STUDIES ON BACTERIAL BLIGHT OF COWPEA CAUSED BY *Xanthomonas axonopodis* pv. *vignicola* (Burkh.) Vauterin *et al.*

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1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] (2n=22) commonly called as Lobia, is one of the most ancient human food sources and short duration multipurpose pulse crop grown extensively in tropical and subtropical countries. It belongs to family Fabaceae. The name cowpea originated from the fact that the plant was an important source of hay for cows in the south-eastern United States and in other parts of the world (Timko *et al.,* 2007). It is native to Africa, as wild cowpeas only exist in Africa and Madagascar (Steele 1976). It was introduced to the Indian sub-continent from Africa approximately 2000 to 3500 years ago.

It is generally more of robust annual herb reaching a height up to 80 cm with a strong taproot and many spreading lateral roots in the surface soil. Growth forms vary and include erect, trailing, climbing, or bushy, usually indeterminate growers under favourable conditions. Fruits are pods containing seeds that vary in size, shape, colour and texture. Pods may be held erect, crescent - shaped or coiled. They are usually yellow when ripe, but may also be brown or purple. The flowers are arranged in racemose or intermediate inflorescence at the distal ends of 5-60 cm long peduncles. Flowers are conspicuous, mostly self-pollinating, borne on short pedicels and the corollas may be white, dirty yellow, pink, pale blue or purple in colour. Flowers open in the early day and close at approximately mid day.

Cowpea forms an important component of farming system, being cultivated for seeds (shelled green or dried), pods or leaves that are consumed as green vegetable or for pasture, hay, silage and green manure. It fits well in a variety of cropping systems and is grown as a cover crop, mixed crop, catch crop or green manure crop in different parts of India like Punjab, Delhi and Haryana.

It is called as vegetable meat due to high amount of protein in grain with better biological value on dry weight basis. On dry weight basis, cowpea grain contains 23.4 per cent protein, 1.8 per cent fat and 60.3 per cent carbohydrates and it is rich source of calcium and iron (Gupta, 1978). It is usually the first crop harvested before the cereal crops are ready and therefore is referred to as "hungry-season crop". Apart from this, cowpea forms excellent forage, give a heavy vegetative growth and cover the ground so well that it checks the soil erosion. As a leguminous crop, it fixes about 70-240 kg per hectare of nitrogen per year and is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it.

It has been estimated that the total world pulse requirement for consumption is around 23 million tonnes and cowpea is grown in 10.73 million hectares with the productivity of 387 kg /ha and production of 3.84 million tonnes. In India, cowpea is grown in an area of about 1.5 million hectares with productivity of 567 kg per ha and production of 0.5 million tonnes. The productivity potential of the crop in Karnataka is 420 kg per ha (Anon, 2011). In Karnataka, cowpea varieties such as C-152 and TVX- 1839-9E are mainly grown for seed purpose while S-488, Pusa- Barsathi for vegetable purpose.

However, it has not been possible to achieve green revolution in pulses as in cereals because of its confinement to poor and marginal soils and due to its relatively high susceptibility to diseases and insect pests. The overall grain yields of cowpea in the present traditional systems is low (Singh *et al*., 1997) due to a complex of biotic and abiotic factors. The abiotic factors that cause yield reduction include poor soil fertility, drought, temperature extremes, excessive moisture, late maturity, acidity and stress due to intercropping with cereals. The biotic factors include insect pests, parasitic flowering plants, as well as viral, fungal, bacterial and nematode diseases.

Among these several constraints, losses due to pests and diseases are very high. Although, 25 to 30 per cent of total cost of production is being spent on plant protection especially pesticides, the biotic constraints could not be managed effectively. Among the diseases infecting cowpea, the bacterial disease popularly known as ‘bacterial blight’ caused by *Xanthomonas axonopodis* pv. *vignicola* (Burkholder,1944) Vauterin *et al*., 1995 formerly *X. campestris* pv. *vignicola* (Burkholder, 1944) Dye is a major production constraint.

The bacterial blight of cowpea was first reported by Burkholder (1944) from USA and in India it was first reported by Patel and Diwan (1950) from Pune and subsequently Chakravarti *et al.* (1972) and Gupta (1978) reported the disease from Rajasthan.

The first symptoms appear on cotyledons of seedlings emerging from infected seed and look reddish and wrinkled. First necrotic lesions are formed on leaves and later the stem is attacked. The pathogen reaches vascular bundles and the disease becomes systemic. The growing tip of the infected plant is killed and the plant ultimately dies. Cankers are often developed on the stem near the union of cotyledons and first leaves. Such stems are unable to bear the load of the plant and easily break in strong winds.

Secondary infection on leaves appeared as light yellow circular spots which are 4 to 10 mm in diameter and scattered on the lamina. The centre of these spots is necrotic and brown. The veins are red in color. On pods, deep green or water-soaked streaks are formed. Such pods become yellow, shrivel and die. The diseased pods produce smaller, wrinkled and infected seeds. These infected seeds serve as the source of primary inoculum. Secondary spread is by rains, insects and implements. The disease is serious in soils with poor drainage. It causes yield loss of 2.7 - 92.2 per cent depending on susceptibility of the variety (Kishun, 1989).

For the successful management of any disease under normal conditions, sanitation, eradiation of primary source and chemical protection at initial stages are some of the measures recommended. However, these measures are not enough, whenever the outbreak of disease occur. Hence, thorough understanding of the disease management strategies and concrete package is necessary to address the menace effectively, so as to save the crop at large.

Now, it is learnt that, bacterial blight of Cowpea is wide spread and is a major production constraint. The disease prevailed during *Kharif* season with varying degree of severity. Systematic survey for the incidence and severity of the disease in different growing regions is essential to design the appropriate management strategy. Hence, in the present investigation, it was intended to take up roving survey in major affected areas of northern Karnataka to generate the information on disease prevalence along with its intensity.

The seed borne inocula initiate epidemic of a number of bacterial plant diseases and play an important epidemiological role in bacterial diseases of leguminous crops. The primary damage due to the disease is through high mortality in the seedlings (Patel and Jindal, 1970). Shekhawat and Patel (1977) reported for the first time seed transmission of *Xanthomonas axonopodis* pv*. vignicola* (Burkh.) Vautrein *et al*., from India. They recorded as high as 62 per cent incidence in summer from the inoculum load of 1 per cent infested seed. The pathogen is seed-borne and secondary spread is by wind-driven rain, soil, insects and infected plant debris (Anon., 1981; Kaiser and Vakili, 1978). Since then few workers have attempted to develop suitable methods for the detection of the pathogen in the seeds.

Gitaitis and Nilakhe (1982) for the first time devised a method of detecting the bacterium in naturally infected seeds which considered of extracting the bacterium from seed coupled with plant bioassay method. Later several workers have succeeded in detecting the bacterium in the seed; their detection method suffers from a number of inherent and practical constraints.

Another major problem in the detection of bacterium from naturally infected seeds is competition from fast growing saprophytic bacteria which out number the pathogen and also it may be complicated by seed borne fungi that can confuse symptoms expression with bacterial infection. Although selective medium (SX agar) exist for *Xanthomonas campestris* pv. *campestris* and certain other pathovars. Complete media such as YDCA (Schaad and Stall, 1988) are used for detection of *X. axonopodis* pv. *vignicola*. No specific medium for the isolation of *X. axonopodis* pv. *vignicola* has been reported till date. Then Wydra *et al.* (2004) developed a semi-selective medium for the isolation and enumeration of *Xanthomonas axonopodis* pv *.vignicola* from cowpea plant and soil samples. So there is a need to evaluate different selective and semi- selective media for the detection of seed borne *Xanthomonas axonopodis* pv*. vignicola* pathogen.

Watkins (1943) reported 60 per cent mortality in California Black Eye variety. Patel *et al*. (1972) reported 15 to 20 per cent seedling mortality in the certified seed production area. Losses ranging between 2.66 to 92.24 per cent at various stages of infection have been observed by Kishun (1989) in various cowpea cultivars.

The use of resistant cowpea varieties remains the most practical long-term method for controlling cowpea bacterial blight because the method does not have a negative impact on the environment and economics of adoption is also minimal. The identification and evaluation of cowpea varieties for resistance have relied mainly on disease incidence and symptoms severity in the fields and greenhouse. Therefore there is a need to screen different varieties of cowpea to identify resistant lines.

Even though information available on chemical management of bacterial blight of cowpea, but there are large numbers of chemicals available in the market as bactericides and many fungicides also reported to inhibit bacterial diseases hence, their bioefficacy and suitability needs are to be verified by *in vitro* and field studies, so as to incorporate the effective ones in the management package.

In recent years, there has been a major thrust on residue free organic cowpea production. Taking the task into consideration, efficient botanicals and bioagents need to be explored to fit into the management schedule. Research on this aspect needs to be triggered to find out actual integrated management strategies in bacterial blight suppression. Keeping all these aspects in view, the investigation was undertaken with the following objectives.

1. Survey for the bacterial blight disease of cowpea.
2. To detect the pathogen in seeds by non serological methods.
3. To screen the varieties/germplasm lines against bacterial blight of cowpea.
4. To test the bioefficacy of chemicals and antibiotics against bacterial blight of cowpea.
5. To develop an integrated management for bacterial blight of cowpea through hot water treatment, bactericides, botanicals and bioagents.

2. REVIEW OF LITERATURE

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the important pulse crop grown for vegetable, seed and fodder purposes. Among the diseases, bacterial blight induced by *Xanthomonas axonopodis* pv. *vignicola* (Burkh.) Vauterin *et al.,* is the most widespread disease of cowpea reported from all regions of the world (Emechebe and Florini, 1997).

The literature pertaining to various aspects on bacterial blight of cowpea disease are reviewed and presented in this chapter. Present knowledge on the aspects such as disease severity, pathogen involved, pathogenicity studies, detection of pathogen in seeds, screening of varieties for resistance, *in vitro* evaluation of antibacterial chemicals, botanicals and bioagents and the integrated management of the disease with special reference to *Xanthomonas axonopodis* pv. *vignicola* (Burkh.) Vauterin *et al.* (1995) has been summarized in this chapter.

2.1 Survey

This disease was observed around 1919 as a destructive disease of cowpea in Indiana province of U.S.A. (Gardner and Kendrick, 1923). They reported the symptoms of the disease on leaves, stems and pods. Watkins (1943) reported 60 per cent mortality in California Black Eye variety of cowpea infected with bacterial blight disease. Similar organism was reported from Kansas province as *Phytomonas vignae* and it was found to be completely destroying the seedlings of certain varieties of cowpea (Anon., 1944).

Burkholder (1944) from United States reported the bacterial blight of cowpea and named pathogen as *Xanthomonas vignicola* causing stem canker of 'Chinese Red' cowpeas in Texas, which proved extremely virulent in inoculation experiment on Red kidney beans (*Phaseolus vulgaris*). Later researchers have made isolations from specimens of 'Blackeye' cowpea sent from Texas and found both yellow and white bacteria and concluded that only the yellow organism proved to be pathogenic.

Hoffmaster (1944) described the causal agent of the disease as *Bacterium vignae* and described the symptoms as sternum of diseased plants bearing prominent swollen cracked canker.

Preston (1948) reported the bacterial canker of cowpea caused by *Xanthomonas vignicola* from Oklahoma and reported that the pathogen was carried within the seed which spreads in the field by moisture propelled wind.

Patel and Diwan (1950) were first to report the occurrence of *Xanthomonas axonopodis* pv.*vignicola* from India and described the symptoms of disease on cowpea. The organism was found to infect all parts above ground but was mostly confined to leaves and pods. Light yellow, irregular to round spots, measuring 4 to 10 mm in diameter with necrotic brown centers appeared initially and increased irregularly until the whole leaf was covered. Severely affected leaves became straw coloured and dropped at the slightest touch.

Later Rangaswami and Gowda (1963) also confirmed its occurrence from Tamil Nadu. Patel and Jindal (1970) reported this disease from Delhi, Punjab, Haryana, Uttar Pradesh, Madhya Pradesh and Maharashtra.

Patel *et al.* (1972) reported 15 to 20 per cent seedling mortality in the certified seed production area infected by bacterial blight in cowpea and also described the symptoms in detail on the seedling, leaves and pods. Shukla (1978) reported that leaf spot of cowpea was caused by *Xanthomonas vignicola.*

The bacterial blight of cowpea was also reported from Puerto Rico (Kaiser and Vakili, 1978) and from East Africa (Kaiser and Ramos, 1979).

Kishun (1989) observed losses ranging 2.66 to 92.24 per cent at various stages of infection in different cowpea cultivars like Pusa Barsati, Pusa Phalguni, Pusa Dofasli, Banglore local and 7552.

Okechukwu *et al.* (2000) conducted field trails in the Sudan savanna site of IITA Experimental Station, Minjibir, Kano State, Nigeria to assess the effect of bacterial blight on pod, grain and fodder yield of two cowpea varieties artificially inoculated with *Xanthomonas campestris* pv. *vignicola.* One hundred per cent disease incidence was recorded in inoculated plants both in 1996 and 1997. High disease severity scores of 59 and 56 were recorded in inoculated IT82D-889 and 67 and 63 for inoculated IAR-48, in 1996 and 1997 respectively.

Shoaga *et al.* (2001) studied the virulence pattern of Ibadan, Ikenne and Kano isolates of bacterial blight pathogen and their effect on yield components on cowpea cultivars Ife Brown, TVx 3236 and 86D-721 through artificial inoculation in the field. The isolates differed significantly in their virulence in each cultivar causing 6.33 to 33.8 per cent disease incidence. Yield depressions of 62.4, 25.8 and 36.5 per cent in Ife brown; 64.9, 13.1 and 29.4 per cent in TVx 3236; and 62.3, 5.4 and 38.1 per cent in 86D-721 were associated with infections by Ibadan, Kano and Ikenne isolates respectively. Overall, Ibadan isolate was more virulent than other two isolates.

Moretti *et al.* (2007) reported the occurrence of leaf spot on cowpea (*Vigna unguiculata*) caused by *Xanthomonas axonopodis* pv. *vignicola* in Mozambique for the first time on June 2004 as angular, necrotic, pustuliform leaf spots, surrounded by a thin water-soaked hallow on cowpea plants cultivated in the Umbeluzi area (Maputo, Mozambique) with an incidence of 70 to 90 per cent.

2.2 Detection of pathogen in the seed

Wilson (1938) soaked bean seeds in a selective medium to extract *Pseudomonas phaseolicola* and then inoculated bean pods to identify the pathogen. Katznelson and Sutton (1951) used a species specific bacteriophage to detect *Pseudomonas phaseolicola* in macerated seeds.

Kennedy (1969) confirmed the seed borne nature of *Pseudomonas syringae* pv*. glycines* by seedling test by treating seeds with a suspension of crushed infected seeds of soybean. Direct plating of surface sterilized seeds on agar media, helped in the detection of internal seed borne nature of *Pseudomonas syringae* pv*. glycines* in soybean seeds (Nicholson and Sinclair, 1971).

Taylor (1970) developed a routine dilution plate method for the detection of *Pesudomonas phaseolicola* from seed extracts of beans. This method could detect low levels of seed-borne infection.

Parashar and Leben (1972) developed a modified growing on test method by wounding of cotyledons of germinated soybean seeds and allowing them to emerge into water saturated air for successful detection of *Pseudomonas syringae* pv*. glycines* in soybean seed lots.

Schaad and White (1974) developed SX medium for the isolation of *Xanthomonas campestris* pv*. campestris* from soil. Guo Yifen and Liang Zaiquan (1981) developed a selective nigrosine medium for isolation and detection of *Erwinia stewartii* in maize seeds.

Shekhawat and Patel (1977) for the first time reported the seed borne nature of *Xanthomonas axonopodis* pv. *vignicola*. They reported that the blight pathogen causes seedling mortality or bushy and stunted plants as a result of severe stem cankers, secondary spread, however caused leaf blight only.

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

Gupta and Chakravarti (1982) from India demonstrated the transmission of *Xanthomonas axonopodis* pv*. vignicola* (*Xav*) through the seeds by raising the plants from artificially inoculated and uninoculated cowpea seeds under green house conditions.

Thus they stated that bacterium is carried externally as well as internally along with the seed which might serve as a primary source of inoculum. But they failed to detect the presence of *Xav* in cowpea by direct plating surface sterilized and unsterilized seeds on nutrient agar and yeast glucose chalk agar medium till germination.

Gitaitis and Nilakhe (1982) devised a method for detecting *Xanthomonas axonopodis* pv.*vignicola* in cowpea seeds. They infused samples of 100 seeds with 100 ml sterile water by the sudden release of 680 mm mercury vacuum. The seeds were agitated at 150 rpm for 2 hours on an orbital shaker and the suspension was centrifuged at 4000g for 30 min. The pellets were resuspended in 5 ml of supernatant and the mixture was infiltrated with a syringe and a needle into healthy cowpea leaves. The bacterial cells were recovered from lesions that developed in 5 to 20 days and identified as *Xanthomonas axonopodis* pv. *vignicola*.

Thind and Soni (1983) carried out studies on the seed borne nature of *Xanthomonas axonopodis* pv. *vignicola* and reported that both external infestation and internal infection play an important role in the disease development. They stated that a minimum of 10^3 cfu /ml bacterial concentration to be there in the inoculum used for soaking the seeds to get successful transmission.

Thind and Soni (1983) reported that *Xanthomonas campestris* pv. *vignicola* and *Xanthomonas campestris* pv. *vignaradiatae* were recovered by streaking the seed suspension on Potato sucrose peptone rifampin aureofungin agar.

Van vuurde *et al.* (1983) extracted *Pseudomonas syringae* pv. *phaseolicola* from bean seeds by soaking the seeds in water at 5^0 C for 24 hours and isolated by dilution plating onto King's B medium.

Two semi-selective culture media Nutrient Starch Cycloheximide Antibiotic Agar (NSCAA) and Basal Starch Cycloheximide Antibiotic Agar (BSCAA) were developed by Randhawa and Schaad (1982) which were compared with NA a non-selective media for the detection of *Xanthomonas campestris* pv. *campestris* in crucifer seeds. Similarly XTS medium for the isolation of *Xanthomonas campestris* pv. *translucens* from wheat seeds was developed by Schaad and Forster (1985).

Kaun *et al.* (1985) developed a method of detection of *Xanthomonas campestris* pv. *carotae* in carrot seeds by extracting the pathogen at a low temperature (5^0 C) stationary aqueous soak of carrot seed in darkness for 18 hours followed by a vigorous shaking of the seed in water containing Tween-20 and concentrating the bacterial cells by centrifugation and finally plating of dilution series of these cells onto a modified Kado and Heskett's D5 medium.

Srivastava and Singh (1986) compared different methods for the detection of *Xanthomonas campestris* pv. *glycines* in soybean seed and got best results with agar Plate method using water agar.

Soni and Thind (1987) detected *Xanthomonas axonopodis* pv. *vignicola* in the seeds by traditional growing-on test and they reported that the incubation growing-on test was more effective than traditional growing on test for the detection of the bacterium.

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

Anitha *et al.* (1992) reported that, of the four seed health testing techniques *viz*., seed soaking, seed maceration, blotter test and growing-on test used for the detection of *Xanthomonas axonopodis* pv. *vignicola* (Burkh.) Dye from naturally infected seeds of cowpea, seed soaking test was found to be superior to other methods.

Shobha (1998) reported that, the recovery of bacterial colonies of *Xanthomonas axonopodis* pv. *glycines* on NSCAA, the semi-selective media were more (112×10^5 cfu/ml) as compared to nutrient agar (55×10^5 cfu/ml). But, on SX and XTS media, the bacterium produced bigger colony as compared to NSCAA medium.

Wydra *et al.* (2004) developed a semi-selective diagnostic medium, Cefazoline-cellobiose-methionine (CCM) for the isolation and enumeration of *Xanthomonas campestris* pv. *vignicola* from cowpea (*Vigna unguiculata*) plant and soil samples.

Okechukwu *et al.* (2010) made an attempt to ascertain the importance of seed as a primary source of inoculum for bacterial blight disease in cowpea. The study was carried out using seeds of five cowpea varieties (TVx 12349, IT86D-721, IT82D-889, Ife Brown and TVx 3236) artificially inoculated with three bacterial isolates (Ikenne, Kano and Ibadan) and seeds harvested from infected plants. Results showed that seed to plant transmission caused 6 to 24% post emergence seedling mortality and 26 – 49 per cent incidence of blight in plants raised from infected seeds.

2.3 Screening of varieties

Hoffmaster (1944) reported that cultivars like Buff, Victor, Potomac or Calico, Speckled and Crowder showed resistance to bacterial canker of cowpea, whereas cultivar Whippoor-will was almost totally destroyed.

Preston (1949) recommended the use of resistant varieties of cowpea, such as Brabham, New Era, Groit, Iron and Victor to bacterial blight of cowpea caused by *Xanthomonas axonopodis* pv.*vignicola* in USA.

Sherwin and Lefebvre (1951) compared different inoculation methods and recommended stem-stab method for testing large populations. Using this technique they screened 351 genotypes of cowpea and beans through inoculating artificially with *Xanthomonas axonopodis* pv. *vignicola* and observed that the cowpea varieties like Brabham, Buff, Iron, Six weeks-Ala, Suwanne and Victor to be highly resistant whereas Brown crowder and New Era were moderately resistant.

Patel and Jindal (1970) screened 1085 cowpea lines of world germplasm collection for bacterial blight resistance and found 59 lines free of the disease. When 421 of these lines including 59 found free in the field were tested by artificial inoculations with the pathogen, 28 lines were resistant and 15 lines were tolerant to the disease.

Patel *et al.* (1972) reported that all the vegetable types like Pusa Barsathi, Pusa Phalguni, Pusa Dofasli and EC 42712 and fodder type, Chinegra were susceptible. Among the grain varieties, Meshed, Early Ramzhorn, RS-9, Black eye-7 and EC 21622 were susceptible whereas K-11, T-2, T-5286-3 contained some tolerant reaction against cowpea bacterial blight.

Singh *et al.* (1975) described Vita-3 variety of cowpea as immune to *Xanthomonas* spp., *Colletotrichum lindemuthianum*, *Cercospora canescens, C. cruenta* and several isolates of cowpea mosaic virus (CPMV). Preliminary results indicated that it was also resistant to two isolates of CPMV in Brazil, to *X. vignicola* in Puerto Rico and to *Meloidogyne incognita* in Nigeria.

Sreekantaradhya *et al.* (1977) found S-488 derived from Virginia × Iron grey, tolerant to *X. vignicola* in the field. Raj and Patel (1977) reported that of 315 cowpea collections tested in the glass house against *X. vignicola*, cowpea mosaic virus and *Cercospora cruenta*, 44 were resistant to more than one disease.

Tyagi *et al.* (1979) stated that of the 105 crosses and varieties of cowpea, only four were resistant to *Xanthomonas axonopodis* pv.*vignicola* in a pot test. Kishun *et al.* (1980) found TVU 580, 526, 4540 to be highly resistant out of 180 entries of cowpea screened against *Xanthomonas axonopodis* pv. *vignicola.*

Allen *et al.* (1981) evaluated the various screening methods for the identification of resistance to *Xanthomonas axonopodis* pv. *vignicola.* They stated that stem injection and leaf infilteration methods were reliable in the glass house, while foliar spray inoculation was appropriate for both glass house and field experiments and demonstrated hypersensitive resistance in TVU 410 by leaf infilteration method whereas VITA 3 was shown to possess field resistance, expressed as reduced and delayed disease development.

Prakash and Shivashankar (1982) screened 221 genotypes of cowpea for bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* reaction using 0–5 scale. Sixteen genotypes were found to be highly resistant and PLS65/1 was the most resistant line followed by C-20, C-2595, MS-9671, Vita-3, Tvx-201, Tvx-944-02E, P-G 779, MS-9363, Tvx-7-54, V-11, PLS-35, C-2085, MS-9032/2 and Tvx-1836-150G.

Various plant characteristics like seedling vigour, plant height, plant habit, number of days to 50 per cent maturity were found not to be associated with disease severity of genotypes.

Patel (1983) screened 190 cowpea accessions in Tanzania, by stem-stab inoculation with the blight organism, *Xanthomonas axonopodis* pv. *vignicola,* 21 accessions reacted as resistant and 20 as moderately resistant. Comparisons of the reactions in five screening tests carried out in Tanzania, India, Nigeria and Puerto Rico showed differences in the pathogenic behaviour among the cultures of the blight organism prevalent in these countries. Cowpea accessions which could be used as differentials for identification of pathogenic races and monitoring the stability of the varietal resistance were also suggested.

Patel and Singh (1984) reported that bacterial blight of cowpea resistant line No. 1552 consistently performed better than No. 779 or No. 868, when tested at four widely separated locations, indicating that it is adapted to varied agro-climatic conditions.

Gitaitis *et al.* (1986) evaluated four major varieties of cowpea in Georgia where analysis of disease progress curves showed that cultivars like Pinkeye Purple Hull and White Acre were moderately resistant but Mississippi Silver and Coronet were highly susceptible to *Xanthomonas axonopodis* pv. *vignicola*.

Dhiman *et al.* (1989) evaluated 6 cowpea cultivars, Pusa Dofasli, Pusa Barsati, L-1552, Brown Seeded and Red Seeded in field at Ludhiana, India, during the spring and rainy seasons of 1986-1987 for their multiple disease resistance to bacterial blight, cowpea mosaic virus and anthracnose. The mean multiple resistance score for the 2 seasons indicated that Sel-263 ranked as best, followed by Pusa Dofasli, Red Seeded, Pusa Barsati, L-1552 and Brown Seeded cultivars.

Khan (1989) screened twenty cowpea varieties in two locations in Karnataka in which V-105, V-126, V-46, APC-36, Co-4 were found highly resistant and C-152, HG-171, PC-48, Cowpea-1, 2, Pusa barsati showed highly susceptible reaction against bacterial blight pathogen *Xanthomonas axonopodis* pv. *vignicola.*

Shah *et al.* (1990) evaluated 105 lines of cowpea against bacterial blight under field conditions in Rajasthan during *Kharif* and reported that Copusa-1, K-317, S-488, H-2 and EC-1956 having resistant reaction and nine other lines to be moderately resistant. They grouped the remaining lines as moderately susceptible (18), susceptible (37) and highly susceptible (33). They also reported maximum infection index of 82 and 88 per cent in Udaipur local followed by JC-12, RC-215, JC-5 and CO-2.

Amusa and Okechukwu (1998) stated that nine out of 45 cowpea breeding lines evaluated have confirmed resistance to blight. Sixteen breeding lines were found to be resistant to canker induction while twelve were resistant to both blight and canker.

Bua *et al.* (1998) evaluated twenty six cowpea lines for resistance to *Xanthomonas axonopodis* pv. *vignicola* and reported that during the first rains, the highest disease incidence, 55.0 and 54.5 per cent, were recorded in the lines IT82E-12 and SLA 59, respectively. During the second rains, the highest disease incidence (65.9%) was recorded in the line Brown.

Gomez *et al.* (1998) evaluated 40 advanced (F9) lines for bacterial leaf blight disease resistance. The results revealed that experimental lines A-4, M-28-6-6 and TC-9-6 were moderately resistant, while TVx-3871 was resistant.

Sushma-Nema *et al.* (2000) observed that cowpea bacterial blight disease was severe during rainy season. Fifty one cultivars/ lines were screened for their reaction to *Xanthomonas axonopodis* pv. *vignicola* at Jabalpur during 1996-97. None of the entries were resistant to the disease and only IFC-9502 was moderately resistant.

Okechukwu and Ekpo (2004) rescreened 96 varieties evaluated earlier in breeder's field as resistant to *Xanthomonas axonopodis* pv. *vignicola* in field and green house under high inoculum pressure. Results from the field screening showed that there were 69 susceptible, 25 moderately susceptible and 2 resistant varieties. In artificial inoculation in the green house IT81D-1228-14, IT82E-16, IT93K-2271-2-2, TVu 1235 and TVu 4360, which were moderately susceptible in the field showed a susceptible reaction.

TVu 12349 and TVu 15549 gave consistent reactions in the field and in the greenhouse and are therefore good sources of stable resistance to blight pathogen.

Sikirou and Wydra (2004) tested forty-six cowpea genotypes including improved and local genotypes to identify resistance to *Xanthomonas axonopodis* pv. *vignicola* using spray inoculation in the field and leaf infiltration in the glasshouse. Results indicated significant differences among genotypes in disease severity. Under field conditions, 27 of 46 genotypes were selected as resistant. However, under glasshouse conditions, when genotypes were submitted to leaf inoculation, the number of resistant genotypes was reduced from 27 to 22.

Lesly (2005) screened 169 genotypes to bacterial blight during *Kharif* 2004 in Dharwad with 56, 27, 47, 22 and 17 resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible reaction respectively. Resistant genotypes such as IC202823, IC257420 and IC97767 showed high seed yield, harvest index and number of pods per plant.

Ajeigbe *et al.* (2008) screened twenty five cowpea lines and two local varities in Nigeria under field conditions for resistance to Bacterial blight. About 44 per cent of the varieties tested were resistant to bacterial blight while 20 per cent showed moderate resistance and others were susceptible. IT98K-506-1, IT97K-1113-7, IT97K- 1069-6, IT97K-1092-2, IT97K-1069-5, IT98K-131-2 and IT97K-568-18 produced higher grain and fodder yields than the other varieties and showed combined resistance to the diseases like bacterial blight, septoria leaf spot and scab.

2.4 Bioefficacy of chemicals / antibiotics, botanicals and bioagents against bacterial blight of cowpea

2.4.1 Chemicals

Thirumalachar *et al.* (1956) reported that chlroamphenicol, tetramycin, aureomycin and Streptomycin sulphate inhibited *Xanthomonas axonopodis* pv.*vignicola* under *in vitro* conditions in the order of efficiency.

Chakravarti and Rangarajan (1966) studied *in vitro* effect of streptocycline on seven species of *Xanthomonas* and six species of *Erwinia* and one each of *Pseudomonas* and *Agrobacerium.*

Padmanabhan and Jain (1966) pointed out that application of bleaching powder (2 kg per ha) to the standing paddy crop, when the crop is 74 days old, controlled the bacterial leaf blight effectively and the efficacy is on par with five sprays of streptocycline + copper oxychloride.

Valkhov *et al.* (1974) reported that bean seeds treated with Streptomycin solution at 5 ml for one hour were the most effective against *Xanthomonas phaseoli* causing bacterial blight of beans.

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

Sharma *et al.* (1981) studied *in vitro* evaluation of eight chemicals against *X. vesicatoria* by paper disc and turbidometric methods and described the effectiveness of combination of Streptocycline and copper sulphate in inhibiting the growth of pathogenic organism.

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

Marinescu (1982) found that, infection of *Phaseolus vulgaris* seeds by *Xanthomonas campestris* pv. *phaseoli* was reduced by Kocide 50 at 0.25 per cent and Kocide-101 at 0.5 per cent.

Bordeaux mixture (5:5:50) in combination with either streptocycline (500 ppm) or plantomycin (500 ppm) was very effective in reducing the citrus canker caused by *Xanthomonas citri.* Spraying of Bordeaux mixture alone also recorded the least canker incidence in comparison with Bavistin @ 0.1 per cent (Krishna and Nema, 1983).

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

Tsvetkov and Donev (1984) stated that, treatment of *Phaseolus vulgaris* plants at the beginning of anthesis with streptomycin reduced infection by *Xanthomonas campestris* pv. *phaseoli* and increased the yield by 14.4 per cent.

Thapliyal and Dubey (1986) reported that combination of an antibiotic with a copper fungicide *i.e.* agrimycin-100 + blitox-50 (copper oxychloride) gave improved control of *Xanthomonas sojense* on soybean and resulted in higher yields.

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

Krishnan and Madhumeeta (1988) observed that plantomycin, streptocycline and paushamycin each at 500 ppm concentration were equally effective in inhibiting the growth of *Xanthomonas campestris* pv. *dieffenbachiae* causing bacterial blight of anthurium.

Jindal and Thind (1990) evaluated Agrimycin-100 (600 μg/ml), Streptocycline (100 μg/ml), Bavistin [carbendazim] (500 μg/ml), Blitox-50 W.P. [copper oxychloride] (2000 μg/ml), Dithane M-45 [mancozeb] (2000 μg/ml) and 9 combinations of the last 4 chemicals as foliar sprays during Monsoon 1982 for the control of bacterial blight of cowpea caused by *Xanthomonas campestris* pv. *vignicola.* All the treatments significantly controlled the disease but the 2 most effective treatments were: (1) 3 sprays of Streptocycline (100 μg/ml) + Bavistin (500 μg/ml) and (2) 2 sprays of Streptocycline (100 μg/ml) + Bavistin (500 μg/ml) followed by a third spray of Streptocycline (100 μg/ml) + Blitox-50 W.P. (2000 μg/ml). Four seed treatments; hot water (50^0C for 30 min), solar heat, Streptocycline (100 μg/ml) + Agallol (2000 μg/ml) and Streptocycline (100 μg/ml) + captan (2000 μg/ml)), the 2 most effective spraying schedules mentioned above and 8 combinations of seed treatments and spraying schedules were evaluated during Monsoon 1983 and 1984. All the combinations provided significantly better disease control and also gave a higher seed yield.

Thammaiah (1991) reported that seed treatment with sodium hypochlorite for 120 mins was very effective for control of *Xanthomonas axonopodis* pv. *vignicola* in cowpea seeds.

Thrimurthi and Agarwal (1992) stated that spraying a combination of Streptocycline and copper oxychloride was effective against bacterial pustule of soybean.

Ravikumar and Khan (1995) reported that the seed treatment with Streptomycin sulphate or Stretopcycline for 120 min at 300, 400 and 500 ppm eliminated the *Xanthomonas campestris* pv. *vesicatoria* from tomato seeds.

Thammaiah and Khan (1995) found that chemical seed treatment with copper oxychloride for 15 – 60 min completely suppressed seed-transmitted bacterial blight of cowpea.

Borah *et al.* (2000) studied the interaction of three phylloplane bacteria of mungbean (Plb-1, Plb-2 and Plb-3) having potential to antagonize bacterial leaf spot pathogen *Xanthomonas axonopodis* pv. *vignaradiatae* and eight antibiotics and fungicides commonly used to manage plant bacterial diseases. In *in vitro* tests, streptomycin and streptocycline inhibited the growth of all three isolates of phylloplane bacteria of mungbean at a very low concentration of 10 μg ml^{-1}. While agrimycin-100, plantomycin and blitox could inhibit the Plb at 200 μg ml^{-1}.

The results obtained by Manjula (2002) on the *in vitro* efficacy of bactericides against *Xanthomonas axonopodis* pv. *punicae* indicated that Paushamycin (500 ppm) and K cycline (500 ppm) produced the highest inhibition zone followed by bacterinol and bacteriomycin. Copper oxychloride at 2000 ppm was found moderately effective and Kasugamycin at 500 ppm concentration was ineffective.

Yenjerappa *et al.* (2004) noticed the superior efficacy of streptocycline (0.05%) in combination with copper oxychloride (2000 ppm) in checking the bacterial blight menace of pomegranate.

The bacterial leaf spot incidence on grape vine was least in the plots sprayed with streptocycline (0.05%) or streptomycin sulphate (0.05%) by Ravikumar *et al.* (2002).

Pruning of infected parts along with one spray of copper oxychloride followed by four sprays of streptocycline (100 ppm) + Copper oxychloride (0.3%) found very much promising in reducing the incidence of bacterial canker of acid lime (Gopal *et al*., 2004).

Alabi and Emechebe (2004) evaluated the effect of seed treatment fungicides like metalaxyl + mancozeb (RidomilMz), metalaxyl + carboxin + furathiocarb (Apron plus), metalaxyl + copper-1-oxide (Ridomil plus), benomyl (Benlate), maneb + thiophanate-methyl (Labilite) and zinc + maneb (Mycotrin) on seed germination, incidence and severity of seedling bacterial blight in cowpea induced by *Xanthomonas campestris* pv *vignicola* in northern Nigeria under field conditions, Zinc + maneb gave the best control and was closely followed by metalaxyl + carboxin + furathiocarb.

The results on *in vitro* evaluation of chemicals carried by Shukla and Gupta (2004) indicated that, copper oxychloride (1000 ppm) in combination with mancozeb (1000 ppm) was very effective in inhibiting the growth of *Xanthomonas vesicatoria*. Bordeaux mixture (1%) was found next best effective and its efficacy was on par with streptomycin sulphate (100 ppm) + mancozeb (1000 ppm).

Ravikumar and Yenjerappa (2005) investigated that, five sprays of bacterinashak (500 ppm) in combination with copper oxychloride (2000 ppm) was significantly effective in reducing the bacterial blight of pomegranate. Highest yield and maximum benefit cost ratio was recorded with the same treatment.

Dhanya and Mary (2006) evaluated Captan (0.3%) for controlling bacterial blight of anthurium caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* which gave 97 per cent control after five sprays at weekly interval as against turmeric powder and streptocycline which gave 100 per cent control.

Astha Singh *et al.* (2007) tested the efficacy of 12 fungicides and 2 antiseptics by disc plate method against the bacterium, *Xanthomonas axonopodis* pv. *malvecearum* causing cotton bacterial leaf blight in which fungicide, Captan at the concentration of 0.02 per cent was found effective similar to streptocycline.

Kotchoni *et al.* (2007) studied the antibacterial effect of hydrogen peroxide (H_2O_2) and N-heterocyclic pyridinium chlorochromate (PCC) on *Xanthomonas campestris* pv. *vignicola*, the causal agent of bacterial blight of cowpea.

Disease severity in cowpea was analysed after seed and seedling treatment with the chemicals at different stages of growth. Pre-treatment of cowpea seeds and seedlings with 1 mM H_2O_2 or PCC led to a reduced disease severity compared to untreated controls. A combination of H_2O_2 and PCC treatment conferred a better suppression of the disease in greenhouse trials than when H_2O_2 or PCC were used individually.

Vinayak (2011) reported that among the various chemicals tested, Streptocycline 1000 ppm + Copper oxychloride 2000 ppm produced the highest inhibition against *Xanthomonas campestris* pv. *campestris* causing black rot of cabbage followed by Streptocycline alone at 1000 ppm whereas Plant guard was least effective.

2.4.2 Botanicals

Sivasankararao and Nigam (1978) tested the effect of essential oil from the leaves of *Cinnamon zeylanicum* (Breyn), *Cymbopogon flourosus* (Staph), *Eucalyptus citrodora* (Hock) *Mentha* sp. on *Bacillus fumilis*, *Erwinia caratovora*, *Microccocus* sp. *Pseudomonas solanacearum* and *Xanthomonas campestris* ,*Cinnamon zeylanicum* oil showed the highest activity in controlling these bacterial pathogens.

Prasad and Alankararao (1987) evaluated the antimicrobial effects of essential oils of five species of *Ocimum*. All the samples showed antibacterial activity against gram positive and gram negative bacteria.

Hannudin and Djantnika (1989) studied the effect of some plant extracts on the growth of *Pseudomonas solanacearum* under *in vitro* conditions. They evaluated extracts from onion and garlic bulbs, roots and stems of *Crotalaria* sp. and *Tagetus* sp. for suppression of *P. solanacearum* and observed that extracts from garlic bulbs inhibited bacterial growth.

Thammaiah *et al.* (1995) reported that extract of *Adathoda zeylenica* was inhibitory to growth of *Xanthomonas axonopodis* pv.*vignicola* and he was able to get good control of bacterial blight of cowpea by treating the seeds with leaf and root extracts.

Karuna and Khan (1993) reported that plant extracts obtained from *Ocimum*, Eucalyptus, *Citronella*, Neem, Adathoda inhibited the growth of *Ralstonia solanacearum* under *in -vitro* condition. Maximum inhibition zone of 43.9 mm dia. was obtained in extract of eucalyptus followed by *Citronella* (40.4mm) and *Ocimum* (37.2) respectively.

Srinivasachary (1995) found that, *Ocimum* plant extract was more effective in inhibiting the growth of *Xanthomonas campestris* pv. *mori* isolated from mulberry under *in vitro* condition followed by *Bursara, Citronella* and *Cinnamom*, whereas garlic extract was highly inhibitory to the growth of *Xanthomonas campestris* pv. *dieffenbachiae* followed by *Ocimum* and *Citronella* (Sushma, 1997).

Khan *et al.* (2000) evaluated leaf extract of *Datura alba,* seed oil of neem (*Azadirachta indica*), neemseed bitter and nimbokil 60 EC at 1, 2 and 3 per cent concentrations on the growth of *Xanthomonas campestris* pv. *malvacearum, in vitro* and on the greenhouse grown cotton varieties/lines. *Datura alba* significantly retarded the growth of bacterium at 3 per cent concentration followed by nimbokil, neemseed bitter and neemseed oil respectively.

Kiran Kumar (2000) reported that, *Ocimum sanctum* effectively controlled bacterial blight of cowpea caused by *Xanthomonas axonopodis* pv. *vignicola*, when undiluted extract is sprayed onto the crop and effectiveness was decreased under dilution.

Madhiazhagan *et al.* (2002) studied efficacy of five different botanical extracts in controlling the bacterial bight disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* through pot culture experiment. Among the five plant extracts, *Adhatoda vasica* significantly minimized the disease with higher grain yield levels followed by *Curcuma longa* and *Allium cepa*.

Shah and Mali (2002) have studied the efficacy of different plant extracts against *Xanthomonass axonopodis* pv. *citri* both *in vitro* and *in vivo* conditions and reported that extracts of onion bulb, garlic, clove and leaf extract of Neem having inhibitory action against citrus canker.

Ashok (2006) evaluated leaf extracts of ten different plants including obnoxious weeds for their efficacy against bacterial blight of rice. Crude extract (1%) of each of the ten plant species were sprayed on the plants of susceptible rice cultivar Kasturi at the maximum tillering to booting stage of the crop. After 24 hours, sprayed plants were clip inoculated with the bacterial suspension. He observed significant reduction in the disease intensity of bacterial blight in all the treatments however maximum disease control of 58.7 per cent was obtained with *Lantana camera* followed by *Eucalyptus citrodora, E. odenophorum* and *Agave americana*.

Mukesh *et al.* (2008) tested many plant extracts against *Xanthomonas axonopodis* pv*. vignicola* and observed leaf extract of weed plant Datura and tulsi having inhibitory effect.

Govindappa *et al.* (2011) tested the efficacy of plant extracts to have antibacterial activity and used them for treating the seeds to know their effect on seed germination and seedling vigour. Based on the *in vitro* studies they observed *Adathoda vasica* leaf extract significantly reducing the growth of bacterial leaf blight causing pathogen in rice, *X. oryzae* pv*. oryzae*.

Seed treatment was found to be effective in reducing the incidence of the disease under greenhouse condition. Physiological observation of *A. vasica* treated plants indicated that restriction of pathogen colonization or disease development. Further investigation through challenge inoculation revealed that it is due to pronounced production of peroxidase, PAL, β-1, 3-glucanase, polyphenol oxidase and phenols in the plant tissue.

2.4.3 Bio-control agents

Jindal *et al.* (1989) tested the efficacy of *Bacillus* sp. to inhibit the growth of *Xanthomonas axonopodis* pv*. vignicola* and found that *Bacillus subtilis* was most effective in controlling bacterial blight of cowpea.

Bora *et al.* (1992) reported that *Erwinia herbicola* and *Penicillium chrysogenum* has good inhibitory action on *Xanthomonas campestris* pv*.vignaeradiate* of mungbean.

Jindal and Thind (1993) evaluated eight species of bacteria and seven species of fungi obtained from cowpea phylloplane by leaf washing technique against *Xanthomonas campestris* pv*. vignicola* in which *Flavobacterium* sp. among bacteria and *Penicillium oxalicum* among fungi proved most inhibitory under *in vitro* condition whereas, in field condition, *Bacillus* sp., *Erwinia herbicola* and *P. oxalicum* gave significantly better disease control than Streptocycline.

Kantachote *et al.* (1995) evaluated *Bacillus subtilis* B1 and *Pseudomonas fluorescens* M1 against *Xanthomonas campestris pv. oryzae*, the pathogen of bacterial leaf blight of rice. *Bacillus subtilis* B1 showed better inhibition of *Xanthomonas campestris pv. oryzae* than *Pseudomonas fluorescens* M1. They attributed this bio-control activity of *B. subtilis* B1 on the target organism to antibiotic substance production. Antibiotic producing ability of the bioagent was enhanced when it was grown in Czapek Dox medium by adding 1 per cent yeast extract.

Lodha (2001) studied the effect of two phylloplane antagonists, a white sterile fungus and a bacterium *Bacillus subtilis* against bacterial blight of clusterbean caused by *X. axonopodis* pv. *cyamopsidis* and noticed the superiority of white sterile fungus in reducing the disease intensity to the maximum extent followed by *Bacillus subtilis*.

However, combined treatment of both of these antagonists in a sequence could not reduce the blight incidence significantly rather increased it.

The efficacy of *Pseudomonas fluorescens* in reducing the bacterial blight intensity of rice was reported by Kaur and Thind (2002). Among the three isolates of *Pseudomonas fluorescens* tested in glasshouse and field, the isolate *Pseudomonas fluorescens* LR (Ludhiana rhizosphere) proved as most effective antagonist in significantly reducing the disease intensity. Among the different methods of antagonist application tried, seed bacterization followed by two foliar sprays recorded the lowest bacterial blight intensity both in glasshouse and field conditions.

Massomo *et al.* (2004) reported the inhibitory action of *Bacillus subtilis* against the bacterium *Xanthomonas axonopodis* pv. *vignicola* causing bacterial blight of cowpea.

Byrne *et al.* (2005) evaluated three pseudomonads *viz.*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas syringae* against bacterial speck of tomato caused by *Xanthomonas campestris* pv. *vesicatoria*, in which *Pseudomonas syringae* provided the highest mean reduction in disease severity.

Dutta *et al.* (2005) observed that, among the twenty one rhizobacterial and fifty two phylloplane bacterial isolates of mungbean, the isolates MRb-1 (rhizobacteria), Plb-1, Plb-2 and Plb-3 (phylloplane bacteria) exhibited the maximum inhibition of *Xanthomonas axonopodis* pv. *vignaeradiatae* tested by dual culture technique using sucrose peptone agar.

MRb-1 was the potential antagonist followed by Plb-3, Plb-2 and Plb-3. Based on the morphological and biochemical characteristics, MRb-1 isolate was identified as *Pseudomonas fluorescens* and Plb isolates were identified as *Bacillus* spp.

Monteiro *et al.* (2005) investigated eight *Bacillus* isolates against *Xanthomonas campestris* pv*. campestris* causal agent of crucifers black rot and reported that *Bacillus subtilis* R14 and *B. megaterium* pv*. cerealis* RAB7 strains were the most efficient of all antagonists. They also observed that lipopeptides produced by *Bacillus* isolates are responsible for antibiosis.

Velusamy *et al.* (2006) reported that *Pseudomonas fluorescens* inhibited the growth of the devastating rice bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* in laboratory assays and suppressed rice bacterial blight up to 59–64 per cent both in glass house and field experiments by producing the antimicrobial antibiotic compound- 2,4 diacetylphloroglucinol (DAPG).

Salaheddin *et al.* (2010) screened 93 bacterial isolates from the rhizosphere of cotton for their efficacy in inhibiting the growth of *Xanthomonas axonopodis* pv. *malvacearum* causing bacterial blight of cotton *in vitro*. Among them, 21 isolates were found to inhibit the *in vitro* growth of *Xam*. These isolates were identified as *Pseudomonas fluorescens* and *Bacillus subtilis* based on phenotypic characteristics, biochemical properties using 16S-23S intergenic transcribed spacer-Polymerase Chain Reaction (PCR). Talc-based powder formulations of the effective antagonistic isolates of *P. fluorescens* (Pf32, Pf93) and *B. subtilis* (B49) were evaluated individually and in combination both under greenhouse and field conditions. The application of a mixture of Pf32, Pf93 and B49 to seed, soil and foliage significantly reduced the bacterial blight incidence and increased the plant height, number of branches and number of bolls under field conditions.

Issazadeh *et al.* (2012) evaluated many *Bacillus* species for their efficiency as a bio-control agents against phytopathogenic bacteria like *Xanthomonas campestris* pv. *campestris* and *Pectobacterium carotovorum* subsp. *Carotovorum*. Among many isolates, *Bacillus thuringiensis, Bacillus cereus, Bacillus subtilis, Bacillus megaterium* and *Bacillus pumilus* showed good activity against the phytopathogens.

2.4.4 Hot water treatment

Singh and Verma (1973) stated that cotton seeds treated with hot water at 56^0 C for 10 min controlled both external and internal infection of *Xanthomonas malvacearum* from cotton seeds.

Shekhawat *et al.* (1982) reported that the infection of *Xanthomonas campestris* pv. *campestris* causing black rot of cabbage and cauliflower was eliminated by hot water treatment at 50^0 C for 30 minutes.

Kolev (1984) reported that bean (*Phaseolus*) seeds were successfully treated against bacteriosis (*Xanthomonas campestris* pv. *phaseoli*) at temperature up to 60^0 C and 45 to 55 per cent relative humidity which effectively controlled the disease.

Bhardwaj *et al.* (1987) reported that damping off and black rot (*X. campestris*) of cauliflower seedlings were effectively controlled by hot water treatment at 52^0 C for 30 minutes followed by 30 minutes dip in streptocycline at 100 ppm.

Sikirou (1999) reported that seed treatment in hot water at 60^0 C for 30 min or 70^0 C for 10 min or treatment in hot air (65^0 C for 120 – 144 h or 70^0 C for 96 h) eliminated the bacterium from infected cowpea seeds without inhibiting seed germination.

Nega *et al.* (2003) reported that *Xanthomonas campestris* pv. *campestris*, *Xanthomonas campestris* pv. *carotae* on cabbage and carrot respectively were controlled by hot water treatment at 50^0 C for 30 minutes.

Barua *et al.* (2007) showed that hot water treatment at 53^0 C for 15 min was effective in controlling *Xanthomonas campestris* pv. *vignaradiata* and *Pseudomonas syringae* pv. *syringae* which are seed borne pathogenic bacteria causing bacterial infections on mungbean.

Viljoen (2010) reported that *Xanthomonas campestris* pv. *musacearum* causing Xanthomonas wilt, the most significant disease of banana in Africa can be controlled by using the banana suckers treated with boiling water.

2.5 Integrated disease management

Shah *et al.* (1991) conducted field experiment to assess the efficacy of chemicals as seed treatment, foliar spray and combination of the two as well as hot water treatment against the bacterial blight of cowpea during *Kharif* 1986 and 1987 and reported that hot water treatment was found effective but it greatly reduced the germination of seeds whereas, seed treatment with streptocycline supported maximum germination of seeds. They also reported that, combination of seed treatment and foliar spray with streptocycline gave the best control (75 per cent) of the disease with better yield of 6.3 q/ha compared to the control (3.8 q/ha).

David (2005) reported the successful management of leaf blight of onion (*Xanthomonas axonopodis* pv. *alli*) with the integration of different strategies such as use of plant activator, biological and chemical control. He opined that, integration of acibenzolar-S methyl (plant activator), biological control agents (commercial formulations of both *Pantoea agglomerans* strain C9-1 and *Pseudomonas fluorescens* strain A 506) with copper hydroxide in a carefully timed spray program can eliminate the leaf blight disease of onion effectively.

Meena *et al.* (2007) evaluated the partially purified plant extracts and antimicrobial chemicals against bacterial pathogens. Among seven partially purified plant extracts and four antibacterial chemicals tested against *R. solanacearum*, *X. campestris* pv. *campestris*, *X. axonopodis* pv. *citri* and *X. axonopodis* pv. *cyamopsidis* through disc diffusion technique. Only Mahua and Satyanashi plant extract and antibacterial chemical streptocycline were found effective at 1000 ppm.

Yenjerappa (2009) evaluated *in vitro* and *in vivo* efficiency of bactericides. Bronip (0.05%) + COC (0.2%) was highly effective in managing the bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* with higher yield levels and in biological control, *Bacillus subtilis, Pseudomonas fluorescens* and garlic extract (10%) were significantly effective in reducing the disease.

Raju (2010) studied both *in vitro* and *in vivo* efficacy of different chemicals, botanicals and bioagents against bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* and found that streptocycline (0.05%) + COC (0.2%), *Pseudomonas fluorescens* and tulsi leaves were superior in inhibiting the growth of bacteria and reducing the severity of the disease both under *in vitro* and *in vivo* conditions.

Raghu (2011) evaluated antibacterial chemicals in *in vitro* against rhizome wilt of ginger caused by *Ralstonia solanacearum* and stated that streptocycline at 500 ppm was best chemical followed by K-cycline. Among the botanicals tested, soapnut + meswak at 20 per cent gave best results and among bioagents *Pseudomonas fluorescens* were found effective both in mycelial disc and culture filtrate methods.

3. MATERIAL AND METHODS

The present investigation on bacterial blight of cowpea, caused by *Xanthomonas axonopodis* pv. *vignicola* were carried out during 2011-12 in the Department of Plant Pathology, College of Agriculture, University of Agriculture Sciences, Dharwad. During the research, lab studies were conducted at Dept. of Plant Pathology, Agricultural College, University of Agricultural Sciences, Dharwad and field experiments were conducted on fields of Institute of Organic Farming, UAS Dharwad. The details of materials used and methodology followed during the course of investigation are described hereunder.

General laboratory procedure

Glassware and cleaning

In all the experimental studies Borosil and corning glassware were used. The glass wares were kept in a cleaning solution containing 60 g of potassium dichromate ($K_2Cr_2O_7$) and 60 ml of concentrated sulphuric acid (H_2SO_4) in one liter of water for 24 hours. Then they were cleaned by washing with the detergent followed by rinsing with distilled water.

Sterilization

The glassware used in this study were sterilized in an autoclave at 1.1 kg per cm^2 pressure for 20 min and kept for drying in hot air oven at 60^oC for 1 hour. Both solid and liquid media were sterilized at 1.1 kg per cm^2 pressure for 15 min.

Surface sterilization of plant parts

The plant material were surface sterilized with 0.1 per cent sodium hypochlorite solution for 30 seconds and then washed in sterilized distilled water for three times.

Media

The name of the media used, their composition and method of preparation are briefly described here under.

1. Media for isolation of *Xanthomonas axonopodis* pv. *vignicola*

A. YDC agar (Yeast extract Dextrose Calcium carbonate Agar)

Yeast extract	- 10.00 g
D-glucose	- 20.00 g
$CaCO_3$	- 20.00 g
Agar	- 15.00 g
Distilled water	- 1000 ml

All ingredients were mixed except $CaCO_3$ and sterilized in an autoclave. $CaCO_3$ was later added to the media and mixed well.

B. Nutrient Agar (NA)

Peptone	- 5.00 g
Beef extract	- 3.00 g
Agar	- 20.00 g
Distilled water	- 1000 ml

2. Potato dextrose agar (PDA) for *Trichoderma harzianum*

Potato	- 200 g
Dextrose	- 20 g
Agar	- 20 g
Distilled water	- 1000 ml

3. Kings-B medium for isolation of *Pseudomonas fluorescens*

Peptone	- 20 g
Glycerol	- 10 ml
K_2HPO_4	- 0.05 g
$MgSO_4$	- 1.5 g
Agar	- 20 g
Distilled water	-1000 ml

3.1 Survey to assess the disease severity of bacterial blight of cowpea in northern Karnataka

To assess the extent of bacterial blight of cowpea disease severity, an intensive roving survey was conducted in major cowpea growing areas of Belgaum, Dharwad, Gadag and Haveri districts of northern Karnataka during *Kharif* 2011. Then fields were assessed for bacterial blight severity by recording the disease on 0-5 disease ratings scale (Shah *et al.*, 1991). Further PDI was calculated by using the following formula (Wheeler, 1969).

$$\text{Per cent Disease Index} = \frac{\text{Sum of individual disease ratings}}{\text{Total No. of leaves observed x Maximum grade}} \times 100$$

3.1.1 Isolation, identification of causal agent and pathogenicity studies

3.1.1.1 Symptomatology

The infected leaves were collected from major cowpea growing areas of northern Karnataka during *Kharif* 2011-12. *Xanthomonas axonopodis* pv. *vignicola* is an incitant of bacterial blight of cowpea. The first symptoms appear on cotyledons of seedlings emerging from infected seeds which look reddish and wrinkled. First necrotic lesions are formed on leaves and later the stem is attacked. The pathogen reaches vascular bundles and the disease becomes systemic. The growing tip of the infected plant is killed and the plant ultimately dies. Cankers are often developed on the stem near the union of cotyledons and first leaves. Such stem are unable to bear the load of the plant and easily breaks in strong winds.

The secondary infection on leaves appears as light yellow circular spots which are 4 to 10 mm in diameter and scattered on the lamina. The centre of these spots is necrotic and brown. The veins are red in color. On pods, deep green or water-soaked streaks are formed. Such pods become yellow, shrivel and die. The diseased pods produce smaller, wrinkled and infected seeds. These infected seeds serve as the source of primary inoculum.

3.1.1.2 Isolation of *Xanthomonas axonopodis* pv. *vignicola* from infected cowpea leaves

The leaves of cowpea showing typical symptoms of bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* were collected during the *Kharif* season of 2011-12 from major cowpea growing areas of northern Karnataka. The bacterium was isolated by extracting the ooze in sterile distilled water taken in test tubes followed by dilution plate technique on nutrient agar.

Small pieces of infected leaves were cut aseptically from the edge of typical spots along with a little portion of healthy tissue. The infected leaf bits were surface sterilized in 0.1 per cent sodium hypochlorite for 30 seconds and washed in three series of sterile water to remove traces of chemicals. The infected leaf bits were then suspended in a small test tube containing 3 ml sterilized distilled water for 10 min. When water became slightly turbid due to oozing of bacterial cells from the cut ends of the diseased tissue, the bacterial suspension was serially diluted in 9 ml sterile distilled water. Then one ml of the diluted bacterial cell suspension was poured into sterilized petriplates containing nutrient agar. The plates were rotated gently in clockwise and anti-clockwise direction, so as to distribute the bacterial cell suspension uniformly in the plates and to obtain well separated bacterial colonies.

The inoculated plates were incubated at 28 ^{0} C for 72 hours. Observations were made for development of well separated light yellow, convex, small bacterial colonies on the nutrient agar medium.

3.1.1.3 Purification of bacterial culture

The suspected bacterial colonies were picked up with the help of sterilized inoculated loop and streaked onto the surface of Yeast extract Dextrose Calcium carbonate Agar (YDCA), Schaad and Stall (1988). The inoculated plates were incubated at 28 ^{0} C for 72 h and the observations were made for the development of well separated deep yellow coloured bacterial colonies. The purified bacterial colonies were streaked on nutrient agar slants and stored at 5 ^{0} C in refrigerator and also in sterile distilled water taken in small culture tubes, by suspending 2-3 loopful of the bacterial culture which served as a stock culture for further studies.

3.1.2 Pathogenicity on cowpea

The pathogenicity test was carried out to find out whether the isolated bacteria were capable of producing typical symptoms of bacterial blight of cowpea under artificial inoculation condition. One colony per dilution plate was selected from isolations made from infected leaves and naturally infected cowpea seeds cv. C-152. Cowpea plants were raised in steam sterilized potting mixture with soil, sand and FYM in 3:1:1 ratio.

The strain of *Xanthomonas axonopodis* pv. *vignicola* from cowpea was multiplied in nutrient broth taken in Erlenmeyer's flask by inoculating a loopful of pure culture. The inoculated flask was incubated at 28^0 C for 72 h. Bacterial suspension was prepared by adjusting cell concentration to 5×10^6 cfu per ml using spectrophotometer at 420 nm (O.D=0.5) (Spectronic 20 D of Milton and Roy, USA). One month old cowpea plants were pre incubated for 24 hours in humid tent made up of plastic sheets in which humidity was maintained between 60 to 80 per cent before the inoculation.

The leaves of one month old cowpea plants were slightly injured by a sterilized insect pin and bacterial suspension was sprayed on to the surface of leaves and stems with low pressure sprayer. The inoculated plants were kept in the plastic tent in which high humidity was maintained by spraying sterile water inside the tent for five days. The plants were taken out from the plastic tent and kept in glasshouse at 25 to 30^{0}C. Observations were made for the development of symptoms of bacterial blight. Plants similarly, injured and sprayed with sterile water constituted control. After the development of the typical symptoms of the disease, the pathogen was reisolated and compared with original culture to prove the pathogenicity.

3.1.3 Identification of the pathogen

The identification of the pathogen involved in causing bacterial blight in cowpea was determined by conducting studies on its morphological and biochemical features of the pathogen as per standard microbiological procedures.

3.1.3.1 Morphological characters

The morphological characteristics of the pathogen such as cell shape, gram reaction, pigmentation, capsule and spore staining characters were studied as per the standard procedures described by Anon. (1957), Bradbury (1970) and Schaad (1992).

3.1.3.2 Biochemical characters

The biochemical characters such as hydrolysis of starch, gelatin liquefaction, hydrogen sulphide production, catalase and acid production from different sugars *viz.,* Lactose, Sucrose, Maltose, Dextrose and Mannitol by the pathogen were studied as per the methods described by Salle (1961) and Schaad (1992).

a) Gelatin liquefaction

Preparation of the nutrient gelatin medium: Peptone 10.0 g, Beef extract 5.0 g, Gelatin 20.0 g, all the ingredients were mixed and volume was made to 1L with pH 7.0 and heated over a water bath until the gelatin was dissolved and sterilized at 15 lb pressure for 15 min.

The media was cooled and poured to the petriplates and allowed to solidify then spot inoculated with 48 hours growth of the test bacterium and incubated the plates at 20 ^{0}C. Then flood the surface of agar with 0.2 per cent mercuric chloride solution of dilute HCl (20%) and observed for formation of white precipitation.

b) Catalase test

A loopful of 24-48 hrs slant growth of the test bacterium was smeared on a slide and covered it with a few drops of 20 per cent hydrogen peroxide. The reaction is found to be positive if gas bubbles are produced.

c) Hydrogen sulphide (H_2S) production

The peptone water medium comprising of Peptone 10 g, NaCl 5.0 g, water 1000 ml and pH 7.0 was dispensed in 5 ml quantities in tubes and autoclaved. To detect H_2S, the lead acetate test strips were prepared as follows. Whatman No. 1 filter paper was cut into 5 × 50 mm strips which were then soaked in warm saturated solution of lead acetate. The strips were then dried, autoclaved and again dried at 60^{0}C.

The medium in each tube was inoculated with a loopful of 48 hours slant growth of the test bacterium. After inoculation, the test strip was inserted in between the plug and inner wall of the tube, so that it hangs just above the broth. The tubes are incubated at 25^{0}C and the observations were recorded at regular intervals up to 14 days. The blackening of test strip indicates liberation of H_2S.

d) Starch hydrolysis

The medium employed is referred to as starch broth and contains, Peptone (10.0 g), beef extract (5.0 g), starch soluble (2.0 g), agar (20.0 g), water (1000 ml) and pH (7.0). Sterilized the medium by autoclaving and poured into sterilized petri plates. The medium was allowed to solidify and spot inoculated the test culture in four plates. The plates were inoculated at 25 ^{0}C and test for starch hydrolysis, one plate at a time, after 2, 4, 7 and 14 days as follows. Flooded the agar surface with Lugol's iodine and allowed to act for a few minutes for development of colorless zone around the bacterial growth.

e) Lactose utilization

The carbon source (lactose) was filter sterilized and mixed with autoclaved, cooled Dye's medium along with 1.2 per cent purified agar. The pH was adjusted to 7.2. Bacterial isolates were spot inoculated with replica plating method and incubated at 30^{0}C for 3, 7 and 14 days. Growth was compared with control, where carbon source was not supplemented (Schaad, 1992).

f) Acid production from Sucrose, Maltose, Dextrose and Mannitol

The acid production by the pathogen *Xanthomonas axonopodis* pv. *vignicola* was tested by using medium C of Dye. Ten ml of medium C of Dye was dispensed in each test tube and sterilized in an autoclave for 15 min. at 121^{0}C for 15 lb. pressure. To these tubes, filter sterilized carbohydrates *viz*., Sucrose, Maltose, Dextrose and Mannitol were added at 0.14 per cent concentration. The tubes were inoculated with 0.1 ml of 24 hrs old bacterial culture and incubated at room temperature for three days. Change in the colour of the medium confirmed the acid production.

3.2 Detection of pathogen in the seed by non-serological methods

It is reported that *Xanthomonas axonopodis* pv. *vignicola* is seed borne (Shekhawat and Patel, 1977). Therefore, investigations were conducted to find out the nature of transmission of the pathogen through seeds.

3.2.1 Detection of pathogen using selective and semi-selective media

3.2.1.1 Collection of seed sample

Cowpea seed sample comprising cv. C-152 collected from naturally infected plants showing typical symptoms of bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* were used. Twenty five seeds were used to conduct each test.

3.2.1.2 Comparison of the efficiency of the various selective and semi-selective media

The relative efficiency of the various media being evaluated was determined on the basis of the recovery of *Xanthomonas axonopodis* pv. *vignicola* by dilution plating on nutrient agar. Culture of *Xanthomonas axonopodis* pv. *vignicola*, maintained in the Dept. Plant Pathology, UAS, Dharwad was used to assess the efficacy of each media.

3.2.1.3 Isolation media

Eight media were tested for their efficiency in recovery of known isolate of *Xanthomonas axonopodis* pv. *vignicola*. They were SX agar (Schaad and White, 1974), XTS (Schaad and Forster, 1985), Modified D-5 medium (Kaun *et al.*, 1985), NSCAA and BSCAA medium (Randhawa and Schaad, 1982), YDCA (Schaad and Stall, 1988), KML medium (Kim *et al.*, 1982; Carmen, 1985), SIBU agar (Silva and Buddenhagen, 1970). The nutrient agar medium was used as a standard check for the purpose of comparing the growth of the bacteria. All the media used in the present investigation are especially meant for the isolation of plant pathogenic bacteria from seeds.

The composition of the various media used for detection is as follows:

Selective media

SX agar (Schaad and White, 1974)

Starch (Soluble potato)	: 10.0 g
Beef extract	: 1.0 g
Ammonium chloride	: 5.0 g
Dipotassium phosphate	: 2.0 g
Methyl violet 2B	: 1.0 ml*
Methyl green	: 2.0 ml**
Agar	: 15.0 g
Distilled water	: 1000 ml
After autoclaving Cycloheximide was added	: 5.0 ml***

* : 1 per cent solution in 20 per cent ethanol was used- Be sure to use methyl violet 2B and not methyl violet B as the later is more toxic.

** : 1 per cent aqueous solution was used.

*** : 5.0 g was added to 10.0 ml methanol. Volume was made up to 100 ml with water and filter sterilized by 0.22 µm membrane.

KML agar (Kim *et al.*, 1982; Carmen, 1985)

Lactose	: 10.0 g
D (+) Trehalose	: 4.0 g
Thiobarbituric acid	: 0.2 g
Yeast extract	: 0.5 g
NH_4Cl	: 1.0 g
K_2HPO_4	: 0.8 g
KH_2PO_4	: 0.8 g
Agar	: 18.0 g
After autoclaving Vancomycin was added	: 0.5 mg

SIBU agar (Silva and Buddenhagen, 1970)

Yeast extract	: 5.0 g
Bacto peptone	: 5.0 g
Dextrose	: 5.0 g
L-glutamine	: 1.0 g
Agar	: 20.0 g

Semi-selective media

XTS agar (Schaad and Forster, 1985)

Nutrient agar	: 23.0 g
Glucose	: 5.0 g
Distilled water	: 1000 ml

After autoclaving,

Cycloheximide	: 2.0 ml*
Gentamycin	: 0.5 ml**
Cephalexin	: 1.0 ml*** were added.

* : 1.0 g was added to 10 ml of 75 per cent ethanol

** : 50 mg was added to 5 ml of 75 per cent ethanol

*** : 50 mg was added to 5 ml of 75 per cent ethanol

NSCAA medium (Randhawa and Schaad, 1982)

Nutrient agar	: 23.0 g
Starch (Soluble-potato)	: 15.0 g
Agar	: 15.0 g

After autoclaving,

Cycloheximide	: 5.0 ml*
Nitrofurantoin	: 1.0 ml**
Vancomycin	: 1.0 ml*** were added.

* : 5.0 g was added to 10.0 ml methanol, volume was made upto 100 ml with water and filter sterilized (0.22 µm membrane)

** : 50 mg was added to 5 ml of 50 per cent dimethyl formamide.

*** : 12.5 mg was added to 25 ml water and filtered as above.

BSCAA medium (Randhawa and Schaad, 1982)

Starch (Soluble potato)	: 10.0 g
Glycine	: 0.2 g
K_2HPO_4	: 1.0 g
KH_2PO_4	: 1.0 g
$MgSO_4.7H_2O$	: 0.2 g*
Methyl green	: 0.2 g**
Agar	: 15.0 g

After autoclaving cycloheximide was added: 5.0 ml***

* : first it was added and mixed well until it got fully dissolved

** : 1 per cent aqueous solution was added

*** : 5.0 g was added to 10.0 ml methanol, brought to 100 ml with water and filter sterilized (0.22 µm membrane)

Modified D-5 medium (Kaun *et al.*, 1985)

D-cellobiose	: 10.0 g
KH_2PO_4	: 3.0 g
Na_2HPO_4	: 1.0 g
NH_4Cl	: 1.0 g
$MgSO_4.7H_2O$	: 0.3 g
Cycloheximide	: 50.0 mg*
Agar	: 15.0 g

* : Filter sterilized (0.22 µm) D-cellobiose and cycloheximide were added after autoclaving.

YDCA and Nutrient agar media composition has been already mentioned above in this chapter only.

The media were sterilized in autoclave for 15 min. at 121^0C for 15 lb. pressure. All the antibiotics were filter sterilized and added to cooled, sterilized (50^0C) media after autoclaving. The media were poured into sterilized petriPlate s and dried for 2 days at room temperature to eliminate surface moisture before use.

3.2.1.4 Colony assessment

A loopful of 48 hr. old bacterial culture was added to 10 ml sterile water blank and one ml of this suspension was further serially diluted using 9 ml sterile water and 100 µl of 10^5 and 10^6 dilutions was spread on the surface of the medium in each plate using a sterilized spreader under aseptic conditions. The inoculated plates were incubated at 28^0C for 72 hr. Observations were recorded for number of colonies developed and the characters of colonies on each medium.

3.2.3 Detection of *X. axonopodis* pv*. vignicola* in the infected seeds by Van vuurde *et al.* (1983) method

3.2.3.1 Preparation of seeds extracts

The seed lot was divided into two sets containing of 25 seeds each of different germplasm lines *viz*., diseased and healthy seeds of Susceptible, Resistant, Moderately susceptible and Moderately Resistant germplasm lines. One set of seed samples were surface sterilized in 1 per cent Sodium hypochlorite for 10 min. and the other set was not surface sterilized. The seeds were incubated in 25 ml sterilized water taken in 100 ml Erleyenmayer's flask and soaked for 24 hr. at 5^0C. After four hours incubation, the soaking solution of each sample was mixed thoroughly by shaking the flasks by hand. After 24 hr, the soaking solution was sampled for dilution plating, just below the surface of the liquid.

3.2.3.2 Isolation

The presence of *X. axonopodis* pv*.vignicola* in seed extracts was investigated by isolating the bacterial cells by dilution plating (Bradshaw, 1949) on nutrient agar. The inoculated plates were incubated at 28^0C for three days and observed for the production of typical colonies which are mucoid, round, convex, glistening, slimy and yellow.

The suspected colonies were isolated and purified on YDCA (Schaad and Stall, 1988). The colonies from the seeds of cv. C-152 from Dharwad were maintained in the laboratory.

3.2.3.3 Procedure

The surface sterilized seeds using 1 per cent Sodium hypochlorite for 10 min. and unsterilized seeds were used for extraction of the bacterium. The bacterium was extracted as described by Van Vuurde *et al.* (1983) by soaking 25 seeds of the seed sample in 25 ml sterile distilled water in 100 ml Erleyenmayer's flask for 24 hr. The seed extracts were serially diluted to 10^2, 10^3 and 10^4, one tenth of a milliliter of each dilution was plated in triplicate on the media which was found effective with respect to recovery of the pathogen out of eight selective and semi-selective media tested.

The bacterial population was estimated after the inoculated plates were incubated at 28^0C for 3 to 5 days.

3.3 Screening of cowpea varieties/germplasm lines against bacterial blight

Fifteen different entries / germplasm lines were tested at field belonging to Dept. Genetics and Plant Breeding, UAS, Dharwad during *Kharif* 2011-2012 against *X. axonopodis* pv*. vignicola* under natural condition. Thirty days old cowpea plants were observed for disease incidence. The seeds were sown with a spacing of 10 cm between plants and 45 cm row to row. On either side with a single row of susceptible check C-152. All cultivation practices such as fertilizer, spacing, inter cultivation and plant protection were carried out as per the package of practices prescribed by the University of Agricultural Sciences, Dharwad. Plant was exposed to natural infection by *X. axonopodis* pv*. vignicola.*

Observations were recorded at the disease incidence on the severity of bacterial blight of cowpea reaction on 0-5 scale.

The observations were recorded on the severity of the disease on the basis of relative percentage of leaf area covered by the disease using 0 to 5 scale (Shah *et al.*, 1991) and per cent disease index was worked out using Wheeler (1969) formula.

Per cent disease index (PDI) was calculated as follows.

$$\text{Per cent disease index} = \frac{\text{Sum of individual disease ratings}}{\text{No. of leaves examined} \times \text{Maximum grade value}} \times 100$$

Grade	Per cent of leaf area infected	Reaction
0	No visible infection	Immune (I)
1	1 – 5% infection	Resistant (R)
2	6 – 15% infection	Moderately resistant (MR)
3	16 – 30% infection	Moderately susceptible (MS)
4	31 – 50% infection	Susceptible (S)
5	>50% infections	Highly susceptible (HS)

3.4 Evaluation of chemicals/antibiotics

3.4.1 *In vitro* evaluation of different chemicals/antibiotics

Chemicals and antibiotics each at three different concentrations were evaluated for their efficacy against the growth of *X. axonopodis* pv. *vignicola* by inhibition zone method. The details of chemicals/antibiotics used and their concentrations were given hereunder.

Trade name	Chemical name	Concentration (%)
Streptocycline	Streptomycin sulphate IP 90% WW + tetracycline hydrochloride 10% WW	0.05, 0.075, 0.1
Streptocycline + Copper oxychloride	Streptomycin sulphate IP 90% WW + tetracycline hydrochloride10% WW + Copper oxychloride 50% WP	0.05 + 0.1, 0.05 + 0.2, 0.05 + 0.3
Blue copper/ Blitox	Copper oxychloride 50% WP	0.1, 0.2, 0.3
Kocide 101	Copper hydroxide 77% WP	0.1, 0.2, 0.3
Captaf	Captan 50% WP	0.1, 0.2, 0.3
Palntomycin	Streptomycin sulphate 9% WW + tetracycline hydrochloride 1% WW	0.05, 0.075, 0.1
Bacterinashak	2-Bromo-2-Nitro propane-1,3-Diol	0.05, 0.075, 0.1
Bleaching powder	Chlorine 30%	0.5, 0.75 ,1
Bordeaux mixture	Copper sulphate + lime	0.5, 0.75, 1

The bacterium was multiplied by inoculating the culture into 20 ml nutrient broth taken in Erleyenmayer's flask. The inoculated flasks were incubated at 28^0C for 72 hours. The bacterial suspension was then seeded to the lukewarm nutrient agar medium and mixed thoroughly. The seeded medium was poured into the sterilized petriplates and plates were allowed to solidify.

The chemicals/antibiotics solutions were prepared at different concentrations as mentioned in the table. The filter paper discs (Whatman no-41) measuring 5mm in diameter were soaked in the respective chemical/antibiotic concentrations for 5 min and transferred onto the surface of seeded medium in the petriplates. The plates were kept upright for about four hr to allow the diffusion of chemicals into the medium and then after 4 hr. the plates were kept upside down and incubated at 28^{0}C for 72 hours. The observations were made for the production of inhibition zone around the filter paper discs and the results obtained were analyzed statistically.

3.4.2 *In vitro* evaluation of botanicals on the growth of *X. axonopodis* pv *vignicola*

The plant based pesticides which are relatively safe, economical and non-hazardous can be used successfully for the management of bacterial diseases in plants. The present investigation aimed at screening some plant extracts/botanicals for their antibacterial properties against *X. axonopodis* pv. *vignicola.*

The list of botanicals/plant extracts with their concentration is given below.

Name of the botanical	Botanical name	Plant Parts used	Concentration (%)
Neem	*Azadirachta indica*	Leaves	5,7.5,10
Duranta	*Duranta repens*	Leaves	5,7.5,10
Nilgiri	*Eucalyptus globules*	Leaves	5,7.5,10
Lantana	*Lantana camera*	Leaves	5,7.5,10
Tulsi	*Ocimum sanctum* L. cv. purple	Leaves	5,7.5,10
Onion	*Allium cepa*	Bulb	5,7.5,10
Chilli	*Capsicum annum*	Leaves	5,7.5,10
Garlic	*Allium sativum*	Bulb	5,7.5,10
Soapnut	*Sapindus mukorossi*	Pulp	5,7.5,10

The fresh plant material were collected and washed first in tap water followed by distilled water; 100 grams of fresh sample was chopped and macerated in a surface sterilized pestle and mortar by adding 100 ml of sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth, filtrate thus obtained was used as a stock solution. To study the antibacterial mechanism of plant extracts and organics, inhibition zone assay method was followed.

The bacterial suspension (72 hr old) of *X. axonopodis* pv *vignicola*, multiplied in nutrient broth (20 ml) was mixed with molten (50^{0}C) nutrient agar medium (1000 ml) contained in an Erleyenmayer's flask, so as to get the thick growth of bacteria on the medium, 15 to 20 ml of seeded medium was poured onto the sterilized petriplates and allowed to solidify.

Five, 7.5 and 10 per cent each of plant extract was prepared by mixing 5, 7.5 and 10 ml of stock solution with 95, 92.5 and 90 ml of sterilized distilled water, respectively. Filter paper discs (Whatman No. 41) measuring 5 mm in diameter were soaked separately in different plant extracts of desired concentrations for 5 min and then placed onto the surface of seeded nutrient agar medium in the petriplates. Then inoculated plates were incubated at 28^0 C for 72 hours.

At the end of incubation period, observations were recorded for the production of inhibition zone representing the efficacy of plant extracts in inhibiting the growth of pathogen. The inhibition zone in each plate was measured in terms of centimeter in diameter and data obtained was analyzed statistically.

3.4.3 *In vitro* evaluation of antagonistic micro-organisms on the growth of *X. axonopodis* pv. *vignicola*

Four biocontrol agents *viz*., *Trichoderma harzianum, Pseudomonas fluorescens* (5 isolates)*, Bacillus subtilis* and *Methylobacterium* spp. (5 isolates) were evaluated for their efficacy against the growth of *X. axonopodis* pv*. vignicola* by inhibition zone assay method. The cultures of these biocontrol agents were obtained from Department of Plant Pathology, Department of Microbiology, UAS Dharwad and Institute of Organic farming UAS, Dharwad.

A suspension of *X. axonopodis* pv*. vignicola* multiplied in nutrient broth (20 ml) was mixed with lukewarm nutrient agar medium (1000 ml) contained in Erleyenmayer's flask. Fifteen to twenty ml of seeded medium was poured into the sterilized petriplates and allowed to solidify. A loopful culture of the antagonistic organism was placed in the centre of petriplates containing the seeded medium. In case of fungal antagonists, mycelial discs of 5 mm (dia.) size taken from actively growing culture were placed in the centre of the plates. The inoculated plates were then incubated at 28^{0}C for 72 hours.

The observations were recorded for the production of inhibition zone around the antagonistic microorganisms around the growth of the pathogen and the results obtained were calculated and analyzed statistically.

3.4.4 *In vivo* evaluation of different chemicals/bioagents/botanicals against bacterial blight of cowpea caused by *Xanthomonas axonopodis* pv. *vignicola*

This experiment was conducted in glass house conditions during 2011-2012. The trails were laid out in Completely Randomized Design (CRD) with four replications for each treatment with untreated control. Variety grown was C-152, susceptible to bacterial blight.

Initially the pots containing 25 to 30 days old cowpea plants were artificially inoculated with *Xanthomonas axonopodis* pv. *vignicola* by spraying the bacterial suspension of 5×10^{6} cfu/ml. After 6 to 8 days when characteristic bacterial blight symptoms appeared on cowpea plants, the spraying of different chemicals, bioagents and botanicals which were found effective under *in vitro* condition was taken with suitable concentration.

The observations on disease incidence and severity of bacterial blight were recorded and per cent disease index and per cent disease reduction over control was worked out.

% reduction over control was calculated as follows:

$$\text{Disease reduction (\%)} = \frac{\text{PDI in control - PDI in treatment}}{\text{PDI in control}} \times 100$$

Where, PDI – Per cent disease index

3.4.5 Hot water treatment for the management of bacterial blight of cowpea

3.4.5.1 Effect of hot water treatment on *X. axonopodis* pv*. vignicola* artificially inoculated seeds

Cowpea seeds of cv., C-152 were artificially inoculated with *X. axonopodis* pv*. vignicola* with a cell suspension of 5×10^{6} cfu/ml.

3.4.5.1.2 Seed inoculation

The bacterial isolates obtained from the infected leaves and seeds were multiplied on YDC agar plates and the cells were harvested in sterilized water. The concentration of the cell suspension was adjusted to 5×10^{6} cfu/ml using spectrophometer at 420 nm (OD=0.5) (Spectronic 20 D, Milton and Roy).

Apparently healthy cowpea seeds collected from the field were inoculated with bacterial suspension by keeping the seeds in the bacterial suspension contained in 250 ml side arm flask for one hour. The inoculated seeds were air dried over night and used for further studies.

Artificially inoculated seeds were treated in hot water separately at 48, 50, 52, 54 and 56^{0}C for 5 and 10 min. in a water bath. The seeds were transferred immediately to a beaker containing ice cold water. The water was drained out and seeds were air dried in shade for 24 hrs and used for further studies.

Seeds were treated at different temperature levels and kept for germination through roll paper towel method and they were also used to plant in pots of 6" size containing sterilized potting mixture of soil, sand and FYM in 3:1:1: ratio. Untreated inoculated seeds used in both the tests served as positive control. Observations for bacterial blight infection were recorded and then per cent disease incidence for seedlings was calculated.

3.5 Integrated management under field conditions

Effective chemicals, botanicals, bioagents and hot water treatment screened earlier to check the incidence of bacterial blight of cowpea were integrated and used here. This experiment was conducted in *Kharif* 2011-12 (June-August) in experimental plot, Institute of Organic Farming, UAS, Dharwad. The trial was laid out in Randomized completely block design (RCBD) with three replications in each treatment. The variety grown was 'C-152'. The details of the treatments were given below.

Treatments	Treatment details
T_1	Hot water treatment
T_2	T_1 + Seed treatment with effective bioagent
T_3	T_1 + Seed treatment with effective chemical
T_4	T_3 + Foliar spray with first best botanical
T_5	T_3 + Foliar spray with first best chemical
T_6	Hot water treatment + Seed treatment with effective bioagent + Foliar spray of bioagent/botanical spray at 25 days and one chemical spray at 45 days
T_7	Untreated check (Control)

The observations pertaining to the incidence and severity of the disease were recorded and the Per cent Disease Index (PDI) was worked out in each treatment. The data obtained were analyzed statistically.

The data on seed yield in treated and control plots were also recorded and analyzed as per the statistical procedures (Sukhatme and Amble, 1985).

4. EXPERIMENTAL RESULTS

In the present investigation, the laboratory and pot culture experiments were conducted in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad and field experiments were conducted at the Institute of Organic farming, UAS, Dharwad. The results of *in vitro* studies and field experiments conducted during 2011-2012 are presented hereunder.

4.1 Survey to assess the disease severity of bacterial blight disease of cowpea in Belgaum, Dharwad, Haveri and Gadag districts

An intensive survey to record the disease severity was conducted in major cowpea growing areas of Belgaum, Dharwad, Haveri and Gadag districts during *kharif* 2011. The details of locations and number of fields visited are tabulated (Table 1).

The bacterial blight severity was noticed in all the locations surveyed ranging from 2.52 to 28.47 per cent. The mean maximum severity (14.32 PDI) was observed in Belgaum district, followed by Gadag district (13.11 PDI) and Dharwad district (11.36 PDI) whereas, the least PDI (9.58 PDI) was observed in Haveri district (Table 2, Fig. 1, and 2).

4.1.1 Belgaum district

Four talukas were surveyed in Belgaum district *viz.*, Belgaum, Gokak, Raibag and Bailhongal. The disease was observed in all the surveyed area and ranged from 4.34 to 28.47 PDI. The mean maximum Per cent Disease Index (19.43) was observed in Bailhongal taluka followed by Belgaum taluka (14.29 PDI) and Gokak taluka (12.22 PDI). The least PDI was observed in Raibag taluka (11.36 PDI).

In Belgaum taluka, five villages were surveyed. The disease index ranged from 4.34 to 24.03 PDI. The maximum disease index of 24.03 PDI was observed in Hirebagevadi village with a PDI range of 22-50 followed by Kitturu (21.21 PDI with 20-25 PDI range) and the least index was observed in Hulli (4.34 PDI with a range of 0-12 PDI).

In Gokak taluka, five villages were surveyed. The disease index ranged from 8.63 to 16.36 PDI. In Rajapur village, maximum disease index (16.36 PDI) was observed with a PDI range of 15-30 followed by Ghataprabha (14.11 PDI with 10-20 PDI range) and the least disease index was observed in Kalolli (8.63 PDI with 5-15 PDI range).

In Raibag taluka, three villages were surveyed and the disease index ranged from 9.74 to 13.48 PDI. The maximum disesae index (13.48 PDI) was observed in Chinchali village with a PDI range of 10-20 followed by Kudachi (10.87 PDI with 5-15 PDI range) and the least disease index was observed in Kakanavadi (9.74 PDI with 0-15 PDI range).

Four villages were surveyed in Bailhongal taluka where, the disease index ranged from 9.40 to 28.47 PDI. The maximum disease index (28.47 PDI) was observed in Pattihala village with a PDI range of 15-50 followed by Vaderatti (23.56 PDI with 10-40 PDI range) and the least disease index was observed in Gudsa (9.40 PDI with a range of 5-15 PDI).

4.1.2 Dharwad district

Three talukas were surveyed in Dharwad district *viz.*, Dharwad, Kalghatki and Kundagol talukas. The disease index observed in all the surveyed area ranged from 2.52 to 21.33 PDI. The mean maximum disease index (17.87 PDI) was observed in Kalghatki taluka, followed by Dharwad taluka (12.90 PDI) and the least index was observed in Kundagol taluka (4.38 PDI).

In Dharwad taluka, five villages were surveyed. The disease index ranged from 6.67 to 18.78 PDI. The maximum disease index (18.78 PDI) was observed in Narendra village with a PDI range of 15-30, followed by Marewad village (14.00 PDI with a range of 5-20 PDI) and the least disease index was observed in Madihala (6.67 PDI with a range of 3-8 PDI).

Table 1: Survey to assess the disease severity of bacterial blight of cowpea in northern Karnataka

Name of the district	Name of the taluka	Name of the village	Cultivars grown	Stage of the crop	No. of fields visited	Per cent Disease Index
Belgaum	Belgaum	Kitturu	Bailhongal local	Flowering stage	3	21.21
		Hirebagevadi	Bailhongal local	Flowering stage	4	24.03
		M.K. Hubli	Bailhongal local	Grand growth stage	3	12.29
		Benduru	C-152	Pod formation stage	2	9.56
		Hulli	C-152	Pod formation stage	1	4.34
		Mean				**14.29**
	Gokak	Ghataprbha	C-152	Pod formation stage	4	14.11
		Tukkanatti	C-152	Pod formation stage	2	10.27
		Rajapur	Bailhongal local	Grand growth stage	4	16.36
		Mudalgi	Bailhongal local	Pod formation stage	3	11.72
		Kalloli	Bailhongal local	Grand growth stage	2	8.63
		Mean				**12.22**
	Raibag	Kakanavadi	C-152	Flowering stage	2	9.74
		Chinchali	Bailhongal local	Grand growth stage	4	13.48
		Kudachi	Bailhongal local	Grand growth stage	3	10.87
		Mean				**11.36**
	Bailhongal	Vaderatti	Bailhongal local	Flowering stage	4	23.56
		Pattihala	Bailhongal local	Flowering stage	5	28.47
		Tigadi	Bailhongal local	Pod formation stage	3	16.29
		Gudsa	Bailhongal local	Grand growth stage	2	9.4
		Mean				**19.43**

Contd...

Name of the district	Name of the taluka	Name of the village	Cultivars grown	Stage of the crop	No. of fields visited	Per cent Disease Index
Dharwad	Dharwad	Madihala	C-152	Pod formation stage	2	6.67
		Aminabhavi	C-152	Maturity stage	3	8.13
		Narendra	C-152	Flowering stage	5	18.78
		Garag	C-152	Grand growth stage	3	11.55
		Marewada	C-152	Flowering stage	4	14
		Mean				**11.83**
	Kalghatgi	Kalghtagi	C-152	Flowering stage	6	21.33
		Kanavihonnapur	V-118	Grand growth stage	4	18.46
		Hirehonnalli	C-152	Grand growth stage	3	13.82
		Mean				**17.87**
	Kundagol	Thimmapur	C-152	Maturity stage	2	5.46
		Ramapur	C-152	Pod formation stage	1	3.24
		Dummawada	C-152	Grand growth stage	1	2.52
		Devikoppa	V-118	Pod formation stage	3	6.29
		Mean				**4.38**
Haveri	Byadgi	Dummihala	C-152	Pod formation stage	3	11.32
		Kummur	C-152	Grand growth stage	3	10.57
		Masanagi	C-152	Flowering stage	1	5.11
		Anuru	V-118	Pod formation stage	2	4.68
		Kagenali	C-152	Maturity stage	3	9.36
		Mean				**8.21**
	Shiggaon	Bankapura	C-152	Grand growth stage	2	9.75
		Hanumaralli	C-152	Pod formation stage	3	10.38
		Mean				**10.07**

Contd...

Name of the district	Name of the taluka	Name of the village	Cultivars grown	Stage of the crop	No. of fields visited	Per cent Disease Index
	Haveri	Haveri	C-152	Flowering stage	3	14.23
		Devagiri	C-152	Flowering stage	2	12.67
		Gourapura	V-118	Flowering stage	1	8.55
		Hosalli	C-152	Pod formation stage	1	6.38
		Mean				**10.46**
Gadag	Gadag	Harti	C-152	Pod formation stage	2	10.45
		Mulgund	C-152	Maturity stage	5	15.61
		Nagavi	C-152	Pod formation stage	3	11.04
		Lakkundi	C-152	Maturity stage	4	13.53
		Beldadi	C-152	Flowering stage	3	17.17
		Mean				**13.56**
	Shirahatti	Shirahatti	C-152	Flowering stage	2	12.33
		Lakshmeshwara	C-152	Maturity stage	2	11.62
		Magadi	C-152	Grand growth stage	3	14.05
		Mean				**12.67**

Table 2: Severity of bacterial blight of cowpea in northern Karnataka

District	Taluk	Per cent Disease Index
Belgaum	Belgaum	14.29
	Gokak	12.22
	Raibag	11.36
	Bailhongal	19.43
	Mean	**14.32**
Dharwad	Dharwad	11.83
	Kalghatgi	17.87
	Kundagol	4.38
	Mean	**11.36**
Haveri	Byadgi	8.21
	Shiggaon	10.07
	Haveri	10.46
	Mean	**9.58**
Gadag	Gadag	13.56
	Shirahatti	12.67
	Mean	**13.11**

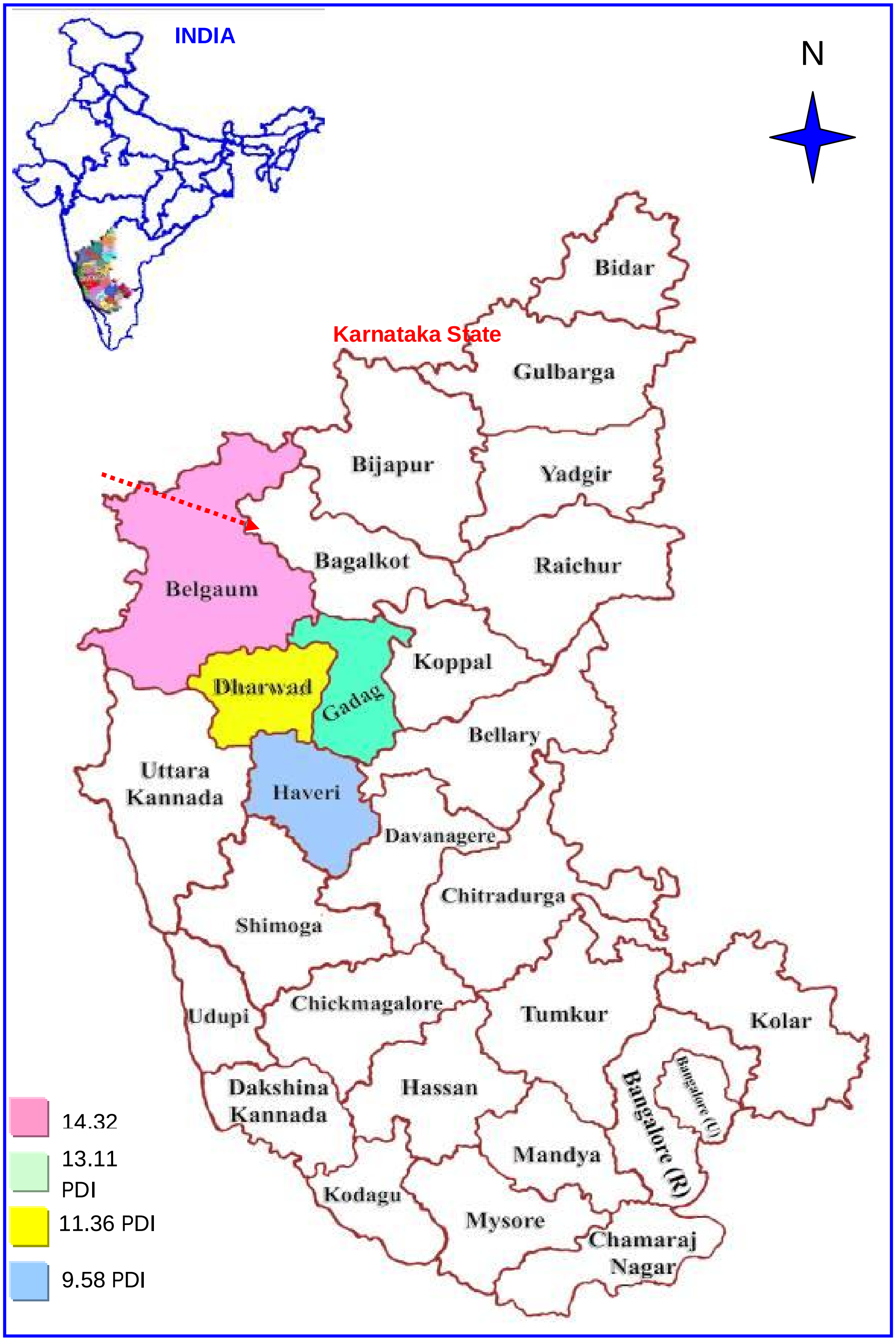


Fig. 1: Map showing the severity of bacterial blight of cowpea in northern districts of Karnataka

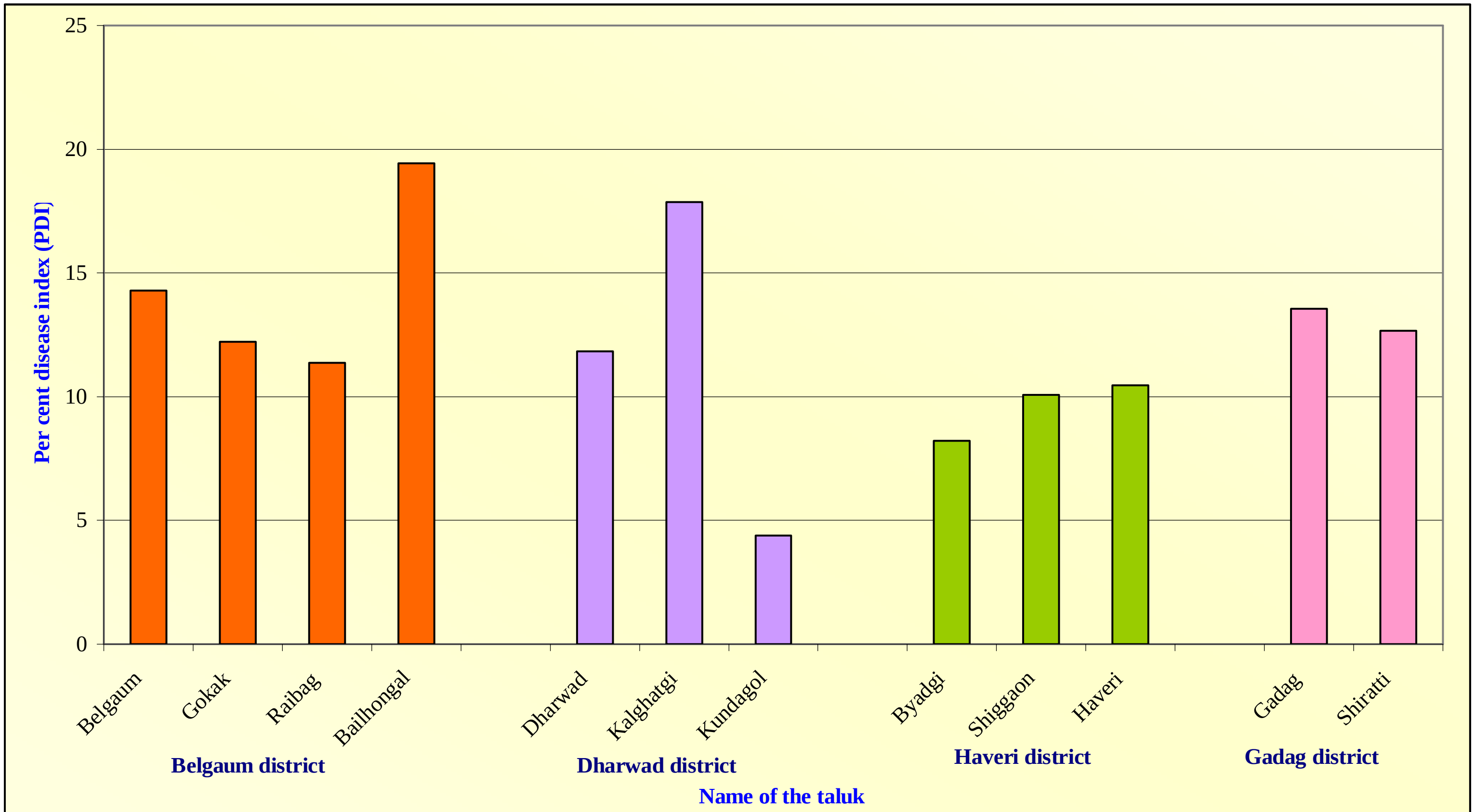


Fig. 2: Average mean incidence of bacterial blight of cowpea in northern districts of Karnataka

Fig**. 2: Average mean incidence of bacterial blight of cowpea in northern districts of Karnataka**

Three villages were surveyed in Kalghatki taluka where the disease index ranged from 13.82 to 21.33 PDI. The maximum disease index (21.33 PDI) was observed in Kalghatki village with a PDI range of 10-30, followed by Kanavihonnapur village (18.46 PDI with a range of 10-25 PDI) and the least disease index was observed in Hirehonnalli (13.82 PDI with a range of 5-15 PDI).

In Kundagol taluka, four villages were surveyed and the disease index ranged from 2.52 to 6.29 PDI. In Devikoppa village, maximum disease index (6.29 PDI) was observed with a PDI range of 0-10, followed by Thimmapur village (5.46 PDI with a range of 0-7 PDI) and the least disease index was observed in Dummawada (2.52 PDI with a range of 0-5 PDI).

4.1.3 Haveri district

Three talukas were surveyed in Haveri district *viz.*, Byadgi, Shiggaon and Haveri. The disease index observed in all the surveyed areas ranged from 4.68 to 14.23 PDI. The mean maximum disease index (10.46 PDI) was observed in Haveri taluka, followed by Shiggaon taluka (10.07 PDI) and the least index was observed in Byadgi taluka (8.21 PDI).

In Byadgi taluka, five villages were surveyed. The disease index was ranged from 4.68 to 11.32 PDI. The maximum disease index (11.32 PDI) was observed in Dummihala with a PDI range of 5-20, followed by Kummur (10.57 PDI with 5-15 PDI range) and the least index was observed in Anuru (4.68 PDI with 0-5 PDI range).

The disease index ranged from 9.75 to 10.38 PDI in Shiggaon taluka where only two villages were surveyed. The maximum disease index (10.38 PDI) was observed in Hanumaralli with a PDI range of 5-15 and the least disease index was observed in Bankapura (9.75 PDI with a PDI range of 3-12).

Among four villages surveyed in Haveri taluka, the disease index ranged from 6.38 to 14.23 PDI. The maximum disease index (14.23 PDI) was observed in Haveri with a PDI range of 10-25, followed by Devagiri (12.67 PDI with a range of 9-20 PDI) and the least index was observed in Hosalli (6.38 PDI with a PDI range of 3-10).

4.1.4 Gadag district

Two talukas were surveyed in Gadag district *viz.*, Gadag and Shirahatti. The disease index observed in all the surveyed areas ranged between 10.45 to 17.17 PDI. The mean maximum disease index (13.56 PDI) was observed in Gadag taluka and the least disease index was observed in Shiratti taluka (12.67 PDI).

Among five villages surveyed in Gadag taluka, disease index ranged from 10.45 to 17.17 PDI. The maximum disease index (17.17 PDI) was observed in Beldadi village with a PDI range of 15-30, followed by Mulgund village (15.61 PDI with a range of 10-25 PDI) and the least disease index was observed in Harti village (10.45 PDI with a PDI range of 5-15).

In Shirahatti taluka, three villages were surveyed and the disease index ranged from 11.62 to 14.05 PDI. The maximum disease index (14.05 PDI) was observed in Magadi village with a PDI range of 11-21, followed by Shirahatti village (12.33 PDI with a range of 6-24 PDI) and the least disease index was observed in Lakshmeshwara village (11.62 PDI with a PDI range of 5-20).

4.1.5 Isolation and identification of the pathogen

4.1.5.1 Collection of diseased samples

Cowpea infected with bacterial blight samples were collected during June to August, 2011 from different cowpea growing regions of Karnataka *viz.*, Belgaum, Dharwad, Haveri and Gadag districts.

4.1.5.2 Symptomatology

The symptoms were observed on leaf, stem and pods. Initially small, water soaked, brown to black coloured lesion were noticed on the upper surface of the leaves. Correspondingly on lower surface, diffused water soaked zone was seen around the spot. Spots were round, angular to irregular in shape.

Angular spots leaves

Brown spots with yellow margin

Angular spots on veins

Blight symptom on leaves

Infection on stem

Infection on green pods

Infection on dry pods

Plate 1. Typical symptoms of bacterial blight on leaves, stem and pods of cowpea

As the disease progressed, these spots grew, increased their size, coalesce and extended upto midrib in a week's time covering the major portion of the leaf lamina. Severely infected leaves turned yellow, became chlorotic and finally fall off.

The cankers were developed on the stem near the union of cotyledons and first leaves. Such stems were unable to bear the load of the plant and easily break in strong winds. On pods, deep green or water-soaked streaks were formed. Such pods became yellow, shriveled and died. The diseased pods produced smaller, wrinkled and infected seed and these served as primary source of inoculum (Plate 1).

4.1.5.3 Isolation of pathogen and maintenance of pure culture

Cowpea showing typical symptoms of bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* (Burkh.) Vauterin *et al.* were collected during the *kharif* season of 2011-12 from major cowpea growing areas of northern Karnataka. The bacterium was isolated by extracting the ooze in sterile distilled water taken in test tubes followed by dilution plate technique on nutrient agar.

Small pieces of infected leaves were cut aseptically from the edge of typical spots along with a little portion of healthy tissue. The infected leaf bits were surface sterilized in one per cent sodium hypochlorite and washed in three series of sterile water to remove traces of sodium hypochlorite. The infected leaf bits were then suspended in a small test tube containing 3 ml sterilized distilled water for 10 min. When water became slightly turbid due to oozing of bacterial cells from the cut ends of the diseased tissue, the bacterial suspension was serially diluted in 9 ml sterile distilled water. Then one ml of the diluted bacterial cell suspension was poured into sterilized petriplates containing nutrient agar. The plates were rotated gently in clockwise and anti-clockwise direction, so as to distribute the bacterial cell suspension uniformly in the plates and to obtain well separated bacterial colonies. The inoculated plates were incubated at 28^0 C for 72 hours. Observations were made for development of well separated light yellow, convex, small bacterial colonies on the nutrient agar medium (Plate 2).

4.1.5.4 Purification of bacterial culture

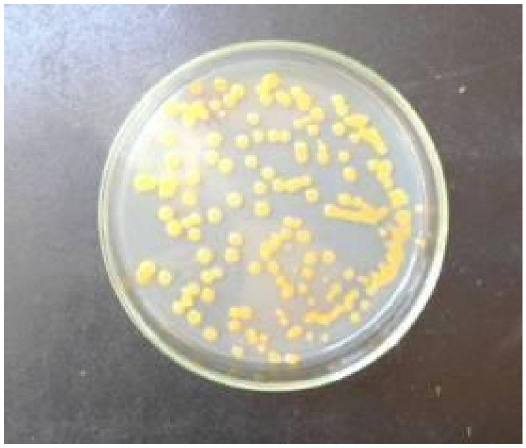
The suspected bacterial colonies were picked with the help of sterilized inoculation loop and streaked on the surface of Yeast extract Dextrose Calcium carbonate Agar (YDCA, Schaad and Stall, 1988). The inoculated plates were incubated at 28^0 C for 48 to 72 h. and the observations were made for the development of well separated light yellow coloured bacterial colonies. The purified bacterial colonies were streaked on nutrient agar slants and stored at 5^0 C in refrigerator. Culture was also matianed in sterile distilled water taken in small culture tubes, by suspending 2-3 loopful of the bacterial culture for future use (Plate 2).

4.1.5.5 Pathogenicity on cowpea

Koch's postulates were followed to prove pathogenic nature of *Xanthomonas axonopodis* pv. *vignicola*. Cowpea plants were raised in sterilized soil in pots. Twenty five to thirty days old plants were used for the purpose of inoculation of the bacterial isolate (Plate 2).

The strain of *Xanthomonas axonopodis* pv. *vignicola* from cowpea was multiplied in nutrient broth taken in Earleyenmayer's flask by inoculating a loopful of bacterial culture to nutrient broth. The inoculated flask was incubated at 28^0 C for 72 hours. Bacterial suspension was prepared by adjusting cell concentration to 5×10^6 cfu per ml in spectrophotometer at 420 nm (Spectronic 20 D, Milton and Roy, USA). The cowpea plants were pre-incubated for 24 hours in humid tent made up of plastic sheets in which humidity was maintained between 60 to 80 per cent before the inoculation.

The leaves of cowpea plants were slightly injured by inserting pin and sprayed with bacterial suspension. The inoculated plants were kept in the plastic tent for two days in which high humidity was maintained by spraying sterile water inside the tent at 25 to 30 ^{0}C. The plants were taken out from the plastic tent and kept in glasshouse. Observations were made for the development of symptoms of bacterial blight. Plants similarly, injured and sprayed with sterile water served as control. The bacterium was reisolated and compared with the original culture of *X. axonopodis* pv. *vignicola* by studying the colony characters, staining and morphology.



Pure culture on nutrientagar medium

Pure culture on Ydca medium

Pot Experiment to prove pathogenicity

Plate 2. Maintenance of pure culture and pathogenicity studies

Plate 3. Disease scale for bacterial blight of cowpea

4.1.5.6 Morphological, physiological and biochemical characteristics

The results of the various morphological, physiological and biochemical tests were conducted as explained in Materials and Methods and results are listed in the Table 3. The bacterium is a rod shaped strictly aerobic, gram negative, oxidase positive and monotrichously flagellated.

It was positive for catalase reaction, utilizes glucose (Dextrose), maltose and sucrose for acid production, positive for liquefaction of gelatin and produced hydrogen sulphide and did not produce indole, in addition, the strain failed to reduce nitrate to nitrite (Plate 4).

Catalase test

After covering with few drops of 20 per cent hydrogen peroxide to a slide smeared by a loopful of test bacteria, there was production of gas bubbles indicating the positive reaction for the test.

Gelatin liquefaction

In this test, a white precipitate was observed around the spot inoculated bacterium when flooded with the solution containing 0.2% mercuric chloride solution in dilute HCl (20%).

Hydrogen sulphide production

It was observed that bacterium produced the gas and it reacted with the lead acetate strips and gave black colour of test strips which indicated the liberation of H_2S. In the present study the bacterium showed positive reaction for this test.

Starch hydrolysis

In this test, a colorless zone around the bacterial growth was observed in contrast to the blue background of the medium.

Acid production from Sucrose, Maltose, Dextrose and Mannitol

The results presented in the Table 3 and Plate 4 indicated the difference in ability to produce acid by *X. axonopodis* pv. *vignicola* from different carbon sources *viz.,* Sucrose, Maltose, Dextrose and Mannitol.

At 24 hrs after inoculation, test tubes inoculated with *Xanthomonas axonopodis* pv. *vignicola* along with sucrose, maltose and dextrose showed very strong acid production which is indicated by the change in colour from dark blue to yellow colour compared to the control in which only Dye C medium was used without any carbon source whereas, mannitol showed negative reaction without producing acid.

Lactose utilization

The result showed yellow coloured colony on the lactose negative medium and pale white coloured colony on the lactose positive medium indicating the utilization of lactose by the bacterial blight pathogen *Xanthomonas axonopodis* pv. *vignicola*.

4.2 Detection of pathogen in seeds by non serological methods

4.2.1 Efficacy of selective and semi-selective media for the growth of *Xanthomonas axonopodis* pv. *vignicola*

Of the various selective and semi-selective media tested for their efficacy in supporting the growth of *Xanthomonas axonopodis* pv. *vignicola,* the NSCAA (Nutrient Starch Cycloheximide Antibiotic Agar) medium was found to be more efficient with highest number of bacterial colonies (132×10^5 cfu/ml) when compared to nutrient agar (75×10^5 cfu/ml).

The next best medium which supported the growth of *X. axonopodis* pv. *vignicola* was SIBU agar medium (126×10^5 cfu/ml colonies) followed by XTS medium (118×10^5 cfu/ml) (Table 4, Fig. 3 and Plate 5).

However, the colony diameter was found to be more in XTS (2-4mm) as compared to NSCAA medium (2-3mm).

Typical colonies appeared within 48 hr. on NSCAA whereas; it took 72 hr. to produce them on XTS and SIBU agar media. On NSCAA medium, the colonies of *X. axonopodis* pv. *vignicola* were round, shiny, raised, yellow coloured with dark centre and translucent hallow. Characters of *X. axonopodis* pv. *vignicola* colonies on various selective and semi-selective media are given in Table 4.

4.2.2 Detection of *Xanthomonas axonopodis* pv. *vignicola* from the seeds of naturally infected cowpea plants by Van vuurde *et al.* (1983) method

The pathogen was isolated from seed extracts obtained by soaking of 25 unsterilized and sterilized cowpea seeds each of cultivars, C-152 (S), DCS 47-1(R), C-152 × IC202709 (MS) and V-118 × IC257422 (MR) in 25 ml sterile distilled water for 24 hr. at 5^0C as explained by Van vuurde *et al.*, 1983, followed by direct plating the seed extract on the surface of nutrient agar and NSCCA media which was found efficient in recovering the maximum no. of bacterial colonies (132×10^5 cfu/ml) in the previous test and also it is specially meant for the isolation of bacteria from seeds.

In this investigation, unsterilized diseased seeds of susceptible variety, C-152 yielded 35×10^2 cfu/ml on NSCAA medium whereas, sterilized seeds yielded 18×10^2 cfu/ml and apparently healthy seeds of same susceptible variety C-152 yielded only 12×10^2 cfu/ml. from unsterilized seeds and sterilized seeds yielded 3×10^2 cfu/ml. Healthy seeds of resistant variety DCS 47-1 not yielded any colonies whereas unsterilized diseased seeds of DCS 47-1 yielded 2×10^2 colonies (Table 5).

4.3 Screening of varieties/germplasm lines against bacterial blight of cowpea

Fifteen different varieties were tested at the Main Agricultural Research Station Dharwad during *Kharif* 2011 against *X. axonopodis* pv. *vignicola* under natural conditions.

The plants showing characteristic blight symptoms on cowpea plants were considered for disease scoring, the scoring was done using 0-5 scale (Shah *et al.*, 1991). The level of infection among the different varieties varied from 2.16 to 62.43 per cent. The reactions of cowpea varieties/genotypes infection during *Kharif* 2011 revealed that out of 15 varieties / genotypes screened two varieties, RC-101(3.55 PDI) and DCS 47-1 (2.16 PDI) showed resistant reaction, another two varieties, V-118 × IC257422 (12.31 PDI) and DC-15 (9.78 PDI) showed moderately resistance reaction. Five varieties namely C-152 ×IC202709 (26.38 PDI), V-118× GL (23.67 PDI), GC-3-300 γ (28.19 PDI), GL-300 γ (25.26 PDI) and Bailhongal Local- 300γ (30.37 PDI) showed moderately susceptible reaction, C-152×IC 208618 (40.25 PDI), GC-3-200 γ (33.04 PDI), GL-200 γ (36.42 PDI), GC-3 (43.36 PDI) and C-152 (48.81 PDI) varieties showed susceptible reaction whereas only one variety C-152 × GL (62.43 PDI) showed highly susceptible reaction (Table 6, Fig. 4 and Plate 3 and 6).

4.4 *In vitro* and *in vivo* evaluation of antibacterial chemicals, plant extracts / organics and antagonistic microorganisms on the growth of *Xanthomonas axonopodis* pv. *vignicola*

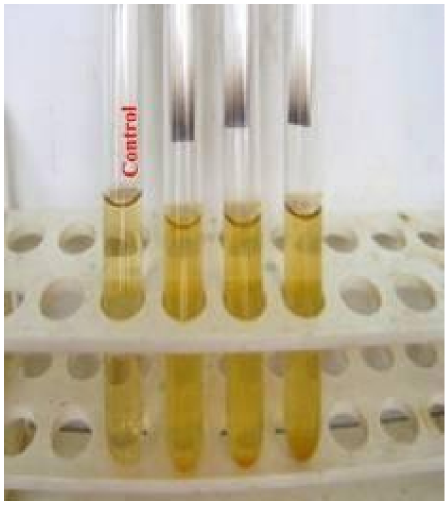
4.4.1 *In vitro* evaluation of antibacterial chemicals on the growth of *Xanthomonas axonopodis* pv. *vignicola*

The investigation was carried out to evaluate commercially available antibacterial chemicals to find out their effectiveness against the growth of *Xanthomonas axonopodis pv. vignicola* under *in vitro* condition and the results were presented in Table 7, Fig. 5 and Plate 7.

The results indicated that streptocycline + copper oxychloride had showed significant superiority over other treatments with highest inhibition (2.97 cm) at 0.05 + 0.3 per cent followed by streptocycline at 0.1 per cent (2.40 cm) and copper oxychloride (2.33 cm). However, all other chemicals *viz.*, bordeaux mixture, copper hydroxide, plantomycin, bacterinashak and bleaching powder were on par with each other, whereas, captan was least effective.

Table 3: Morphological and biochemical characterization of *Xanthomonas axonopodis* pv. *vignicola*

Test	Inference
Colony morphology on YDCA medium	
Configuration	Round
Margin	Entire
Elevation	Convex
Surface	Smooth
Pigment	Yellow
Shape	Rod
Arrangement	Single
Biochemical characterization	
Gram reaction	-
Starch hydrolysis	+
Gelatin liquefaction	+
H_2S production	+
Catalase test	+
Acids from	
a) Maltose	+
b) Sucrose	+
c) Dextrose	+
d) Mannitol	-
Lactose utilization	+



H_2 S production

Acid production from different carbon sources

Gelatin liquefaction

Starch hydrolysis

Lactose utillization

Plate 4. Biochemical tests to characterize *Xanthomonas axonopodis* pv. *vignicola*

The interaction effect among the chemicals and concentrations indicated that streptocycline (0.05%) + copper oxychloride (0.3%) and streptocycline (0.1%) alone were found significantly superior over other treatments with an inhibition zone of 2.97cm and 2.40 cm, respectively.

The effect of copper oxy chloride and bordeaux mixture were on par with each other at all the concentrations. They showed maximum inhibition zone of 2.33cm (0.3 per cent) and 1.97cm (1.0 %) concentrations, respectively followed by copper hydroxide at 0.3 per cent (1.72cm), plantomycin at 0.1 per cent (1.50 cm), bacterinashak at 0.1 per cent (1.47) and bleaching powder at 1.0 per cent (1.13 cm).

The captan was found least effective at higher concentrations also. Captan with 0.1 and 0.2 per cent concentration produced inhibition zone of 0.70 cm and 0.82 cm respectively. It was not effective even at highest concentration (0.3 per cent) with an inhibition zone of 0.87 cm indicating its ineffectiveness against bacterial blight pathogen.

4.4.1.1 Bioefficacy retention of different chemicals

This study was conducted to know the retention capacity of different chemicals in inhibiting the pathogen over a period of time. The results indicated that bleaching powder and captan showed 100 per cent reduction after five days (120 h) of incubation whereas; streptocycline + copper oxy chloride and copper oxy chloride alone showed least per cent reduction *i.e.*, 5.72 and 5.57 per cent respectively even after 120 h (Table 8, Fig. 6).

4.4.2 *In vitro* evaluation of botanicals and organics on the growth of *Xanthomonas axonopodis* pv. *vignicola*

In vitro evaluation of botanicals was carried out with respect to inhibition zone produced due to inhibition of *Xanthomonas axonopodis* pv. *vignicola* at different concentrations as explained in the Material and Methods and the data is presented in Table 9, Fig. 7 and Plate 8.

The results indicated that garlic bulb extract at 10 per cent concentration showed highest inhibition of 1.18 cm followed by soapnut extract at 10 per cent conc. with an inhibition of 1.07 cm and they were on par with each other and found significantly superior over other treatments.

The interaction effect among the botanicals and concentration indicated that garlic extract was found most effective at 10 per cent concentration with an inhibition zone of 1.18 cm. Neem (0.68 cm), tulsi (0.78 cm) and neelgiri (0.77 cm) extracts were moderately effective and were on par with each other at 10 per cent concentration whereas, lantana, durantha, onion and chilli extracts were ineffective in inhibiting the bacteria.

4.4.3 *In vitro* evaluation of bioagents on the growth of *Xanthomonas axonopodis* pv*. vignicola*

The antagonistic microorganisms *viz.*, *Trichoderma harzianum*, *Pseudomonas fluorescens* migula (five different isolates), *Methylobacterium* spp. Whitney and Busy (five isolates) and *Bacillus subtilis* Cohn. were evaluated against *Xanthomonas axonopodis* pv. *vignicola* under *in vitro* condition by inhibition zone method as explained in the Material and Methods. Inhibition zone produced across the antagonistic microorganisms was recorded and presented in Table 10.

The results indicated that the antagonistic microorganism *Pseudomonas fluorescens* resulted in maximum inhibition of the *Xanthomonas axonopodis* pv. *vignicola.* Out of different strains of *Pseudomonas* used, strain No.1 inhibited *X. axonopodis* pv. *vignicola* with an inhibition zone of 2.07 cm followed by strain No.4 with an inhibition zone of 2.03 cm. Other strains *viz.*, strain No. 2(1.80cm), strain No.3 (1.53 cm) and strain No. 5 (1.60 cm) were on par with each other. Treatments involving *Pseudomonas fluorescens* were found significantly superior over other treatments followed by *Bacillus subtilis* (0.82 cm). Out of five *Methylobacterium* strains used, four of the strains were found little effective against *X. axonopodis* pv. *vignicola* with 0.67, 0.55, 0.68 and 0.65 cm inhibition. However the fungal antagonist like *T. harzianum* was found ineffective as it failed to inhibit the growth of *X. axonopodis* pv. *vignicola* (Table 10, Fig. 8 and Plate 9).

Table 5: Detection of *Xanthomonas axonopodis* pv. *vignicola* in naturally infected seeds by Van Vuurde *et al*. (1983) method

Treatment		Population of bacterial colony (cfu/ml)*	
		Unsterilized seeds	Sterilized seeds
Apparently Healthy seeds	C-152	12×10^2	3×10^2
	C-152 × IC202709	6×10^2	1×10^2
	V-118 × IC257422	2×10^2	-
	DCS 47-1	-	-
Diseased seeds	C-152	35×10^2	18×10^2
	C-152 × IC202709	21×10^2	7×10^2
	V-118 × IC257422	5×10^2	1×10^2
	DCS 47-1	2×10^2	1×10^2

*Average of three replications

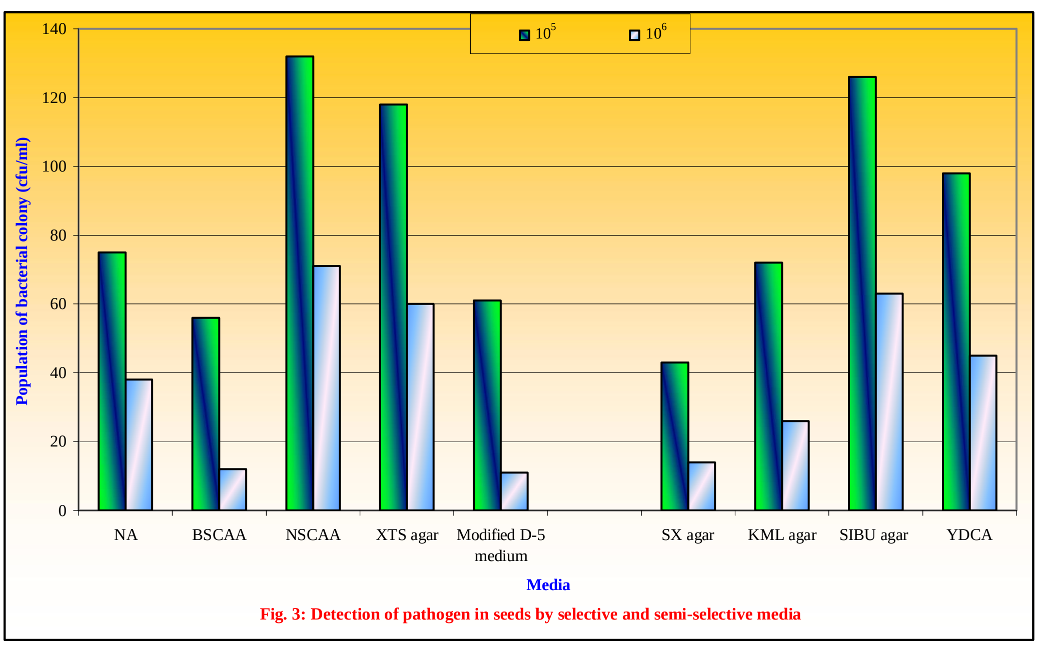
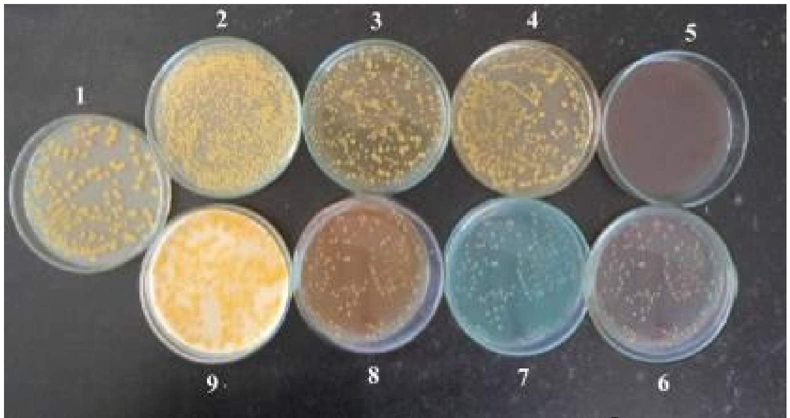
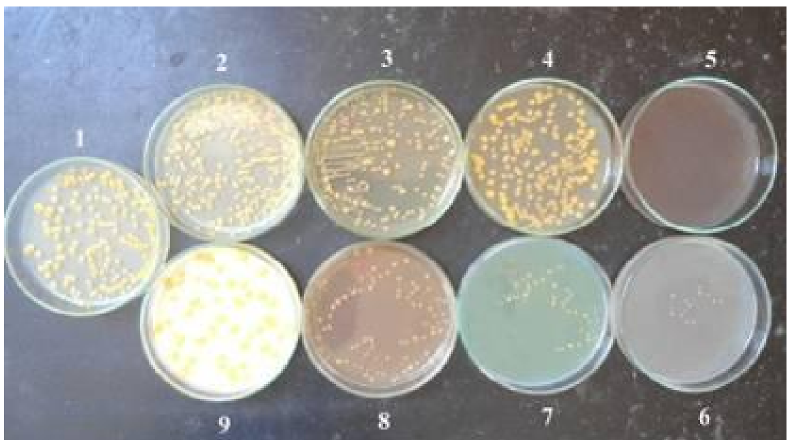


Fig. 3: Detection of pathogen in seeds by selective and semi-selective media



a) X. axonopodis pv. Vignicola colonies at 10^5 dilution



b) X. axonopodis pv. Vignicola colonies at 10^6 dilution

NSCAA medium **SIBU agar medium**

1) NA **2)NSCAA** **3) XTS** **4)SIBU**

5)SX **7) KML** **7)BSCAA** **8)D – 5 media**

9)YDCA

Plate 5. Detection of *X. axonopodis* pv. *vignicola* from seeds using different solid media

4.4.4 *In vivo* evaluation of different chemicals/bioagents/botanicals against bacterial blight of cowpea caused by *Xanthomonas axonopodis* pv. *vignicola*

This experiment was conducted in glass house conditions using different chemicals/bioagents/botanicals which were found superior in inhibiting the growth of bacterial blight pathogen under *in vitro* conditions.

Initially the pots containing 25 to 30 days old cowpea plants were artificially inoculated with *Xanthomonas axonopodis* pv. *vignicola* by spraying the bacterial suspension of 5×10^6 cfu/ml. After 6 to 8 days when characteristic bacterial blight symptoms appeared on cowpea plants, the spraying of different chemicals/ bioagents/ botanicals was taken up with suitable concentration.

The results indicated that minimum Per cent Disease Index (3.11%) was observed in treatment T_1 involving Streptocycline + COC (0.05 + 0.3%) spray followed by treatments T_2 (Streptocycline spray at 0.1%) and T_3 (*Pseudomonas fluorescens* spray at 0.5% conc.) with PDI of 5.37 and 12.14 per cent respectively compared to untreated control (T_7) where the PDI was maximum (28.45%). The results are presented in Table 11, Fig. 9 and Plate 10.

The per cent disease reduction over control was maximum in treatment Streptocycline + COC (0.05 + 0.3%) with 89.07 per cent followed by Streptocycline spray at 0.1 per cent (81.12%). Spray with *Pseudomonas fluorescens* also reduced the disease to the tune of 57.33 per cent.

4.4.5 Effect of hot water treatment on seed germination and seedling infection of cowpea

The artificially inoculated cowpea seeds were subjected to hot water treatment at 48, 50, 52, 54 and 56^0C for 5 and 10 min. This experiment was conducted in roll paper method and pot culture as explained in Materials and Methods. The data are presented in Table 12, Fig. 10a, 10b and Plate 11.

In roll paper method, the seeds treated with hot water at 52^0C for 10 min. were very effective in eliminating the seed borne pathogen as average no. of infected seedlings were 6.33. The germination of seeds was also not much affected (58.00 %) as compared to untreated control (72.00 %). However, average no. of infected seedlings was minimum in 56^0C for 10 min. (1.67) but germination percentage was least (47.00 %).

In pot culture experiment also, the seeds treated with hot water at 52^0C for 10 min was very effective in eliminating the seed borne pathogen as very little infection (3.21 %) was noticed in seedlings raised from such seeds without affecting germination (52.00 %).

The seeds treated at temperatures of 54 and 56^0C though resulted in the effective control of the disease, but these temperature levels had deleterious effect on the germination of seeds than 52^0C. The disease incidence and germination of seeds in the untreated control was 23.28 and 76.00 per cent respectively indicating the internally seed borne nature of the pathogen.

4.5 Integrated management of bacterial blight of cowpea (*Xanthomonas axonopodis* pv. *vignicola*) under field conditions

A field experiment was conducted during *Kharif* 2012 on the integrated management of bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* in the Institute of Organic Farming field located at University of Agricultural sciences, Dharwad.

Treatments were allocated under Randomized Completely Block Design (RCBD) as described under Material and Methods. Observations recorded on the germination percentage, disease severity and yield are presented in Table 13, Fig. 11and Plate 12.

Table 6: Screening of cowpea germplasm lines/varieties for resistance against *Xanthomonas axonopodis pv. vignicola* causing bacterial blight

Germplasm line/ variety name	Per cent Disease Index	Grade (0-5 scale)	Reaction
C-152 ×IC202709	26.38	3	MS
V-118× GL	23.67	3	MS
C-152×IC 208618	40.25	4	S
GC-3-200 γ	33.04	4	S
GC-3-300 γ	28.19	3	MS
GL-200 γ	36.42	4	S
GL-300 γ	25.26	3	MS
V-118 ×IC257422	12.31	2	MR
Bailhongal Local- 300 γ	30.37	3	MS
RC-101	3.55	1	R
DCS 47-1	2.16	1	R
DC-15	9.78	2	MR
GC-3	43.36	4	S
C-152	48.81	4	S
C-152 × GL (F_1)	62.43	5	HS

Note: 1 – R, 2 – MR, 3 – MS, 4 – S, 5 - HS

Table 6a: Grouping of cowpea genotypes based on their reaction for bacterial blight

Disease score	Variety/gerplasm line
0	Nil
1	RC-101, DCS 47-1
2	DC-15, V-118 ×IC257422
3	C-152 ×IC202709, V-118× GL, GC-3-300 γ, GL-300 γ, Bailhongal Local-300 γ
4	C-152×IC 208618, GC-3-200 γ, GL-200 γ, GC-3, C-152
5	C-152×GL (F_1)

*- γ indicates gamma irradiated varieties.

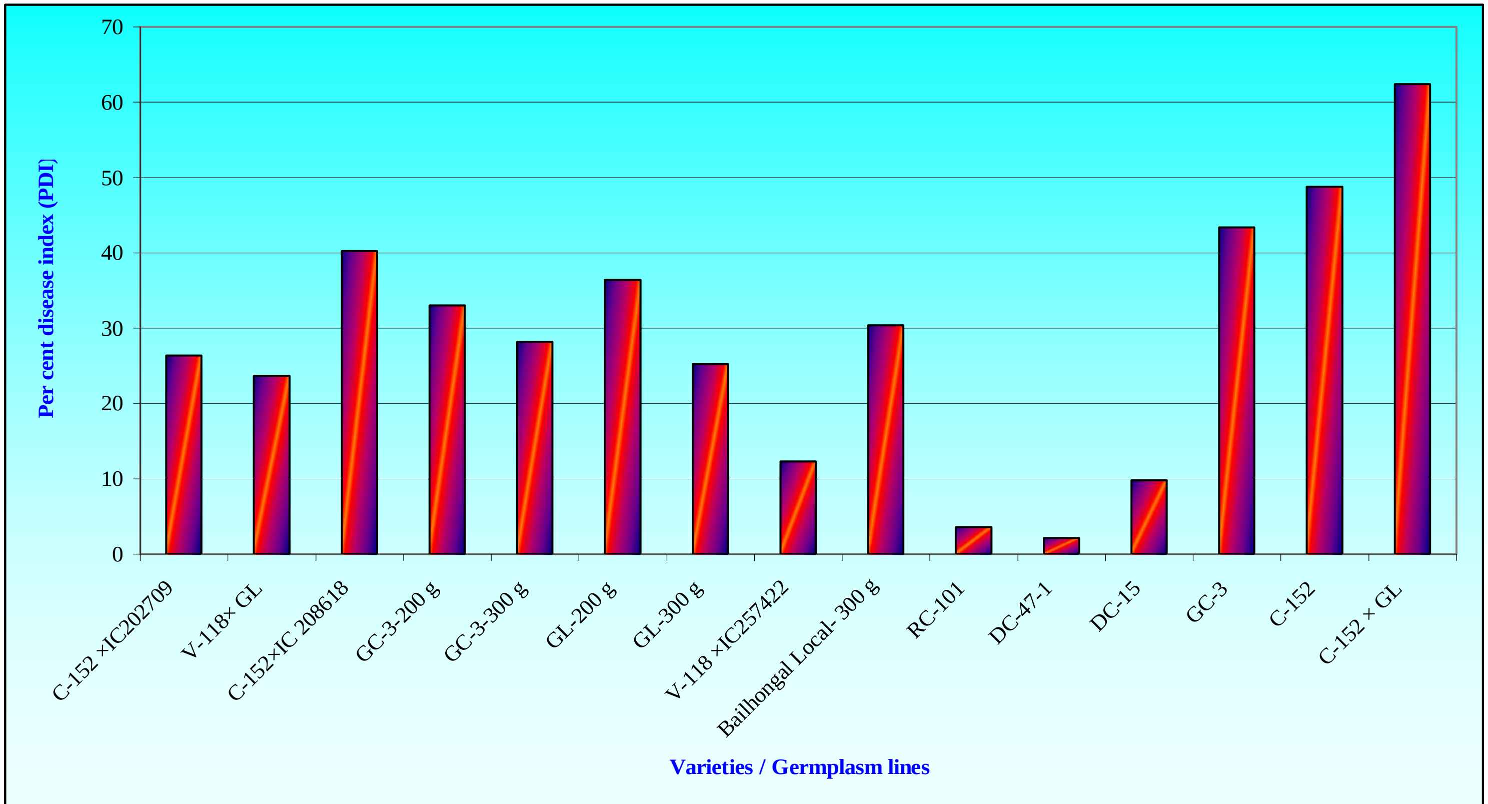


Fig. 4: Screening of cowpea varieties/germplasm lines for resistance against *Xanthomonas axonopodis* pv. *vignicola* causing bacterial blight

Fig. 4: Screening of cowpea varieties/germplasm lines for resistance against Xanthomonas axonopodis pv. vignicola causing bacterial blight

Experimental filed view

Resistant variety (DCS 47 – 1)

Resistant variety (RC – 101)

Susceptible line (C – 152 x GL) F_1

Plate 6. Screening of different germplasm lines/varieties against bacterial blight of cowpea

Table 7: *In vitro* evaluation of antibacterial chemicals on the growth of *Xanthomonas axonopodis* pv. *vignicola*

Name of the chemical	Concentration (%)	Mean diameter of the inhibition zone(cm)
Streptocycline	0.05	1.60 (1.61)*
	0.075	2.37 (1.83)
	0.1	2.40 (1.84)
Streptocycline + Copper oxychloride	0.05 + 0.1	2.17 (1.78)
	0.05 + 0.2	2.37 (1.83)
	0.05 + 0.3	2.97 (1.99)
Copper oxychloride	0.1	1.70 (1.64)
	0.2	2.27 (1.81)
	0.3	2.33 (1.83)
Copper hydroxide	0.1	1.37 (1.54)
	0.2	1.60 (1.61)
	0.3	1.72 (1.65)
Captan	0.1	0.70 (1.30)
	0.2	0.82 (1.35)
	0.3	0.87 (1.37)
Plantomycin	0.05	1.00 (1.41)
	0.075	1.17 (1.47)
	0.1	1.50 (1.58)
Bleaching powder	0.5	0.67 (1.29)
	0.75	0.90 (1.38)
	1.0	1.13 (1.46)
Bacterinashak	0.05	1.10 (1.45)
	0.075	1.13 (1.46)
	0.1	1.47 (1.57)
Bordeaux mixture	0.5	1.53 (1.59)
	0.75	1.63 (1.62)
	1.0	1.97 (1.72)
Control	-	0.00 (1.00)
Factor	**SEm±**	**CD at 1%**
Chemicals	0.0087	0.0327
Concentration	0.0048	0.0180
Interaction	0.0151	0.0568

*- Figures in the parenthesis are $\sqrt{x+1}$ transformed values.

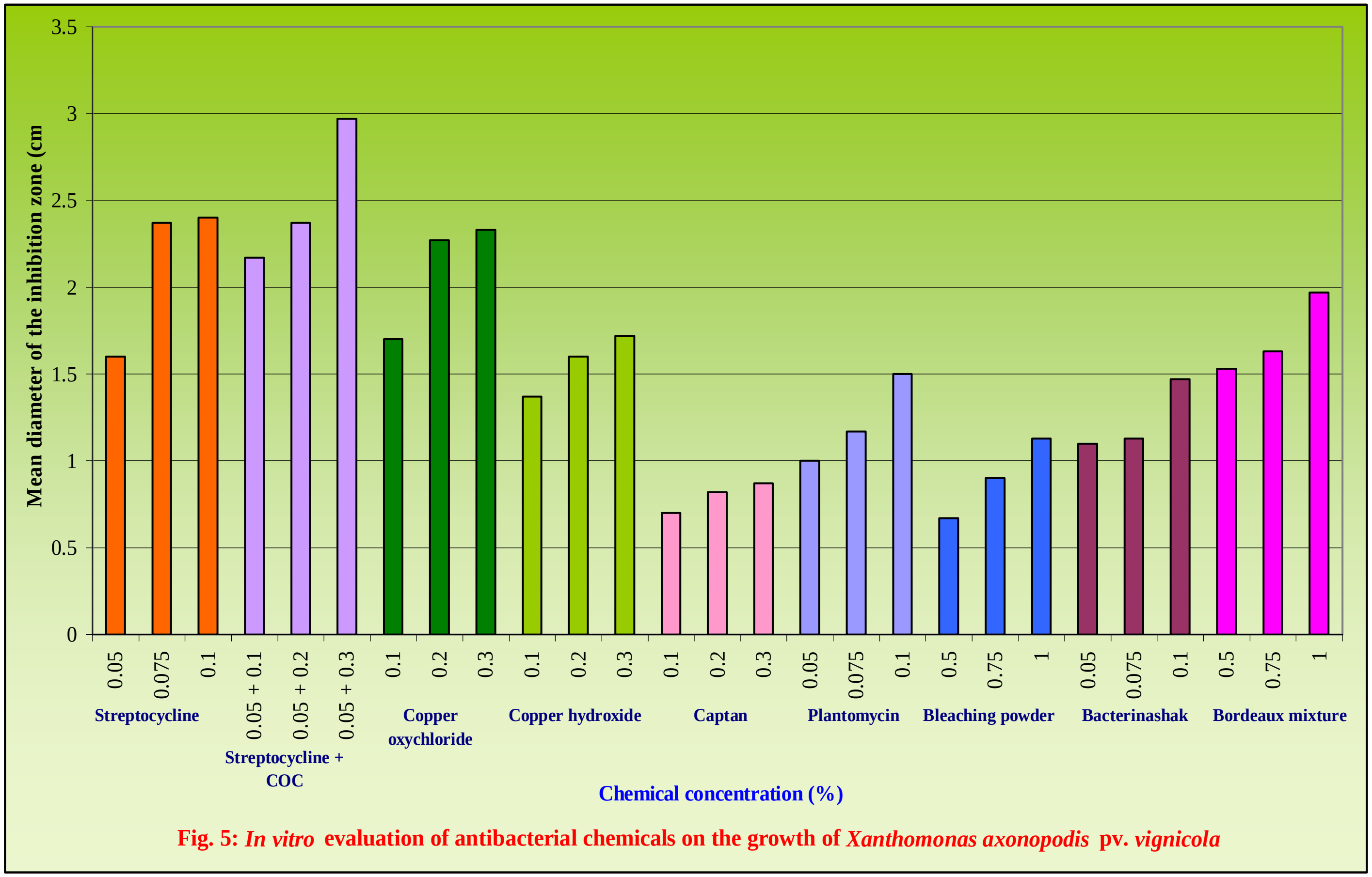


Fig. 5: In vitro evaluation of antibacterial chemicals on the growth of Xanthomonas axonopodis pv. vignicola

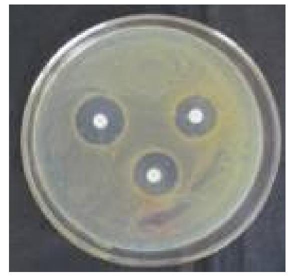
Captan (0.1%)

Captan (0.2%)

Captan 0.3%)

Bordeaux mixture (0.5%)

Bordeaux (0.75%)



Bordeaux mixture (1.0%)

Plantomycin (0.05%)

Plantomycin (0.075%)

Plantomycin (0.1%)

Streprocycline (0.05%)

Streprocycline (0.075%)

Steprocycline (0.1%)

Steprocycline (0.05%)
+ COC(0.1%)

Steprocycline (0.05%)
+ COC(0.2%)

Steprocycline (0.05%)
+ COC(0.3%)

Plate 7. *In vitro* evaluation of antibacterial chemicals on the growth of *X. axonopodis* pv. *Vignicola*

Plate 7. Cond……….

Copper oxychloride (0.1%)

Copper oxchloide (0.2%)

Copper Oxychloride (0.3%)

Copper hydroxide (0.1%)

Copper hydroxide (0.2%)

Copper hydroxide (0.3%)

Bacterinashak (0.05%)

Bacterinashak (0.07%)

Bacterinashak (0.1%)

Bleaching powder (0.5%)

Bleaching (0.75%)

Bleaching powder(1.0%)

Control

Table 8: Studies on bioefficacy retention of different chemicals over a period of time

Chemical	Inhibition zone (cm) at									% Reduction
	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h	
Streptocycline	2.40	2.35	2.35	2.33	2.32	2.30	2.27	2.20	2.20	8.33
Streptocycline + COC	2.97	2.95	2.95	2.92	2.90	2.86	2.85	2.82	2.80	5.72
Copper oxychloride	2.33	2.32	2.32	2.30	2.29	2.25	2.24	2.21	2.20	5.57
Copper hydroxide	1.72	1.70	1.70	1.68	1.63	1.60	1.60	1.57	1.55	9.88
Captan	0.87	0.86	0.86	0.84	0.80	0.65	0.50	0.00	0.00	100
Plantomycin	1.50	1.49	1.49	1.45	1.43	1.42	1.40	1.36	1.31	12.66
Bleaching powder	1.13	1.12	1.12	1.10	1.05	1.00	0.76	0.50	0.00	100
Bacterinashak	1.47	1.46	1.46	1.40	1.38	1.35	1.32	1.30	1.26	14.28
Bordeaux mixture	1.97	1.95	1.95	1.93	1.92	1.90	1.88	1.81	1.72	11.16

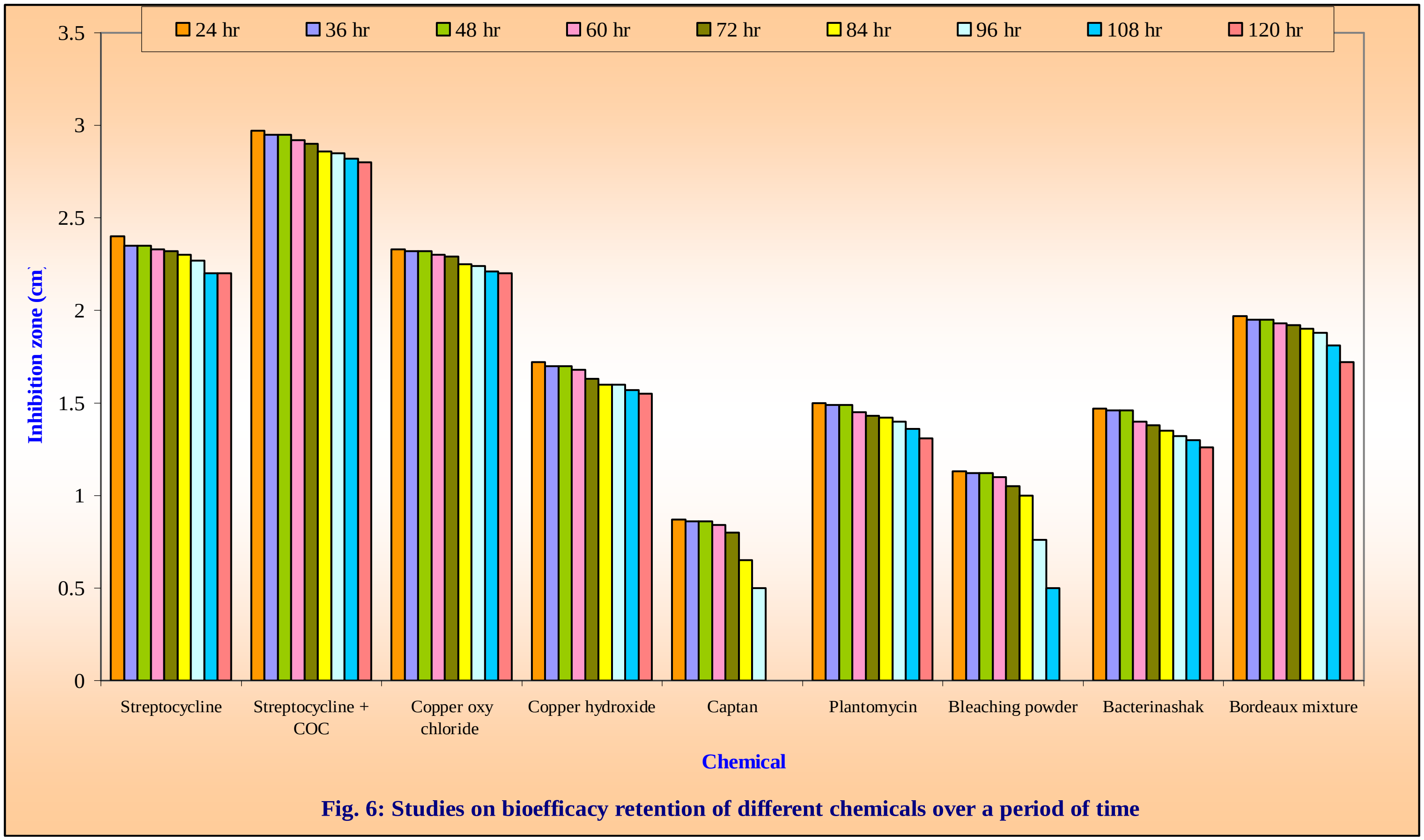


Fig. 6: Studies on bioefficacy retention of different chemicals over a period of time

Table 9: *In vitro* evaluation of botanicals against the growth of *X. axonopodis* pv. *vignicola*

Name of the Botanical	Botanical name	Plant Part used	Mean diameter of inhibition zone (cm) at different concentrations (%)		
			5	**7.5**	**10**
Lantana	*Lantana camera*	leaves	0.00 (1.00)*	0.00 (1.00)	0.00 (1.00)
Garlic	*Allium sativum*	bulb	0.67 (1.29)	0.83 (1.35)	1.18 (1.48)
Neem	*Azadirachta indica*	leaves	0.00 (1.00)	0.00 (1.00)	0.68 (1.30)
Soapnut	*Sapindus mukorossi*	pulp	0.63 (1.28)	0.77 (1.33)	1.07 (1.44)
Durantha	*Durantha repens*	leaves	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Onion	*Allium cepa*	bulb	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Tulsi	*Ocimum sanctum* L. cv. Purple	leaves	0.00 (1.00)	0.55 (1.24)	0.78 (1.34)
Nilgiri	*Eucalyptus globules*	leaves	0.00 (1.00)	0.63 (1.28)	0.77 (1.33)
Chilli	*Capsicum annum*	leaves	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control			0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Factors			**Botanicals**	**Concentration**	**Interaction**
SEm±			0.0050	0.0188	0.0086
CD at 1%			0.0027	0.0101	0.0323

*- Figures in the parenthesis are $\sqrt{x+1}$ transformed values.

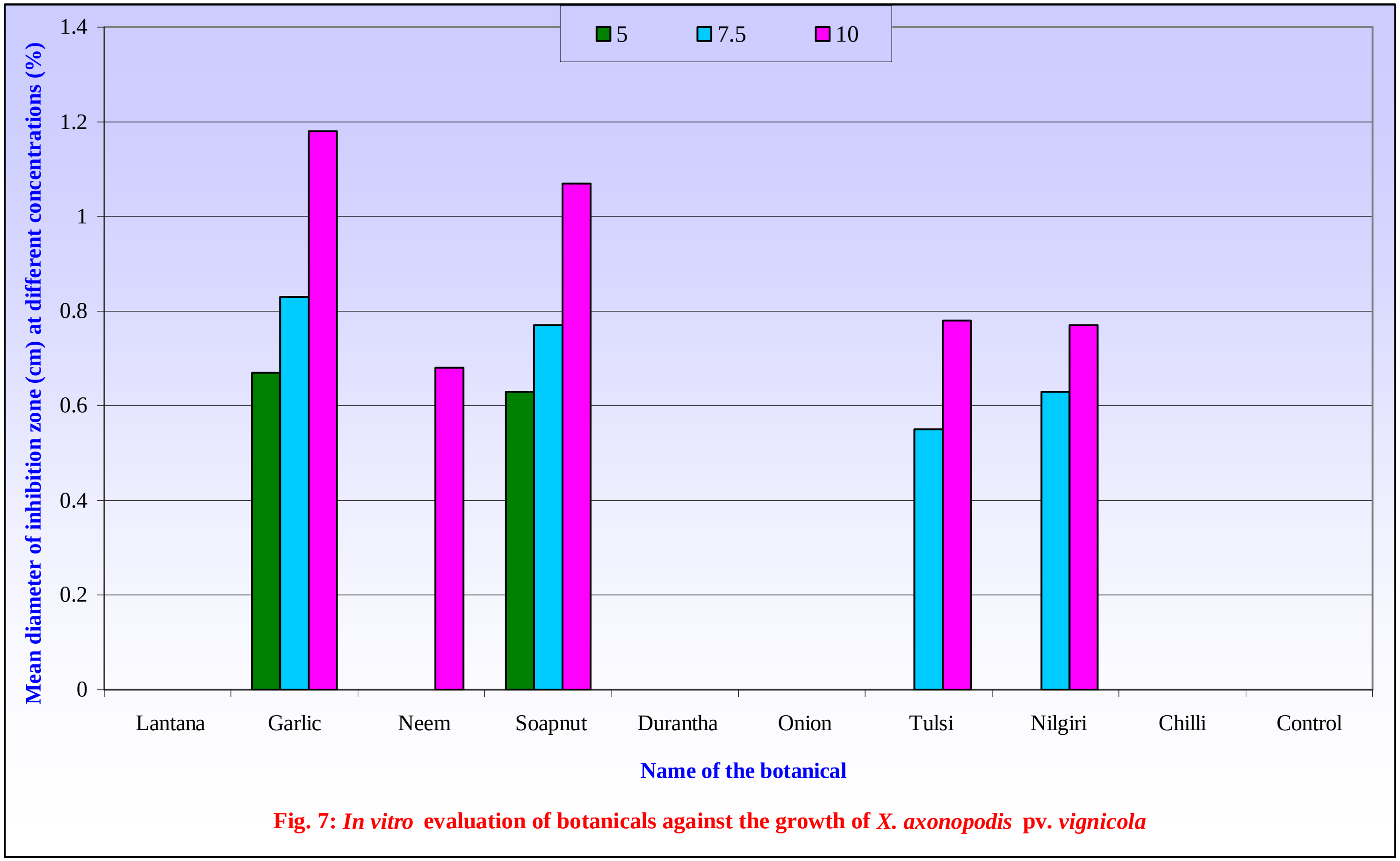


Fig. 7: In vitro evaluation of botanicals against the growth of X. axonopodis pv. vignicola

Garlic

Tulsi

Duranbtha

Neem

Onion

Lantana

Nilgiri

Soapnut

Chilli

Contorol

Plate 8. *In vitro* evaluation of botanicals on the growth of *X. axonopodis* pv. *vignicola*

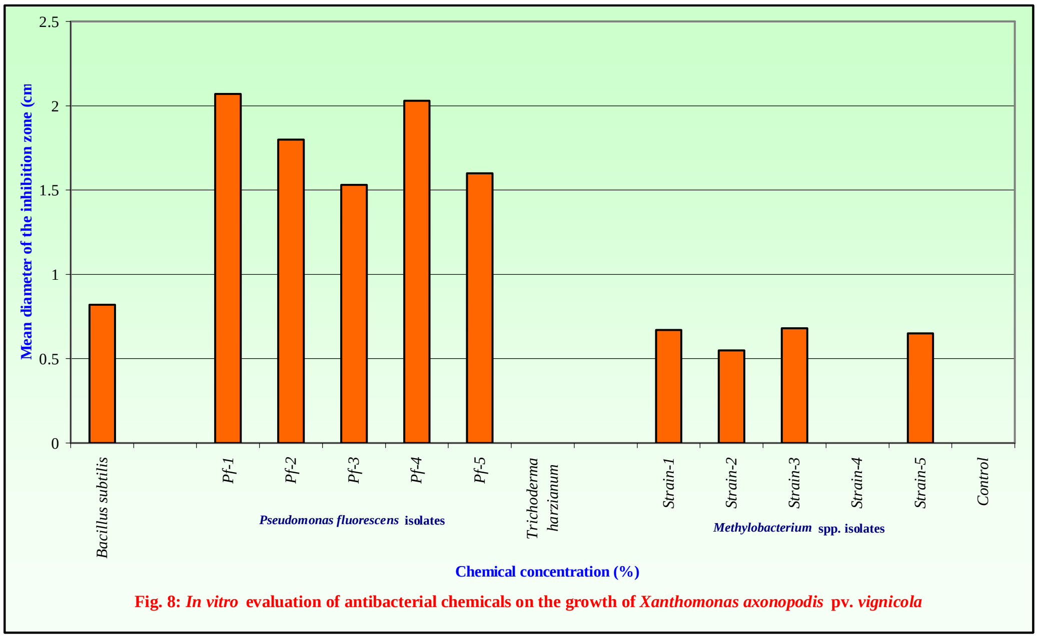


Fig. 8: In vitro evaluation of antibacterial chemicals on the growth of Xanthomonas axonopodis pv. vignicola

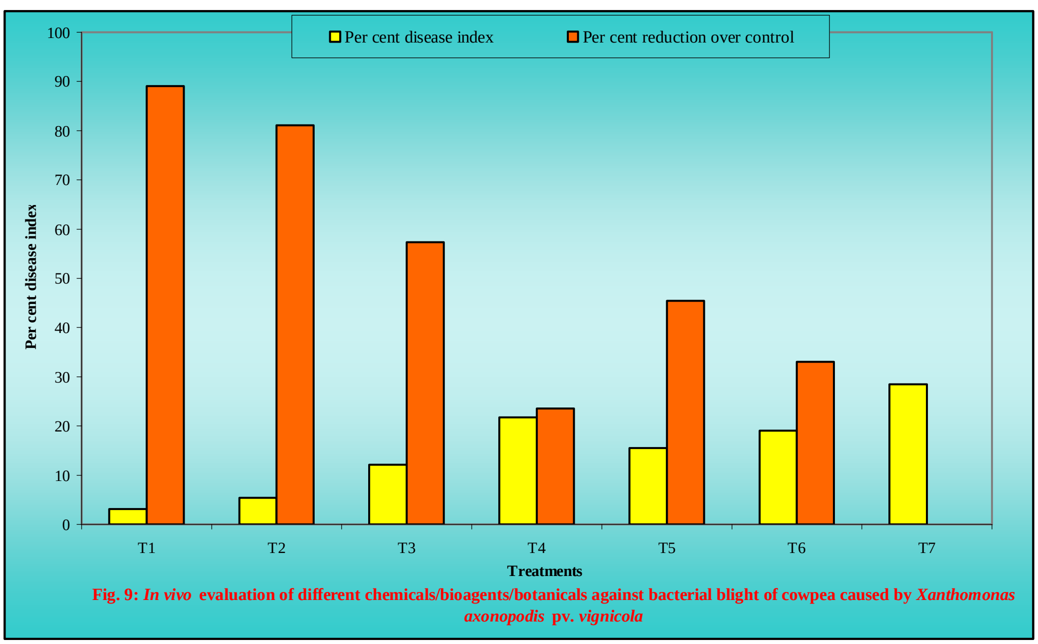
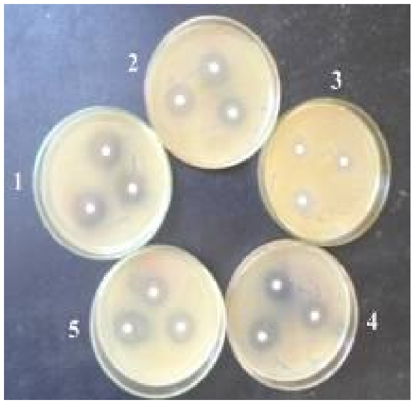


Fig. 9: In vivo evaluation of different chemicals/bioagents/botanicals against bacterial blight of cowpea caused by Xanthomonas axonopodis pv. vignicola

Table 11: *In vivo* evaluation of different chemicals/bioagents/botanicals against bacterial blight of cowpea caused by *Xanthomonas axonopodis* pv. *vignicola*

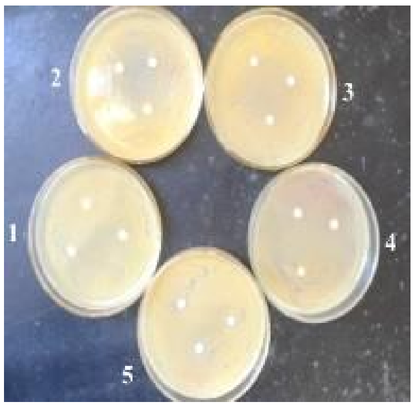
Treatment	Chemical/bioagent/botanical name	Trade/common name	Dosage (%)	Per cent disease index	Per cent reduction over control
T_1	Streptomycin sulphate 90% + tetracycline hydroxide 10 % + copper oxychloride	Streptocycline + Blitox	0.05 + 0.3	3.11 (10.16)*	89.07
T_2	Streptomycin sulphate 90% + tetracycline hydroxide 10 %	Streptocycline	0.1	5.37 (13.39)	81.12
T_3	*Pseudomonas fluorescens*	*Pseudomonas fluorescens*	0.5	12.14 (20.39)	57.33
T_4	*Bacillus subtilis*	*Bacillus subtilis*	0.5	21.75 (27.80)	23.55
T_5	*Allium sativum*	Garlic	10.0	15.53 (23.21)	45.41
T_6	*Sapindus mukrorossi*	Soapnut	10.0	19.06 (25.88)	33.01
T_7	Untreated Control	-		28.45 (32.23)	-
	SEm±			0.38	-
	CD @ 5%			1.13	

* Figures in the parenthesis are arcsine transformed values.



Pseudomonas fluorescens

Bacillus subtilis



Methylobacterium spp

Trichoderma harzianum

Plate 9. *In vitro* evaluation of bioagents on the growth of *X. axonopodis* pv. *vignicola*

Plate 10. *In vivo* (Pot experiment) evaluation of different chemicals/ bioagents/botanicals against bacterial blight of cowpea caused by *X. axonopodis* pv. *vignicola*

Table 12: Effect of hot water treatment on seed germination and seedling infection of artificially inoculated cowpea seeds by *Xanthomonas axonopodis* pv. *vignicola*

Treatment		**Roll paper towel method**		**Pot culture experiment**		
Temperature (^{0}C)	Time (min)	Germination (%)	Average no. of infected seedlings	Germination (%)	Per cent seedling infection	Per cent leaves infection per plant
48	5	71.00 (57.42)*	25.00	72.00 (58.05)	19.26 (26.03)	7.57 (15. 97)
	10	70.00 (56.79)	21.33	67.00 (54.94)	13.45 (21.52)	6.66 (14.45)
50	5	67.00 (54.94)	16.00	63.00 (52.54)	10.78 (19.17)	5.34 (13.37)
	10	65.00 (53.73)	11.33	60.00 (50.77)	8.13 (16.57)	5.00 (12.92)
52	5	62.00 (51.94)	9.67	55.00 (47.87)	5.62 (13.71)	4.26 (11.91)
	10	58.00 (49.60)	6.33	52.00 (46.15)	3.21 (10.33)	2.12 (8.38)
54	5	52.00 (46.15)	4.67	49.00 (44.43)	1.43 (6.87)	1.00 (5.74)
	10	50.00 (45.00)	3.67	46.00 (42.71)	0.00 (0.00)	0.00 (0.00)
56	5	49.00 (44.43)	2.33	44.00 (41.55)	0.00 (0.00)	0.00 (0.00)
	10	47.00 (43.28)	1.67	40.00 (39.23)	0.00 (0.00)	0.00 (0.00)
Control	-	72.00 (58.05)	31.00	76.00 (60.67)	23.28 (28.85)	10.33 (18.75)
SEm ±		1.01	0.33	1.07	0.36	0.09
CD at 1%		4.01	1.33	4.27	1.42	0.36

* - Figures in the parenthesis are arcsine transformed values.

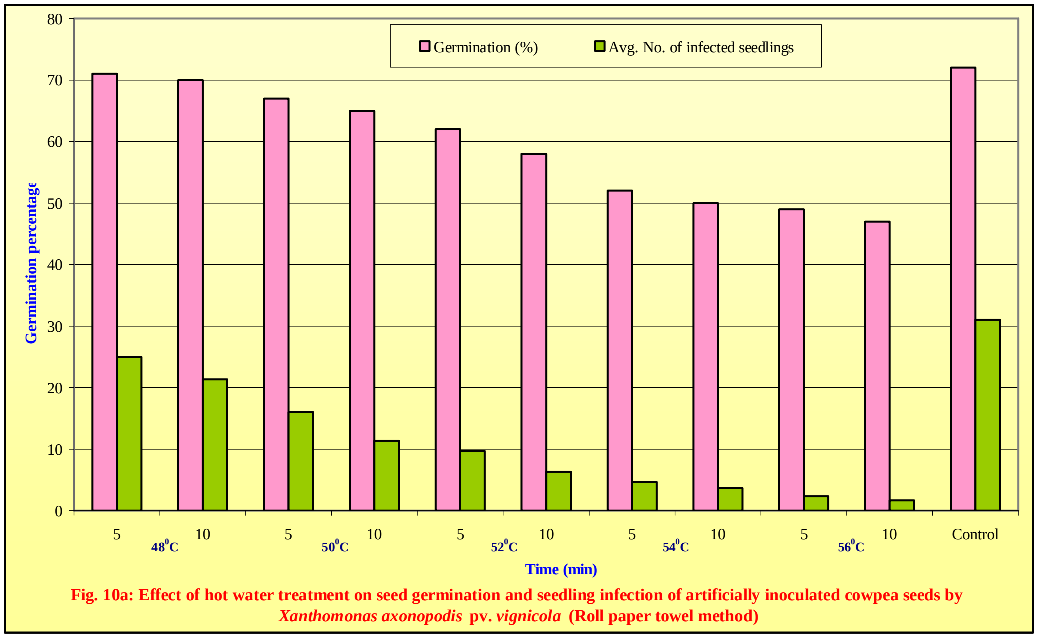


Fig. 10a: Effect of hot water treatment on seed germination and seedling infection of artificially inoculated cowpea seeds by Xanthomonas axonopodis pv. vignicola (Roll paper towel method)

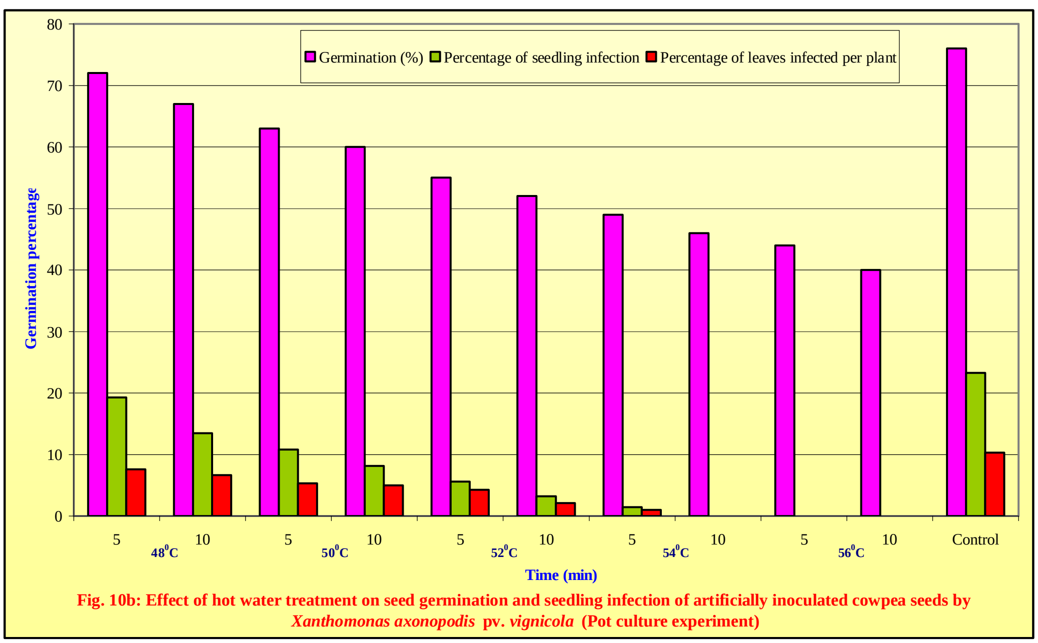


Fig. 10b: Effect of hot water treatment on seed germination and seedling infection of artificially inoculated cowpea seeds by Xanthomonas axonopodis pv. vignicola (Pot culture experiment)

52^{0}C for 10 min

Control

T_1 - 48°C for 5 min. T_2 - 48°C for 10 min. T_3 - 50°C for 5 min. T_4 - 50°C for 10 min.
T_5 - 52°C for 5 min. T_6 - 52°C for 10 min. T_7 - 54°C for 5 min. T_8 - 54°C for 10 min.
T_9 - 56°C for 5 min. T_{10} - 56°C for 10 min. C - Control

Plate 11. Effect of hot water treatment on seed germination and seedling infection of artificially inoculated cowpea seeds by *Xanthomonas axonopodis* pv. *vignicola*

Highest germination per cent (72.00 %) was recorded in T_6 involving Hot water treatment + Seed treatment with *Pseudomonas fluorescens* (0.5%) + Foliar spray of *Pseudomonas fluorescens* at 25 days and Streptocycline + Copper oxy chloride (0.05 + 0.3%) spray at 45 days followed by T_2 involving hot water treatment + Seed treatment with *Pseudomonas fluorescens* (0.5%) (70.17 %) compared to T_7 (control) in which germination percentage was 75.33 %.

Among the different treatments, treatment (T_6) involving Hot water treatment + Seed treatment with *Pseudomonas fluorescens* (0.5%) + Foliar spray of *Pseudomonas fluorescens* at 25 days and Streptocycline + Copper oxy chloride (0.05 + 0.3%) spray at 45 days recorded very less disease incidence (9.25 PDI) compared to control (68.72 PDI). The next best treatment was T_5 involving Hot water treatment + Seed treatment with Streptocycline + Copper oxy chloride (0.05 + 0.3%) + Foliar spray with Streptocycline + Copper oxy chloride (0.05 + 0.3%) which recorded a disease incidence of 21.10 PDI.

4.5.1 Yield

The yield was significantly higher in T_6 *i.e.*, Hot water treatment + Seed treatment with *Pseudomonas fluorescens* (0.5%) + Foliar spray of *Pseudomonas fluorescens* at 25 days and Streptocycline + Copper oxy chloride (0.05 + 0.3%) spray at 45 days (8.03 q/ha) followed by T_5 *i.e.*, Hot water treatment + Seed treatment with Streptocycline @ 0.05% + Copper oxy chloride @ 0.3% + Foliar spray of Streptocycline @ 0.05% + Copper oxy chloride @ 0.3% (6.71 q/ha) when compared to untreated control (4.90 q/ha) (Table 13, Fig. 11).

Table 13: Integrated management of bacterial blight of cowpea under field conditions

Treatment	Treatment details	Germination percentage	Percent Disease index	Yield (q/ha)
T_1	Hot water treatment (52^0C for 10 min)	67.50 (55.24)*	35.87 (36.79)	5.90
T_2	T_1+ Seed treatment with *Pseudomonas fluorescens* (0.5%)	70.17 (56.89)	32.50 (34.76)	6.32
T_3	T_1 + Seed treatment with Streptocycline + Copper oxychloride (0.05 + 0.3%)	68.67 (55.96)	29.03 (32.60)	6.16
T_4	T_3 + Foliar spray with Garlic extract (10.0%)	69.33 (56.37)	28.60 (32.33)	6.29
T_5	T_3 + Foliar spray with Streptocycline + Copper oxychloride (0.05 + 0.3%)	69.83 (56.68)	21.10 (27.35)	6.71
T_6	Hot water treatment + Seed treatment with *Pseudomonas fluorescens* (0.5%) + Foliar spray of *Pseudomonas fluorescens* at 25 days and Streptocycline + Copper oxychloride (0.05 + 0.3 %) spray at 45 days	72.00 (58.05)	9.25 (17.70)	8.03
T_7	Control	75.33 (60.22)	68.72 (55.99)	4.76
	SEm±	0.75	0.90	0.10
	CD @ 5%	2.30	2.76	0.31
	CV %	2.27	4.57	2.73

- \- Figures in the parenthesis are arcsine transformed values.

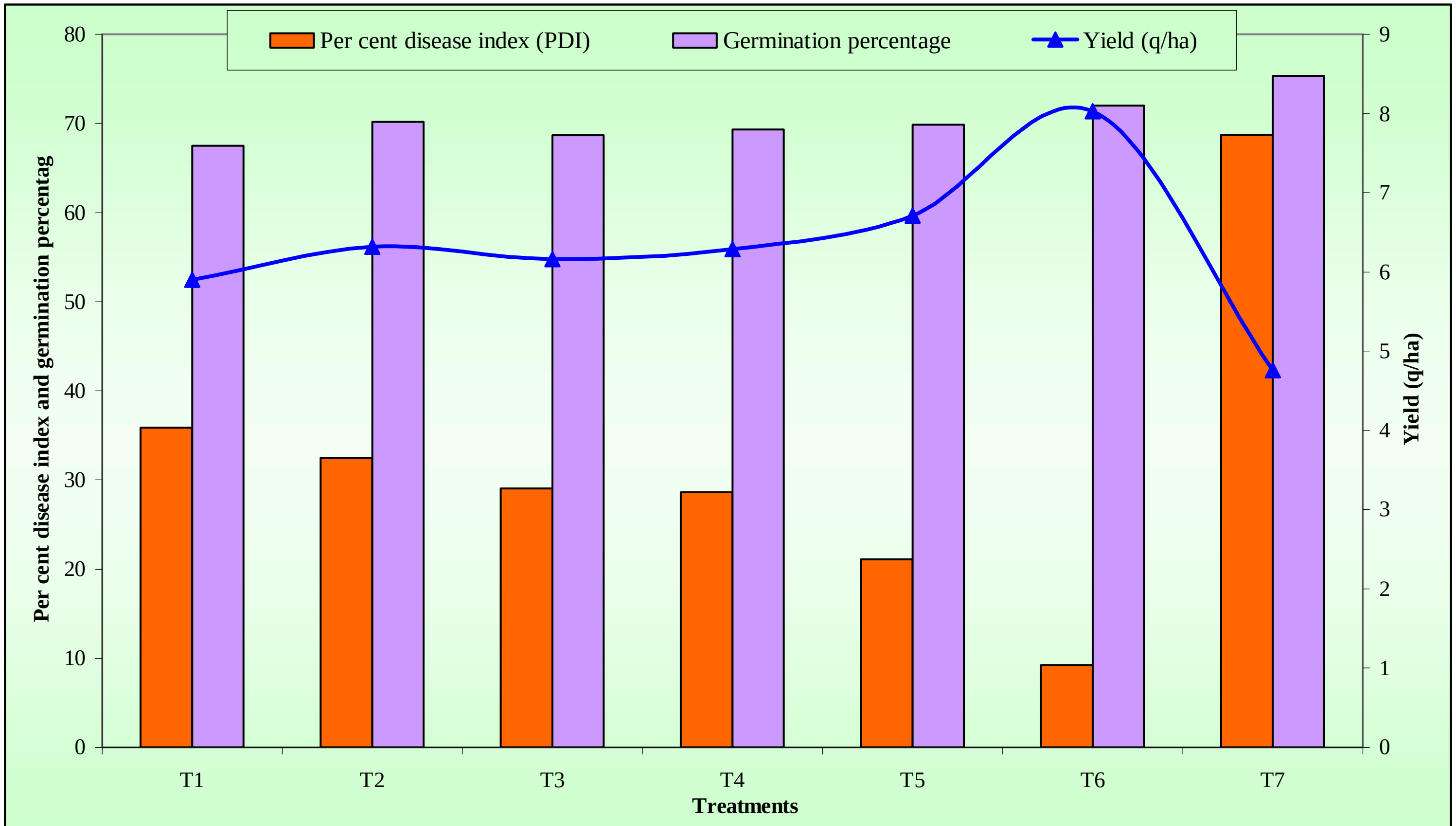


Fig. 11: Integrated management of bacterial blight of cowpea under field conditions

Fig. 11: Integrated management of bacterial blight of cowpea under field conditions

General field view

T6 – Hot water treatment + seed treatment with Pseudomonas fluorescens (0.5%) + foliar spray of Pseudomonas fluorescens at 25 days Streptocycline + COC (0.05 + 0.3%)spray at 45 days

Control

Plate 12. Integrated management of bacterial blight of cowpea under field conditions

5. DISCUSSION

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the important pulse crop grown in India. It forms an important component of farming system, being cultivated for seeds, pods or leaves that are consumed as green vegetable or for pasture, hay, silage and green manure. It is called as vegetable meat as it contains good amount of proteins, vitamins, fat and carbohydrates. Hence, it is consumed by most of the people in the world as supplementary diet.

Cowpea suffers from many diseases; among these, bacterial blight is the major disease causing severe reduction in yield. The disease is caused by the bacterium *Xanthomonas axonopodis* pv. *vignicola* (Burkholder, 1944) Vauterin *et al.* (1995) which is present in almost all cowpea growing regions of the world as it is both externally and internally seed borne pathogen.

In India, it was first reported by Patel and Diwan (1950) from Pune and subsequently Chakravarti *et al.* (1972) and Gupta (1978) reported the disease from Rajasthan. Later during 1963, Rangaswami and Gowda also confirmed its occurrence from Tamil Nadu. This disease was also reported from Delhi, Punjab, Haryana, Uttar Pradesh, Madhya Pradesh and Maharashtra by Patel and Jindal in 1970.

5.1 Survey for disease incidence of bacterial blight in Dharwad, Belgaum, Gadag and Haveri districts

The survey and surveillance work would help to identify the hotspots in different districts of northern Karnataka. The detailed investigations were carried out on various aspects of management of bacterial blight of cowpea. As it is a seed borne pathogen, bacterial blight disease is an important constraint in all cowpea growing areas in Karnataka. So there is an urgent need to educate the farmers for managing this important disease.

To know the incidence of bacterial blight in northern Karnataka, a survey was conducted during *Kharif* 2011-12 in major cowpea growing districts, *viz.*, Dharwad, Belgaum, Gadag and Haveri.

The survey revealed that bacterial blight incidence was noticed in all the locations surveyed with a range from 2.52 to 28.47 per cent (Table 1). The mean maximum disease index of 14.32 per cent was noticed in Belgaum district, followed by Gadag district (13.11%) and Dharwad district (11.36%) whereas, the least incidence was observed in Haveri (9.58%) (Table 2, Fig.1 and 2).

The disease incidence varied from locality to locality, because of cropping pattern, environmental conditions, use of different varieties and build up of inoculum load. The higher disease severity may be attributed to heavy rainfall and high temperature which favoured the bacterial blight disease. Further, negligence of crop as it is hardy crop and growing in marginal lands without proper management practices also aggravated the disease.

It was observed during the survey that incidence of disease was negligible up to July. This clearly indicated that the disease intensity depends on factors like location, cultural practices followed by use of infected seeds of susceptible variety, improper drainage and meteorological factors like temperature, relative humidity and rainfall. In general, it was observed that disease incidence was maximum during July-August which coincides with heavy rains and cool weather.

In Karnataka, cowpea has been grown in marginal lands which have poor physical characteristics with low productivity which impose stress on the plant and favours the activities of the pathogens. During survey it has been observed that Bacterial blight incidence was more in *Kharif* season *i.e.*, wherever rainfall was more during the season. The disease incidence was more because of suitable environmental parameters for development of the pathogen. Incidence of the disease was less wherever improved varieties were used and early sowing has been practiced indicating the importance of proper selection of cropping season and disease free seeds helping in managing bacterial blight disease of cowpea.

Similar observations were made by Preston (1948) who reported the bacterial canker of cowpea caused by *Xanthomonas vignicola* from Oklahoma and opined that the pathogen was carried within the seed and spreads in the field by moisture propelled wind. Kishun (1989) observed losses ranging from 2.66 to 92.24 per cent at various stages of infection in different cowpea cultivars substantiating the results of the present survey wherein incidence varied from genotype to genotype.

Moretti *et al.* (2007) reported the occurrence of leaf spot on cowpea (*Vigna unguiculata*) caused by *Xanthomonas axonopodis* pv. *vignicola* in Mozambique for the first time on June 2004 *i.e.*, during *Kharif* season as angular, necrotic, pustuliform leaf spots, surrounded by a thin water-soaked hallow on cowpea plants with an incidence of 70 - 90 per cent.

From survey, the studies revealed that the bacterial blight incidence was present in almost all districts wherever the cowpea was cultivated. The incidence of bacterial blight varied from place to place with a range of 2.52 to 28.47 per cent. Survey also revealed that Belgaum and Gadag districts as hot spots for the bacterial blight disease of cowpea (Table 1 and Fig.1).

5.1.1 Symptomatology

During the survey, characteristic symptoms of bacterial blight were recorded and the pathogen was identified as *Xanthomonas axonopodis* pv. *vignicola* using different morphological and biochemical tests. The symptoms appeared on cotyledons of seedlings emerging from infected seed which were reddish and wrinkled. First necrotic lesions were formed on leaves and later they appeared on stem. When pathogen reached vascular bundles, the disease became systemic, later the growing tip of the infected plant was killed. Cankers often developed on the stem near the union of cotyledons and first leaves. Such stem were unable to bear the load of the plant and easily got shattered in strong winds.

Secondary infection on leaves appeared as light yellow circular spots which were 4 to 10 mm in diameter and scattered on the lamina. The centre of these spots was necrotic brown and veins turned red in colour. Deep green or water-soaked streaks were formed on pods and such pods became yellow, shriveled and died. The diseased pods produced smaller, wrinkled and infected seeds (Plate 1).

Similar kind of symptoms on leaves were described by Patel and Diwan (1950) as light yellow, irregular to round spots, measuring 4 to 10 mm in diameter with necrotic brown centers appeared and increased irregularly until the whole leaf was involved. Severely affected leaves became straw coloured and dropped at the slightest touch.

Gardner and Kendrick (1923) also reported similar symptoms of bacterial blight of cowpea on leaves, pods and stem, confirming the findings of present investigation.

Further, the bacterial blight symptoms on cowpea described by various workers from different parts of the country *viz.*, Rangaswami and Gowda (1963) from Tamil Nadu, Patel and Jindal (1970) from Delhi, Punjab, Haryana, Uttar Pradesh, Madhya Pradesh and Maharashtra, Khan (1989) and Thammaiah (1991) from Karnataka were having resemblance with the symptoms observed during present investigation.

5.1.2 Isolation of *Xanthomonas axonopodis* pv. *vignicola* and pathogenicity

The causal agent *Xanthomonas axonopodis* pv. *vignicola* was isolated from infected leaves, stem and seeds of cowpea showing typical characteristics symptom of the disease. Isolation method adopted was both serial dilution and streak plate method as explained in material and methods. The media used was Yeast extract Dextrose Calcium carbonate Agar (YDCA) medium. Well separated colonies were picked up and streaked on YDCA medium. The plates were incubated at 30^0C for 72 hr. The pathogen produced pale yellow to dark yellow colonies with mucoid and convex appearance. The obtained pure culture was stored in the refrigerator at 5^0C in NA slants and also in sterilized distilled water. The cultures were renewed by sub-culturing once in a fortnight on YDCA medium (Plate 2).

The pathogenicity of the bacterium was proved by inoculating bacterial suspension (5 x 10^6cfu/ml) to 25 to 30 days old plants of susceptible cowpea variety C-152.

The plants were injured by using sterilized insect pins and syringe and the bacterial suspension was sprayed to the plants. The characteristic symptoms appeared after 6 to 8 days of inoculation in the form of small, water soaked, brown to black coloured lesions, which later developed into angular to irregular shaped spots on leaves, even veins turned red in colour. Reisolation from artificially inoculated cowpea plants yielded the bacterial colonies similar to the original culture (Plate 2).

Shekaraiah (1981) isolated the bacterial pathogen from infected cowpea leaves and proved pathogenicity. Infection was readily seen by him on tender leaves artificially inoculated plants in seven to ten days of incubation. Isolation and pathogenicity studies were also carried out in a similar fashion by Khan (1989) by using both infected leaves and seeds of susceptible variety.

The pathogen

The causal agent of bacterial blight of cowpea was named as *Xanthomonas vignicola* by Burkholder during 1944. In the same year Hoffmaster described it has *Bacterium vignae*. It was also reported as *Phytomonas vignae* from Kansas province (Anon., 1944). Presently it is confirmed as *Xanthomonas axonopodis* pv. *vignicola* (Burkh.) Vauterin *et al.*, 1995.

5.2 Detection of pathogen in seeds by non serological methods

Ever since, Shekhawat and Patel (1977) reported the transmission of *Xanthomonas axonopodis* pv. *vignicola* through the seeds, few reports have appeared on the detection of the pathogen in seed. Due to cumbersome and time consuming nature of work, detection of seed borne pathogen was limited. Hence, efforts have been made in the present investigation to apply, modify and develop the most suitable, rapid, sensitive and accurate techniques in the detection of the pathogen in naturally infected seeds.

Seed samples

Cowpea seed samples of cultivars C-152 (S), DCS 47-1(R), C-152 × IC202709 (MS) and V-118 × IC257422 (MR) were used in the detection of the pathogen by two different techniques.

5.2.1 Detection of pathogen from seeds using selective and semi-selective media

Few attempts have been made in the past to detect the presence of *Xanthomonas axonopodis* pv. *vignicola* in cowpea seeds on agar medium. Gupta and Chakravarti (1982) failed to isolate *X. axonopodis* pv. *vignicola* by direct plating the surface sterilized and non-sterilized seeds on Nutrient agar and Yeast extract glucose chalk agar and they also failed to isolate the bacteria by direct plating the seeds on plain agar medium till they germinated. Later Thind and Soni (1983) were able to recover the bacterium from individual seeds collected from artificially inoculated and naturally infected seeds on Potato- Sucrose-peptone rifampin aureofungin agar.

In the present investigation, NSCAA (Nutrient Starch Cycloheximide Antibiotic Agar) medium was found more efficient with recovery of 132 $\times 10^5$ cfu/ml followed by SIBU agar medium with 126 $\times$ 10^5 cfu/ml of *X. axonopodis* pv. *vignicola* cells whereas SX and BSCAA media were found to be less efficient (Table 4, Fig. 3 and Plate 5).

The results are in confirmation with the findings of Khan (1989) who reported that, SIBU agar a semi-selective medium as most efficient medium with 95 per cent recovery of *Xanthomonas axonopodis* pv. *vignicola* cells followed by KML agar medium with 82.25 per cent and NSCAA with 59.28 per cent whereas XTS and SX were found to be less efficient. Shobha (1998) also reported the maximum recovery of bacterial colonies of *Xanthomonas axonopodis* pv. *glycines* on NSCAA medium with 112 $\times 10^5$ cfu/ml as compared to the nutrient agar with only 55×10^5 cfu/ml.

5.2.2 Detection of *Xanthomonas axonopodis* pv. *vignicola* by Van vuurde *et al.* (1983) method

Both unsterilized and sterilized seeds of different germplasm lines which were screened in the present investigation against *X. axonopodis* pv. *vignicola* and found resistant, moderately resistant, susceptible and moderately susceptible in their reaction for bacterial blight disease were used to know the efficiency of Van vuurde *et al.* (1983) method and confirm the seed borne nature of the pathogen. The results indicated that, diseased unsterilized seeds of susceptible variety C-152 yielded 35×10^2 cfu/ml on NSCAA medium which was found effective in recovering maximum no. of bacterial colonies and specially meant for isolation of bacteria from seeds whereas, seeds from resistant and moderately resistant line DCS 47-1 and V-118 × IC257422 respectively not yielded any colonies on NSCAA medium indicating that the pathogen is not carried internally in these lines. Also number of cfu/ml of the extract varied from cultivar to cultivar. This variation may be due to the difference in the degree of susceptibility of cultivars tested against *Xanthomoans axonopodis* pv. *vignicola* as evident from the results of screening of varieties/lines for their resistance to bacterial blight (Table 5).

Further, the colonies obtained from Van vuurde *et al.* (1983) method were confirmed as *Xanthomonas axonopodis* pv. *vignicola* on the basis of morphological, cultural and biochemical studies. Gupta and Chakravarti (1982) failed to isolate the pathogen from the surface sterilized and unsterilized seeds by direct plating the seeds on Nutrient agar while Khan (1989) isolated the pathogen both from surface sterilized and unsterilized seeds collected from naturally infected plants by soaking the seeds in sterile water for 24 h at 5^0C as explained by Van vuurde *et al.* (1983). Similarly, Anitha *et al.* (1992) also reported that, of the four seed health testing techniques *viz.*, seed soaking, seed maceration, blotter test and growing-on test used for the detection of *Xanthomonas axonopodis* pv. *vignicola* from naturally infected seeds of cowpea, seed soaking test was superior than other methods.

5.3 Screening of varieties

Differential reaction among the cowpea lines towards *Xanthomonas axonopodis* pv. *vignicola* was recorded as early as 1944 by Hoffmaster. This was further confirmed by Preston (1949) who advocated the use of resistant varieties such as Brabham, New Era, Groit, Iron and Victor. Later Sherwin and Lefebvre (1951) found Buff, Six week- Alaska and Suwanne to be highly resistant among the 351 cowpea lines tested.

In the present study, fifteen different entries / genotypes were tested at fields of Genetics and Plant breeding, UAS, Dharwad during *Kharif* 2011-2012 against *X. axonopodis* pv. *vignicola* under natural conditions.

The reactions of cowpea varieties / germplasm lines for *X. axonopodis* pv. *vignicola* infection during *Kharif* 2011 revealed that out of 15 varieties/ genotypes screened under field (0-5 scale), two varieties, RC-101 and DCS 47-1 showed resistance reaction, two varieties, DC-15, V-118×IC257422 were moderately resistant, five varieties were moderately susceptible and another five varieties showed susceptible reaction and only one germplasm line C-152 × GL was highly susceptible (Table 6, Fig. 4 and Plate 3, 6).

Variety GC-3 showed similar susceptible reaction (43.36%) as reported by Lesly (2005) whereas, variety C-152 which showed susceptible reaction during the present investigation was in tune with the results obtained by Khan (1989).

Prakash and Shivashankar (1982) screened 221 genotypes of cowpea for bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* reaction using 0–5 scale. They reported sixteen genotypes to be highly resistant among them, PLS65/1 was the most resistant line followed by C-20, C-2595, MS-9671, Vita-3, Tvx-201, Tvx-944-02E, P-G -779, MS-9363, Tvx-7-54, V-11, PLS-35, C-2085, MS-9032/2 and Tvx-1836-150G.

About 105 lines of cowpea were screened by Shah *et al.* (1990), against bacterial blight under field conditions in Rajasthan during *Kharif* and reported that Copusa-1, K-317, S-488, H-2 and EC-1956 having resistant reaction and nine other lines to be moderately resistant. They grouped the remaining lines as moderately susceptible (18), susceptible (37) and highly susceptible (33).

They also reported maximum infection index of 82 and 88 per cent in Udaipur local followed by JC-12, RC-215, JC-5 and CO-2, indicating the proness of local varieties for the infection by *X. axonopodis* pv. *vignicola*.

Similarly many workers screened different cowpea varieties for resistance against bacterial blight at various places and reported their differential host preference (Dhiman *et al*., 1989 and Sushma-Nema *et al*., 2000).

5.4 Evaluation of bioefficacy of chemicals/antibiotics, botanicals and bioagents

5.4.1 Evaluation of chemicals and antibiotics

In vitro evaluation of chemicals provides the preliminary information about the efficacy of particular chemical in a shortest period of time and therefore it serves as a basis for further field assay. Thirumalachar *et al.* (1956) reported the bioefficacy of streptomycin sulphate in inhibiting *Xanthomonas axonopodis* pv. *vignicola* under *in vitro* conditions.

Among the different chemicals evaluated in the present investigation, Streptocycline + Copper oxy choride exhibited superior efficacy in inhibiting *Xanthomonas axonopodis* pv. *vignicola* with an inhibition zone of 2.97 cm followed by Streptocycline (2.40 cm) and Copper oxy chloride (2.33 cm) alone. However both the treatments were on par with each other. All other chemicals *viz*., copper hydroxide; Bordeaux mixture, Plantomycin and Bacterinashak were found to be moderately effective but were significantly different from each other. Bleaching powder and Captan were less effective and they were also significantly different from each other. In respect of concentration, efficacy of each chemical varied significantly and chemicals bioefficacy was higher at relatively higher concentrations (Table 7, Fig. 5 and Plate 7).

The results of present investigation also revealed that copper containing compounds were effective in controlling bacterial blight of cowpea such as Bordeaux mixture (1.97 cm), copper hydroxide (1.72 cm) and copper oxy chloride (2.33 cm) used in the present investigation inhibited the growth of the pathogen with better inhibition zone.

The present findings are in agreement with Sharma *et al.* (1981), who reported that, the combination of streptocycline and copper sulphate was most effective in inhibiting the growth of *Xanthomonas vesicatoria* causing bacterial leaf spot of tomato as assessed by *in vitro* paper disc and turbidometric method. Ravikumar and Khan (1995) also reported that the seed treatment with Streptomycin sulphate or Stretopcycline for 120 min at 300, 400 and 500 ppm eliminated the *Xanthomonas campestris* pv. *vesicatoria* from tomato seeds.

While studying the bioefficacy of different antibiotics, Borah *et al.* (2000) reported that under *in vitro* conditions, streptomycin and streptocycline inhibited the growth of all three isolates of phylloplane bacteria of mungbean having potential to antagonize bacterial leaf spot pathogen *Xanthomonas axonopodis* pv. *vignaradiatae* at a very low concentration of 10 μg ml^{-1}. While agrimycin-100, plantomycin and blitox could inhibit the Phylloplane bacteria (Plb) at 200 μg ml^{-1}.

Vinayak (2011) reported similar the findings while working with various chemicals against black rot of cabbage and observed that, Streptocycline 1000 ppm + Copper oxy chloride 2000 ppm producing the highest inhibition against *Xanthomonas campestris* pv. *campestris* followed by Streptocycline alone at 1000 ppm.

The results on *in vitro* evaluation of chemicals carried by Shukla and Gupta (2004) indicated that, copper oxy chloride (1000 ppm) in combination with mancozeb (1000 ppm) was very effective in inhibiting the growth of *Xanthomonas vesicatoria* causing bacterial leaf spot of tomato. They also reported that, Bordeaux mixture (1%) to be next best effective and its efficacy was on par with streptomycin sulphate (100 ppm) + mancozeb (1000 ppm). Supporting the results of the present study wherein, Bordeaux mixture was found effective against *X. axonopodis* pv. *vignicola*.

5.4.1.1 Bioefficacy retention capacity of different chemicals

The study conducted to know the retention capacity of different chemicals in inhibiting the pathogen over a period of time revealed that bleaching powder and captan showed 100 per cent reduction after five days (120 h) of incubation whereas; Streptocycline + Copper oxy chloride and Copper oxy chloride alone showed least per cent reduction *i.e.*, 5.72 and 5.57 per cent respectively even after 120 h (Table 8, Fig. 6).

This study may help us to know the most effective chemical which can inhibit the pathogen over a longer period of time indicating its better bioefficacy retention capacity in controlling the pathogen and further the disease development for longer time and thus help the farmers with better option while choosing a chemical for spraying to control the disease.

5.4.2 *In vitro* evaluation of botanicals

Botanicals are safe, ecofriendly and cost effective means of managing the crop diseases effectively. In the present study, among the nine different plant extracts/botanicals tested against *X. axonopodis* pv. *vignicola*, none of the botanicals were found to inhibit the growth of the pathogen completely. However, some of these botanicals exhibited considerable amount of inhibition.

Among the various plant extracts/botanicals evaluated, garlic bulb extract followed by soapnut pulp extract was found effective which were on par with each other and significantly superior over other treatments. However, Neem, Tulsi and Nilgiri extracts were moderately effective against *X. axonopodis* pv. *vignicola*. The other botanicals *viz.,* lantana, durantha, onion and tulsi extracts failed to inhibit the growth of the pathogen even at higher concentration of 10 per cent (Table 9, Fig. 7 and Plate 8).

The results of the present investigation are in confirmation with the results obtained by Hannudin and Djantnika (1989) while studying the effect of some plant extracts on the growth of *Pseudomonas solanacearum* causing bacterial wilt of tomato under *in vitro* conditions. They evaluated extracts from onion and garlic bulbs, roots and stems of *Crotalaria* sp. and *Tagetus* sp. for suppression of *P. solanacearum* and found that extracts from garlic bulbs inhibiting the bacterial growth.

Mukesh *et al.* (2008) tested many plant extracts against *Xanthomonas axonopodis* pv*. vignicola* and stated that leaves extract of weed plant Datura and tulsi to be effective against bacterial blight of cowpea supporting the results of the present study with respect to tulsi as it showed moderate inhibition against *Xanthomonas axonopodis* pv*. vignicola*. The antibacterial activity of garlic has been known for a long time. Allicin was initially considered to be the principle compound responsible for inhibitory activity but it was later proved that it is a derivative from alliin-allinase system present in raw garlic.

Shah and Mali (2002) have tried the efficacy of different plant extracts against *Xanthomonass axonopodis* pv. *citri* both *in vitro* and *in vivo* and reported that onion bulb, garlic, clove and leaf extract of neem having effective inhibitory action against citrus canker. Similar results obtained in the present investigation confirms the efficacy of garlic and neem extract against *X. axonopodis* pv. *vignicola*.

5.4.3 *In vitro* evaluation of bioagents

The ecological consequences of overuse and misuse of chemicals/bactericides in plant disease management became a serious threat in inducing environmental contamination. Hence, the biological control as an alternate option is gaining importance and awareness among farming community as the approach is ecofriendly and cost effective. Under biological control of plant diseases, various antagonistic organisms have been identified which suppress the pathogen by different mechanisms *viz.,* antibiosis, hyperparasitism, competition, hypovirulence and by inducing systemic resistance by acting as elicitors (Vidyasekaran, 1999).

Among the different antagonists tried as biocontrol agents in the present study, *Pseudomonas fluorescens* and *Bacillus subtilis* were found significantly superior over other antganosists in inhibiting the growth of the pathogen. The fungal biocontrol agent, *Trichoderma harzianum* and *Methylobacterium* spp. were found totally ineffective (Table 10, Fig. 8 and Plate 9).

These findings are similar to the results reported by Jindal *et al.* (1989) who tested the efficacy of *Bacillus* spp. to inhibit the growth of *Xanthomonas axonopodis* pv. *vignicola* and found that *Bacillus subtilis* was effective in controlling bacterial blight of cowpea.

Massomo *et al.* (2004) also reported the inhibitory action of *Bacillus subtilis* against the bacterium *Xanthomonas axonopodis* pv. *vignicola* causing bacterial blight of cowpea which is similar to the observations recorded in the present study.

The results of the present study indicating the bioefficacy of *Pseudomonas fluorescens* are in tune with the reports of Laha *et al.* (1992) stating that fluorescent pigments produced by *Pseudomonas* to be the sequester Fe^{3-} (Ferric) ions and are termed as siderophores, which act as inhibitors for the growth of some phytopathogenic bacteria and fungi.

5.4.4 *In vivo* evaluation of bactericides/antibacterial chemicals, botanicals and biogents

The study was conducted in glass house condition to assess the efficacy of chemicals/ botanicals /bioagents which were found effective under *in vitro* condition for the management of bacterial blight of cowpea.

The results indicated that, Streptocycline + Copper oxy chloride (0.05% + 0.3%) and Streptocycline (0.1%) alone were significantly effective than other treatments. Of the two bioagents tested, *Pseudomonas fluorescens* was found more effective than *Bacillus subtilis*. Among botanicals, both garlic and soapnut were moderately effective in minimizing the disease incidence and were on par with each other (Table 11, Fig. 9 and Plate 10).

Tsvetkov and Donev (1984) opined that, treatment of *Phaseolus vulgaris* plants at the beginning of anthesis with streptomycin reduced infection by *Xanthomonas campestris* pv. *phaseoli* and increased the yield by 14.4 per cent.

Thrimurthi and Agarwal (1992) tried similar combinations and stated that spraying a combination of streptocycline and copper oxychloride was effective against bacterial pustule of soybean caused by *Xanthomonas axonopodis* pv. *sojense* thus the present study confirmed the results obtained in this case.

Byrne *et al.* (2005) evaluated three pseudomonads *viz.*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas syringae* against bacterial speck of tomato caused by *Xanthomonas campestris* pv. *vesicatoria*, in which *Pseudomonas syringae* provided the highest mean reduction in disease severity.

Similar bioefficacy of *Pseudomonas fluorescens* was observed in reducing the bacterial blight intensity of rice as reported by Kaur and Thind (2002). Among the three isolates of *Pseudomonas fluorescens* tested in glasshouse and field, the isolate *Pseudomonas fluorescens* LR (Ludhiana rhizosphere) proved as most effective antagonist in significantly reducing the disease intensity. Among the different methods of antagonist application tried, seed bacterization followed by two foliar sprays recorded the lowest bacterial blight intensity both in glasshouse and field conditions. In the present investigation also *Pseudomonas fluorescens* (Pf-1) spray moderately minimized the disease severity in glass house conditions.

Madhiazhagan *et al.* (2002) studied the field efficacy of five botanical extracts in controlling the bacterial bight disease of rice caused by *Xanthomonas oryzae* pv. *oryzae* through pot culture experiment and mentioned that, among the five plant extracts, *Adhatoda vasica* significantly minimized the disease with higher grain yield levels. *Curcuma longa* and *Allium cepa* were the next best effective treatments. Similarly, Kiran Kumar (2000) reported that, *Ocimum sanctum* effectively controlled bacterial blight of cowpea caused by *Xanthomonas axonopodis* pv. *vignicola*, when undiluted extract is sprayed onto the crop and observed decreased effectiveness upon dilution.

5.4.5 Hot water treatment

The seed borne nature of bacterial blight of cowpea was first reported by Shekhawat and Patel (1977).

Later Gupta and Chakravarti in 1982 also demonstrated the transmission of *Xanthomonas axonopodis* pv. *vignicola* through the seeds and stated that, the bacterium is both externally and internally seed borne. Hence the present study was conducted to know the better temperature for eliminating the seed borne pathogen without affecting the seed germination. As hot water treatment is eco-friendly, cost effective and removes the pathogen from seed efficiently hence, it can be included in integrated management of seed borne plant pathogenic bacteria.

The results in the present study indicated that 52^0 C for 10 min was effective in controlling the bacterial blight of cowpea both in roll paper towel method and pot culture experiment with minimum no. of infected seedlings (6.33) and percentage of seedling infection (3.21%) respectively. Germination was also not much affected at 52^0C for 10 min (58.00 % and 52.00 %) as compared to control (72.00 % and 76.00 %). Even though 54 and 56^0C were effective in eliminating the pathogen but it had deleterious effect on germination of seeds (Table 12, Fig. 10a, 10b and Plate 11).

The present findings are in confirmation with Singh and Verma (1973) who opined that cotton seeds treated with hot water at 56^0 C for 10 min controlled both external and internal infection of *Xanthomonas malvacearum*. Later in 1999, Sikirou reported that seed dipping in hot water at 60^0 C for 30 min or 70^0 C for 10 min or treatment in hot air (65^0 C for 120 – 144 h or 70^0 C for 96 h) was eliminating the bacterium from infected cowpea seeds without inhibiting seed germination. However, temperature range depends on the strength of outer coat and water imbibing nature of the seed.

Barua *et al.* (2007) showed that hot water treatment at 53^0 C for 15 min was effective in controlling *Xanthomonas campestris* pv. *vignaradiatae and Pseudomonas syringae* pv. *syringae* which are seed borne pathogenic bacteria causing bacterial infections on mungbean, indicating results of the present study to be more and more accurate.

5.5 Integrated management under field conditions

A field experiment was conducted during *Kharif* 2012 on the integrated management of bacterial blight of cowpea *caused by Xanthomonas axonopodis* pv. *vignicola* in the Institute of organic farming field located at UAS, Dharwad. The treatments were allocated under Randomized Completely Block Design (RCBD). Observations were recorded on per cent disease index and yield which are presented in the Table 13, Fig. 11 and Plate 12.

Among the different treatments implemented on the field condition, treatment T_6 (Hot water treatment + Seed treatment with *Psuedomonas fluorescens* + Foliar spray of *Psuedomonas fluorescensat* 25 days and Streptocycline at 0.5 g/lit + Copper oxy chloride at 3.0 g/lit at 45 days was found to be the best with minimum disease index of 9.25 per cent with highest yield of 8.03 q per ha followed by treatment T_5 (Hot water treatment + Seed treatment with Streptocycline at 0.5 g/lit + Copper oxy chloride at 3.0 g/lit + Foliar spray with Streptocycline (0.5 g/lit) + Copper oxy chloride (3.0 g/lit) with 21.10 per cent disease and yield of 6.71 q per ha and were statistically superior compared to control.

Highest germination per cent (72.00 %) was recorded in T_6 involving Hot water treatment + Seed treatment with *Pseudomonas fluorescens* (0.5%) + Foliar spray of *Pseudomonas fluorescens* at 25 days and Streptocycline + Copper oxy chloride (0.05 + 0.3%) spray at 45 days followed by T_2 involving Hot water treatment + Seed treatment with *Pseudomonas fluorescens* at 0.5% concentration (70.17 %) compared to treatment T_7 (control) in which germination per cent was 75.33 %.

Similar findings were reported by Shah *et al.* (1991) who assessed the efficacy of chemicals as seed treatment, foliar spray and combination of the two as well as hot water treatment against the bacterial blight of cowpea during *Kharif* 1986 and 1987 and opined that hot water treatment was found effective but it greatly reduced the germination of seeds whereas, seed treatment with streptocycline supported maximum germination of seeds. They also reported that, combination of seed treatment and foliar spray with streptocycline gave the best control (75%) of the disease with better yield of 6.3 q/ha.

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

Similarly the yield obtained in the present study sprayed with Streptocycline + Copper oxy chloride (6.71 q/ha) was also more when compared to the control (4.90 q/ha).

The results of the present investigation are in confirmation with the findings of Jindal and Thind (1990) who reported that, three sprays of Streptocycline (100 μg/ml) + Bavistin (500 μg/ml) and 2 sprays of Streptocycline (100 μg/ml) + Bavistin (500 μg/ml) followed by a third spray of Streptocycline (100 μg/ml) + Blitox-50 W.P. (2000 μg/ml) and hot water treatment (50^0C for 30 min) provided significantly better disease control with higher seed yield against bacterial blight of cowpea under field conditions.

Ravikumar and Khan (1995) reported that the seed treatment with Streptomycin sulphate or Stretopcycline for 120 min at 300, 400 and 500 ppm eliminated the *Xanthomonas campestris* pv. *vesicatoria* from tomato seeds. In the present investigation was also seeds were treated with Stretopcycline + Copper oxychloride reduced the disease to greater extent (29.03%) compared to the control (68.72%) with moderate yield of 6.16 q/ha.

Integration of various management practices have resulted in minimum PDI (9.25%) with good germination (72.00%) and highest yield (8.03 q/ha). Hot water treatment eliminated internally present bacterium, whereas *Pseudomonas fluorescens* suppressed the inoculum present on the outer surface of the seed and also *Pseudomonas fluorescens* has grown on the phylloplane rendering protection against air borne bacterium. However, remaining bacterial propagules were killed through spraying Streptocycline + Copper oxychloride at 45 days of crop which was susceptible stage of the crop. Efficiency of integrated approach (9.25%) was conspicuous in the present study looking to the disease pressure in the control plot (68.72%) which was very much reflected in highest yield of 8.03 q/ha compared to control (4.90 q/ha).

Future line of work

1. Studies on detection of pathogen using molecular markers.
2. Bioagents and botanicals may be tested in large scale.
3. Micronutrient application may be incorporated with other integrated measures for better efficacy.
4. Epidemiological factors need to be studied with respect to spread and survival of the pathogen to develop good management strategies.

6. SUMMARY AND CONCLUSIONS

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important multipurpose pulse crop grown extensively in tropical and subtropical countries of the world. It is called as vegetable meat due to amount of proteins present in grains. This crop is subjected to various diseases, among them bacterial blight is one of the important diseases caused by *Xanthomonas axonopodis* pv. *vignicola* (Burkh.) Vauterin *et al.,* 1995 which is known to cause severe reduction in grain yield as it is both internally and externally seed borne pathogen.

The present investigation was carried out during *Kharif* 2011-2012 in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad and in the field of Institute of Organic farming, UAS, Dharwad which included, survey to assess the disease severity in Belgaum, Dharwad, Gadag and Haveri districts, isolation, symptoatology and pathogenicity studies, detection of pathogen in seeds using non serological methods, *in vitro* evaluation of chemicals, bioagents and botanicals and finally the integrated management of bacterial blight of cowpea involving hot water treatment, effective chemical, botanical and bioagent under field conditions. The results obtained are summarized hereunder.

Considering the magnitude of the disease and its resultant losses, the investigation was undertaken to study the disease and pathogen thoroughly to bring out an appropriate management aspects to mitigate the problem effectively.

The survey conducted during *Kharif* 2011-12 revealed that the disease was present in all the districts surveyed and the disease intensity ranged from 2.52 to 28.47 per cent. The maximum per cent disease index was recorded in Belgaum district (14.32 PDI) followed by Gadag (13.11 PDI) and Dharwad district (11.36 PDI) due to heavy rainfall and susceptible cultivars used in these regions whereas; the least per cent disease index of 9.58 PDI was recorded in Haveri district.

The causal organism was isolated from the infected leaf, stem and seeds by following the serial dilution plating technique using nutrient agar medium. Culture was purified by streaking suspected single colony on the Yeast extract Dextrose Calcium carbonate Agar medium.

The pathogenicity test was conducted by inoculating 25 to 30 days old cowpea plants by spraying bacterial suspension of 5×10^6 cfu/ml of *Xanthomonas axonopodis* pv. *vignicola* and the results revealed that the characteristic symptoms of water soaked spots developed after seven days after inoculation which later turned into angular to irregular spots along the margins, veins of leaf lamina.

The detection of *Xanthomonas axonopodis* pv. *vignicola* in seeds by using different selective and semi-selective media revealed that NSCAA media was more efficient in recovering the colonies of seed borne bacteria *Xanthomonas axonopodis* pv. *vignicola* with 132×10^5 cfu/ml as compared to 75×10^5 cfu/ml of colonies on nutrient agar. The next best medium was SIBU agar medium with 126×10^5 cfu/ml colonies followed by XTS medium (118×10^5 cfu/ml). All these media used are specially meant for isolation of plant pathogenic bacteria from seeds. Hence, this revealed that the cowpea bacterial blight organism *Xanthomonas axonopodis* pv. *vignicola* is seed borne in nature.

Another method employed to detect pathogen in seeds has been developed by Van vuurde *et al.* (1983), results of this method revealed that unsterilized seeds of susceptible cultivar C-152 yielded more no. of colonies 35×10^2 cfu/ml by direct plating the seed extract on NSCAA whereas the resistant germplasm, DCS 47-1 yielded very less no. of colonies 2×10^2 from diseased unsterilized seeds.

Of the 15 cowpea germplasm lines screened for resistance under field conditions, two germplasm lines such as RC-101 and DCS 47-1 were found resistant, two were moderately resistant, five were moderately susceptible and five were susceptible whereas, germplasm C-152 × GL was found highly susceptible.

In vitro evaluation of chemicals/antibiotics revealed that Streptocycline + Copper oxy chloride (0.05 + 0.3%) with an inhibition zone of 2.97 cm exhibited superior efficacy against the growth of *X. axonopodis* pv. *vignicola* followed by Streptocycline (2.40 cm) alone and Copper oxy chloride (2.33cm).

Results also indicated that the copper compounds *viz.*, Bordeaux mixture, Copper hydroxide used in the present investigation found to be effective against the growth of *X. axonopodis* pv. *vignicola.*

In vitro evaluation of botanicals tested at different concentration of 5, 7.5 and 10 per cent on the growth of *Xanthomonas axonopodis* pv. *vignicola* revealed that garlic extract was found effective with an inhibition zone of 1.18 cm at 10 per cent followed by soapnut extract (1.07%) at 10 per cent conentration.

Among the biocontrol agents, *Pseudomonas fluorescens* and *Bacillus subtilis* were significantly superior to other antagonistic microorganisms under *in vitro* conditions in inhibiting the growth of the pathogen.

The evaluation of different effective chemicals, botanicals and biogents in pot experiment indicated that Streptocycline + Copper oxy chloride (0.05 + 0.3%) was most effective in reducing the disease severity followed by Streptocycline alone. However, all other bioagents and botanicals used in the study were found moderately effective in minimizing the disease severity.

Hot water treatment by both roll paper towel method and pot culture experiment revealed that 52^0C for 10 min. was found effective in eliminating seed borne bacteria *Xanthomonas axonopodis* pv. *vignicola* without affecting seed germination to a greater extent.

In field experiment, treatment (T_6) involving Hot water treatment + Seed treatment with *Pseudomonas fluorescens* (0.5%) + Foliar spray of *Pseudomonas fluorescens* at 25 days and Streptocycline + Copper oxy chloride (0.05 + 0.3%) spray at 45 days recorded maximum yield of 8.03 q/ha with least disease index of 9.25 per cent followed by T_5 (Hot water treatment + Seed treatment with Streptocycline at 0.05 per cent + Copper oxy chloride at 0.3 per cent + Foliar spray of Streptocycline at 0.05 per cent + Copper oxy chloride at 0.3 per cent) with 6.71 q/ha and 21.10 per cent disease index.

Conclusion

1. Maximum per cent disease index was found in Belgaum district.
2. Pathogen can be easily detected in seeds by using different selective and semi-selective media.
3. Streptocycline (0.05%) in combination with Copper oxychloride (0.3%) was found to be the most efficient in controlling the pathogen both under *in vitro* and *in vivo* conditions.
4. Among the botanicals, garlic (10 %) was effective followed by soapnut (10 %) in inhibiting the pathogen compared to other botanicals.
5. Among the bioagents, *Pseudomonas fluorescens* was effective followed by *Bacillus subtilis* which was moderately effective.
6. In hot water treatment, 52^0C for 10 min. was found effective in eliminating seed borne pathogen both in roll paper method and pot culture without having deleterious effect on germination of seeds.
7. Under field conditions, hot water treatment + seed treatment with *Pseudomonas fluorescens* (0.5%) + foliar spray of *Pseudomonas fluorescens* at 25 days and Streptocycline + Copper oxychloride (0.05 + 0.3%) spray at 45 days was the most effective strategy in minimizing the disease severity with highest yield.

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** - Original papers not seen.*

Appendix I: Composition of media used in different biochemical tests

Dye's medium

$NH_4H_2PO_4$	: 0.5 g
K_2HPO_4	: 0.5 g
$MgSO_4.\ 7H_2O$	: 0.2 g
NaCl	: 5.0 g
Yeast extract	: 1 g
Distilled Water	: 1000 ml
pH	: 7.0

Dye's medium C

$NH_4H_2PO_4$	: 0.5g
K_2HPO_4	: 0.5 g
$MgSO_4.\ 7H_2O$	: 0.2 g
NaCl	: 5.0 g
Yeast extract	: 1 g
Bromocresol purple	: 0.7 ml of 1.5 % alcohol solution
Distilled Water	: 1000 ml
pH	: 7.0

STUDIES ON BACTERIAL BLIGHT OF COWPEA CAUSED BY *Xanthomonas axonopodis* pv. *vignicola* (Burkh.) Vauterin *et al*.

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ABSTRACT

Cowpea bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* has become major constraint in all the cowpea growing regions of northern Karnataka. Survey data revealed that the highest disease severity (14.32 PDI) was observed in Belgaum district followed by Gadag and Dharwad districts. Lowest disease severity (9.58 PDI) was observed in Haveri district.

Detection of pathogen using different selective and semi-selective media revealed NSCAA as best medium for isolating the pathogen from seeds with maximum number of bacterial colonies(132×10^5 cfu/ml) followed by SIBU agar medium (126×10^5 cfu/ml). Van vuurde *et al.* method opined that diseased seeds of susceptible variety C-152 yielded more number of bacterial colonies (35×10^2) compared to the diseased seeds of resistant variety DCS 47-1 which yielded 2×10^2 colonies.

Screening of varieties/gerplasm lines against bacterial blight of cowpea revealed DCS 47-1 and RC-101 varieties as resistant and C-152 × GL (F_1) as highly susceptible. *In vitro* evaluation of chemicals indicated that, Streptocycline (0.05%) + Copper oxy chloride (0.3%) exhibited superior efficacy followed by Streptocycline (0.1%) and Copper oxy chloride (0.3%) alone. Among botanicals garlic and soapnut extracts at 10 % concentration were found inhibitory. Among bioagents, *Pseudomonas fluorescens* (isolate 1) was superior in inhibiting growth of bacteria followed by *Bacillus subtilis. In vivo* studies indicated that Streptocycline (0.05%) + COC (0.3%) and Streptocycline (0.05%) alone found effective in reducing the disease severity of 89.07 and 81.12 per cent respectively. Hot water treatment conducted in roll paper towel method and pot culture revealed that 52^0 C for 10 min was found effective. Integrated management under field conditions revealed hot water treatment + Seed treatment with *Pseudomonas fluorescens* (0.5%) + Foliar spray of *Pseudomonas fluorescens* at 25 days and Streptocycline + COC (0.05 + 0.3%) spray at 45 days was effective (9.25 PDI and yield 8.03 q/ha).