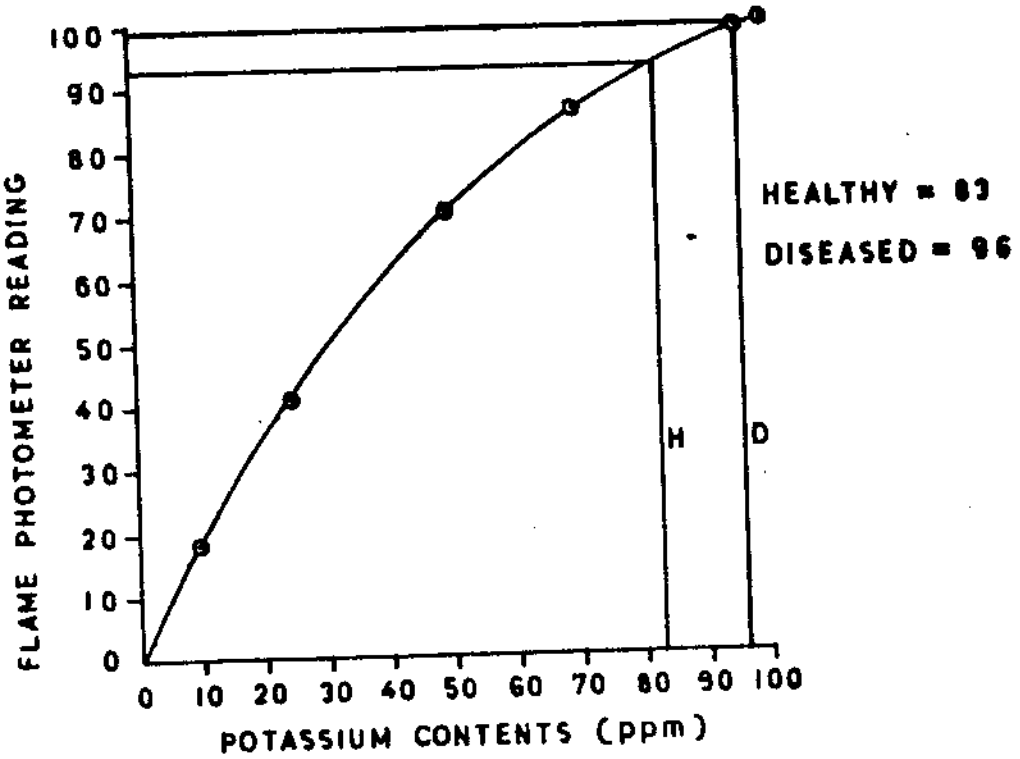
Contd...

1	2	3	4	5	6
Feb. 24-Mar. 2, 1982	11.59	24.33	35.71	64.86	1.0
March 3-9,1982	9.02	24.20	23.57	59.43	-
March 10-16, 1982	10.51	27.46	17.14	55.86	-
March 17-23, 1982	15.70	33.16	17.86	60.71	-
March 24-30, 1982	16.29	32.79	19.43	55.43	-
March 31-April 6,1982	18.24	35.23	20.17	44.71	-
April 7-13, 1982	16.63	35.01	10.14	29.00	-
April 14-20, 1982	19.21	36.21	17.29	44.00	0.7
April 21-27, 1982	20.70	34.11	27.29	62.00	11.7
April 28-May 4,1982	22.59	36.23	17.86	43.43	-
May 5-11, 1982	21.10	31.59	52.00	73.57	85.8
May 12-18, 1982	22.64	33.96	28.00	60.57	-
May 19-25, 1982	23.04	37.30	19.86	48.43	2.5
May 26-June 1,1982	25.96	37.61	29.43	54.57	-
June 2-8, 1982	26.98	39.28	13.86	35.86	-
June 9-15, 1982	26.61	36.09	37.14	63.00	-

APPENDIX - III

STANDARD CURVE FOR ESTIMATION OF SODIUM AND POTASSIUM BY FLAME PHOTOMETER

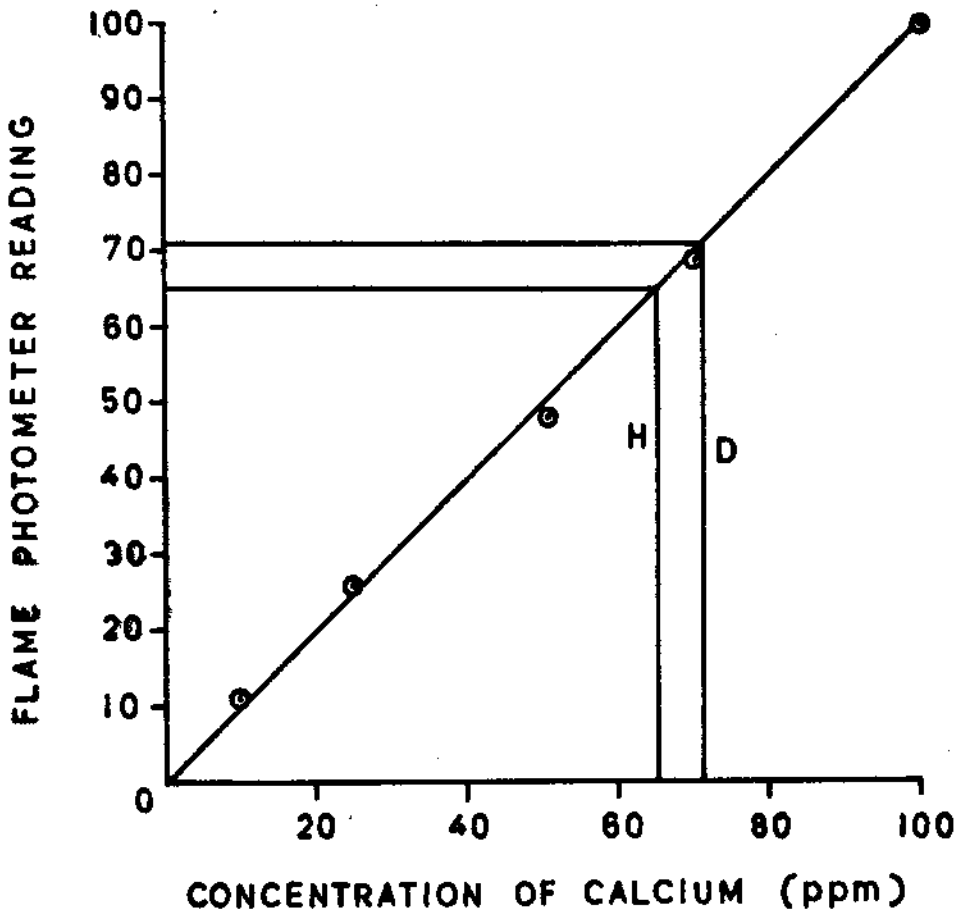




APPENDIX - IV

STANDARD CURVE FOR CALCIUM ESTIMATION BY FLAME PHOTOMETER

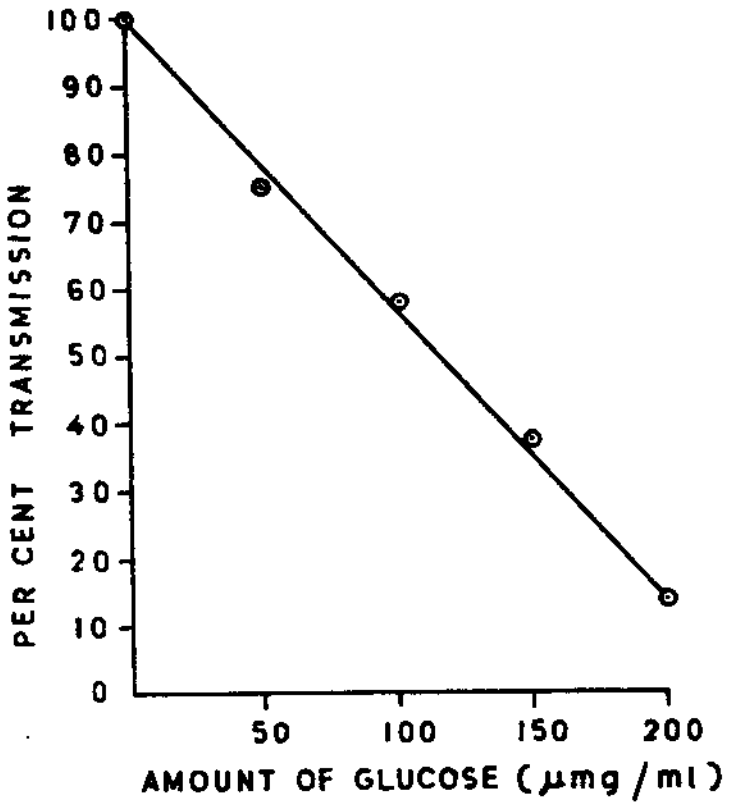
HEALTHY = 66
DISEASED = 72



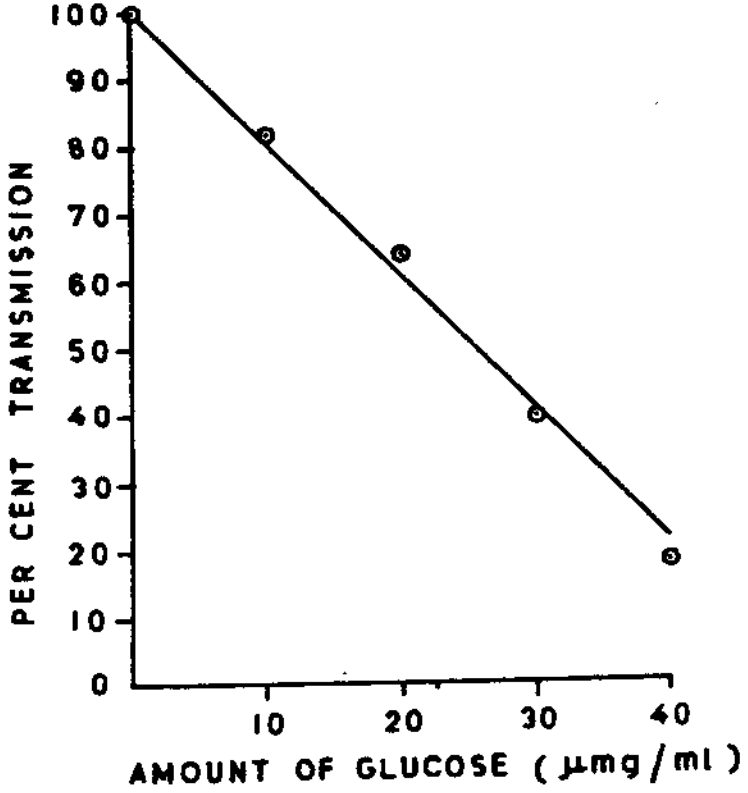
APPENDIX - V

STANDARD LINEAR GRAPH FOR ESTIMATION OF REDUCING AND TOTAL SUGARS

REDUCING SUGAR

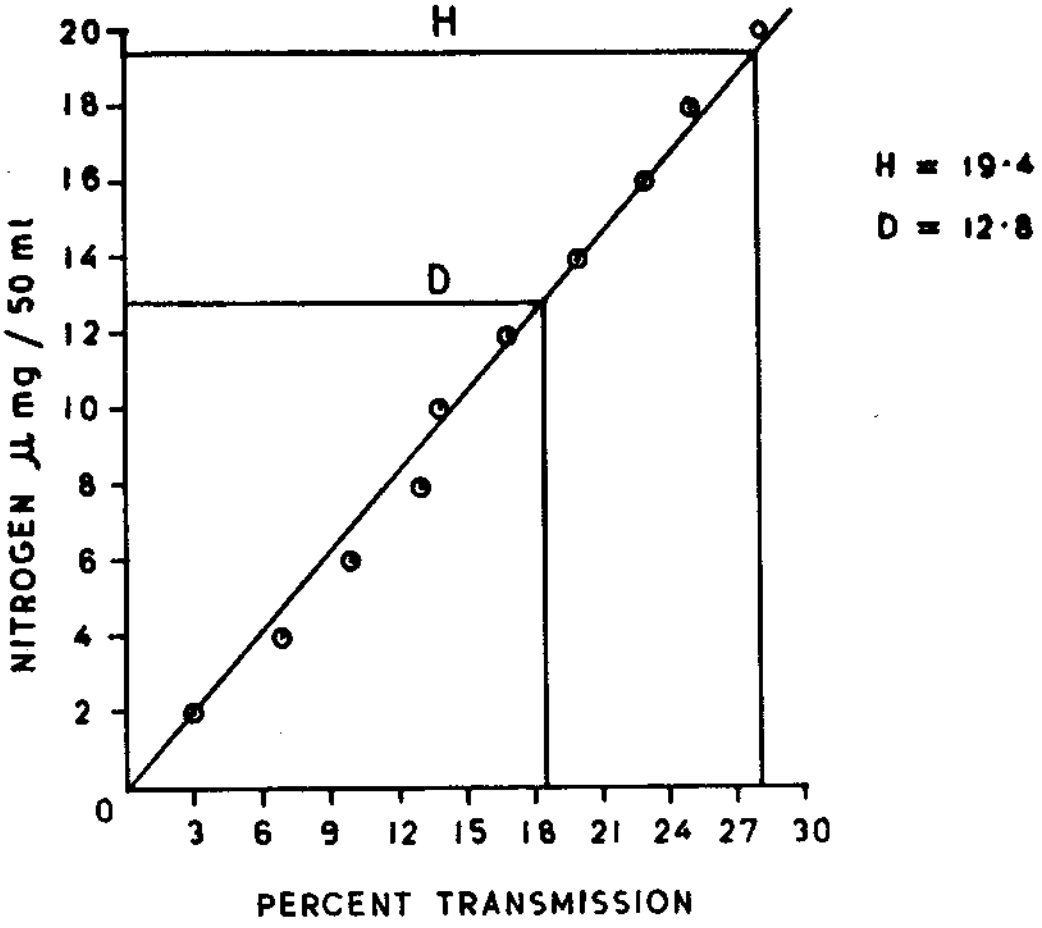


TOTAL SUGAR



APPENDIX - VI

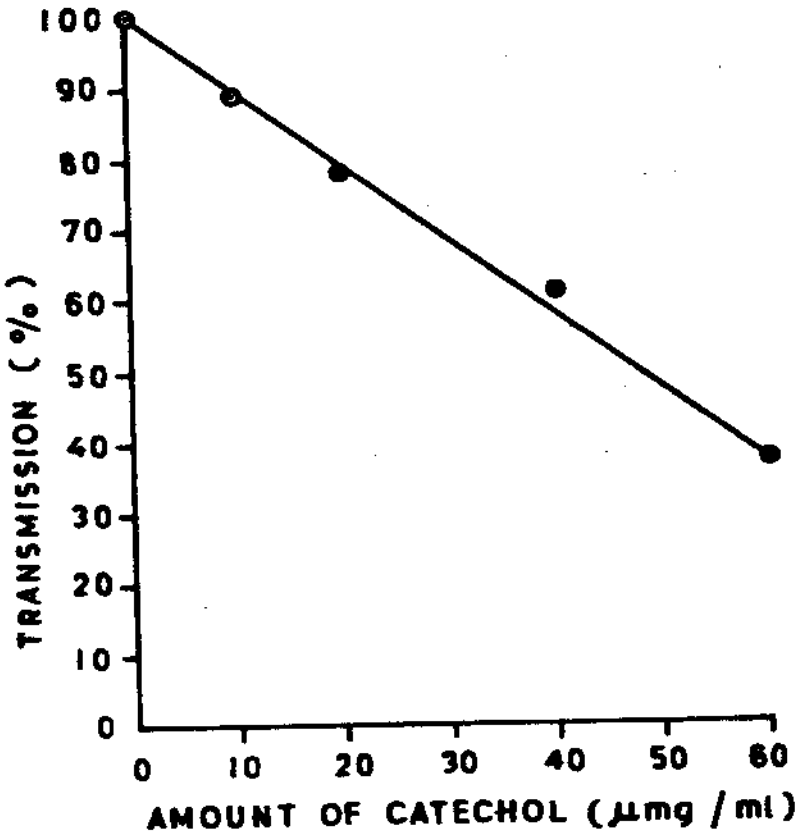
STANDARD LINEAR GRAPH FOR CONCENTRATION OF NITROGEN IN μ mg / 50 ml AND PER CENT TRANSMISSION



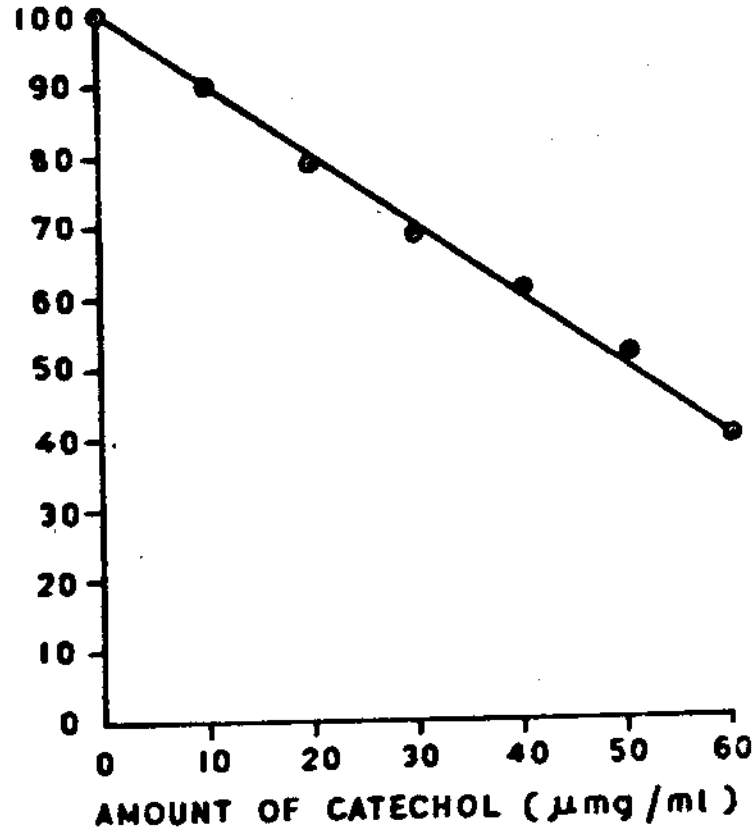
APPENDIX – VII

STANDARD LINEAR GRAPH FOR TRANSMISSION (%) AND CONCENTRATION OF CATECHOL (μ mg / ml) FOR PHENOLS AND ORTHODIHYDROXY PHENOLS

TOTAL PHENOLS



O-DIHYDROXY PHENOLS



APPENDIX - IX

STANDARD LINEAR GRAPH FOR TRANSMISSION (%) AND GLUTAMIC ACID CONCENTRATION FOR PROTEASE ESTIMATION