STUDIES ON TIP-OVER DISEASE OF BANANA CAUSED BY *Erwinia carotovora* subsp. *carotovora* (Jones) Holland AND ITS MANAGEMENT

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CONTENTS

Sl. No.	Chapter Particulars
	CERTIFICATE
	ACKNOWLEDGEMENT
	LIST OF TABLES
	LIST OF FIGURES
	LIST OF PLATES
1.	INTRODUCTION
2.	REVIEW OF LITERATURE
	2.1 Occurrence of Tip-over
	2.2 Symptoms
	2.3 Host range studies
	2.4 *In vitro* evaluation of antibacterial chemicals, botanicals/ organic product and antagonistic micro organisms on the growth of *Erwinia carotovora* subsp. *carotovora*
3.	MATERIAL AND METHODS
	3.1 Survey: To assess disease incidence in Belgaum, Dharwad, Bijapur and Bagalkot districts
	3.2 Isolation and identification of causal agent and pathogenicity studies
	3.3 Host range studies of pathogen
	3.4 *In vitro* evaluation of anti bacterial chemicals, botanicals/organic products, and bio agents on the growth of *Erwinia carotovora* subsp. *carotovora*
4.	EXPERIMENTAL RESULTS
	4.1 Survey for the disease incidence of Tip-over of banana in Bagalkot, Belgaum, Bijapur and Dharwad districts
	4.2 Symptomatology, isolation, identification and pathogenicity studies
	4.3 Host range studies
	4.4 *In vitro* evaluation of antibacterial chemicals, botanicals / organics and bioagents on the growth of *Erwinia carotovora* subsp. *carotovora*
5.	DISCUSSION
	5.1 Survey for the disease incidence of Tip-over of banana in Belgaum, Dharwad, Bijapur and Bagalkot districts
	5.2 Symptomatology, isolation and pathogenicity studies
	5.3 Host range studies
	5.4 *In vitro* evaluation of antibacterial chemicals, botanicals and bioagents on the growth of *Erwinia carotovora* subsp. *carotovora*
6.	SUMMARY AND CONCLUSIONS
	REFERENCES

LIST OF TABLES

Table No.	Title
1.	Survey for the disease incidence of Tip-over of banana in Belgaum, Dharwad, Bijapur and Bagalkot districts.
2.	Mean disease incidence of Tip-over of banana in different districts of Karnataka
3.	Morphological, physiological, and biochemical characteristics of *Erwinia carotovora* subsp. *carotovora*
4.	Host range studies of *Erwinia carotovora* subsp. *carotovora*
5.	*In vitro* evaluations of chemicals on the growth of *Erwinia carotovora* subsp. *carotovora*
6.	*In vitro* evaluation of combination of chemicals /antibiotics on the growth of *Erwinia carotovora* subsp. *carotovora*
7.	*In vitro* evaluation of botanicals/organics against the growth of *Erwinia carotovora* subsp. *carotovora*
8.	*In vitro* evaluation of bioagents against the growth of *Erwinia carotovora* subsp. *carotovora*
9.	Pot culture studies against *Erwinia carotovora* subsp. *carotovora*

LIST OF FIGURES

Figure No.	Title
1.	Survey for the disease incidence of Tip-Over of Banana in different districts of northern Karnataka
2.	Mean disease incidence of Tip-over of banana in different districts of northern Karnataka
3.	*In vitro* evaluations of antibacterial chemicals on the growth of *Erwinia carotovora* subsp. *carotovora*
4.	*In vitro* evaluation of combination of chemicals /antibiotics against the growth of *Erwinia carotovora* subsp. *carotovora*
5.	Pot culture studies against *Erwinia carotovora* subsp. *carotovora*

LIST OF PLATES

Plate No.	Title
1.	Survey for the disease incidence of Tip-over of banana in different districts of northern Karnataka
2.	Symptoms of Tip-over disease of banana
3.	Isolation and identification of causal agent and pathogenicity studies
4.	Biochemical test for *Erwinia carotovora* subsp. *carotovora*
5.	Host range studies for *Erwinia carotovora* subsp. *carotovora*
6.	*In vitro* evaluations of chemicals on the growth of *Erwinia carotovora* subsp. *carotovora*
7.	*In vitro* evaluation of combination of chemicals/antibiotics on the growth of *Erwinia carotovora* subsp. *carotovora*
8.	*In vitro* evaluation of botanicals / organic products on the growth of *Erwinia carotovora* pv. *carotovora*
9.	*In vitro* evaluation of bioagents on the growth of *Erwinia carotovora* subsp. *carotovora*
10.	Pot culture studies against *Erwinia carotovora* subsp. *carotovora*

1. INTRODUCTION

Banana (*Musa* spp.) is one of the oldest cultivated tropical fruit crops in India next to mango in both area and production. It was known from the time immemorial as cheapest, plentiful and most nourishing of all the fruits. Banana is generally grown throughout the country all round the year. It is not a season bound crop and grows in warm humid climates better than in cold dry climates.

Banana and plantain constitute a major staple food crop for millions of people in developing countries. In most tropical countries, green (unripe) bananas used for cooking represent the main cultivars. Bananas are cooked in ways that are similar to potatoes. Both can be fried, boiled, baked, or chipped and have similar taste and texture when served. One banana provides the same calories equivalent to a single potato. The best known bananas of commercial type all over the world belong to the pure *Musa acuminata* AAA group.

Banana plant is one of the most versatile plants that every part of the plant is useful to cure different types of disorders. Further, banana is the one of the oldest and best known fruits of the world. Banana is delicious and seedless and is available in all seasons and it is very hygienic and nutritious. Banana is a staple starch for many tropical populations. Depending upon cultivar and ripeness, the flesh can vary in taste from starchy to sweet and texture from firm to mushy. Both skin and inner part can be eaten raw or cooked. Bananas' flavor is due, amongst other chemicals to isoamyl acetate which is one of the main constituents of banana oil.

Banana belongs to family Musaceae of order Scitaminae. The centre of origin of this tropical fruit has been considered to be the Malayan archipalego also called Garden of Paradise.

Banana contains nearly all the essential nutrients including minerals, vitamins and has several medicinal properties. It is a rich source of energy and each banana weighing around 100g would provide the energy requirement (2400 cal/day) of a sedentary man. It contains 27 per cent carbohydrates, 70 per cent moisture, little amount of proteins and fat.

The banana plant has long been a source of fiber for high quality textiles. Banana fibre is used in the production of banana paper. Banana sap from the pseudostem, peelings or flesh may be sufficiently sticky for adhesive uses. The large leaves may be used as umbrellas when the pseudostems are tied together to form a floatation device. Banana peel may have capability to extract heavy metal contamination from river water, similar to other purification materials. Banana peel has displayed antioxidant activity in vitro, especially from unripe extracts.

Several factors are attributed to the constraints for high production of banana and the major being the occurrence of diseases. Banana is subjected to many serious debilitating diseases caused by fungi, viruses, bacteria, nematodes and non-parasitic diseases. Among the bacterial diseases, Tip-over or bacterial rhizome rot of banana is gaining importance in recent years in Karnataka though earlier the disease was considered as of minor importance.

Wardlaw (1950) for the first time reported the bacterial nature of Bacterial head rot/ rhizome rot of banana from Allahabad in Uttara Pradesh. Khan and Nagaraj (1998) reported the same disease from several banana growing regions of Karnataka. There are conflicting reports pertaining to the exact identity of the causal organisms. Several workers in the past have reported it to be *Erwinia carotovora* (Jones) Holland and *Erwinia chrysanthemi* from across the world. While, Khan and Nagaraj (1998) from Karnataka in their preliminary work, have reported it as *Erwinia carotovora*. But Chattopadhay and Mukherjee (1986) from West Bengal implicated *Erwinia chrysanthemi* as the casual agent.

The leaves in the diseased plants appeared pale yellow, lusterless, with marginal necrosis. The young central leaves/ shoots appeared scorched giving a burnt appearance when such plants were uprooted along with the rhizomes, the large part of the rhizome was found massive soft, rotted and turned brown to black discolouration. The rotted area showed tunneling effect and emitted foul smell. Whenever, a strong wind or mild knock was given to severely affected plant, the plant toppled down at much rotted portion just below the soil line, hence the name Tip-over.

There is an urgent need to address this problem and management of this disease effectively, keeping these points in view, the present investigation was undertaken with the following objectives.

1. Survey to assess disease incidence in Belgaum, Dharwad, Bijapur and Bagalkot districts.
2. Symptomatology, isolation and pathogenicity studies.
3. Host range studies of pathogen.
4. *In vitro* evaluation of chemicals, botanicals and bio agents against the pathogen.

2. REVIEW OF LITERATURE

2.1 Occurrence of Tip-over

Stover (1959) reported that a rhizome rot of bananas, prevalent in most banana growing areas of LaLima, Honduras was found to be caused by *Pectobacterium carotovorum* (Jones) Waldiesyn *Erwinia carotovora* (Jones) Holland.

Llanos and Carmen (1967) recorded a new disease on banana characterized by rot and collapse of the pseudostem since 1964. Isolation and inoculation showed that an unidentified bacterium was the primary cause.

Fernandez-Borrero (1967) reported that rot of the banana is new to Xolumbia and pathogenicity of the *Erwinia* sp. was proved experimentally.

Fernandez-Borrero and Selma (1970) isolated *Erwinia paradiasica* from the pseudostem of banana plants from Columbia.

Zutra and Volcani Zafriria (1971) detected bacterial soft rot of banana plants caused by *Erwinia carotovora* for the first time at Rosh Hanikra in 1965 and has since been recorded from other parts of Israel.

Edward *et al.* (1973) noticed “Tip-over” or “bacterial head rot ” in a small piece of land with alluvial soil located close to Yamuna river for the first time on variety Basarai banana in 1968 from India and isolated a strain of *Erwinia carotovora* from rotting parts of rhizome.

Shillingford (1974) observed bacterial rhizome rot on a plant of Valery cultivar at Orange River experimental station, Jamaica early in 1970.

Ordosgoitty *et al.* (1974) reported a watery rot of pseudostem of plantain from Venezuela and isolated *Erwinia carotovora* from diseased plants.

Rivera docando (1978) reported two new bacterial of banana pseudostem caused by *Erwinia chrysanthemi* and a corm rot in bananas caused by *Erwinia carotovora* subsp. *carotovora* from Cuba.

Dickey and Victoria (1980) reported that the soft rot of the plantain (*Musa paradisiaca* L.) was initially observed in Puerto Tejada, Columbia, a small town near California and later spread into the Cauca valley and eventually into other areas of Columbia. They proposed that the bacterial strains isolated from plantains (*Musa paradisiaca* L.) were designated as *Erwinia chrysanthemi* subsp *.paradisiaca*.

Chattopadhyay and Mukherjee (1986) detected a bacterial soft rot disease on the pseudostem and corm of Giant Governor Cultivar of Cavendish banana in India in 1983 in the orchards of Mondouri farm of the ViswaVidhyalaya. Lakshmanan and Mohan (1986) observed Tip-over or head rot caused by *Erwinia carotovora* in Tamil Nadu in North Arcot and Trichy districts.

Choi *et al.* (1988) reported a new disease on banana fruits grown under vinyl house condition in Taejon area from Korea and isolated seven bacterial isolates from infected fruits, out of which three isolates were identified as *Erwinia carotovora* subsp. *carotovora*. Periara and Nunes (1988) reported soft rot of the rhizome and pseudostem of banana (*Musa accuminata*) caused by *Erwinia carotovora* from Brazil.

Hassanzadeh (1990) isolated ten strains of *Erwinia carotovora* from diseased banana trees in different locations in Chabahar and compared with an isolate from rotted cucumber fruit.

Cedeno *et al.* (1990) for the first time reported *Erwinia carotovora* subsp. *atroseptica* as the casual agent of the soft rot disease of Harton plantain stems in Venezuela.

Khan and Nagaraj (1998) reported the incidence of Tip-over or Rhizome rot of banana for the first time in Kolar and Bangalore districts in Karnataka state.

2.1.1 Survey of Tip-over

Wardlaw (1950) observed that the disease caused serious losses in La Laima district on banana and abaca in Panama, Costa Rica and Guatemala and thought that the disease is probably of wide distribution.

Stover (1959) reported that the rhizome rots in the entire major banana growing areas of Central America and the Carribean islands is possibly a single disease.

Shillingford (1974) conducted a survey in collaboration with the Banana Growers Association reported that the bacterial rhizome rot was present in at least 50 per cent of Valery fields. The disease was therefore, widespread and present in all the important banana growing areas.

Dickey and Victoria (1980) reported that soft rot of plantains was initially observed in Puerto Tejada, Columbia, a small town near California and the disease later spread into the Cauco valley and eventually into other areas of Columbia. Later, the pathogen and disease was identified and reported only in Columbia.

Rivera and Garcia (1981) observed that watery rot of the pseudostem of the banana is widespread, occurring in all but three provinces surveyed and incidence of the disease was found to be high.

Chattopadhyay and Mukherjee (1986) undertook a survey on the occurrence of the disease in West Bengal and reported that the disease has been prevalent in Karimpur area of Nadia district for some time and spread to many other villages through seed materials.

Chattopadhyay (1987) detected the rhizome rot of banana and the survey revealed that it is prevalent in the alluvial tracts of West Bengal.

Volvas *et al*. (1994) conducted a survey of banana diseases in sucker propagated plantation and isolated three pectinolytic bacteria, *Erwinia* sp. and *Pseudomonas* sp., from nematode infected plants.

Snehalatharani (2001) reported the Tip-over incidence in southern Karnataka Bangalore, Tumkur and Kolar districts and West Godavari and Konnur districts of AP.

Henz *et al.* (2006) undertook a survey to identify the pectolytic bacteria associated with soft rot of arracacha roots in Brazil. From 1998 to 2001, 227 isolates of *Erwinia* spp. were obtained from arracacha roots and identified by biochemical and physiological tests (pectolytic activity, lecithinase, a-methyl glucoside, phosphatase, erythromycin sensivity, growth at 37ºC). Of these isolates, 89.9% were identified as *E. chrysanthemi* (*Ech*), 9.7% as *E. carotovora* subsp. *carotovora* (*Ecc*) and 0.5% as *E. carotovora* subsp. *atroseptica*.

2.2 Symptoms

Wardlaw (1950) for the first time observed Head rot and reported the complex nature of the disease on the basis of the following evidences (i) discolouration and soft rotting of the main rhizome and suckers (ii) a very severe infestation of the rhizomes and corms by the weevil borer (*Cosmopolites sordidus*), which was almost invariably present and (iii) scanty, diseased root systems, showing die back of the main roots, local lesions along these roots with associated nematodes and discoloured diseased rootlets, the collective effect being described by farm stall as “Short root”. Head rotted plants were easily pushed over being either uprooted or broken across the much rotted and perforated rhizome about soil-level. Cross sectional destruction of the rhizome at soil level was almost complete. In badly affected areas, plants which had attained to a considerable height suffered greatly from wind action. The disease typically developed to its full severity contemporaneously with the first crop of fruit that is in plantations about one year old.

Chattopadhyay (1987) reported that the disease generally appeared at the very primary stage of establishment of the plantation. The soft rot is characterized by a massive soft, odorous rot at the center or a portion of the rhizome. The rot progresses up the pseudostem, destroying the growth point and causing internal decay, often with vascular discolouration. Externally, the symptoms sometimes resemble those of the fusarium wilt. Yellowing and wilting of the leaves are the characteristic symptoms.

Saygili *et al.* (2005) reported the disease incidence of tomato soft rot was approximately 20-25 and 80-90 per cent in greenhouses of Mersin and Antakya, respectively. Symptoms of the disease were observed on tomato stem, pith and fruits. Two different symptoms on stem were observed. One of them (type 1) was characterized by wilting of the whole plant, water-soaking areas on the stem, hollowing of the pith, browning of the vascular tissue and maceration of stem and fruits. The other one (type 2) was characterized by brown-black spots limited on the pruning sites of the stem and maceration of fruits, but not of stem. Non-fluorescent, white-cream colored bacteria and fluorescent, yellow-colored, mucoid bacteria were consistently isolated on the media from symptom type 1 and type 2, respectively. Ten selected representative bacterial strains from each symptom group were identified and characterized on the basis of morphological, physiological, biochemical, pathogenicity tests and fatty acid methyl ester (FAME) analysis. Strains isolated from symptom type 1 were identified as *Erwinia carotovora* subsp. *carotovora* and *E. chrysanthemi*. Strains isolated from symptom type 2 were identified as *Pseudomonas viridiflava.*

Thammaiah *et al.* (2005) reported that the infected rhizomes show pockets of dark brown or yellow water soaked areas with dark peripheral rings in abundance in the cortex region in the initial stages and then throughout the rhizomes producing decay leading to cavity formation. In advanced stage of infection, splitting of the pseudostem was the prominent symptom.

Çetinkaya-Yildiz and Aysan (2007) reported that Stem rot diseases of tomato, caused by *Erwinia carotovora* subsp. *carotovora*, are a serious problem in greenhouses in Turkey. Pathogen causes wilting of the whole tomato plants, water-soaking areas on stem, browning of vascular tissue, hollowing of pith, and soft rotting stem and fruits. The symptom development starts from root or crown region of seedlings in greenhouses.

2.2.1 Pathogenicity studies

Wardlaw (1950) reported that the bacterium inoculated into healthy rhizome tissue caused the characteristic disease symptoms, however the other inoculations were unsuccessful. This has been related to the fact that the bacterium quickly loses its pathogenicity in culture.

Stover (1959) revealed that disease symptoms on the above ground parts appearing 8-10 weeks after inoculated rhizomes were planted in pots. Wounding increased disease severity.

Ordosgoitty *et al.* (1974) revealed that inoculation of *Musa* spp. and hybrids demonstrated the high virulence of the pathogen.

Shillingford (1974) established the pathogenicity of the bacterium by inoculating bacterial culture into the banana rhizome and observed characteristic internal symptoms. Further inoculations of vegetables were achieved by placing a loopful of the bacterial culture on the sterilized surface and stabbing through the centre with a sterile needle. Tomatoes rotted completely within 48 hours, leaving the skin more or less intact. Potatoes are also rotted rather quickly but carrots and onions were slow to rot.

Dickey and Victoria (1980) demonstrated the pathogenicity by multiplying the bacterium on yeast extract dextrose calcium carbonate agar for 24 hours at 27 ^{0} C and inoculated the culture by dissecting needle and into small pieces (4x8cm) of plantain pseudostem from healthy mature plants. Each piece was placed in a closed petridish with wetted filter paper. In addition, cells were collected on the tips of sterile toothpicks and were inserted near the base of 60-80 day old plantain seedlings. The control treatment comprised of an insertion by a sterile needle into a pseudostem piece. The disease initiation started 12 days after inoculation.

Chattopadhyay and Mukherjee (1986) reported that on inoculation with bacterial suspensions of $3x10^{3}$ cfu/ml by injection, the disease symptoms on healthy banana were produced successfully within 14 days, thus confirming the identity of the bacteium.

Periara and Nunes (1988) demonstrated the pathogenicity tests with tomato plants and potato tubers and suggested that the pathogen was an *Erwinia* sp. probably of the carotovora group.

Hassanzadeh (1990) compared ten strains of *Erwinia carotovora* from diseased banana plants with an isolate from rotted cucumber plant. Biochemical and pathogenicity tests indicated that isolates were intermediate between *Erwinia carotovora* and *Erwinia chrysanthemi*

Lakshmann and Mohan (1992) reported that the causal bacterium, *Erwinia carotovora* was isolated and the pathogenicity was proved by soaking the suckers in a bacterial suspension of virulent strain for 15 minutes.

Tomlinson *et al.* (2010) identified *Erwinia chrysanthemi* as the causal organism of a serious corm and rhizome rot of banana in lowland Papua New Guinea. The pathogen was repeatedly isolated from diseased tissue and soil surrounding diseased plants, but not from healthy corms or field soil. *E. carotovora* subsp. *carotovora* was often a co□isolate with *E. chrysanthemi* but pathogenicity tests indicated a minor role for this organism in the disease. Observations of disease incidence in 10 banana cultivars indicated significant differences in disease susceptibility between newly planted corms and established plants, diploids versus triploids and AAA *versus* ABB genotypes.

2.3 Host range studies

Rivera *et al.* (1980) reported that isolates of Erwinia from both banana and maize were pathogenic to potato, pineapple*, Diffenbachia. Pennisetum purpureum* and *Bracharia mutica.* The banana strains also infected banana, plantain, canna, capsicum, *Poinsettia* and *Begonia*. Banana strains were more virulent, induced symptoms earlier and on more species. Disease development, particularly in *Begonia* and *Diffenbachia* was retarded by low temperatures.

Lakshmanan and Mohan (1992) reported that twelve intercrops *viz., Cajanus cajan* (L.) Mill., *Phaseolus vulgaris*, *Dolichos lablab*, *Vigna sinesis*, *Phaseolus aureus, Phaseolus mungo*, *Arachis hypogeae*, *Lycopersicon esculentum* Mill., *Beta vulgaris* L., *Allium cepa, Brassica oleracea* var*. capitata* L., and *Daucas carota* L., were tested for their reaction to the bacterium by inserting the inoculum into the stems at soil level with the help of sterile tooth picks. Among these intercrop, *L. esculentum, B. vulgaris, A. cepa, B. oleraceavar. capitata and D. carota* were readily infected within 10 days after inoculation.

Schuerger and Batzer (1993) conducted experiments for their identification and host range of *Erwinia* pathogen causing stem rot of hydrophonically grown plants. Eighteen different plant hosts were screened for resistance to the pathogen. *Phaseolus vulgaris* cv. Glastada, *Momordica charantia* cv. Park 5188 and *Luffa aegyptiaca* were the most resistant. Plants within the cruciferae and cantaloupe melon cv. Chilton were the most susceptible. Cultural activities that increased wounding of plants generally increased the incidence of disease.

Arsenijevic and Obradovic (1996) reported soft rot and wilt symptoms on two year old cabbage plants. Arsenijevic *et al*. (1996) reported bacterial soft rot of cabbage heads from Yugoslavia. They investigated that the strains caused soft rot of inoculated slices of potato, carrot, parsley, parsnip, watermelon and melon, fruits of cucumber, capsicum, tomato, fragments of cabbage leaves and onion bulbs, wilting and stem rot on sunflower.

Alippi *et al.* (1997) reported tomato as a host of *Erwinia carotovora* subsp. *carotovora* in Argentina.

Rajeh and Hamed Khalif (2000) studied the host range of soft rot disease of vegetables in Jordan. *Erwinia carotovora* subsp. *carotovora* was collected from various crops including cabbage, cauliflower, chard, common mallow, gladiolus spp., lettuce, onion, pepper, potato, spinach, squash, sweet melon and tomato. The bacterial isolates were categorised into five groups based on their capacity to produce acid from different carbon sources.

Halfeld-Vieira and Nechet (2008) for the first time reported of *Pectobacterium carotovorum* subsp. *carotovorum* (*Erwinia carotovora* subsp. *carotovora*) causing soft rot of bunching onion (*Allium fistulosum*) plants in Roraima, Brazil. Its occurrence was reported only in Distrito Federal.

Bhat *et al.* (2010) reported seven different vegetable species *viz.* carrot (*Dacus carota*), cucumber (*Cucumis sativa*), onion (*Allium cepa*), potato (*Solanum tuberosum*), knol khol (*B. oleracea var caulorapa*), cauliflower (*B. oleracea var botrytis*), tomato (*Lycopersicon esculentum*), belonging to different families and tested for host range studied of the pathogen. Studies on host range of the bacterium under artificial inoculation revealed that all the seven tested vegetable species, representative of the families, were susceptible to soft rot.

2.4 *In vitro* evaluation of antibacterial chemicals, botanicals/ organic product and antagonistic micro organisms on the growth of *Erwinia carotovora* subsp. *carotovora*

2.4.1 Chemical control

Mahmoud *et al.* (1981) reported that in disc diffusion test, isolates of *Erwinia carotovora* from potato were sensitive to Streptomycin, Tetracycline, Ampicillin and Chloramphenicol but not to Penicillin. Haloes produced by Ampicillin and to a lesser extent by Streptomycin and Tetracycline showed mutants resistant to antibiotics.

Saini and Parashar (1981) reported that stable bleaching powder at 1000 ppm was inhibitory to *Erwinia carotovora* subsp. *carotovora in vitro* and in test with potato tubers. Further, soil drenching at 12.50 kg/ha was better than tuber treatments (500 and 1000 ppm) and gave maximum tuber germination , best disease control and highest yield obtained irrespective of whether inoculated tubers were sown in uninfected soil or healthy tubers were sown in infested soil.

Tsuyumu (1982) reported that carboxylic acid was found to be pectinase inhibitors and most of the compounds tested also inhibited both the maceration and disease development by *Erwinia carotovora* subsp. *carotovora*.

Farag *et al.* (1984) reported that *Erwinia carotovora* subsp. *carotovora* was highly sensitive to streptomycin and ampicillin followed by tetracycline, penicillin and chlorophenicol under *in vitro* conditions. Application of streptomycin to seed pieces gave good control followed by chlorophenicol.

Saleh and Huang (1997) isolated five isolates of pectolytic bacterium from tomato exhibiting soft rot symtoms. Based on biochemical and physiological characteristics, pathogenicity tests and fatty acid composition analysis, the pathogen was identified as *Erwinia cartovora* subsp. *carotovora*. Further, they stated that benzoic acid and sodium benzoate @ one, five and ten µm inhibited bacterial growth and these two chemicals were effective in controlling the disease in both tomato and potato tubers.

Nagaraj *et al.* (2002) evaluated various bactericides and antibiotics under *in vitro*, conduction methoxy ethyl mercuric chloride 2000 ppm, copper sulphate @ 4000 ppm, steptocycline @750 ppm and norfloxin @ 750 ppm were found effective in inhibiting the growth of *Erwinia carotovora* subsp. *carotovora.*

Thammaiah *et al.* (2005) made an *in vitro* evaluation of chemicals against *Erwinia chrysanthemi* by modified paper disc method with seven treatments. The results revealed that the combination of streptocycline (1000 ppm) + copper oxychloride (2000 ppm) recorded the maximum inhibition of *Erwinia chrysanthemi* (24.00 mm) followed by copper oxychloride 4000 ppm (23.33).

Thammaiah *et al.* (2006) evaluated different chemicals against rhizome rot of banana *in vitro* and reported that streptocycline @ 500 ppm and COC @ 2000 ppm recorded maximum inhibition.

Abd El-Khair and Haggag (2007) evaluated the bactericides, i.e., streptomycin sulfate, Starner and Micronite Soreil for controlling the soft rot disease causing by *Erwinia carotovora* subsp. *carotovora in vitro*. The results showed that the Starner and streptomycin sulfate reduced the cellulolytic enzyme.

Tao *et al.* (2011) evaluated the potential of neomycin to suppress the development of economically important plant pathogenic bacteria.

The *in vitro* antibacterial activity against *Xanthomonas campestris* pv*. citri, Erwinia carotovora* subsp*. carotovora*, *Xanthomonas oryzae* pv. *oryzae* and *Ralstonia solanacearum* was evaluated. Studies revealed that post-infectional spraying with neomycin significantly inhibited the development of citrus bacterial canker caused by *X. campestris* pv*. citri*, cabbage soft rot caused by *E. carotovora* subsp*. carotovora*, ginger bacterial wilt caused by *R. solanacearum* and rice bacterial blight caused by X. oryzae pv.oryzae gave 80.51, 77.55, 77.54 and 69.07% disease reduction, respectively

2.4.2 Biological control

Vijay Pal *et al*. (1993) studied the bactericidal activity of nine common weed species *in vitro* against *Erwinia carotovora* subsp. *carotovora*, *Erwinia chrysanthemi*. Extracts of *Cenchrus cilaris*, *Calotropis procera*, *Solanum surattense* and *Cannabis sativa* inhibited the growth of the bacteria.

Bdliya and Dahiru (2006) tested the efficacy of some plant extracts for the control of potato tuber soft rot caused by *Erwinia carotovora* ssp. *carotovora* in the laboratory conditions. Neem leaf and seed aqueous extracts significantly reduced the incidence and severity of tuber soft rot, while ironweed and Siamese cassia aqueous leaf extracts gave moderate control of the disease.

Dusko *et al.* (2006) evaluated water, ethanol and ethyl acetate extracts of 12 plants from the family Apiaceae for antibacterial activities against selected phytopathogenic bacteria. Following plants were tested: *Aegopodium podagraria, Angelica silvestris, Chaerophyllum bulbosum, Daucus carota* subsp*. carota, Foeniculum vulgare, Heracleum sphondylium, Pastinaca sativa, Peucedanum cerevaria, Peucedanum oreoselinum, Pimpinella saxifraga, Sanicula europea, Torilis anthriscus* against *Pseudomonas glycinea* and *Erwinia carotovora*. The most active were the extracts of *Torilis anthriscus* especially in relation to *Pseudomonas glycinea. Aegopodium podagraria*, *Daucus carota*, *Heracleum sphondyilium* and *Pimpinella saxifraga* showed significant antibacterial properties*.*

Bhardwaj and Laura (2008) screened aqueous extracts of twenty plants for their antibacterial activity against *Erwinia carotovora* subsp. *carotovora*. The strongest inhibitory effect was noticed in the leaf extracts of *Camella sinesis,* and bark extracts of *Acacia arabicae*a and *Acacia catechu.* The inhibitory effect was also shown by leaf extracts of *Azadirachta indica,* root extracts of *Asparagus racemosus,* seed extracts of *Acacia fernesiana* and fruit extracts of *Aegle marmelos.*

El-Zemity *et al.* (2008) evaluated 13 plant essential oils namely: caraway, chenopodium, cinnamon, clove, eucalyptus, garlic, geranium, lemon, matrecary, peppermint, rose, rosemary and thyme and fourteen of their monoterpenoidal constituents: borneol, camphor, carvacrol, carveol, carvone (R and S), chlorothymol, cineol, cinnamaldehyde, citronellol, eugenol, geraneol, menthol, and thymol were investigated for their antibacterial activity against *Erwinia carotovora var carotovora*. New N-methyl carbamate derivatives based on monoterpenoids were introduced. Cinnamon, clove, chenopodium, caraway, rosemary and thyme oils proved to possess good antibacterial activity. Thymol, chlorothymol, and carvacrol were potent against the tested bacteria and quite close to the effect of the standard. Conversion of menthol into its carbamate derivative gave good enhancement in the bactericidal activity at all concentrations compared to menthol itself. Also, the carbamate derivatives of b-citronellol and borneol improved the bactericidal activity against *E. carotovora*. Synergistic antibacterial activity of the tested monoterpenoids was observed when combined with each of the three synergists particularly with Triton X-100.

Saadoun *et al.* (2008) evaluated extracts of 3 Orobanche species, *O. cernua*, *O. crenata* and *O. egyptiaca* for their antibacterial activity against local isolates of crown gall (Agrobacterium) and soft rot (Erwinia) phytopathogens. Extract of *O. cernua* showed the highest activity against both pathogens with clear inhibition zones of 8-24 mm and 12-16 mm in diameters, respectively. When the inhibitory action of 100 mg/ml concentration of the 3 Orobanche species extract on local isolates of Erwinia was compared with each other, data revealed the ability of *O. cernua* to exhibit more activity than the others and disability of *O. egyptiaca* to inhibit any of these pathogens.

Snehalatharani and Khan (2009) tested for the efficacy of six plant species like Ocimum, Eupatorium, Meswak, Kalongi, Isoqbul and Garlic for their antagonistic and antibacterial effect on *Erwinia carotovora* subsp *carotovora/ Erwinia chrysanthemi*. Of all plant extracts tested, Garlic was found to be highly effective in inhibiting the growth of banana *Erwinia* species followed by Isoqbul.

Bhakt *et al.* (2011) screened the susceptibility of nine microbial species to an antimicrobial extract from *Eclipta alba* using the well diffusion assay. Three different volumes (24, 30 and 36 µl/well) were tested. Analysis of the data revealed that all extracts from *Eclipta alba* showed antimicrobial activities. An N-butanol fraction showed inhibitory activities against all nine microbial species. Samples extracted with petroleum ether, dichloromethane, methanol or water had varying levels of inhibition against some of these microorganisms. *Erwinia carotovora* was the most susceptible Gram-negative bacterium among the other gram negative bacteria.

Seyed *et al*. (2011) reported that flowers and leaves of *Malva sylvestris* extracts exhibited strong antibacterial effects against *Erwinia carotovora*, with MIC value of 128 and 256 µg/ml, respectively. The flowers extract also showed high antibacterial effects against some human pathogen bacteria strains such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Entrococcus faecalis*, with MIC value of 192, 200 and 256 µg/ml, respectively. The plant methanol extracts had relatively high cytotoxic activity against MacCoy cell line.

Kavita and Satish (2011) used nine medicinal plants *viz*., *Acacia nilotica* (Leaf), *Acorus calamus* (Rhizome), *Carum copticum* (seeds), *Emblica officinalis* (Leaf), *Eupatorium odaratum* (Leaf), *Hyptis suaveolens* (Leaf), *Millingtonia hortensis* (Leaf), *Ocimium gratissium* (*Leaf) and Pedalium murex*. (Leaf and fruits) was screened for antibacterial activity against important phytopathogenic bacteria such as *Xanthomonas campestris* pv*. vesicatoria, Xanthomonas axonapodis* pv. *malvacearum, Xanthomonas oryzae* pv. *oryzae,* and *Erwinia carotovora* (MTCC 1428). Among different solvent extracts tested, methanol and ethanol extract of *Emblica officinalis, Acacia nilotica,* and *Carum copticum* recorded significant inhibitory activity against all the test pathogens followed by *Pedalium murex, Hyptis suaveolens*, *Millingtonia hortenesis* and *Eupatorium odaratum*.

Moussa *et al.* (2010) investigated activity of 4 different extracts of 25 local plant species belonging to 17 families against two phytopathogenic bacteria, *Erwinia carotovora* and *Ralstonia solanacearum* the pathogens of soft rot and brown rot diseases of important cultivated plants. Bioactivity-guided separation of the bioactive methanol extract of the dried leaves of *Myrtus communis* resulted in the isolation of a chromatographically pure compound. Based on spectroscopic methods (1H, 13C-NMR and MS) as well as chemical methods (detection tests and acidic hydrolysis). The active compound which isolated for the first time from this plant was characterized as 3-methoxy myricetin 7-O-α-L-rhamnopyranoside. This compound exerted a bactericidal activity against both *Erwinia carotovora* and *Ralstonia solanacearum* with MLC values of 200 and 100 µg ml^{-1} respectively.

2.4.3 Bioagents

Visser *et al.* (1986) evaluated many of lactic acid bacteria, isolated from plant surfaces and plant-associated products, were found to be antagonistic to test strains of the phytopathogens *Xanthomonas campestris*, *Erwinia carotovora*, and *Pseudomonas syringae*. Effective *in vitro* inhibition was found both on agar plates and in broth cultures.

Laha *et al*. (1992) stated that the fluorescent pigments produced by *Pseudomonas fluorescens* were sequester Fe^{3+} and were considered as siderophores which inhibits a large number of phytopathogenic bacteria and fungi, whereas biochemical studies by Valasubramanian *et al* (1994) showed that efficient strains of *Pseudomonas fluorescens* produced an antibiotic Phenazine-1-carboxylic acid (PAC) which hinders the growth of plant pathogenic bacteria.

Vanneste *et al.* (1994) conducted *in vitro* studies, in which *Erwinia herbicola* Eh252 inhibited all 52 strains of *Erwinia chrysanthemi*, *Erwinia carotovora* subsp. *carotovora* and *Erwinia carotovora* subsp. *atroseptica.* They suggested that antibiotic production as one of the mechanism of pathogen inhibition, even though some other factors were also evolved.

Irda Safni (2003) concluded that *Psedomonas fluorescens* may also be able to inhibit the growth of bacteria *E. carotovora*, which cause soft rot disease. Firstly, this is because it has several specific characteristics, namely colonization of host plants' root system, secretes metabolite substances (siderophore), has ability to compete with nutrients and space, is easily found in the ecosystem, and it is not toxic for a variety of crops. Secondly, *E. carotovora* is treated as soil-borne pathogen to which the bacterium *P. fluorescens* can be effectively applied.

Cladera-Olivera (2006) reported that the soft rot bacterium *Erwinia carotovora* was inhibited by a novel bacteriocin-like substance (BLS) produced by *Bacillus licheniformis* P40. The BLS caused a bactericidal effect on *E. carotovora* cells at 30 $\mu g\ mL^{-1}$. *Erwinia carotovora* cells treated with BLS were analyzed by FTIR showing differences in the 1390 cm^{-1} and 1250-1220 cm^{-1} bands, corresponding to assignments of membrane lipids. BLS was effective in preventing *E. carotovora* spoilage on potato tubers, reducing the symptoms of soft rot at 240 $mg\ mL^{-1}$ and higher concentrations. Soft rot development was completely blocked at 3.7 mg mL^{-1}.

Hajhamed *et al.* (2007) reported that the isolates of *P. fluorescens* (Pf1), *S. marcescens* (Sm1) and *P. aeruginosa* (Pa1) were moderately effective against the bacterial soft rot caused by *Erwinia* spp. when they were applied before, after and at the same time of inoculation with the pathogen, respectively.

Abd El-Khair and Haggag (2007) evaluated the two bioagents, *Trichoderma harzianum* and *Bacillus subtilis* for controlling the soft rot disease causing by *Erwinia carotovora* subsp. *carotovora in vitro*. *In vitro* results showed that the Starner*, B. subtilis* and *T. harzianum* reduced the pectolytic enzymes (PG and PME enzymes).

Snehalatharani and Khan (2009) reported that the efficacy of three antagonistic microorganisms *viz. Pseudomonas fluorescens*, *P. aeruginosa* and *Bacillus subtilis*. Among antagonistic microorganisms, *P. aeruginosa* was found to be the most effective *in vitro* conditions followed by *P. fluorescens.*

Karkouri *et al.* (2010) reported the isolation of an actinomycete strain from a Moroccan biotope that inhibits the growth of *Erwinia chrysanthemi* 3937VIII. PCR amplification and DNA sequencing of the 16S ribosomal RNA gene allowed the identification of this strain as *Streptomyces cinereoruber*. The concentrated culture supernatant of this actinomycete strain exhibited activity against the growth of *Erwinia chrysanthemi* 3937VIII and two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus amylolitiquefaciens*, but had no effect on other Gram-negative bacteria tested (*Erwinia carotovora* 197stp ^{R}, *Escherichia coli* TG1 and *Pseudomonas* sp.), suggesting that this actinomycete strain secretes into the extracellular medium a substance that inhibits selectively the growth of other bacteria, especially the phytopathogenic *Erwinia chrysanthemi* 3937 VIII.

Cruz-Quiroz *et al.* (2011) evaluated antagonistic capacity of newly isolated *Pseudonomas fluorescens* strains against three important phytopatogenic bacteria (*Clavibacter michiganensis, Xanthomonas axonopodis* and *Erwinia carotovora*). Their results demonstrated that cell free extracts exhibited a limited antagonist capacity in comparison of those extracts with cells, which showed an excellent capacity to inhibit the growth of *C. michiganensis, X. axonopodis* and *E. carotovora*, demonstrating the intracellular nature of the bioactive metabolites associated to bacterial growth inhibition.

2.4.4 *In vivo* evaluation of Tip-over disease of banana caused by *Erwinia* spp.

Stover (1959) reported that an Agrimycin drench proved ineffective in greenhouse tests at 100 ppm concentration. Loos (1962) reported that paring of rhizomes and chemical dip of rhizomes with Bordeaux-Nemagon, Triton 100 and two per cent Dithane D-40 had no effect on the incidence of rhizome rot or on sprouting of rhizomes.

Nirmaljit Singh *et al.* (1979) reported that decay of potato tubers due to *Erwinia carotovora* was completely checked by Streptocycline followed by Agrimycin-100 and stable bleaching powder. Blitox -50 and Dithane M-45 provided less protection as compared to other compounds.

Further they stated that 100 ppm of streptocycline gave cent per cent control while 50, 20 and 10 ppm concentration afforded 47.50, 18.75 and 13.00 per cent protection, respectively.

Lakshmanan and Mohan (1986) conducted a field trial at different locations of North Arcot district in Robusta banana during 1984-85 to evolve control measures. Methoxy ethyl mercuric chloride at 0.1 per cent, applied six times to the soil 30 days after planting at monthly interval gave best control of *Erwinia carotovora.*

Chattopadhyay (1987) reported that soil and plant drenching with bleaching powder @ 2g/l water at an interval of 10-15 days is effective in controlling the disease. Drenching is required up to the run-off stage, to ensure that all the plants parts are covered by the bleaching powder solution. He also suggested that dipping of corm in chlorine water before planting proved effective.

Salazar and Duque (1994) reported that Streptomycin sulphate @ 0.5g and iodine as (Q-2000) @ 6 ml/l of water gave better control of *Erwinia chrysanthemi* pv. *Paradisiaca* on plantain than Copper oxychloride @ 4 g/l and commercial iodine @ 5 g/l.

Anonymous (2002) reported that Carbofuran @ 40 g/plant, drenching of COC 500 ppm and dipping of suckers in 5000 ppm of COC for 30 min., reduced rhizome rot in banana.

Nagaraj *et al.* (2002) conducted two field trials to evaluate the efficacy of various bactericides and antibiotics. Three times drenching with streptocycline either alone or in combination with copper sulphate completely suppressed the disease (100%) and increased the yield by 143.37%. In another field trial, norfloxin plus copper sulphate gave very good control of disease (100%) followed by streptocycline plus copper sulphate.

Thammaiah *et al.* (2005) conducted an experiment at Arabhavi and found that maximum survivability of plant in field against *Erwinia chrysanthemi* was noted with sucker treatment with 2000 ppm bleaching powder for 30 min dip (60.00 mm), bleaching powder for 30 min dip soil application of Furadan 40g/sucker (60.00 mm), bleaching powder for 60 min dip (53.33 mm).

Kannan *et al.* (2006) reported that soil drenching of sodium hypochorite (0.5%) and streptomycin sulphate (500 ppm) performed well and reduced the rhizome rot incidence.

Abd El-Khair and Haggag (2007) evaluated the bactericides, i.e., streptomycin sulfate, Starner and Micronite Soreil and two bioagents, *Trichoderma harzianum* and *Bacillus subtilis* were applied for controlling the soft rot disease causing by *Erwinia carotovora* subsp. *carotovora in vitro* and in field. *In vitro* results showed that Starner*, B. subtilis* and *T. harzianum* reduced the pectolytic enzymes (PG and PME enzymes), while Starner and streptomycin sulfate reduced the cellulolytic enzyme (Cx). The tested materials were also proved to be powerful bactericide against the bacterial soft rot pathogen. Streptomycin sulfate*, T. harizanum* and *B. subtilis* prevent the soft rot disease in daughter potato tubers and increased the vegetative characters, plant height and number of leave per plant. Results show that plant tubers yield and the average of tuber weight has been increased when the above bactericides were applied, comparing with un-treated plants. Starner and Micronite Soreil proved to be of a moderate effect in reducing the incidence of soft rot disease, while a positive effect on tuber weight and plant tuber yield has been recorded compared to control.

Anonymous (2008) reported that sucker dip in COC at 4 g/l for 45 minutes followed by spraying of streptocycline (0.03%) and suckers dip in *Pseudomonas fluorescens* (1:1) for 45 minutes recorded better growth and lower disease infection

Patel *et al.* (2011) carried out an experiment at Fruit Research Station, Navsari Agricultural University, Gandevi, Gujarat during years 2006-07 to 2007-08. For the control of rhizome rot disease of banana, ten different chemicals were used as soil application, drenching and dipping of suckers. From the Studies it was revealed that paring + 0.15% Acephate dip + 1% B.M. and suckers dip in mancozeb 0.3% for 10 min. were found effective and economical in reducing the rhizome rot and also gave higher fruit yield.

3. MATERIAL AND METHODS

The present investigation on isolation, cultural and biochemical variability of the pathogen were carried out in the Department of Plant Pathology, College of Agriculture, UAS, Dharwad. The details of materials used and methodology followed during the course of investigation described here under.

General laboratory procedure

Glassware cleaning

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

Sterilization

All glassware used in the 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 ^{o} C for 1 hour. Both solid and liquid media were sterilized at 1.1 kg per cm^2 pressure for 15 min.

3.1 Survey: To assess disease incidence in Belgaum, Dharwad, Bijapur and Bagalkot districts.

Survey on the disease incidence was done to know the status of the disease in major banana growing areas of northern Karnataka *viz.*, Belgaum, Dharwad, Bijapur and Bagalkot districts. Infected plant parts showing typical symptoms of Tip-over disease of banana were collected from the above mentioned districts.

The survey is conducted by Rovers method in the major banana growing areas of the above mentioned districts of Northen Karnataka.

Per cent Disease Incidence (PDI) can be calculated by,

$$\text{Per cent disease incidence} = \frac{\text{No. of plants infected}}{\text{Total No. of plants examined}} \times 100$$

3.2 Isolation and identification of causal agent and pathogenicity studies

3.2.1 Isolation

Banana plants showing typical sympyoms of rhizome rot or soft rot were selected from the above mentioned districts of Dwarf Cavendish var. Grand-Naine. The affected rhizomes in the initial stages of rotting were selected and collected in polythene bags and closed with rubber band. Then the material brought to laboratory for the purpose of isolation.

Small pieces of rhizome showing mild rotting were cut into small pieces aseptically along with the healthy tissues. The infected tissue pieces were surface sterilized in 70 per cent alcohol and washed three times in sterile distilled water so as to remove traces of alcohol. CVP medium is the diagnostic media for the Erwinias, so pour plate method was done to get single colonies. With the help of sterilized inoculation loop, the suspension will be streaked onto the surface of nutrient agar medium contained in sterilized petriplates. The inoculated plates were incubated at 28^{o} C for 48 hours. Observations were made for the development of well separated out bacterial colonies.

3.2.2 Pathogenicity studies

The ability of the isolates to infect and cause rotting of banana plant was tested by inoculating the isolates to banana seedlings in order to induce symptoms under artificial conditions.

3.2.2.1 Proving the Koch postulates

Forty days old banana plants has to be inoculated with 48 hours old bacterial cultures. Inoculation done through root dip method, pseudo stem injection using hypodermic syringe and soil application by scraping soil surface and pouring the suspension.The inoculated plants were kept in glass house and were watered regularly.The plants similarly injured but inoculated with sterile water served as control.

Observations were recorded for the development of symptoms of Tip-over disease such as pale yellow and brown discoloured necrotic leaves and development of rotting at the rhizome region.

Media used are

1. Crystal Violet Pectate (CVP) medium

2. Nutrient Agar (NA)

Peptone	: 5.0 g
Beef extract	: 3.0 g
Agar	: 20 g
Distill 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	: 0 ml

3.2.3 Morphological and biochemical characterization of the bacterium

The physiological and biochemical characters of the isolate of the bacterium was studied for colony morphology on Nutrient agar medium, Crystal Violet Pectate (CVP) medium and Kings B medium (KBM) and the biochemical characters like hydrolysis of starch, gelatin liquefaction, hydrogen sulphide production, acid production from sucrose, maltose and glucose, catalase production. The tests were conducted as per the methods described by Bradbury (1986) and Schaad (1988).

a) Gelatin liquefaction

The nutrient gelatin medium was used for this study. Peptone 10.0 g, Beef extract 5.0 g, Gelatin 20.0 g, Water 1000 ml and pH 7.0. All the ingredients were mixed and heated over a water bath until the gelatin has dissolved, and sterilized at 15 lb pressure for 20 min. Then cooled the media and poured to the petriplates and allowed to solidify then spot inoculated with 48 hours growth of the test bacterium incubated the plates at 20^{o} C. Then flood the surface of agar with 0.2% mercuric chloride solution 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 ml hydrogen peroxide. The reaction is would be positive if gas bubbles are produced.

c) Hydrogen sulphide (H_2S) production

The peptone water medium was used which comprised of Peptone 10 g, NaCl 5.0 g, water 1000 ml and pH 7.0. The medium 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 are then soaked in warm saturated solution of lead acetate. The strips were then dried, autoclaved and again dried at 60^{o}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 ^{o} C and the observations were recorded at regular intervals upto 14 days. The blackening of test strip indicates liberation of H_2S.

d) Starch hydrolysis

The medium employed here 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). the medium was sterilized 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. Later the agar surface was flooded with Lugol's iodine and allowed to act for a few minutes for development of colorless zone around the bacterial growth.

e) Lactose utilization

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 ^{o} C for 3, 7 and 14 days and then observed.

3.3 Host range studies of pathogen

The ability of the bacterium to infect different host and crop plants was tested by inoculating pathogen to the vegetables and fruits under aseptic condition. For this study clean fresh healthy fruits and vegetables were washed in running tap water to remove dust and soil particles, if any, and then surface sterilized in one per cent sodium hypochlorite for 30 seconds to eliminate surface contaminants. The traces of sodium hypochlorite has to be removed by washing the fruits and vegetables in three series of sterile water and the fruits and vegetables are blot dried

The following are the plants that were used for the study of host range of pathogen *viz.*, carrot, potato, tomato, onion, cabbage, brinjal, groundnut and citrus

3.4 *In vitro* evaluation of anti bacterial chemicals, botanicals/organic products and bio agents on the growth of *Erwinia carotovora* subsp. *carotovora*

3.4.1 *In vitro* evaluation of chemicals on the growth of *Erwinia carotovora* subsp. *Carotovora*

Trade name	Chemical name	Concentration (ppm)
Streptocycline	Streptomycin sulphate 90% + tetracycline hydroxide 10%	100, 250, 350, 500
Bromopol	2-Bromo-2-Nitro propane-1-3-Diole	100, 250, 350, 500
Plantamycin	Streptomycin sulphate 9% + tetracycline hydroxide 1%	100, 250, 350, 500
Bleaching powder	Chlorine 30%	1000, 2000, 3000, 4000
Kocide	Copper hydroxide	1500, 2000, 2500, 3000
Copper sulphate	Copper sulphate	1500, 2000, 2500, 3000
Blue copper	Copper oxy chloride	1500, 2000, 2500, 3000
Control	-	-

In vitro evaluation of combination of antibiotics and chemicals on the growth of *Erwinia carotovora* subsp. *Carotovora*

Treatments	Concentration (ppm)
Streptocycline + Copper oxychloride	500 + 3000
Streptocycline + Copper hydroxide	500 + 3000
Streptocycline + Copper sulphate	500 + 3000
Plantamycine + Copper oxychloride	500 + 3000
Plantamycine + Copper hydroxide	500 + 3000
Plantamycine + Copper sulphate	500 + 3000
Bromophol + Copper oxychloride	500 + 3000
Bromophol + Copper hydroxide	500 + 3000
Bromophol + Copper sulphate	500 + 3000

The bacterium was multiplied by inoculating the culture into 20 ml of nutrient broth taken in "Erleynemayers" flask. The inoculated flasks were incubated at 30^{o}C for 48 hours. The bacterial suspension was then seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized petriplates and plates were allowed to solidify.

The bactericides were prepared at different concentrations as mentioned in the table.3 The filter paper discs (Whatman no-44) measuring 5mm in diameter were soaked in the respective chemical concentrations for 5 min and transferred onto the surface of seeded medium in the petriplates.

The inoculated plates were kept in refrigerator at 5^{o}C for 4 hours to allow for the diffusion of chemicals into the medium. The plates were then incubated at 30^{o}C for 72 hours. The observations for the production of inhibition zone around the filter paper discs. The results obtained were analyzed statistically.

Chemicals each at four concentrations were evaluated for their efficacy against the growth of *Erwinia carotovora* subsp. *carotovora* by inhibition zone method. The details of chemicals used and their concentrations were given hereunder.

The chemicals were prepared at different concentrations as mentioned in the above table. The filter paper discs (Whatman no-44) measuring 5mm in diameter were soaked in the respective chemical concentrations for 5 min and transferred onto the surface of seeded medium in the petriplates. The inoculated plates were kept in refrigerator at 5^{o}C for 4 hours to allow for the diffusion of chemicals into the medium.

The plates were then incubated at 30^{o}C for 72 hours. The observations for the production of inhibition zone around the filter paper discs. The results obtained were analyzed statistically.

3.4.2 *In vitro* evaluation of botanicals/organic products on the growth of *Erwinia carotovora* subsp. *carotovora.*

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 and commercial products for their antibacterial properties against *Erwinia carotovora* subsp. *carotovora.*

The list of plant extracts and commercial products with their concentration are given below.

Common name	Scientific name	Economic plant part	Per cent concentration
Tulasi	*Ocimum santum*	Leaves, shoot	5, 7.5, 10
Eupatorium	*Eupatorium adenophorum*	Leaves	5, 7.5, 10
Meswak	*Salvadora persica*	Roots	5, 7.5, 10
Kalongi	*Nigella sativa*	Seeds	5, 7.5, 10
Soapnut	*Sapindus mukorossi*	Seeds	5, 7.5, 10
Garlic	*Allium sativum*	Bulbs	5, 7.5, 10
Cow dung + Lime	-	Extract	10g + 1g

Fresh plant materials were collected and washed first in tap water and then in distilled water; 100grams 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 used as a stock solution. To study the mechanism of plant extracts and organics, inhibition zone assay method was followed.

A heavy suspension (72 hours old) of *Erwinia carotovora* subsp. *carotovora* multiplied in nutrient broth (20 ml) was mixed with molten (50^{o}C) nutrient agar medium (1000 ml) contained in an Erleyenmayer's flask, the bacterial suspension was then seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized petriplates and plates were allowed to solidify.

Five (5), Seven point Five (7.5) and Ten (10) per cent each of plant extract was prepared by mixing 5, 10, and 20 ml of stock solution with 95, 92.5 and 90 ml of sterilized distilled water, respectively.

The filter paper discs (Whatman No .44) measuring 5 mm in diameter were soaked in the respective concentrations for 5 min and transferred onto the surface of seeded medium in the petriplates. The inoculated plates were kept in refrigerator at 5^{o}C for 4 hours to allow the diffusion of chemicals into the medium. The plates were then incubated at 30^{o}C for 72 hours. The observations for the production of inhibition zone around the filter paper discs. The results obtained were analyzed statistically.

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 millimeter in diameter and data obtained was analyzed statistically.

3.4.3 *In vitro* evaluation of bioagents on the growth of *Erwinia carotovora* subsp. *carotovora*

Six biocontrol agents *viz.*, *Trichoderma harzianum, Trichoderma viride, Trichoderma koenigii, Trichoderma virescens, Pseudomonas fluorescens,* and *Bacillus subtilis* were evaluated for their efficacy against the growth of *Erwinia spp* by inhibition zone assay method. The cultures of these bio control agents were obtained from Department of Plant Pathology, UAS Dharwad and Institute of Organic Farming, University of Agricultural Sciences, Dharwad.

A heavy suspension of *Erwinia spp* multiplied in nutrient broth (20 ml) was mixed with lukewarm nutrient agar medium (1000 ml) contained in "Erleynemayers" flask. The inoculated flasks were incubated at 30^{o}C for 72 hours.

The bacterial suspension was then seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized petriplates and plates were allowed to solidify.

Loop ful culture of the antagonistic organism was placed on the 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 30°C for 72 hours. The observations for the production of inhibition zone around the antagonistic microorganisms was calculated and analyzed statistically.

3.4.4 Pot culture studies

The results showing best in the *in vitro* condition will be studied in the pot culture. Chemicals were screened for their efficacy to check the incidence of rhizome rot or Tip-over of banana. This experiment was conducted in glass house of Plant Pathology, UAS Dharwad. The trial was laid out in Randomized completely block design (RCBD) with three replications in each treatment and each replication had three plants of Grand Naine. Chemicals were drenched after the occurrence of disease and it was drenched twice. Formula for the Per cent Disease reduction over Control is given below.

$$PDC = \frac{PDIC - PDIT}{PDIC} \times 100$$

PDC = Per cent disease reduction over control

PDIC = Per cent Disease Incidence in control

PDIT = Per cent Disease Incidence in treatment

The details of the treatments were given below.

Pot culture studies against *Erwinia carotovora* subsp. *Carotovora*

Treatment	Treatment details
T_1	Streptocycline + Copper oxychloride
T_2	Streptocycline + Copper hydroxide
T_3	Streptocycline + Copper sulphate
T_4	Bromopol + Copper oxychloride
T_5	Bromopol + Copper hydroxide
T_6	Plantamycin + Copper oxychloride
T_7	*Pseudomonas flourescens* + Garlic
T_8	Control

The observations pertaining to the disease reduction over the control were recorded using the formula. The data obtained were analyzed statistically.

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 Soybean glasshouse respectively. The results including *in vitro* studies and pot culture experiments were conducted during 2011-12 are presented hereunder.

4.1 Survey for the disease incidence of Tip-over of banana in Bagalkot, Belgaum, Bijapur and Dharwad districts

Roving survey was conducted during 2011-12 to assess the disease incidence of tip over of banana in major banana growing areas of northern Karnataka in districts Belgaum, Dharwad, Bijapur and Bagalkot shown in Plate 1.

The per cent disease incidence was noticed in all the locations surveyed, with a range from 4.20 to 65.28 per cent presented in Table 1 and shown in Fig. 1. The mean maximum disease incidence (46.61%) was observed in Bijapur district followed by Bagalkot district (23.72%). Whereas, the least incidence (8.87%) was observed on Belgaum district and in Dharwad district very least incidence (5.36%) was recorded is presented in Table 2 and Fig. 2.

Bagalkot district

Two taluks were surveyed in Bagalkot district *viz.*, Bagalkot and Bilagi taluks. The disease incidence observed in all the surveyed area was ranged from 10.25 to 45.80 per cent. The mean maximum disease (28.02%) was observed in Bilagi taluk and the least incidence was observed in Bagalkot taluk (19.42%).

In Bilagi Taluk, two villages were surveyed. The incidence was ranged from 10.25 to 45.80 per cent. The maximum incidence (45.80%) was observed in Badagandi, and the least incidence was observed in Chikkasangama (10.25%).

In Bagalkot Taluk, two villages were surveyed. The incidence ranged from 16.25 to 22.35 per cent. The maximum incidence (22.35%) was observed in Govinakoppa, and the least incidence was observed in Kaladagi (16.25%).

Bijapur district

Two taluks were surveyed in Bijapur district *viz.*, Bijapur and Basavan Bagewadi taluks. The disease incidence observed in all the surveyed area was ranged from 15.33 to 65.28 per cent. The mean maximum disease (65.28%) was observed in Basavan Bagewadi taluk and the least incidence was observed in Bijapur taluk (27.92%)

In Bijapur Taluk, two villages were surveyed and the incidence was ranged from 15.33 to 40.50 per cent. The maximum incidence (40.50 %) was observed in Jumnal and the least incidence was observed in Utnal (15.33%)

In Basavan Bagewadi Taluk, one village was surveyed and the incidence was (65.28%) recorded in Hoganahalli.

Belgaum district

Three taluks were surveyed in Belgaum district *viz.*, Saundatti, Raibagh and Bailhongal. The disease incidence observed in all the surveyed area ranged from 4.20 to 15.00 per cent. The mean maximum disease (11.32%) was observed in Saundatti taluk, followed by Bailhongal taluk with (7.29 %) and the least incidence was observed in Raibagh taluk (5.05%)

In Saundatti Taluk, two villages were surveyed. The incidence was ranged from 6.28 to 16.36 per cent. The maximum incidence (16.36%) was observed in Sogal, and the least incidence was observed in Gurlahosur (6.28%)

In Bailhongal Taluk, two villages were surveyed. The incidence ranged from 4.25 to 10.00 per cent. The maximum incidence (10.00%) was observed in Sampagoan, and the least incidence was observed in Bevinakoppa (4.25%).

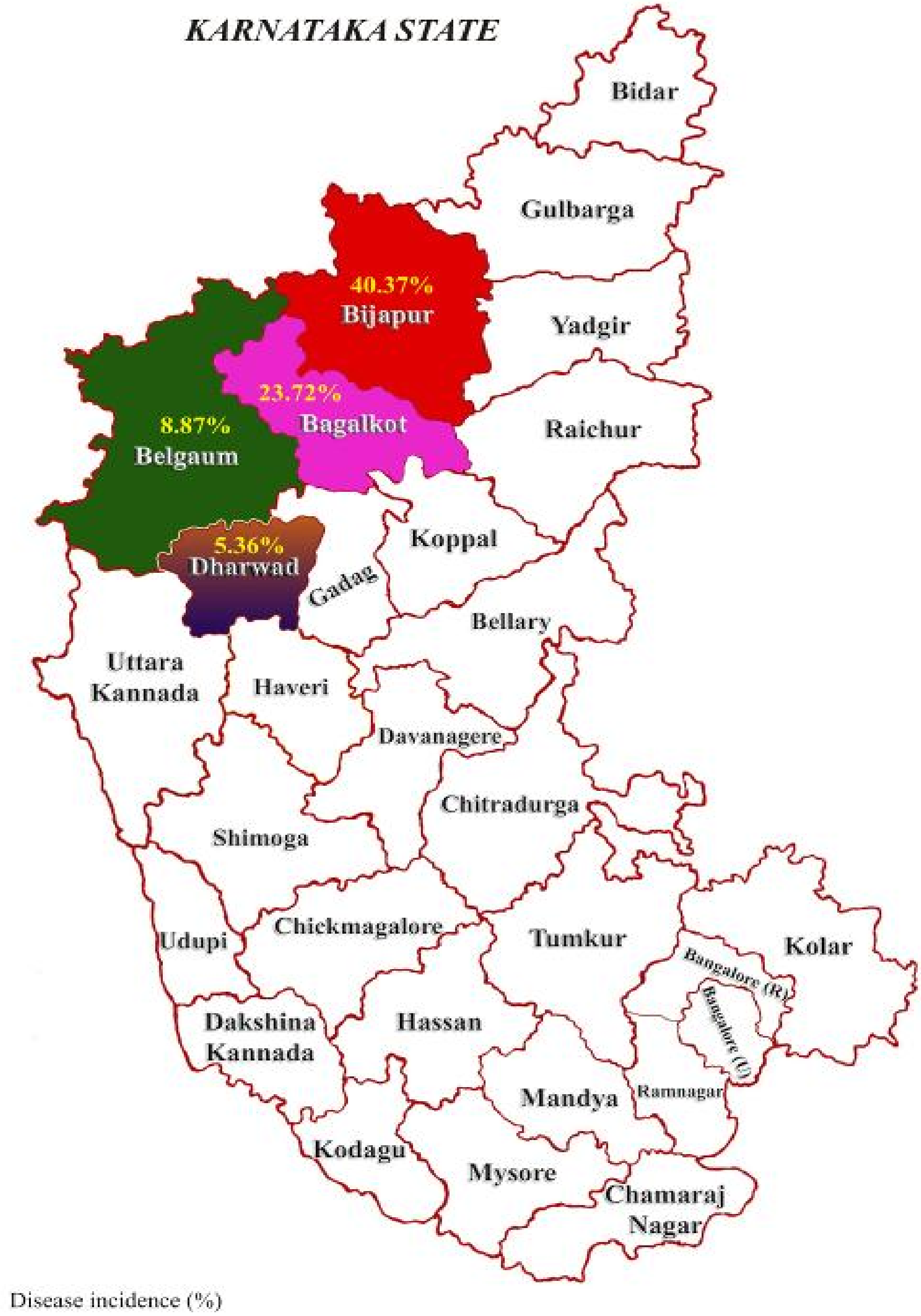


Plate 1: Survey for the disease incidence of Tip-over of banana in different districts of northern Karnataka

Plate 1. Survey for the disease incidence of Tip-over of banana in different districts of northern Karnataka

Table 1: Survey for the disease incidence of Tip-over of banana in Belgaum, Dharwad, Bijapur and Bagalkot districts.

Sl. No.	District	Taluk	Village	Variety	No. of orchards	Disease Incidence (%)
1	Bagalkot	Bagalkot	Govinkoppa	TC	2	22.35
			Kaladgi	TC	3	16.50
			Gaddankeri	TC	2	19.42
		Bilagi	Badagandi	TC	4	45.80
			Chikkasangama	TC	3	10.25
2	Bijapur	Bijapur	Jumnal	TC	6	40.50
			Utnal	TC	2	15.33
			Kanmadi	TC	3	27.95
			Korthi	TC	4	18.87
		Basavan Bagewadi	Hoganahalli	TC	5	65.28
3	Dharwad	Kalghatagi	Gudihal	Local	4	5.36
4	Belgaum	Saundatti	Sogala	TC	2	16.36
			Gurlahosur	Local	4	6.28
		Raibagh	Niralkodi	TC	3	11.23
			Mugalkod	TC	5	5.05
		Bailhongal	Bevinakoppa	Local	5	4.25
			Sampagaon	Local	3	10.00

TC-Tissue Culture

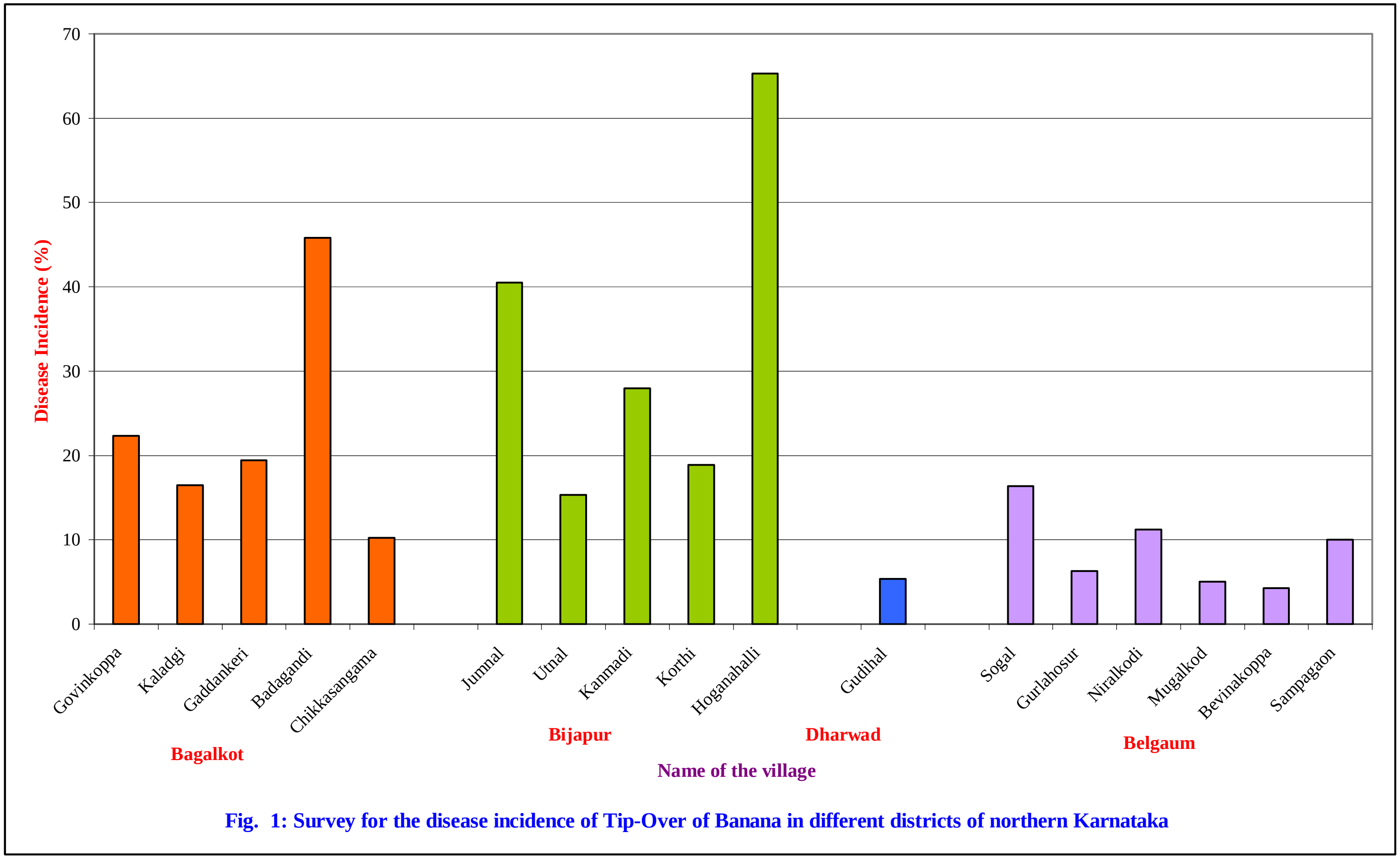


Fig. 1: Survey for the disease incidence of Tip-Over of Banana in different districts of northern Karnataka

Table 2: Mean disease incidence of Tip-over of banana in different districts of Karnataka

District	Taluk	Disease incidence (%)
Bagalkot	Bagalkot	19.42
	Bilagi	28.02
	Mean	23.72
Bijapur	Bijapur	27.95
	Basavana bagewadi	65.28
	Mean	40.37
Dharwad	Kalghatagi	5.36
	Mean	5.36
Belgaum	Saundatti	11.32
	Raibagh	8.18
	Bailhongal	7.12
	Mean	8.87

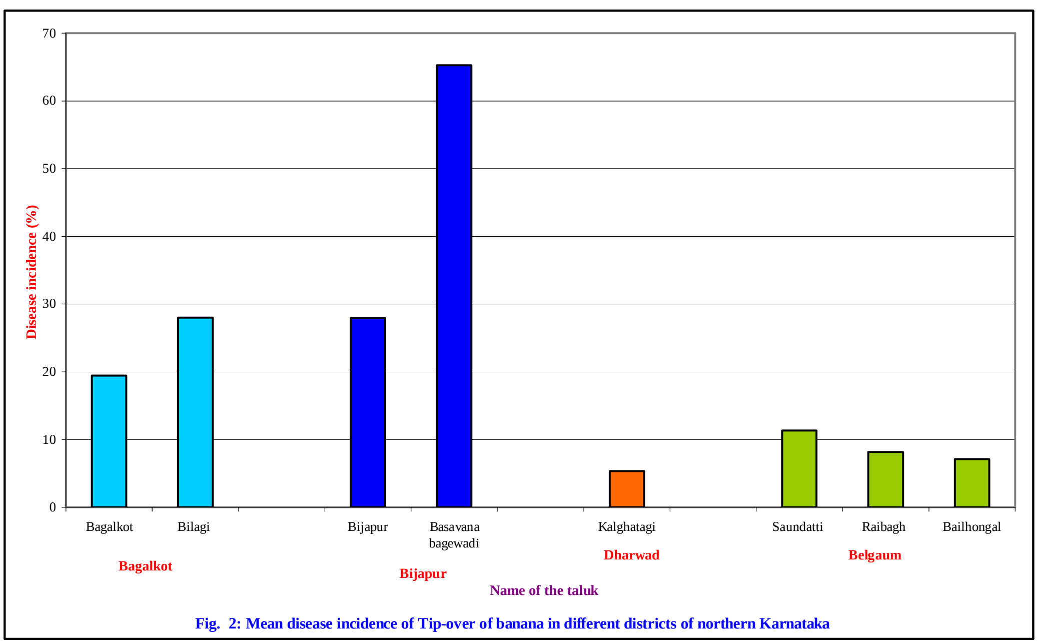


Fig. 2: Mean disease incidence of Tip-over of banana in different districts of northern Karnataka

In Raibagh taluk, two villages were surveyed. The incidence ranged from 5.05 to 11.23 per cent. The maximum incidence (11.25%) was observed in Niralkodi, and the least incidence was observed in Mugalkod (5.05).

Dharwad district

One taluk was surveyed in Dharwad district *i.e.*, Kalghatagi. The disease incidence observed was 5.36 per cent in the Gudihal village.

4.2 Symptomatology, isolation, identification and pathogenicity studies

4.2.1 Symptomatology

Symptoms were observed on rhizome, pseudostem and leaf. The above ground portion of infected plants appeared weak, thrifty with pale yellow lustreless leaves with marginal necrosis or scorching in older leaves. The plants appeared dwarf and such plants when uprooted along with rhizomes, showed rotting, tissue becoming soft and tunnelling dark brown to black and at this stage tunnelling or cavities was also observed in the rotted portions of the rhizomes.

The soft rot was characterized by a massive soft, odorous rot of the center or a portion of the rhizome. The rot progressed up the pseudostem, destroying the growth point and causing internal decay, often with vascular discolouration. There was discoluration and soft rotting of the main rhizome and suckers. The internal symptoms of the disease were rotting of the rhizome with brown discolouration starting from the pheripheral region and extended laterally towards the core of the rhizome (Plate 2). Soon after, the rotting extended to large and deeper part of the rhizome, the tissue became massive soft, watery and dark brown to black and at these cavities in the rotted tissue had become porous like without solid tissue because of degradation by pathogen.

4.2.2 Isolation of pathogen and maintenance of pure culture

The causal organism *Erwinia carotovora* subsp. *carotovora* was isolated from infected rhizome showing typical symptoms of tip over. Isolation was done by pour plate method, as explained in material and methods on Crystal Violet Pectate medium as it is a diagonostic media for Erwinias. Well separated single colony was picked and streaked on Nutrient Agar medium at 28^0 C for 48 hours. The pure colonies obtained were again streaked on slants and pure colonies put in sterilized vials containing water too. The cultures so obtained were stored in the refrigerator at 5^0C, which served as a stock culture for further studies. The cultures were renewed by sub-culturing once in a fortnight Nutrient Agar slants.

4.2.3 Proving pathogenicity for *Erwinia carotovora* subsp. *carotovora*

Koch's postulates were followed to prove pathogenic nature of *Erwinia carotovora* subsp. *carotovora*. For proving pathogenicity banana plants of forty days old are raised on steam sterilized standard potting mixture with soil, sand, and FYM in 3:1:1 ratio. Inoculation was done with with 48 hours old bacterial culture through root dip method, pseudo stem injection using hypodermic syringe and soil application by scraping soil surface and pouring the suspension and a control treatment inoculation with sterile water was maintained and is presented in Plate 3.

Observations were recorded for the development of symptoms of Tip-over disease such as pale yellow and brown discoloured necrotic leaves and development of rotting at the rhizome region. After the development of the typical symptoms of the disease, the pathogen was reisolated. The reisolated culture resembled the original mother culture and thus pathogenicity test was confirmed.

4.2.4 Morphological, physiological and biochemical characteristics

The results of the various morphological, physiological and biochemical tests are given in Table 3 and Plate 4. The bacterium is a rod shaped facultatively anaerobic, gram negative, oxidase negative and peritrichously flagellated.

Field view : wilting of rhizome

Dislodging of infected plant

Wilting st seedling stage

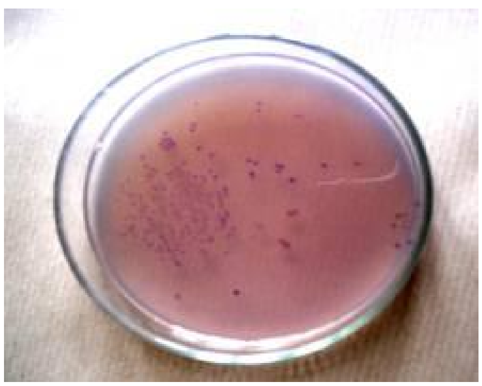
Splitting of the pseudostem

Massive degradation of tissue

Dark brown discolouration of pseudostem

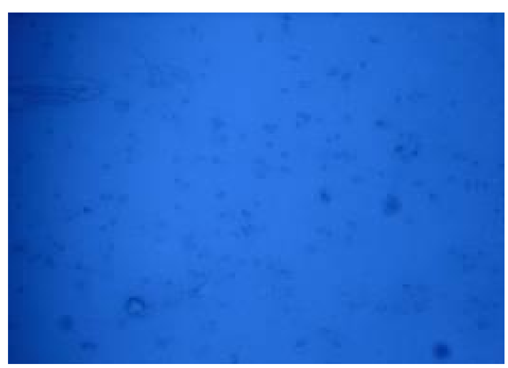
Plate 2. Symptoms of Tip-over disease of banana

Pathogenicity test



Single colonies of Erwinia carotvara Supsp. Carotovora on CVP medium

Erwinia carotovora subsp. Carotovora on Nutrient agar



Microscpic view (1000 w)

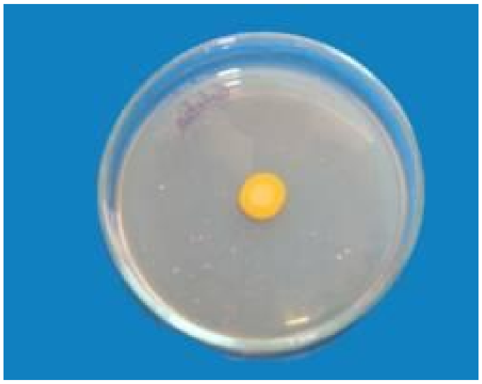
Plate 3. Isolation and identification of causal agent and pathogenicity studies

Table 3: Morphological, physiological, and biochemical characteristics of *Erwinia carotovora* subsp. *carotovora*

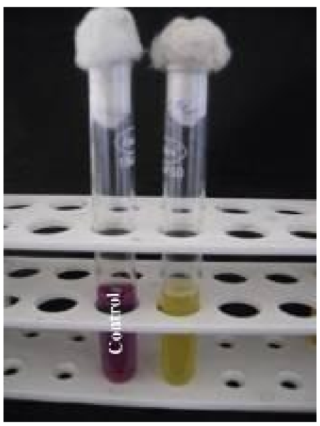
Test	Inference
Colony morphology on Nutrient Agar	
Shape	Round
Margin	Entire
Elevation	Convex
Surface	Smooth
Pigment	Yellow
Cell Shape	Rod
Arrangement	Single
Biochemical Characterization	
Gram reaction	-
Starch hydrolysis	+
Gelatin liquefaction	+
H_2S production	+
Potato soft rotting	+
Levan production	–
Tobacco hypersensitivity	+
Catalase test	+

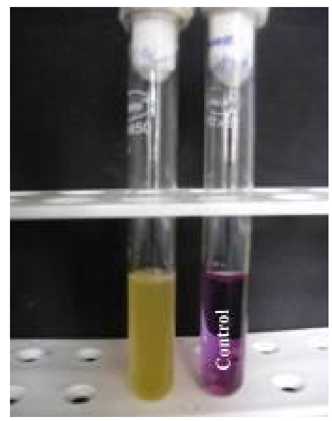
- : Negative

+ : Positive



Gelatin liquefication **Starch bydrolysis**





Sucrose **Maltose** **Glucose**

H_2S Production **Lactose utilization**

Plate 4. Biochemical test for *Erwinia carotovora* subsp. *carotovora*

It was negative for catalase reaction but utilized glucose, fructose, 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 nitrites.

4.2.4.1 Catalase test

After covering with few drops of 20 volume hydrogen peroxide to a slide smeared by a loopful of test bacteria, there was no production of gas bubbles indicating the negative reaction for the test.

4.2.4.2 Gelatin liquefaction

This test revealed that a white precipitate was formed around the spot inoculated bacterium when flooded with the solution containing 0.2% mercuric chloride solution in dilute HCl (20%).

4.2.4.3 Hydrogen sulphide production

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

4.2.4.4 Starch hydrolysis

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

4.2.4.5 Acid production from maltose, glucose, sucrose and dextrose

There was change in the colour from pink to yellow that confirmed the positive for acid production from maltose, glucose, sucrose and dextrose.

4.2.3.6 Hypersensitive reaction on tobacco

The pure culture of *Erwinia carotovora* subsp. *carotovora* injected into intercellular spaces of tobacco leaves (*Nicotiana tobaccum* var. *samsun*) produced characteristics water soaked lesions within 16 to 20 hours of inoculation. Further, after 24 hours the light yellow area started collapsing and formed a desiccated light brown necrotic area.

4.3 Host range studies

The following were the plants used for the study of host range of pathogen carrot, potato, tomato, onion, cabbage, brinjal, groundnut and citrus. They were inoculated as mentioned in material and methods. All the plants above mentioned were infected by the pathogen. However time taken for expression of the symptoms varied and its given in Table 4 and Plate 5. In this study, host range of *Erwinia carotovora* subsp.*carotovora* was drawn out. Tomato showed after two days, cabbage and onion took three days for first symptom expression, while brinjal and groundnut took five days. Potato and Carrot showed symptoms after two days. No symptoms were observed in Citrus.

On tomato it produced soft rot with brown discolouration of leaves and subsequent drying of leaves was observed. Onion produced typical symptoms of soft rot and oozing of putrefied liquid at the point of inoculation. On cabbage, brown-blackish discolouration of leaves and subsequent drying of leaves was observed.

4.4 *In vitro* evaluation of antibacterial chemicals, botanicals / organics and bioagents on the growth of *Erwinia carotovora* subsp. *carotovora*

4.4.1 *In vitro* evaluation of chemicals on the growth of *Erwinia carotovora* subsp. *carotovora*

Table 4: Host range studies of *Erwinia carotovora* subsp. *carotovora*

Hosts	Common name	No. of days taken for symptom expression	Symptoms
Lycopersicon esculentum	Tomato	2	Typical soft rot and drying of leaves
Solanum melongena	Brinjal	5	Brownish discolouration
Allium cepa	Onion	3	Drying of leaves
Solanum tuberosum	Potato	2	Brownish discolouration
Daucas carota	Carrot	2	Typical soft rot and black discolouration
Brassica oleracea	Cabbage	3	Brown to blackish discolouration
Arachis hypogea	Groundnut	5	Brownish discolouration
Citrus sp	Citrus	-	No symptoms

Tomato

Brinjal

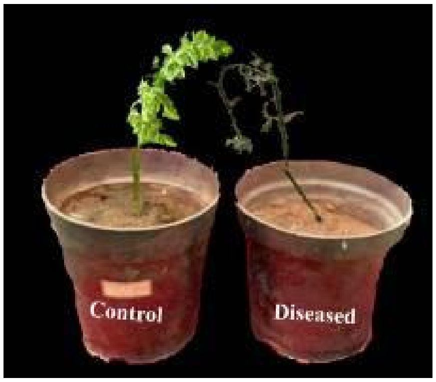
Groundnut

Cabbage

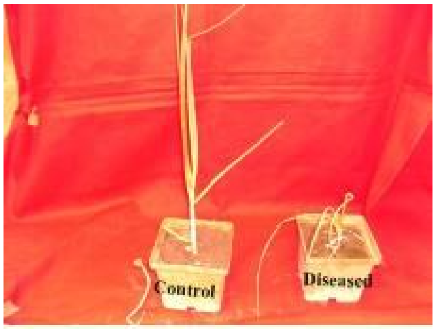
Plate 5. Host range studies for *Erwinia carotovora* subsp. *carotovora*

Plate 5. Cond……….

Potato



Carrot



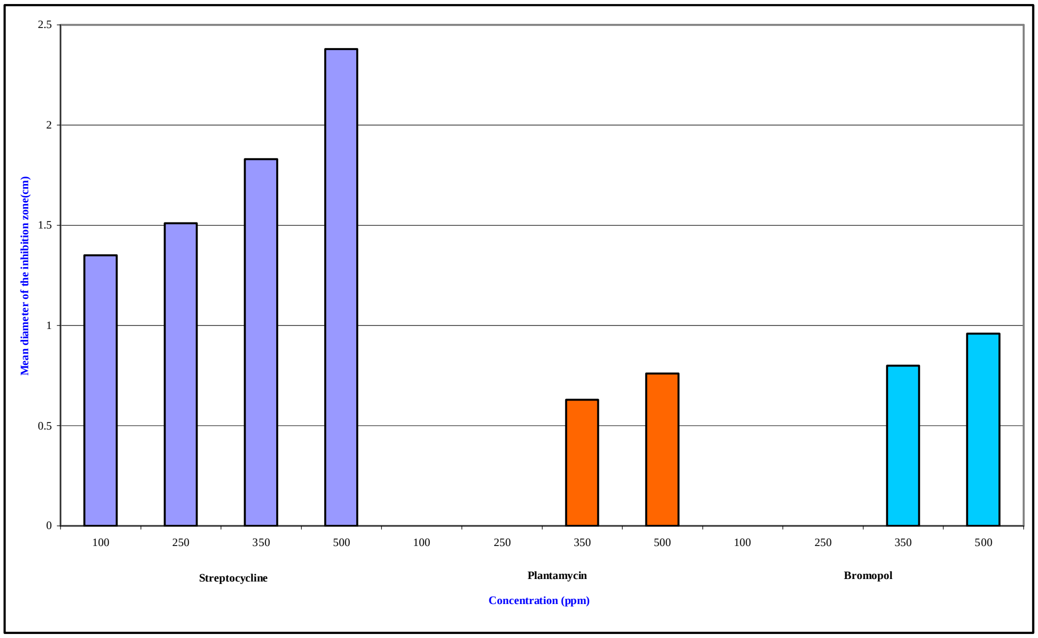
Onion

Citrus

Table 5: ***In vitro*** **evaluations of chemicals on the growth of** ***Erwinia carotovora*** **subsp.** ***carotovora***

Sl. No	Name of the chemical	Concentration (ppm)	Mean diameter of the inhibition zone (cm)
1	Streptocycline	100	1.35 (1.53)
		250	1.51 (1.58)
		350	1.83 (1.69)
		500	2.38 (1.84)
2	Plantamycin	100	0.00 (1.00)
		250	0.00 (1.00)
		350	0.63 (1.27)
		500	0.76 (1.33)
3	Bromopol	100	0.00 (1.00)
		250	0.00 (1.00)
		350	0.80 (1.34)
		500	0.96 (1.40)
4	Bleaching powder	1000	0.00 (1.00)
		2000	0.00 (1.00)
		3000	0.00 (1.00)
		4000	0.75 (1.32)
5	Copper Oxychloride	1500	1.35 (1.53)
		2000	1.44 (1.56)
		2500	1.60 (1.61)
		3000	1.72 (1.65)
6	Copper Hydroxide	1500	1.21 (1.49)
		2000	1.33 (1.53)
		2500	1.49 (1.58)
		3000	1.59 (1.61)
7	Copper Sulphate	1500	0.74 (1.32)
		2000	0.85 (1.35)
		2500	0.92 (1.39)
		3000	1.01 (1.43)
Control			0.00
Factor		S.Em±	CD at 1%
Chemicals		0.0029	0.0103
Concentration		0.0022	0.0082
Interaction		0.0058	0.0216

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



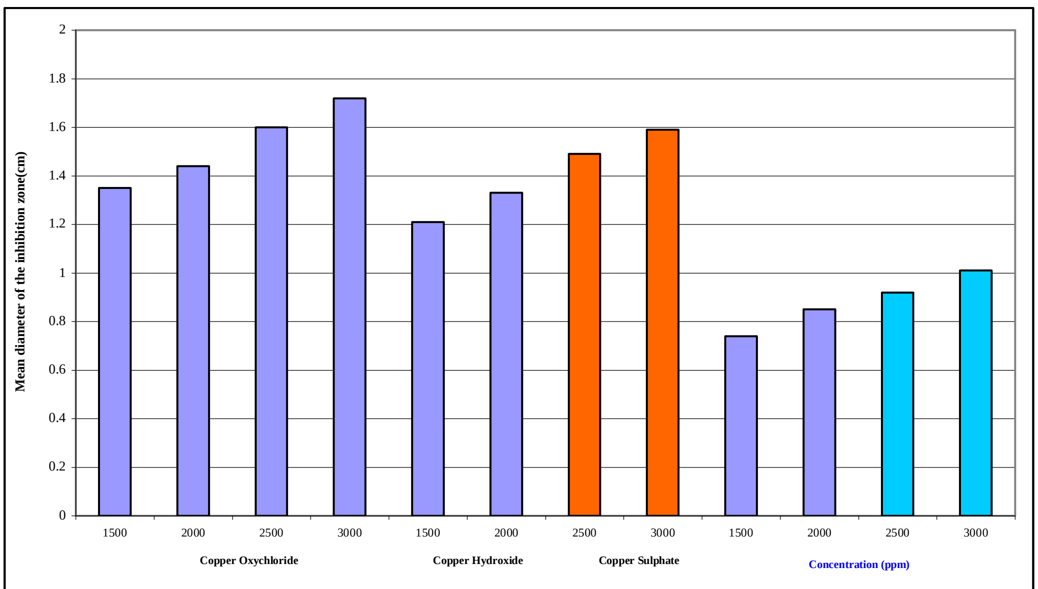
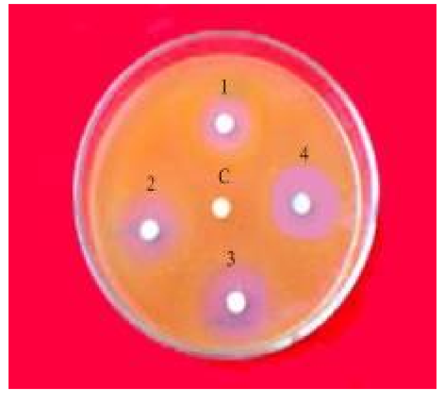
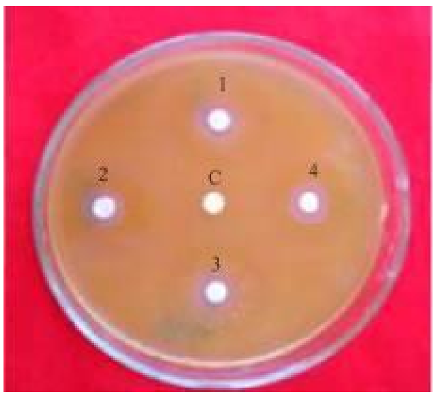


Fig. 3: *In vitro* evaluations of antibacterial chemicals on the growth of *Erwinia carotovora* subsp. *carotovora*

Fig. 3: In vitro evaluations of antibacterial chemicals on the growth of Erwinia carotovora subsp. carotovora



Copper hydroxide



Copper sulphate

Copper oxychloride

C - Control 1 – 1500ppm 2 – 2000ppm

3 – 2500ppm 4 – 3000ppm

Plate 6. *In vitro* evaluations of chemicals on the growth of *Erwinia carotovora* subsp. *carotovora*

Plate 6 Contd……..

Plantamycin

C - Control 1 - 100 ppm 2 - 250 ppm
3 - 350 ppm 4 - 500 ppm

Streptocyline

Bromopol

Bleaching powder

C - Control 1 - 1000 ppm 2 - 2000 ppm
3 - 3000 ppm 4 - 4000 ppm

Table 6: *In vitro* evaluation of combination of chemicals /antibiotics on the growth of *Erwinia carotovora* subsp. *carotovora*

Sl. No.	Name of the chemical	Concentration (ppm)	Mean diameter of inhibition zone (cm)
1	Streptocycline + Copper oxychloride	500 + 3000	2.83 (1.95)
2	Streptocycline + Copper hydroxide	500 + 3000	2.56 (1.88)
3	Streptocycline + Copper sulphate	500 + 3000	2.13 (1.76)
4	Plantamycin + Copper oxychloride	500 + 3000	1.73 (1.65)
5	Plantamycin + Copper hydroxide	500 + 3000	1.63 (1.62)
6	Plantamycin + Copper sulphate	500 + 3000	1.06 (1.43)
7	Bromopol + Copper oxychloride	500 + 3000	2.03 (1.74)
8	Bromopol + Copper hydroxide	500 + 3000	1.93 (1.65)
9	Bromopol + Copper sulphate	500 + 3000	1.26 (1.50)
		S.Em±	0.33
		CD at 1%	1.36

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

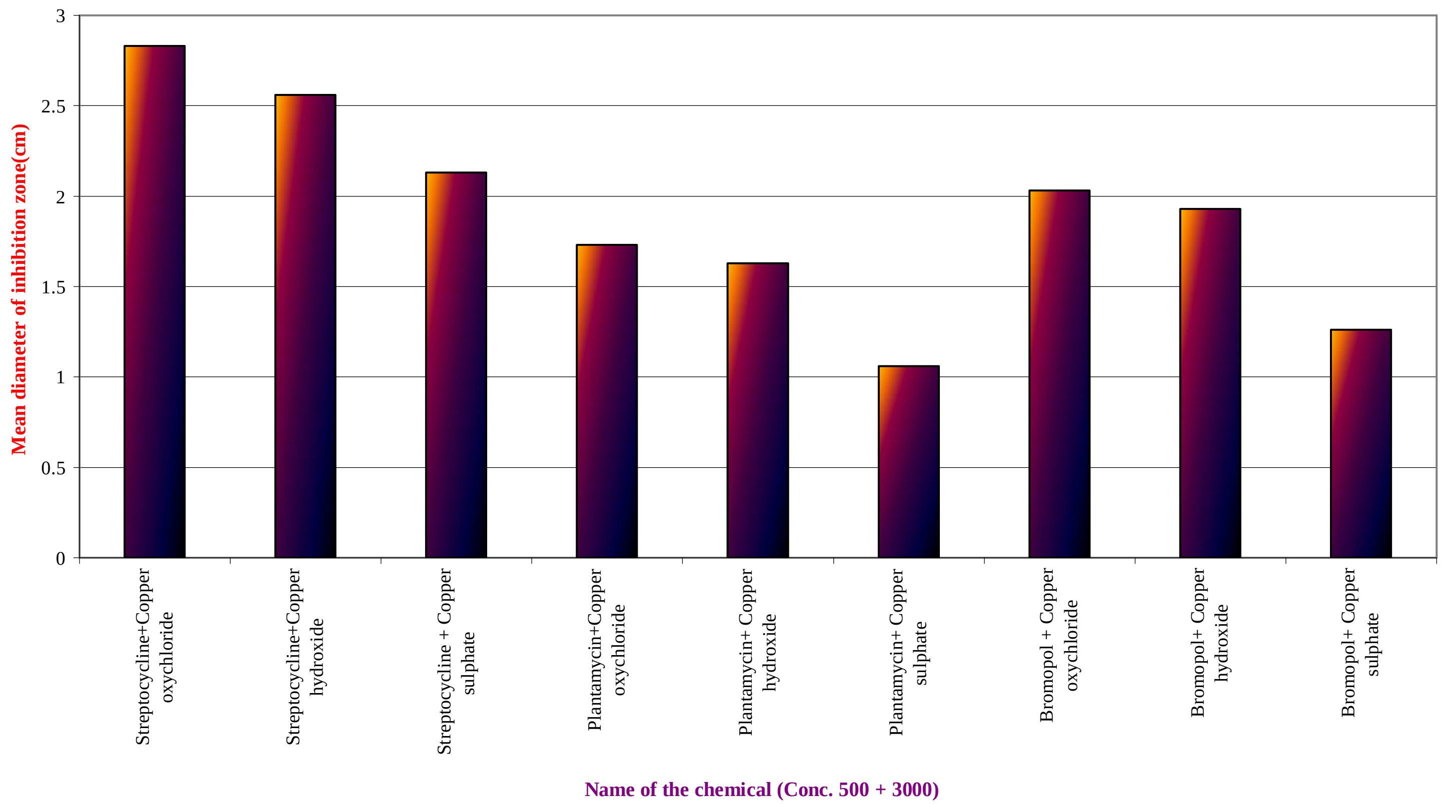
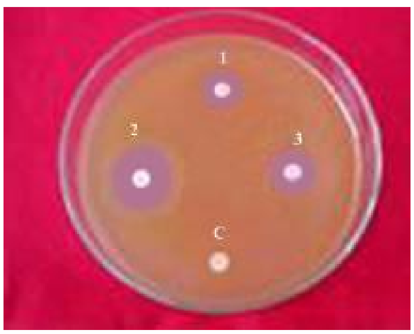


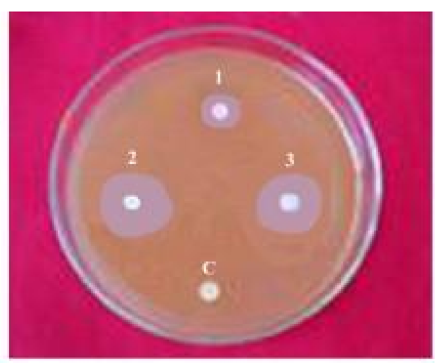
Fig. 4: *In vitro* evaluation of combination of chemicals /antibiotics against the growth of *Erwinia carotovora* subsp. *carotovora*

Fig. 4: In vitro evaluation of combination of chemicals /antibiotics against the growth of Erwinia carotovora subsp. carotovora

Streptocycline



Plantamycin



1 - Copper sulphate (3000 ppm)
2. Copper oxychloride (3000 ppm)
3. Copper hydroxide (3000 ppm)

Bromopol

Plate 7. *In vitro* evaluation of combination of chemicals/antibiotics on the growth of *Erwinia carotovora* subsp. *carotovora*

Present investigation was carried out to evaluate commercially available antibacterial chemicals to find out their effectiveness against the growth of *Erwinia carotovora* subsp. *carotovora* under *in vitro* condition and the results were presented in Table 5, Fig. 3 and Plate 6.

Results indicated that streptocycline proved significantly superior over other treatments with highest inhibition (2.38 cm) at 500 ppm followed by Copper oxychloride at (1.72 cm) and Copper hydroxide (1.21 cm) both at 3000 ppm. Next best was Copper sulphate which showed inhibition of (1.01 cm) at 3000 ppm. Bromopol and Plantamycin were on par with each other, at 500 ppm concentrations they showed (1.40 cm) and (1.33 cm) respectively, where both the chemicals showed no effect at 100 and 250 ppm Bleaching powder showed least effective only at higher concentration at 4000 ppm. However, all other chemicals *viz.*, copper oxychloride and copper hydroxide were on par with each other.

Interaction effect among the chemicals and concentrations indicated that streptocycline (500 ppm) and copper oxychloride (3000 ppm) were found significantly superior over other treatments with an inhibition zone of 2.38 cm and 1.72 cm, respectively.

Among the different antibacterial chemicals in combination with copper chemicals evaluated against the *Erwinia carotovora* subsp. *carotovora* under *in vitro*. Results revealed that, copper oxychloride and copper hydroxide both (3000 ppm) each in combination with Streptocycline (500 ppm) The results indicated that streptocycline (500 ppm) in combination with Copper oxychloride (3000 ppm) found an inhibition zone of 2.83 mm (1.95 cm) which exhibited superior followed by streptocycline (500 ppm) with copper hydroxide than with copper sulphate both at 3000 ppm as given in Table 6 and Fig. 4 Plate 7.

Bromopol and Plantamycin, each in combination with 3000 ppm of copper oxychloride formed an inhibition zone of 2.03 and 1.73 cm respectively. Next chemicals in same combination with copper hydroxide and copper sulphate has shown inhibition zone which were on par with each other.

4.4.2 *In vitro* evaluation of botanicals/organic products on the growth of *Erwinia carotovora* subsp.*carotovora.*

In vitro evaluation of botanicals was carried out with respect to inhibition zone produced due to inhibition of *Erwinia carotovora* subsp. *carotovora* at different concentrations as explained in the Material and Methods and the data is presented in Table 7, Plate 8.

Six botanicals were selected for the evaluation Tulsi, Garlic, Eupatorium, Meswak, Kalongi, Soapnut. Out of six botanicals evaluated, it was found that only Garlic was effective at 10 per cent concentration, rest of them were found ineffective. Cowdung and lime (10 g + 1 g) was also found ineffective.

4.4.3 *In vitro* evaluation of bioagents on the growth of *Erwinia carotovora* subsp. *carotovora*

The antagonistic microorganisms *viz.*, *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma koningii* Oudem, *Trichoderma virens* Miller Gidden., *Pseudomonas fluorescens* migula and *Bacillus subtilis* Cohn were evaluated against *Erwinia carotovora* subsp. *carotovora* under *in vitro* conditions by inhibition zone method, Mycelial disc method and Culture filtrate method as explained in the Material and Methods. Inhibition zone produced across the antagonistic microorganisms was recorded.

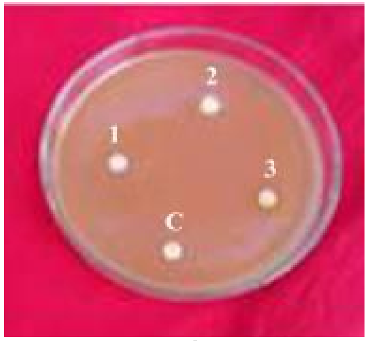
The results indicated given in Table 8 and Plate 9 that the antagonistic microorganism *Pseudomonas fluorescens* resulted with an inhibition zone of 1.85 cm which was found significant over *Bacillus subtilis* which did not produced any inhibition zone. Whereas, the fungal antagonists like *T. harzianum*, *T. viride*, *T. virens* and *T. koengii* were found ineffective.

Table 7: *In vitro* evaluation of botanicals/organics against the growth of *Erwinia carotovora* subsp. *carotovora*

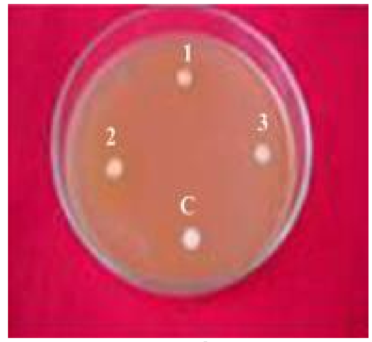
Sl. No.	Common name	Scientific name	Economic plant part	Mean diameter of inhibition zone (cm) at different concentrations (%)		
				5	7.5	10
1	Tulsi	*Ocimum santum*	Leaves, shoot	0.00 (1.00)*	0.00 1.00	0.00 1.00
2	Eupatorium	*Eupatorium adenophorum*	Leaves	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
3	Meswak	*Salvadora persica*	Roots	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
4	Kalongi	*Nigella sativa*	Seeds	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
5	Soapnut	*Sapindus mukorossi*	Seeds	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
6	Garlic	*Allium sativum*	Bulbs	0.00 (1.00)	0.00 (1.00)	0.92 (1.39)
7	Cow dung + Lime	-	Extract	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control				0.00		

Factor	S.Em±	CD at 1%
Botanicals	0.0011	0.0041
Concentration	0.0017	0.0064
Interaction	0.0029	0.0110

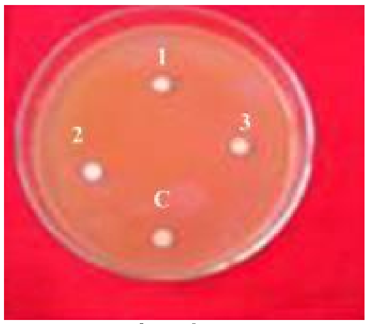
* - Figures in the parenthesis are $\sqrt{x + 1}$ transformed values.



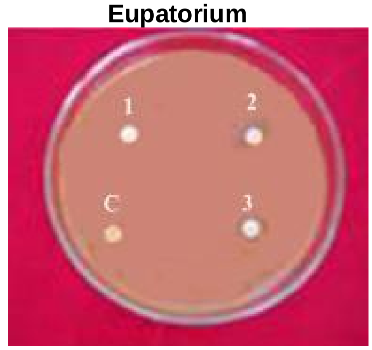
Tulsi



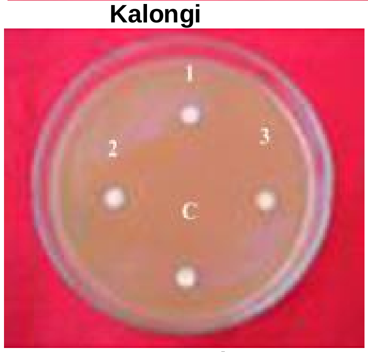
Eupatorium



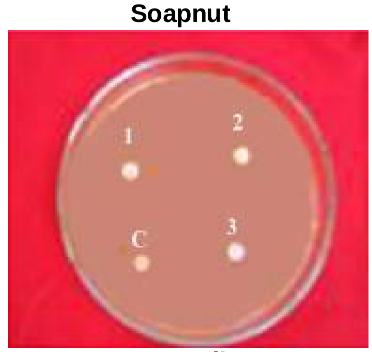
Kalongi



Soapnut



Meswak



Garlic

Garlic

1 - 5%
2 -7.5%
3 - 10%
C - Control

Plate 8. *In vitro* evaluation of botanicals / organic products on the growth of *Erwinia carotovora* pv. *carotovora*

Table 8: *In vitro* evaluation of bioagents against the growth of *Erwinia carotovora* subsp. *carotovora*

Sl. No.	Name of the bio agent	Mean diameter of inhibition zone (cm)
1	*Trichoderma harzianum*	0.00 (1.00)
2	*Trichoderma viride*	0.00 (1.00)
3	*Trichoderma koenigii*	0.00 (1.00)
4	*Trichoderma virens*	0.00 (1.00)
5	*Pseudomonas flourescens*	1.03 (1.42)
6	*Bacillus subtilis*	0.00 (1.00)
7	Control	0.00
	S.Em±	0.02
	CD at 1%	0.08

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

P - *Pseudomonas fluorescens*
B - *Bacillus subtilis*
C - Control

Inhibition Zone

Mycelial disc method

1) *Trichoderma harzianum*

2) *Trichoderma viridae*

3) *Trichoderma koningii*

4) *Trichoderma virens*

C - Control

Culture filtrate method

Plate 9. *In vitro* evaluation of bioagents on the growth of *Erwinia carotovora* subsp. *carotovora*

4.4.4 Pot culture studies

The chemicals which performed best under *in vitro* condition were studied in the pot culture. Chemicals were screened for their efficacy to check the incidence of rhizome rot or tip over of banana. This experiment was conducted in glass house of Plant Pathology, UAS, Dharwad. The trial was laid out in Randomized Completely Block Design (RCBD) with three replications in each treatment and each replication had three plants of Grand Naine. The details of the treatments were given below and in Table 9, Fig. 5 and Plate 10.

In vivo evaluation of the integrated management of the disease was taken as pot culture studies. The design adopted was Randomized Completely Block Design (RCBD) with three replications, each replication comprised of three plants. Results revealed that the T_1 (streptocycline 500 ppm + copper oxychloride 3000 ppm) gave 61.94 per cent disease reduction over the control shown in Plate 10. T_2 (streptocycline 500 ppm + copper hydroxide 3000 ppm) gave 56.74 per cent next followed by T_3 (streptocycline 500 ppm + copper sulphate) and T_4 (bromopol 500 ppm + copper oxychloride 3000 ppm) 53.88 and 50.61% respectively. T_5 (bromopol 500 ppm + copper hydroxide 3000 ppm), T_6 (plantamycin 500 ppm + copper oxychloride 3000 ppm) and T_7 (*Pseudomonas fluorescens* + garlic 10%) showed least disease reduction 42.37, 32.80 and 23.68 per cent, respectively. Treatment T_8 (untreated control) showed 100 per cent death of banana plants.

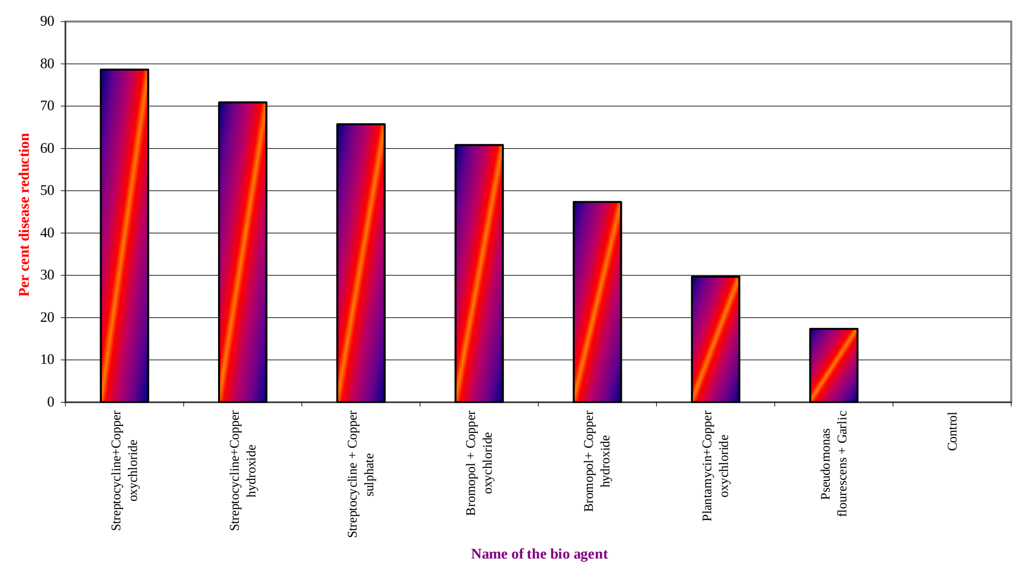


Fig. 5: Pot culture studies against *Erwinia carotovora* subsp. *carotovora*

Fig. 5: Pot culture studies against Erwinia carotovora subsp. carotovora

Invivo evaluation of chemicals and Biogents at initial stage

In vivo evaluation of one month after Chemical treatment

Streptocycline + copper oxychloride

Plate 10. Pot culture studies against *Erwinia carotovora* subsp. *Carotovora*

Table 9: Pot culture studies against *Erwinia carotovora* subsp. *carotovora*

Treatment	Treatment details	Per cent disease reduction
T_1	Streptocycline + Copper oxychloride	(78.62) 61.94
T_2	Streptocycline + Copper hydroxide	(70.89) 56.74
T_3	Streptocycline + Copper sulphate	(65.72) 53.88
T_4	Bromopol + Copper oxychloride	(60.78) 50.61
T_5	Bromopol + Copper hydroxide	(47.37) 42.37
T_6	Plantamycin + Copper oxychloride	(29.64) 32.80
T_7	*Pseudomonas flourescens* + Garlic	(17.34) 23.68
T_8	Control	0.00
S.Em ±		0.40
CD at 5%		1.22

* - Figures in the parenthesis are corresponding arcsine transformed values.

5. DISCUSSION

Banana is the one of the oldest and best known fruits of the world. Banana is delicious and seedless and is available in all seasons. Banana plant is one of the most versatile plants that every part of the plant is useful to cure different types of disorders or conditions. Banana is very hygienic and nutritious and most widely consumed food in the world. Banana and plantain constitute a major staple food crop for millions of people in tropical populations.

Several factors are attributed to the constraints for high production of banana and the major being the occurrence of diseases. Banana is subjected to many serious debilitating diseases caused by fungi, viruses, bacteria, nematodes and non-parasitic diseases. Among the bacterial diseases, tip-over or bacterial rhizome rot of banana is gaining importance in recent years in Karnataka through earlier the disease was considered as of minor importance.

For the first time in India, Wardlaw (1950) reported the bacterial nature of bacterial head rot/ rhizome rot of banana from Allahabad in UP. Later Chattopadhyay and Mukherjee (1986) detected a bacterial soft rot disease on the pseudostem and corm of Giant Governor cultivar of Cavendish banana in India in 1983 in the orchards of Mondouri farm of the Viswa Vidhyalaya.

Lakshmanan and Mohan (1986) observed tip-over or head rot caused by *Erwinia carotovora* in Tamil Nadu in North Arcot and Trichy districts. Khan and Nagaraj (1998) reported the incidence of tip-over or rhizome rot of banana for the first time in Kolar and Bangalore districts in Karnataka state.

5.1 Survey for the disease incidence of Tip-over of banana in Belgaum, Dharwad, Bijapur and Bagalkot districts

Roving survey was conducted during 2011-12 to assess the disease incidence of tip over of banana in major banana growing areas of Northern Karnataka in districts Belgaum, Dharwad Bijapur and Bagalkot.

The disease incidence was noticed in all the locations surveyed, with a range from 4.20 to 65.28 per cent. The mean maximum disease incidence (46.61%) was observed in Bijapur district followed by Bagalkot district (23.72%). Whereas, the least incidence (8.87%) was observed on Belgaum district and in Dharwad district very least incidence (5.36%). The survey is in line with the studies conducted by Snehalatharani (2001) and Nagaraj and Khan (2002)

Snehalatharani (2001) reported the disease incidence in southern parts of Karnataka Bangalore, Tumkur and Kolar districts and West Godavari and Konnur districts of Andhra Pradesh.

Nagaraj and Khan (2002) were conducted survey and reported the incidence of Tip-over for the first time in Karnataka.

5.2 Symptomatology, isolation and pathogenicity studies

5.2.1 Symptomatology

Symptoms were observed on rhizome, pseudostem and leaf. The above ground portion of infected plants appeared weak, thrifty with pale yellow lustreless leaves with marginal necrosis or scorching in older leaves. The leaves in the diseased plants appeared pale yellow, lusterless, with marginal necrosis. The young central leaves/ shoots appeared scorched giving a burnt appearance when such plants were uprooted along with the rhizomes, the large part of the rhizome was found massive soft, rotted and turned brown to black discolouration.

The internal symptoms of the disease are rotting of the rhizome with brown discolouration starting from the pheripheral region and extending laterally towards the core of the rhizome. Soon after, the rotting extended to large and deeper part of the rhizome, the tissue becomes massive soft, watery and dark brown to black and at this cavities in the rotted tissue had become porous like without solid tissue because of degradation by pathogen.

The rotted area showed tunneling effect and emitted foul smell. Whenever a strong wind or mild knock was given to severely affected plant, the plant toppled down at much rotted portion just below the soil line, hence, the name Tip over.

Similar kinds of symptoms were described by Chattopadhyay (1987) that the soft rot is characterized by a massive soft, odorous rot of the center or a portion of the rhizome. The rot progresses up the pseudostem, destroying the growth point and causing internal decay, often with vascular discolouration. There was discoluration and soft rotting of the main rhizome and suckers.

Wardlaw (1950) discoluration and soft rotting of the main rhizome and suckers. Head rot plants were easily pushed over being either uprooted or broken across the much rotted and perforated rhizome about soil-level. Cross sectional destruction of the rhizome at soil level was almost complete. In badly affected areas, plants which had attained to a considerable height suffered greatly from wind action.

5.2.2 Isolation of pathogen and proving pathogenicity

The causal organism *Erwinia carotovora* subsp. *carotovora* was isolated from infected rhizome showing typical symptoms of Tip-over. Isolation was done by pour plate method, as explained in material and methods on crystal violet pectate medium as it is a diagonostic media for Erwinias. Well separated single colony was picked and streaked on nutrient agar medium at 28^{0} C for 48 hours.

Inoculation was done with 48 hours old bacterial culture through root dip method, pseudo stem injection using hypodermic syringe and soil application by scraping soil surface and pouring the suspension for the forty days old banana plants. After the development of the typical symptoms of the disease like pale yellow and brown discoloured necrotic leaves and development of rotting at the rhizome region, the pathogen was reisolated. The reisolated culture resembled the original mother culture and thus pathogenicity test was confirmed

. Lakshmann and Mohan (1992) reported that the causal bacterium, *Erwinia carotovora* was isolated and the pathogenicity was proved by soaking the suckers in a bacterial suspension of virulent strain for 15 minutes.

Isolation and pathogenicity studies were also carried out in a similar fashion by Tomlinson *et al.* (1987) Biochemical and pathogenicity tests identified *Erwinia chrysanthemi* as the causal organism of a serious corm and rhizome rot of banana in lowland Papua New Guinea. The pathogen was repeatedly isolated from diseased tissue and soil surrounding diseased plants.

5.3 Host range studies

The following are the plants used for the study of host range of pathogen carrot, potato, tomato, onion, cabbage, brinjal, groundnut and citrus. They were inoculated as mentioned detail in material and methods. All the plants above mentioned were infected by the pathogen. However, time taken to express the symptoms varied. In this study, host range of *Erwinia carotovora* subsp. *carotovora* was drawn out. Carrot, potato and tomato showed after two days, cabbage and onion took three days for first symptom expression, while brinjal and groundnut took five days. Citrus did not show any symptoms.

Alippi *et al.* (1997) reported tomato as a host of *Erwinia carotovora* subsp. *carotovora* in Argentina. Bhat *et al.* (2010) Seven different vegetable species *viz.* carrot (*Dacus carota*), cucumber (*Cucumis sativa*), onion (*Allium cepa*), potato (*Solanum tuberosum*), knol khol (*B. oleracea var caulorapa*), cauliflower (*B. oleracea var botrytis*), tomato (*Lycopersicon esculentum*), belonging to different families were tested for host range studied of the pathogen.

Studies on host range of the bacterium under artificial inoculation revealed that all the seven tested vegetable species, representative of the families, were susceptible to soft rot. Here the present findings are also exhibited same situation as earlier workers are show the same line.

5.4 *In vitro* evaluation of antibacterial chemicals, botanicals and bioagents on the growth of *Erwinia carotovora* subsp. *carotovora*

5.4.1 *In vitro* evaluation of antibacterial chemicals on the growth of *Erwinia carotovora* subsp.*carotovora*

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.

Present investigation was carried out to evaluate commercially available antibacterial chemicals to find out their effectiveness against the growth of *Erwinia carotovora* subsp. *carotovora* under *in vitro* condition

Results indicated that streptocycline had showed significantly superior over other treatments with highest inhibition (2.38) at 500 ppm followed by copper oxychloride at (1.72) and copper hydroxide (1.59) both at 3000 ppm. Next was copper sulphate which showed inhibition of (1.01 cm) at 3000 ppm. Bromopol and plantamycin were on par with each other, at 500 ppm concentrations they showed (1.40 cm) and (1.33 cm) respectively, where both the chemicals showed no effect at 100 and 250 ppm bleaching powder showed least effective only at higher concentration at 4000 ppm. However, all other chemicals *viz*., copper oxychloride and copper hydroxide were on par with each other.

Thammaiah *et al.* (2006) evaluated different chemicals against rhizome rot of banana *in vitro* and reported that streptocycline 500 ppm and copper oxychloride 2000 ppm recorded maximum inhibition. Under field condition Kannan *et al* (2006) reported that soil drenching of sodium hypochorite (0.5%) and streptomycin sulphate (500 ppm) performed well and reduced the rhizome rot disease incidence.

Nagaraj *et al.* (2002) evaluated various bactericides and antibiotics under *in vitro*, methoxy ethyl mercuric chloride 2000 ppm, copper sulphate @4000 ppm, steptocycline @750 ppm and norfloxin @ 750 ppm were found effective in inhibiting the growth of *Erwinia carotovora* subsp. *carotovora.*

Thammaiah *et al.* (2005) made an *in vitro* evaluation of chemicals against *Erwinia chrysanthemi* by modified paper disc method with seven treatments. The results revealed that the combination of streptocycline 1000 ppm + copper oxychloride 2000 ppm recorded the maximum inhibition of *Erwinia chrysanthemi* (24.00 mm) followed by copper oxychloride 4000 ppm (23.33).

5.4.2 *In vitro* evaluation of botanicals on the growth of *Erwinia carotovora* subsp. *carotovora*

Botanicals next to bioagents are safe, ecofriendly and cost effective means of managing the crop diseases effectively. In the present study, among seven different plant extracts were screened along with one organic product against *Erwinia carotovora* subsp. *carotovora* none of the plant extracts were found to inhibit the growth of the pathogen completely.

Six botanicals were taken for the evaluation Tulsi, Garlic, Eupatorium, Meswak, Kalongi, Soapnut. Out of six botanicals evaluated it was found that only Garlic was effective at 10 per cent concentration, where as rest found ineffective. Cowdung and lime (10g + 1g) was also found ineffective.

Snehalatharani and Khan (2009) tested for the efficacy of six plant species like Ocimum, Eupatorium, Meswak, Kalongi, Isoqbul and Garlic for their antagonistic and antibacterial effect on *Erwinia carotovora* subsp *carotovora/ Erwinia chrysanthemi* were studied. Of all plant extracts tested, Garlic was found to be highly effective in inhibiting the growth of banana *Erwinia* species followed by Isoqbul.

5.4.3 *In vitro* evaluation of bioagents on the growth of *Erwinia carotovora* subsp. *carotovora*

In the light of present day constraints with the use of chemical pesticides in plant disease management, biological control is increasingly occupying the minds of scientists all over the world as they are ecofriendly and cost effective. In the present study, among the different antagonistic microorganism *Pseudomonas fluorescens* was found significant over *Bacillus subtilis* which did not produced any inhibition zone. Whereas, the fungal antagonists like *T. harzianum*, *T. viride*, *T. virens* and *T. koengii* were found ineffective.

Similar type of results have been obtained by Vanneste *et al.* (1994) conducted *in vitro* studies, in which *Erwinia herbicola* Eh252 inhibited all 52 strains of *Erwinia chrysanthemi*, *E. carotovora* subsp. *carotovora* and *Erwinia carotovora* subsp. *atroseptica.* They suggested that antibiotic production, as one of the mechanism of pathogen inhibition, even though some other factors were also evolved

Irda safni (2003) concluded that *P. fluorescens* may also be able to inhibit the growth of bacteria *E. carotovora*, which cause soft rot disease. Firstly, this is because it has several specific characteristics, namely colonization of host plants' root system, secretes metabolite substances (siderophore), has ability to compete with nutrients and space, is easily found in the ecosystem, and it is not toxic for a variety of crops. Secondly, bacteria *E. carotovora* is included in soil-borne pathogens to which the Bacteria *P. fluorescens* can be effectively applied.

Snehalatharani and Khan (2009) reported that the efficacy of three antagonistic micro organisms *viz. Pseudomonas fluorescens*, *P. aeruginosa* and *Bacillus subtilis*. Among antagonistic microorganisms, *P. aeruginosa* was found to be most effective *in vitro* conditions followed by *P. fluorescens*

Laha *et al.* (1992) stated that the fluorescent pigments produced by *Pseudomonas fluorescens* were sequester Fe^{3+} and were considered as siderophores which inhibits the large number of phytopathogenic bacteria and fungi, whereas biochemical studies by Valasubramanian *et al.* (1994) showed that efficient strains of *Pseudomonas fluorescens* produced an antibiotic Phenazine-1-carboxylic acid (PAC) which hinders the growth of plant pathogenic bacteria.

5.4.4 Pot culture studies

In vivo evaluation of chemicals, botanicals and bioagents were taken as pot culture studies. The design adopted was Randomized Completely Block Design (RCBD) with three replications, were each replication compiled three plants. Results revealed that the T_1 (drenching of streptocycline 500 ppm + copper oxychloride 3000 ppm) gave 61.94 per cent disease reduction over the control shown in Plate 9. T_2 (streptocycline 500 ppm + copper hydroxide 3000 ppm) gave 56.74 per cent next followed by T_3 (streptocycline 500 ppm + copper sulphate) and T_4 (bromopol 500 ppm + copper oxychloride 3000 ppm) 53.88 and 50.61% respectively. T_5 (bromopol 500 ppm + copper hydroxide 3000 ppm), T_6 (plantamycin 500 ppm + copper oxychloride 3000 ppm) and T_7 (*Pseudomonas fluorescens* + Garlic 10%) showed least disease reduction 42.37%, 32.80% and 23.68% respectively. T_8 (untreated control) showed 100% death of banana plants.

The similar results were obtained by Anonymous (2008) reported that sucker dip in COC at 4 g/l for 45 minutes followed by spraying of streptocycline (0.03%) and suckers dip in *Pseudomonas fluorescence* (1:1) for 45 minutes recorded better growth and lower disease infection

Kannan *et al.* (2006) reported that soil drenching of sodium hypochorite (0.5%) and streptomycin sulphate (500 ppm) performed well and reduced the rhizome rot disease incidence.

Anonymous (2002) reported that Carbofuran @ 40 g/plant, drenching of COC 500 ppm and dipping of suckers in 5000 ppm of COC for 30 min., reduced rhizome rot in banana.

6. SUMMARY AND CONCLUSIONS

Banana (*Musa* spp.) is an important fruit crop in India. The crop is subjected to various diseases and bacterial Rhizome rot or Tip-over disease caused by *Erwinia carotovora*.subsp. *carotovora* is one of the important disease, which caused great loss in recent years.

The present investigation was carried out during 2010-12 in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad. For that the survey work was carried to assess the disease incidence in Bagalkot, Bijapur, Belgaum and Dharwad districts. Isolation, symptomatology and pathogenicity studies, host range studies, *in vitro* evaluation of antibacterial chemicals, plant extracts / organic products and bioagents and finally the integrated management of Rhizome rot or Tip-over of banana taken in pot culture studies were undertaken. 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 bring out appropriate management aspects to mitigate problem effectively.

Survey conducted during 2011-12 revealed that the incidence of Tip-over of banana was ranged from 4.25 to 65.28 per cent in all the locations surveyed, with the highest disease incidence in the villages of Bijapur district followed by Bagalkot and Belgaum districts. Lowest disease incidence was observed in the villages of Dharwad district.

Characteristic symptoms of rhizome rot were noticed during survey and the samples were collected for isolation of pathogen. The causal organism was isolated from infected rhizome and pseudostem by following the serial dilution plating technique using nutrient agar medium. Culture of each isolate was purified by streaking suspected single colony on the Nutrient agar and Crystal Violet Pectate medium.

Pathogenicity test for *Erwinia carotovora* subsp. *carotovora* was conducted by inoculating a bacterial suspension of 5×10^8 cfu per ml to banana plants by root dip method which were 40–45 days old growing in pots. The symptoms were developed and reisolation work was carried out. The culture was compared with original culture and Koch's postulates were proved.

Pathogenic test revealed that the *Erwinia carotovora* subsp. *carotovora* was pathogenic to banana and produced typical symptoms. The leaves in the diseased plants appeared pale yellow, lusterless, with marginal necrosis. The young central leaves/ shoots appeared scorched giving a burnt appearance when such plants were uprooted along with the rhizomes, the large part of the rhizome was found massive soft, rotted and turned brown to black discolouration.

Host range study of *Erwinia carotovora* subsp.*carotovora* was carried out and the plants used for the study are carrot, potato, tomato, onion, cabbage, brinjal, groundnut, and citrus. All the plants above mentioned were infected by the pathogen except citrus. However, time taken to express the symptoms varied. In this study, host range of *Erwinia carotovora* subsp.*carotovora* was drawn out. Tomato, potato and carrot showed symptoms after two days, cabbage and onion took three days for first symptom expression, while brinjal and groundnut took five days.

Among the different chemicals tested *in vitro* streptocycline at 500 ppm has shown highest inhibition zone of 2.38 mm (1.84 cm), next best inhibition was 1.72 mm (1.65 cm) of copper oxychloride at 3000 ppm, followed by copper hydroxide at 3000 ppm which showed the inhibition of 1.59 mm (1.61 cm). Bromopol at 500 ppm showed inhibition of 0.96 (1.40) and Plantamycin 0.76 (1.33) where both the chemicals were ineffective at 100 and 250 ppm concentrations. Copper sulphate showed the inhibition zone of 1.01 (1.43) at 3000 ppm.

Among the different antibacterial chemicals in combination with copper chemicals evaluated against the *Erwinia carotovora* subsp. *carotovora* under *in vitro*. Results revealed that, Copper oxychloride and copper hydroxide both (3000 ppm) each in combination with Streptocycline (500 ppm)

The results indicated that streptocycline (500 ppm) in combination with Copper oxychloride (3000 ppm) found an inhibition zone of 2.83 mm (1.95 cm) which exhibited superior followed by streptocycline (500 ppm) with copper hydroxide than with copper sulphate both at 3000 ppm.

In vitro evaluation of plant extracts / organics tested at different concentration of 5, 7.5 and10 per cent on the growth of *Erwinia carotovora* subsp. *carotovora*. Among the seven botanicals only Garlic at 10% concentration produced the inhibition zone of 0.92 mm (1.39 cm).

In biological control, the *Pseudomonas fluorescens* was found effective antagonistic microorganism under *in vitro* conditions on the growth of the pathogen.

In vivo evaluation of the integrated management of the disease was taken as pot culture studies. The design adopted was Randomized Completely Block Design (RCBD) with three replications, were each replication compiled three plants. Results revealed that the T_1 (streptocycline 500 ppm + copper oxychloride 3000 ppm) gave 61.94 per cent disease reduction over the control. T_2 (streptocycline 500 ppm + copper hydroxide 3000 ppm) gave 56.74 per cent next followed by T_3 (streptocycline 500 ppm + copper sulphate) and T_4 (bromopol 500 ppm + copper oxychloride 3000 ppm) 53.88 and 50.61% respectively. T_8 (untreated control) showed 100% death of banana plants.

Conclusions

1. The disease incidence was high in Bijapur district and it was major in tissue cultured banana plants.
2. In host range studies, no symptoms observed in citrus, while all vegetables were susceptible and groundnut showed slight symptoms.
3. Streptocycline (500 ppm) in combination with copper oxychlride (3000 ppm) was found to be the most effective in controlling the pathogen both under *in vitro* and *in vivo* conditions.
4. Among plant extracts, only Garlic gave good result under *in vitro*.
5. Among the bio-agents tested, *Pseudomonas fluorescens* was found effective.

Future line of work

1. Exploration of resistant varieties which are high yielding and location specific.
2. Further, studies on molecular aspects of *Erwinia carotovora* subsp. *carotovora*, to identify the race present in India.
3. Outbreak of this disease is more in tissue culture plants; hence it needs detailed investigation about mechanism.

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STUDIES ON TIP-OVER DISEASE OF BANANA CAUSED BY *Erwinia carotovora* subsp. *carotovora* (Jones) Holland AND ITS MANAGEMENT

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ABSTRACT

Banana (*Musa* spp.) is an important fruit crop of India. Survey conducted during 2011-12 revealed that the incidence of Tip over of banana was ranged from 4.25 to 65.28 per cent in all the locations surveyed, with the highest disease incidence in the villages of Bijapur district followed by Bagalkot and Belgaum districts. Lowest disease incidence was observed in the villages of Dharwad district and was severe in tissue cultured banana plants. The causal organism was isolated from infected rhizome and pseudostem by following the serial dilution plating technique using nutrient agar medium. Pathogenic test revealed that the *Erwinia carotovora* subsp. *carotovora* was pathogenic to banana and produced typical symptoms. The leaves on the diseased plants appeared pale yellow, lustreless with marginal necrosis. The young central leaves/shoots appeared scorched giving a burnt appearance, when such plants were uprooted along with the rhizomes, the large part of the rhizome was found massive soft, rotted and turned brown to black discolouration. The bacterium is a rod shaped, facultatively anaerobic, gram negative. It utilized glucose, fructose, and sucrose for acid production, positive for gelatin liquefaction and produced hydrogen sulphide. In host range studies, carrot, potato, tomato, onion, cabbage, brinjal and groundnut expressed similar symptoms. However time taken for symptoms expression varied. Citrus plants did not show any symptoms. Under both *in vitro* and *in vivo* conditions streptocycline (500 ppm) in combination with copper oxychloride (3000 ppm) was found to be the most effective in inhibiting the pathogen. Among the seven plant extracts tested, only garlic at 10 per cent concentration gave maximum inhibition zone under *in vitro*. Among the bio-agents tested, *Pseudomonas fluorescens* was found effective, whereas, *Trichoderma* spp. were found to be ineffective.