STUDIES ON FROG EYE LEAF SPOT OF BIDI TABACCO CAUSED BY Cercospora nicotianae Ell. &Eve.

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By
MALLAPPA PRAKASH

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, DHARWAD
UNIVERSITY OF AGRICULTURAL SCIENCES
DHARWAD -580005

JULY-2007

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(P.V.KENCHANAGOUDAR)

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1. INTRODUCTION

Tobacco (*Nicotiana tabacum* L.), which belongs to the family solanaceae, is believed to be introduced into India from its native Central America by Portuguese in 1603. It is a major commercial crop of India, grown throughout the country. India is one of the principal tobacco producing countries of the world, and tobacco has attained its commercial importance in India. Since, 1930 tobacco cultivation has become a way of life as well as an industry and has made great strides, while playing a key role in Indian economy despite its disapproval due to its alleged association with human health, tobacco has thrived well. It has a significant contribution to Indian economy through its earning by way of central excise and foreign exchange.

India occupies second place in area and third place in production accounting for 10 per cent of world area and about 9 per cent of tobacco production by using just 0.3 per cent arable land. During 2005-06 the area covered was 0.4 mha and the production was 700 mkg (Anon., 2006). India is one of the leading exporter of tobacco occupying fourth place in overall exports and ranks fifth in the export of flue cured virginia (FCV) tobacco after Brazil, Zimbabwe, China and USA. Tobacco accounts 4 per cent (204 million US Dollars) of India's agricultural exports and 12 per cent (Rs.8182 crores) of total excise revenue. Further, it is livelihood for about 35 million people including six million farmers as well as others in direct or indirect manner.

The genus *Nicotiana* encompasses over 66 species, out of which only two species, *viz*., *Nicotiana tabacum* L. and *Nicotiana rustica* L. are cultivated in India. *N. tabacum* is grown throughout the country, where as hardly five to six per cent of total area under tobacco cultivation is accounted for by *N. rustica* varieties. Various types of tobacco grown in the country are mainly Flue Cured Virginia (FCV), bidi, hookah, chewing, lanka, cigar filler, cigar wrapper, snuff, natu, cheroot and rustica.

Major tobacco growing states of India are Andhra Pradesh, Gujarat, Karnataka, Uttar Pradesh, West Bengal, Tamil Nadu, Orissa and Maharashtra. Among all these Andhra Pradesh, Gujarat and Karnataka are important tobacco growing states. In Karnataka, the principal types of FCV (about 70,000 ha) and bidi tobacco (about 20,000 ha) are grown with good quality leaf of which the farmer is raised as a monsoon crop on light soil areas of Southern transition zone of Mysore, Hassan, Shimoga and Chikkamagalur districts, while the later is grown as a rainfed crop mainly on heavy to medium soils of Nipani tract of Belgaum district, depended on conserved soil moisture. People from all walks of life, rich and poor, young and old, educated and illiterate, great men and not so great men have enjoyed tobacco in one form or the other over centuries. Different users of tobacco use for different reasons some smoke or chew tobacco because it relaxes them, some to concentrate and some enjoy it, whatever be the reason it is certain that tobacco users derive certain benefits. Apart from these, tobacco is a source of medicine, edible protein oil, pesticide and organic acids.

The tobacco environment has often provided ideal conditions for spread and multiplication of organisms, which are later, adapted as tobacco parasites. Bidi tobacco suffers from many abnormalities caused by a wide range of pathogens, *viz*., fungi, nematodes, bacteria, viruses, flowering plant parasites and phytoplasma (Lucas, 1975). The losses due to these diseases are estimated to be in range of 5 to 15 per cent depending on their intensity. Among all foliar diseases, frog-eye leaf spot caused by *Cercospora nicotianae* Ell. and Eve. has become a major threat in recent years. The lower and matured leaves are affected first by the disease. The spots are brown with ash grey centres; often the centres may turn white and dry up. The typical spot has a white center, surrounded by black margin, resembling the eye of frog. The spot size may vary from about five to 10 mm in diameter.

The frog eye leaf spot disease was first reported by Ellis and Everhart (1883) from North Carolina, United States of America (U.S.A). They named the organism as *Cercospora nicotianae*, which is now accepted by mycologists throughout the world. The causal entity belongs to the class- Deuteromycetes, order- Moniliales and family- Dematiaceae. *Cercospora nicotianae* is regarded as a weak parasite and it is a soilborne and airborne in nature. From India, it was first reported from Patansagar (M.P) during 1894 (Vasudeva, 1963). Much of our knowledge of frog-eye leaf spot disease of tobacco has been derived from

investigations of Jochems (1931), Meura (1932), Mandelson (1933), Hill (1936) and Hopkins (1939). Frog-eye leaf spot of tobacco has been reported to cause considerable damage to the crop in Queensland, Rhodesia, Sumatra and Malaya (Thomson, 1923, Jochems, 1931, Mandelson, 1933, Hopkins, 1939) respectively. Epiphytotics due to this disease also occurred in Indonesia, Nyasaland and Panama (Lucas, 1975). In Queensland, it is said to be second in importance as a tobacco disease. Frog-eye lesions may sometimes be confused with brown spots, wild fire, black fire or any of the several physiological leaf spots. The disease occurs commonly in bidi tobacco in the nursery as well as in the field.

Humid and warm weather during August – September is highly congenial for development of the disease. The heavy and continuous rains after one month of crop growth including high atmospheric relative humidity for longer periods resulted in leaf spot and increases to epiphytotic proportions (Mandelson, 1933). Avoidable loss due to this disease has been estimated to the tune of 21 per cent in bidi tobacco field under normal monsoon conditions in Gujarat (Patel *et al*., 2001). Epidemiology and management of bidi tobacco diseases has been reviewed by Srikant Kulkarni *et al.* (2005). Not only it reduces yield, the produce prepared from infected leaf is of poor quality. Also the heavy infection leads to reduction in total alkaloids (18%), reducing sugar (46%) and total phenol (32%), in FCV tobacco (Stavely and Chaplin, 1972). Carbendazim is generally recommended to minimize the losses due to the disease (Anjaneyulu and Nagarajan, 1981; Shah *et al*., 1984; Patel *et al*., 1991; Reddy *et al*., 1992 and Patel *et al*., 2001, Hundekar *et al.,* 2005). At present no other effective management measures are known or worked out. Since, leaf is the final produce, alternative approaches to the use of chemicals would be a better proposition with regard to its management.

Different types of tobacco are being cultivated in India under different agro-climatic conditions. Among these, Nipani area of Belgaum district in Karnataka is known for production of excellent quality bidi tobacco in our country. In recent years, frog-eye leaf spot has become a major production constraint in bidi tobacco growing areas (Anon., 2004). Hence, the present study is aimed at a systematic study on survey, etiology and management of the disease so as to help to produce better quality bidi tobacco. Keeping these points in view, the present investigation was undertaken with the following objectives.

1. Survey and surveillance for frog-eye leaf spot in major bidi tobacco growing areas of Karnataka.
2. Isolation, identification and pathogenicity studies.
3. To study the morphological, physiological and cultural characters of the pathogen.
4. Bioassay of new fungicides and bio-agents against *Cercospora nicotianae.*
5. Management of frog-eye leaf spot through fungicides, bioagents, plant extracts and host plant resistance.
6. Economic and quality parameter analysis in the management of frog eye leaf spot of bidi tobacco.

2. REVIEW OF LITERATURE

The first report on frog-eye leaf spot was made in the North Carolina (U.S.A.) in 1883 by Ellis and Everhart and they named the organism as *Cercospora nicotianae*.

2.1 Survey and Surveillance for frog eye leaf spot

Avoidable loss due to this disease has been estimated to the tune of 21 per cent in bidi tobacco field under normal monsoon conditions in Gujarat (Patel *et al*., 2001). Frog-eye leaf spot has become a major production constraint in bidi tobacco growing areas (Anon., 2004)

2.1.1 Symptomatology

The lower and more matured leaves are affected first by the disease. The spots are brown with ash grey centers. Often the centers may turn white and dry up. The typical spot has a white center, surrounded by black margin, resembling the eye of frog, hence the name. The spot size may vary from about five to 10 mm in diameter. One to over hundred spots may appear on each leaf blade and several spots may coalesce towards the leaf tip and margin causing the leaf to dry up from the margin and wither prematurely. Both the yield and quality of the tobacco are reduced by this disease (Rangaswami, 1972, Patel *et al.* 2001). Eleven different genera were isolated from surface sterilized disks from field leaves of various ages from the tobacco var. Toleza, of these only *Cercospora nicotianae* and *Alternaria longipes* produced visible symptoms (Norse, 1972).

The phase of the frog-eye leaf spot showed during curing is called barn spot [also known as green spot, black barn spot, pole burn and leaf burn]. In the seed bed and the field (more commonly on lower leaves) greenish brown leaf spots appear with somewhat indefinite margins form. They become reddish-brown to brown with a characteristic center which becomes progressively paler to a dingy grey and bleached and parchment like. The developed spot has a narrow dark brown margin and a well defined outline, 2-15 mm diameter. Seedlings and the lower leaves of field plants can be destroyed. Depending on growing conditions, variations in spotting can occur, generally larger brown lesions with more irregular outlines, sometimes with zonate markings and chlorotic halos. In the barn after harvest greenish or greyish spots are formed. On the flue cured leaf small black, circular lesions develop, these may be extremely numerous and coalesce to form irregular, dark blotches. This barn spot phase can develop later on leaves, which show no visible symptoms when reaped (Mulder and Holliday, 1974).

Older leaves of the Cv. Vam-Hicks were more susceptible to infection by *Cercospora nicotianae* than younger leaves [Tsay and Chen, 1974]. The spots caused by *Cercospora nicotianae* increased slowly with age of crop for up to one month. Subsequent heavy and continuous rain resulted in leaf spot becoming epidemic. However, such rain did not cause a high disease incidence when the crop was less than one month old. Heavy rain on a single day or continuous light rain over a few days did not favor heavy spot increase, even on a maturing crop (Krishnamurty and Elias, 1975).

2.2 Isolation and proving pathogenicity

Lanetskii *et al*. (1976) used the luminescent microscopy method for identification of the fungus *Cercospora nicotianae* infection in plant tissue. Hartill (1976) reported the spore distribution of *Erysiphe cichoracearum*, *Alternaria longipes* and *Cercospora nicotianae* was similar to that of dark coloured spores of non-pathogenic fungi. The size and shape of spores did not markedly affect their distribution on the leaf but this altered with time in relation to changes in leaf orientation. More spores were found on lower than on upper leaves.

Pululu and Corbaz (1989) reported that *Physalis angulata* was the primary source of inoculum for infection of field grown tobacco by *C. nicotianae*. *Physalis pubescens* was reported as another occasional host plant from the province of Shaba. *C. nicotianae* was also isolated from infected leaves of *Solanum nigrum, Datura stramonium*, *Lycopersicon esculentum*, *Solanum melongena* and *Nicandra physalodes*. Pathogenicity was confirmed by

inoculations on *Physalis angulata*, *Nicotiana tabacum* and *Physalis pubescens* plants. It is concluded that elimination of *P. angulata* and *P. pubescens* plants from and around tobacco fields may help to control *C. nicotianae*.

2.2.1 Morphology of Fungus

Vasudeva (1963) in his compilation on 'Indian *Cercosporae*' described the morphological characters of *C. nicotianae* as conidiophores in clusters, which are tufted, amphigenous, 75- 100 x 4- 5µ in size, 2- 3 times geniculate, dark brown, septate and arise through stomata. The conidia are slender, slightly curved, thin walled, hyaline, multiseptate i.e., 3- 6 septe and measure 40- 75 µ x 3- 5 µ in size.

2.3 Cultural studies

2.3.1 Growth on solid media

Mandelson (1933) and Yen (1956) grew the fungus C*ercospora nicotianae* on Potato dextrose agar (PDA) medium. Hill (1936) reported that no conidia were produced in single spore culture of *C. nicotianae* grown on several types of media including potato dextrose agar and plain tobacco agar.

Diachun and Valleau (1941) obtained sporulation on tobacco leaf decoction agar medium and also reported several isolations of C*. nicotianae* made direct from tobacco frog eye spots on this medium. Most of these isolates produced conidia and conidiophores six days after inoculation on the medium. The fungus C. *nicotianae* grew well on synthetic nutrient solution with sucrose as carbon source but sporulation was not observed (Steinberg, 1950).

Kilpatric and Johnson (1956) observed good growth and sporulation on Carrot leaf decoction agar. However, Carrot leaf did not induce any sporulation. Stavely and Nimmo (1968) observed maximum radial growth of *C. nicotianae* on an agar medium containing 1.6 g DL-Leucine, 50 g sucrose and 2-4 g yeast extract per litre and mineral nutrient and concluded that sucrose was the best source of carbon for the growth. Further, they tested ten different types of media and reported maximum growth of *C. nicotianae* on carrot leaf decoction agar while growth was minimum on PDA.

Alasoadura and Fajola (1970) observed abundant conidia production on tobacco decoction agar when *C. nicotianae* culture was subjected to darkness and high relative humidity. Liu (1971) reported that differences in spore production between 99 isolates of *C. nicotianae* indicated that distinct strains exist.

Verma and Agnihotri (1972) found maximum growth of *C. cruenta* and *C. beticola* on Czapek (dox) agar followed by Carrot leaf decoction agar media. Schneider *et al.* (1973) reported that non sporulating cultures of *Cercospora* spp. were able to sporulate when grown on antibiotic potato dextrose carrot agar medium (APDCA).

Fajola (1978) observed that the sporulation of *C. nicotianae, C. ricinella, C. canescens, C. arachidicola* and *C. (Cercosporidium) henningsii* was best in continuous darkness at relative humidity (RH) 100 per cent and 20^0C. Increase in conidiophores and conidia in length and septation was observed with increase in RH. The effect of light was not very significant.

Queiroz and Menezes (1993) obtained the sporulation of *C. nicotianae* by plating a spore suspension on dishes containing different culture media. Incubation was carried out at 27±1º C under an alternating light (12 h light/12 h darkness) regime or continuous darkness for five days. The greatest sporulation was obtained on V-8 juice-$CaCO_3$ agar, followed by coconut milk agar and tomato juice – $CaCO_3$ agar under the alternating light regime. Sporulation was very low on dry tobacco leaves – $CaCO_3$ agar, dry tobacco leaves agar, PDA and PDA-panvit media under both the light regimes tested.

2.3.2 Growth on Liquid media

2.3.2.1 Growth Phase

Lily and Barnett (1951) while discussing growth pattern of fungi outlined the following growth phases: 1. Stationary phase, 2. Phase of accelerated growth, 3. Phase of declining acceleration, 4. Maximum stationary phase and 5. Phase of decline or autolysis. They attributed these phases of fungus to the environmental and nutritional condition in which it grows.

Kanti (1975) reported the maximum growth of C*ercospora moricola* Cooke at 20 days after inoculation in potato dextrose broth. Lakshminarayana (1981) obtained maximum growth of *Cercospora solani-melongenae* Chupp on 22nd day of incubation in potato dextrose broth. Dinesha (1984) harvested maximum mycelial growth of *Cercospora sorghi* Ell. and Eve. on 16th day of incubation in potato dextrose broth.

2.3.3 Growth on liquid media

Dange and Patel (1968) obtained maximum growth of *Cercospora beticola* Sacc in Czapek (dox) broth. Growth was moderate in Richard's broth and Carrot leaf decoction and poor in Asthana and Hawker's broth.

Raghunathan (1969) found that Richard's solution was the best liquid medium for the growth of *C. canescens* and *C. dolichi infecting Dolichos lablab* Linn. *Dolichos* leaf extract was the least favorable for growth, although it supported good and early sporulation. The sporulation was spares in Czapek (dox) and Leonian's solution. He used Asthana and Hawker's solution 'A' as a basal medium.

Chen *et al*. (1979) reported that in liquid media, the maximum growth (dry mycelial weight) of the fungus *C. kikuchii* was obtained on V-8 juice broth and Potato dextrose broth and the least growth occurred in sterile distilled water.

2.4 Nutritional studies

2.4.1 Carbon Utilization

Rangaswami and Chandrasekharan (1962) reported that the media rich in sugars were found to favour good growth of *Cercospora* species on cucurbitaceous hosts. Dange and Patel (1968) recorded the best growth of *Cercospora beticola* when glucose was used as a carbon source. Verma and Agnihotri (1972) reported that Sucrose was the best carbon source for *C. cruenta* to sporulate.

Lakshminarayana (1981) obtained maximum growth of *C. solani-melongenae* when sucrose was supplied as a source of carbon for its growth. Dinesha (1984) also found that Sucrose was the best carbon source for the growth of *C. sorghi*. Khandar *et al.* (1985) opined that glucose supported the best growth of *C. canescens* Ell. and Mart. followed by sucrose, maltose and fructose.

2.4.2 Nitrogen Utilization

Berger and Hanson (1963) obtained the best growth of *C. zebrins* in glutamic acid in culture. They found that asparagine, urea and potassium nitrate as other good sources. Dayal and Ram (1968) obtained the good growth of *C. jasminicola* by urea, peptone, glycine and potassium nitrate as a nitrogen sources.

Dange and Patel (1968) reported that calcium and potassium nitrate supported good growth of *C. beticola*. Stavely and Nimmo (1968) obtained good growth of *C. nicotianae*, when they used DI-Leucine as a source of nitrogen.

Dinesha (1984) obtained maximum growth of *C. sorghi* when sodium nitrate was used as a source of nitrogen. Lakshminarayana (1981) found that L-asparagine supported maximum growth of *C. solani-melongenae* followed by potassium nitrate and L-methionine. Siddaramaiah (1986) obtained the best growth of *C. moricola* when asparagine was used as nitrogen source.

2.5 Physiological studies

2.5.1 Temperature

Dayal and Ram (1968) observed the higher percentage of germination of *C. jasminicola* was recorded at an optimum temperature of 28^0C. Makadia *et al.* (1979) reported that 27^0C temperature was the optimum for maximum growth and spore production of *C. nicotianae*. Dinesha (1984) recorded maximum spore germination of *C. sorghi* at 25^0C. Khandar *et al.* (1985) observed the best germination of *C. canescens* conidia was at 27^0C followed by 30^0C and 20^0C. Jenns *et al.* (1989) observed that cercosporin accumulation was regulated by temperature in *Cercospora* species, higher levels accumulating at 20^0C than at 30^0C.

2.5.2 Hydrogen ion concentration (pH)

Chandrasekhar and Rangaswamy (1960) reported that *C. cruenta* and *C. beticola* growth was found best at pH 6.0. Dayal and Ram (1968) recorded the higher percentage of spore germination of *C. jasminicola* at pH 5.5.

Dange and Patel (1968), Raghunathan (1969) reported two optimum pH 6.5 and 8.0 to be most favourable for the growth of *C. canascens* and *C. dolichi* respectively. Verma and Agnihotri (1972) reported pH 6.8 and pH 7.0 to be most favourable for the growth of *C. cruenta* and *C. beticola*. Makadia *et al.* (1979) reported that the optimum pH for maximum growth and sporulation of *C. nicotianae* was 4.5 and 6.5 respectively.

2.6 Management

2.6.1 Through fungicides

Peptines (1960) reported that out of five tested fungicides against frog-eye disease of tobacco, Semesan and Mansate at 2.4 gm/lit gave effective control. Rosa (1963) obtained the best control of *C. nicotianae* by spraying with Maneb at 10 gm/lit. twice weekly. Pandico and Bayubay (1965) reported that among Mansate, Parasite, Orthocide 50 and Gycop-33, Parasite was found the most effective in the control of frog eye spot disease on tobacco. Bagoyo *et al.* (1967) observed that the Mansate at 2 lb per 100 gallon proved most effective against *C. nicotianae* followed by Shell Cu, Glyodin and Miller fungicide.

Anonymous (1972) reported that three sprays of Benomyl at 1 or 2 g/lit at intervals of 10 and 16 days from nine weeks after planting helped in effective management of *Cercospora nicotianae*. Fajola and Alasoadura (1973) reported that Dithane M-45 (80 % Mancozeb) sprayed weekly or monthly at 500 ppm during three growing seasons gave complete control of *C. nicotianae* on tobacco. There was no phytotoxicity at the highest concentration tested and no significant difference between the two frequencies of spraying, Verdasan (PMA, 3% Hg) gave complete control at 500 ppm but caused severe leaf damage at that concentration.

Reddy (1978) reported that Benlate (Benomyl) at 500 g/ha and Cercobin (Thiophanate) at 750 g/ha gave the best control of *Cercospora nicotianae* in a field trial. Chandwani and Lal (1979) said that the best control of *C. nicotianae* was achieved with NF-48 (Thiofaminet) at 500 g/ha and 2 MBC [Carbendazim] formulations [Bavistin at 500 or 1000 g/ha and Hindustan Minerals at 1000 g/ha] in the field condition.

Wajid *et al.* (1986) opined that foliar spray of 0.025 per cent Bavistin [Carbendazim] or Topsin-M (Thiophanate-methyl) applied 45 days after transplanting tobacco significantly decreased frog-eye spot [*C. nicotianae*] infection on green leaves and spotting on cured leaves and increased bright grade and TBLE [Total bright leaf equivalent] yield.

Anjeneyulu *et al.* (1988) reported the Carbendazim, Rovaral (Iprodione), Daconil (Chlorothalonil) Dithane Z-78 (Zineb) RH-2161 and Difolatan (Captafol) alone and Captafol in combination with Blitox (Copper oxychloride) and Zineb were evaluated for control of *C. nicotianae* on tobacco. The most effective control was given by two sprays of Carbendazim at 150 and 300 g ai/ha giving 80 and 88 % control, respectively followed by Chlorothalonil at 900 and 1100 g ai/ha which gave 55 and 63 % reductions in disease incidence, respectively.

Murty and Nagarajan (1986) observed that frog eye spot disease was effectively controlled by spraying Difolatan @ 0.1 % or Thiram @ 0.1 %. The next best were Bordeaux mixture @ 0.4 % and Bayer 5072 @ 0.05 % plus Thiram @ 0.05 %. Patel *et al.* (1987) reported that Metalaxyl in combination with Carbendazim was equally effective as Bordeaux mixture in controlling both damping off and *Cercospora* leaf spot in bidi tobacco nursery.

Patel *et al.* (1991) reported that Carbendazim gave the most effective disease control followed by Thiophanate-methyl, Mancozeb and Bordeaux mixture but Guazatine and Metalaxyl + Ziram were less effective. Reddy *et al.* (1992) reported that two sprays of Carbendazim (Bavistin 50 %) @ 0.04 % or 0.025 % concentration were the most promising and economical in getting effective control of frog eye spot leading to higher yield of cured leaf in burley tobacco.

Ahmad and Shafique (1994) reported that, Benlate [Benomyl] was the best for reducing disease severity while Rubigan (Fenarimol) and Derosal (Carbendazim) were intermediately and equally effective. Hundekar *et al.* (2005) reported that spraying with Hexaconazole (0.1%) or Propiconazole (0.1%) or Carbendazim (0.05%) were found to be better in managing the frog-eye leaf spot disease of bidi tobacco in Karnataka.

2.6.2 Through plant extracts

Shekhawat and Prasad (1971) observed that extract of *Vernonia cinera* and *Beta vulgaris* significantly inhibited all the pathogenic fungi viz., *Alternaria tenuis* Nees, *Curvularia penniseti* (Nitra) Boadun and *Helminthosporium* sp isolated from bean pearl millet and Watermelon, respectively. Narain and Satapathy (1977) reported that leaf extract of *Vinca rosea* effectively inhibited the spore germination and mycelial growth of *Helminthosporium nodulosum* Berk and Curt. *Sclerotium rolfsii* (Sacc) Curzi, *Pestolatia* spp., *Fusarium oxysporum* Schelcht. *Colletotrichum* sp and *Aspergillus niger* Van Tieghem.

Sheik and Agnihotri (1977) studied the antifungal activity of some plant extracts against *Alternaria brassicae*, *Collectrichum papayae* and *Helminthosporium* sp. *in vitro*. They observed that the extracts prepared from *Lawsonia alba, Datura stramonium* and *Mentha piperita* were effective against all the three test fungi, where as pieces of the scales of *Allium cepa* caused maximum inhibition when tested against *A. brassicae* and *Colletotrichum papayae.*

Chary *et al.* (1984) found that phytoextract of *Arthobotrytis odoratissimum* (leaves and flowers), *Delonix regia* (flowers, leaves and bark), *Euphorbia microphylla* (leaves), *Oxalis corniculata* (petiole and bulb), *Physeolus autropurpurens* (leaves) were inhibitory to the spore germination of *Alternaria alternata* (tr.) Veissler and *Curvalaria lunata* (walker) Boedijn.

Tewari and Dath (1984) reported that leaf extract of *Ocinum sanctum, Lawsonia inermis, Nyctanthes arbortristis* and piper beetle showed the strong antifungal activity against rice pathogens viz., *Pyricularia oryzae* Sacc. *Drechslera oryzae* Van Bredade Hann and *Corticium sasakii* (Shiral) Matsumato. Ghewade (1989) reported leaf extracts of *Azadiracta indica* juss and *Lawsonia inermis* L. were effective in controlling *Cercospora* sp. (leaf spot disease) and rust of groundnut.

Singh *et al.* (1999) observed that plant quaternary alkaloid Δ^3-alstovenine inhibited the spore germination of most of the *Cercospora* spp. It was most sensitive as 100 per cent inhibition of spore germination was observed at 250 mg/lit *in vitro*. Hussain *et al.* (1999) reported that ginger, garlic and neem extracts gave excellent control of seed borne *C. kikuchii.* The fungus was completely inhibited by dipping seeds for five minutes.

2.6.3 Through bioagents

Siddaramaiah (1986) reported that *Trichoderma harzianum* showed hyperparasitic reaction on *C. moricola*. He also reported that *Bacillus subtilis* showed high degree of inhibition to the growth of *C. moricola* in bioassay studies.

Lazzaretti and Bettiol (1997) treated soybean seeds with a biological product containing *Bacillus subtilis* cells (60 g) and metabolites (60 g) and observed the best disease control of *C. kikuchii*. The root nodulation of soybean by nitrogen fixing bacteria was not

affected when the product was applied simultaneously with *Rhizobium* and *Bradyrhizobium japonicum*. Further, the product also did not affect seedling emergence.

Drehmel and Chilton (2002) reported that the non-protein amino acid 2- amino-3-cyclopropylbutanoic acid that was isolated from the mushroom *Amanita cakeri* was found to be toxic to the fungus *C. kikuchii.* Satyaprashanth (2004) reported that *Trichoderma viride* showed the maximum inhibition of the *C. kikuchii* and followed by *T. koningii*.

2.7 Quality parameters

Stavely and Chaplin (1972) reported that the plants, which were heavily infected by *C. nicotianae* averaged 18, 46 and 32 per cent less total alkaloid, reducing sugar and total phenol respectively, and 6.5 per cent more total nitrogen than non infected leaves. Gopalakrishna and Hanumantharao (1980) reported that the chemical character such as total N, Soluble N, and nicotine have related with the quality of natu tobacco.

Patel *et al.* (1989) reported that the significant positive correlation with different forms of nitrogen (Total-N, protein-N, ammonical-N, nitrate-N and nicotine). Among these, total-N and ammonical-N had maximum correlation with quality score, while nicotine had maximum correlation with valuation in two different locations of Anand (Gujarat) and Nipani (Karnataka).

Patel *et al.* (2001) observed that the *C. nicotianae* infected plants were less nicotine content (46.7%) and less reducing sugars (24.3%) compared to healthy leaves of the tobacco plant.

2.8 Host plant resistance

Stavely (1971) reported that of 990 tobacco introductions tested against *Cercospora nicotianae* none was immune but 31 of the 47 most resistance under glasshouse selection developed fewer lesions than three tobacco Cvs., Burley 11 B, Burley 21 and Coker 187-Hicks in field tests Stavely *et al.* (1973) reported that the factors controlling immunity to *C. nicotianae* and *Meloidogyne javanica* were transferred from *M. repanda* to *N. tabacum*. Wu and Lin (1971) reported that all 80 varieties tested were susceptible to *C. nicotianae* but incidence of the disease was relatively low on Chathan India, Oxford 26 A, SC 66, Speight G 3, Isabella A and Twist Bud white Burley.

Stavely (1971) evaluated more than 1000 tobacco introductions for their resistance to different pathogens. Six introductions, 540, 154, 264, 1049, 301 and 1057 were most resistant to *C. nicotianae*, Stavely *et al*. (1972) reported that resistance to *C. nicotianae* was transmitted from *N. repanda* to the progeny of autotetraploid *N. tabacum* X amphidiploid *(N. repanda X N. Sylvastris*). These hybrids were sterile but after treatment with colchicine some fertile plants resulted, which were then successfully back crossed with *N. tabacum,* producing 3 and 12 % of progeny having resistance to *C. nicotianae*.

Nagarajan *et al.* (1978) reported that among 32 species screened under artificial conditions, *N. alata, N. debneyi, N. nesophila* and *N. nudicaulis* were immune and *N. longiflora, N. plumbaginifolia, N. repanda* and *N. undulate* were resistant to *C. nicotianae*.

Nagarajan and Reddy (1982) evaluated a total of 700 entries of *N. tabacum* and *N. rustica* and 31 wild *Nicotiana* species were screened in the glasshouse for resistance to several pathogens. Four wild species were immune and four were resistant to *Cercospora nicotianae*.

Screening of 829 breeding materials (bidi tobacco type) against leaf spot diseases (*Cercospora* and *Alternaria*) under field conditions revealed only three cultures/lines viz., F_5[A2X(A2-10 X 22-5-22-34)] 74-35-38, F_5(144 X 14-126-38-30) 107-13-33 and F_7(3-58-38-32-83 X GT4) 154-57-40-38-37 to be free from these leaf spot diseases. The same cultures, when examined the following year under field conditions, exhibited infection by the leaf spot organisms (Anonymous, 1983 & 1984).

Tobacco entries were evaluated against the frog eye disease at seedling stage. FCV special was susceptible, L 1494 was moderately susceptible. The lines 22-5-1 and 27-2-31 while lines 4-1-22 and 6-6-17 were moderately resistant. At adult stage, same lines reported

as moderately susceptible while L 1494 and FCV special were highly susceptible (Anonymous, 1984). None of the 139 breeding materials screened against *Cercospora* leaf spot disease under field conditions at Anand (Gujarat) was resistant (Anonymous, 1987).

Anjeneyulu *et al.* (1985) observed that out of 593 tobacco accessions screened under field conditions, the air-cured varieties Ewtimo seme, Little dutch Ragondorf and Molovata were resistant. Out of 32 species screened in the greenhouse, *N. alata, N. debneyi, N. nesophila and N. nudicaulis* were immune, while *N. longiflora, N. plumbaginifolia, N. repanda* and *N. undulate* were resistant and *N. occidentalis* was moderately resistant.

Goy *et al*. (1992) reported that *N. glutinosa* was very susceptible to *Cercospora nicotianae*. Ahmed and Yaqub (1994) evaluated five cultivars of *Nicotiana rustica* in a plot trial, Rustica swabi and Naswari were resistant, while the others being susceptible or highly susceptible.

Narayanaswamy *et al.* (2000) evaluated 34 tobacco germplasm collections (cultivars, exotics, genotypes and inbred lines) screened for resistance to frog eye disease. Only two exotic lines (NLS-4 and NLS-5) and inbred lines, viz, KST-25, 1099/2/4, 1117/2, PCT-7, Thrupthi (KST-19), II-1619 and V-3884 were found to be resistant. Other FCV tobacco derivatives (1204-4, II-1308, FCH-145, V-3542, V 3643, 1099/2/1, Cv. Bhavya, II 1623, II 1624, Gold streak, Gold line, 135/9, 95/4, 1276/3/1 and 1290/1) were moderately resistant. FCH-154, V-3885, V-3886, 134/6, 136/3, 95/13, 95/15, 95/5 and ILTD special were moderately susceptible while FCV special was highly susceptible. Shamarao Jahagiradar *et al.* (2005) reported multiple disease resistant lines against frog eye leaf spot, brown leaf spot and tobacco mosaic virus in bidi tobacco.

3. MATERIAL AND METHODS

Present investigations were carried out during the period 2006-07. Laboratory and glass house experiments were carried out in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka while field experiments were taken up at Agriculture Research Station (ARS), Nipani, Tq: Chikkodi, Dist: Belgaum, University of Agricultural Sciences, Dharwad, Karnataka.

Nipani is situated in northern transitional tract of Karnataka representing zone-8 at 16.2º North latitude and 74.2º East longitude and at an altitude of 610 meters above mean sea level. It has a mild subtropical rainy climate with a mean annual rainfall of about 689mm, well distributed over a period of seven to eight months (May-November) with two prominent peaks in July and October. However, the period 2006-07 has been above normal rainfall year. Temperature ranged from 18 to 35^0C. The hottest months were March, April and May with a mean maximum temperature at around 35^0C. December and January were the coldest months with a mean minimum temperature of around 18^0C. The relative humidity fluctuates between 55 and 85 per cent.

3.1 Survey and surveillance

A rowing survey was conducted to know the per cent incidence of frog eye leaf spot disease in bidi tobacco growing areas of Nipani during 2006-07. Survey was taken up for four months starting from September to December.

3.2 Isolation of the pathogen

The causal organism *Cercospora nicotianae* was isolated from tobacco leaves showing the typical frog eye symptom of the disease. The infected leaves were cut in to leaf bits and surface sterilized with one per cent Sodium hypochlorite solution for two minutes. and repeatedly washed in sterilized distilled water. Then the infected leaf bits were transferred on to Petri dishes [1-2 leaf bits per Petri dish] containing Potato dextrose agar with the help of a sterile forceps and incubated at 25^0C for 15 days. Further purification and subculturing were done on Potato dextrose agar slants and Petri dishes.

3.2.1 General procedure

3.2.1.1 Glassware and Cleaning

For all the studies, only acid washed corning glassware were used. The glass ware were kept submerged overnight in the cleaning solution prepared by dissolving 60 g of potassium dichromate ($K_2Cr_2O_7$) and 60 ml of concentrated sulphuric acid (H_2SO_4) in a litre of distilled water. Then, they were washed with Vim powder followed by cleaning in running tap water and when needed for physiological studies, rinsed in distilled water.

3.2.1.2 Sterilization

All the glassware used in the studies were sterilized in an autoclave at 1.1 kg cm^{-2} pressure for 20 min. and then dried in a hot air oven at 55^0C. The soil for pots was sterilized at 1.4 kg cm^{-2} pressure for 30 min.

3.2.2 Identification of the pathogen

The culture obtained was compared with the original descriptions of the fungus. It was also sent to Agharkar Research Institute, Pune for further confirmation.

3.2.2.1 Maintenance of the culture

The fungus was subcultured on Potato dextrose agar slants and allowed to grow at 25^0C for 15 days and such slants were preserved in a refrigerator at 4^0C and subcultured once in 30 days.

3.2.3 Pathogenicity test

Pathogenicity study was conducted on tobacco plants Cv. A-119. Seedlings were raised by sowing the seeds in pots and watering was done twice a week. Sixty day old plants were sprayed with the suspension containing mycelial bits of the fungus prepared in sterilized distilled water. Such inoculated plants were covered with polythene bags and kept in dark for 12 hour and then transferred to a growth chamber, which was maintained at 25^0C and 100 per cent relative humidity. The pots were removed from the growth chamber after 48 hours and kept in glass house. Regular observations were made for the appearance and development of symptoms. Controls were maintained by spraying the plants only with distilled water. The fungus was reisolated from the infected leaves and the culture obtained was compared with the original to confirm the identity.

3.3 Cultural studies

3.3.1 Growth on Solid Media

The growth characters of the fungus were studied on eleven different solid media. All the media were sterilized at 1.1 kg cm^{-2} pressure for 20 minutes to carry out the study. 20 ml of each of the medium was poured into 90 mm diameter Petridishes. Such plates were inoculated with five mm disc of culture and incubated at 25^0C. Each treatment was replicated thrice. Colony diameter was recorded by averaging the linear growth of the colony in two directions for each plate at 19^{th} day after inoculation. The fungal colony colour, surface elevation and sporulation were also noticed at the end of the incubation period. The data on radial growth was analyzed statistically. The preparation of various media was done following the procedure given by Ainsworth (1971) and Tuite (1969). Composition of each medium used is furnished below.

1. Potato Dextrose Agar:

Peeled and sliced potatoes	- 200g
Agar-agar	- 20g
Dextrose	- 20g
Distilled water	- 1000ml

The potatoes were boiled in 500 ml of distilled water and the extract was collected by filtering through a muslin cloth. Agar-agar was melted separately in 500 ml of distilled water. The potato extract was mixed in the molten agar and 20g of dextrose was added to the mixture. The volume was made up to 1000 ml with distilled water and sterilized at 1.1 kg cm^{-2} pressure for 20 min.

2. Malt Extract Agar:

Malt extract	-	25g
Agar-agar	-	20g
Distilled water	-	1000 ml

Malt extract was dissolved in 500 ml of distilled water. Agar-agar was melted separately in 500 ml of distilled water. Both the solutions were mixed thoroughly. Volume was made upto 1000 ml with distilled water and sterilized at 1.1 kg cm^{-2} pressure for 20 min.

3. Oat Meal Agar:

Oat flakes	-	60g
Agar-agar	-	20g
Distilled water	-	1000ml

Oat flakes were boiled in 500ml of distilled water for 20 min. and the extract was filtered through a muslin cloth. Agar-agar was melted separately in 500ml of distilled water. Both the solutions were made upto 1000 ml with distilled water and sterilized at 1.1 kg cm^{-2} pressure for 20 min.

4. Richard's Agar:

Sucrose	- 50 g
Potassium dihydrogen phosphate	- 5 g
Potassium nitrate	- 10 g
Magnesium sulphate	- 2.5 g
Ferric chloride	- 0.02 g
Agar-agar	- 20 g
Distilled water	- 1000 ml

All the above ingredients except potassium dihydrogen phosphate and agar-agar were dissolved in 450 ml of distilled water. Agar-agar was melted separately in 500 ml of distilled water and was mixed with the above solution. The volume was made up to 950 ml. Potassium dihydrogen phosphate was dissolved in 50 ml of distilled water. The two solutions were autoclaved and subscequently mixed together.

5. Czapek (dox) agar:

Sucrose	- 30 g
Sodium nitrate	- 2 g
Potassium dihydrogen phosphate	- 1 g
Magnesium sulphate	- 2.5 g
Potassium chloride	- 0.5 g
Ferrous sulphate	- 0.01 g
Agar-agar	- 20 g
Distilled water	- 1000 ml

Agar-agar was melted in 500 ml of distilled water. All the ingredients were dissolved in 500 ml of distilled water. Both the solutions were mixed thoroughly and sterilized at 1.1 kg cm^{-2} pressure for 20 min.

6. V-8 Juice agar:

V-8 juice agar	-	44.3 g
Distilled water	-	1000 ml

44.3 g of V-8 juice agar was suspended in 1000 ml distilled water and the contents were sterilized at 1.1 kg cm^{-2} pressure for 20 min.

7. Sabouard's agar:

Peptone	-	10 g
Dextrose	-	20 g
Agar-agar	-	20 g
Distilled water	-	1000 ml

Agar-agar was melted in 500 ml of distilled water. All the ingredients were dissolved in 500 ml of distilled water. Both the solutions were mixed thoroughly and sterilized at 1.1 kg cm^{-2} pressure for 20 min.

8. Asthana and Hawker's agar:

Glucose	- 5 g
Potassium nitrate	- 3.5 g
Potassium dihydrogen phosphate	-1.75 g
Magnesium sulphate	- 0.75 g
Agar-agar	- 20 g
Distilled water	- 1000 ml

Agar-agar was melted in 500 ml of distilled water. All the ingredients were dissolved in 500ml of distilled water. Both the solutions were mixed thoroughly and sterilized at 1.1kg pressure for 20 min.

9. Host extract agar:

Tobacco leaves	-	200 g
Agar –agar	-	20 g
Distilled water	-	1000ml

Tobacco leaves were cut into small bits, washed and boiled in 500 ml of distilled water for 20 min. and extract was collected by filtering through a muslin cloth. Agar-agar was melted separately in 500 ml of distilled water and both solutions were mixed. The volume was made up to 1000 ml with distilled water and sterilized at 1.1 kg cm^{-2} pressure for 20 min.

10. Host extract dextrose agar:

Tobacco leaves	-	200 g
Dextrose	-	20 g
Agar-agar	-	20 g
Distilled water	-	1000 ml

Tobacco leaves were cut into small bits, washed and boiled in 500 ml of distilled water for 20 min. and extract was collected by filtering through a muslin cloth. Agar-agar was melted separately in 500 ml of distilled water and both solutions were mixed. Then added 20 grams of dextrose and volume was made upto 1000 ml with distilled water and sterilized at 1.1 kg cm^{-2} pressure for 20 min.

11. Carrot leaf decoction agar:

Carrot leaves	-	300 g
Agar-agar	-	20 g
Distilled water	-	1000 ml

Carrot leaves were boiled in 500 ml of distilled water for 20 min. and the extract was collected by filtering through a muslin cloth. Agar-agar was melted separately in 500 ml of distilled water. Both the solutions were mixed thoroughly. Volume was made upto 1000 ml and sterilized at 1.1 kg cm^{-2} pressure for 20 min.

3.3.2 Growth studies in Liquid Media

3.3.2.1 Growth Phase

Twenty ml of potato dextrose broth was poured into each of the 100 ml conical flasks. These were sterilized and inoculated with five mm mycelial disc from the periphery of the 15 days old culture and incubated at 25^0C. A set of three flasks were harvested every 48 hour starting from fifth day of inoculation upto 29^{th} day. Cultures were filtered through Whatman No. 42 filter paper, which were previously dried to a constant weight in hot air oven at 60^0C. The mycelial mat on the filter paper was thoroughly washed with distilled water to leach out any salts associated with the mycelium. Subsequently, the filter papers along with the mycelial mat were dried to a constant weight, cooled in dessicator and weighed on an electronic balance. The data was analyzed statistically.

3.3.2.2 Selection of basal media

The composition and preparation of different liquid media used were same as that of the solid media excluding use of agar-agar. Twenty ml of each medium was poured in each of the 100 ml flasks. These flasks were sterilized and inoculated with five mm mycelium discs cut from the periphery of the 15 days old culture and incubated at 25^0C. Each treatment was replicated thrice. After 19 days, the mycelium growth was harvested and filtered through Whatman No.42 filter paper and weighed as described earlier. The data were analyzed statistically. The best synthetic and non-synthetic media were found out and used for further studies, as basal media.

3.4 Nutritional studies

3.4.1 Carbon Utilization

The carbon requirement of the fungus was studied in Czapek (dox) broth, the quantity of carbon compounds added was determined based on their molecular weights. So as to provide equivalent amount of carbon as sucrose present in basal medium. Carbon sources used in the study were dextrose, fructose, cellulose, sucrose, lactose, glucose and starch and one set was maintained as control without adding carbon source. All the compounds were dissolved properly and sterilized at 1.1 kg cm^{-2} pressure for 20 min. Each treatment was replicated thrice. The flasks were inoculated as described earlier and incubated at 25^0C for 19 days. Later, at the end of 19 days mycelial growths were harvested and dry mycelial weights were obtained. The data was analyzed statistically.

3.4.2 Nitrogen Utilization

The nitrogen requirement of the fungus was studied in Czapek (dox) broth. The quantity of nitrogen compounds added was determined on the basis of their molecular weights, so as to provide an equivalent amount of nitrogen as sodium nitrate in the basal medium. The nitrogen sources used were ammonium chloride, ammonium sulphate, aspergine, ammonium orthophosphate, sodium nitrate and urea. One set was maintained as control without adding any nitrogen source to the Czapek (dox) broth. All the nitrogen sources were dissolved properly and then sterilized at1.1 kg cm^{-2} pressure for 20 min. Each treatment was replicated thrice. The flasks were inoculated as described earlier and incubated at 25^{0C} for 19 days. The mycelial growth was harvested and recorded the dry mycelial weight. The results were analyzed statistically.

3.5 Physiological studies

3.5.1 Temperature Requirement

Potato dextrose broth was used in this experiment. Conical flasks of 100 ml capacity containing 20 ml of each liquid medium were inoculated and incubated at different temperature levels. The different temperature levels tested for the growth of *C. nicotianae* were 5, 10, 15, 20, 25, 30, 35, 40°C and room temperature. Each treatment was replicated thrice. The dry mycelial weight at each temperature level was recorded after incubating for 19 days and the data were analyzed statistically.

3.5.2 Hydrogen-Ion concentration (pH)

Potato-dextrose broth was used as the basal medium. The pH of the liquid medium was adjusted using 0.1 N alkali (NaOH) or 0.1 N acid (HCl). According to the schedule of Vogel (1955), the medium was buffered to already adjusted pH by adding 50 ml of disodium hydrogen phosphate, Citric acid buffer for required pH. The different hydrogen ion concentrations used were 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. Conical flasks of 100 ml capacity and each containing 20 ml of the medium were inoculated and incubated at 25^0C. Each treatment was replicated thrice. The dry mycelial weight was obtained after incubating for 19 days as described earlier and the results were analyzed statistically.

3.6 *In vitro* evaluation of fungicides

The bio-efficacy of seven systemic fungicides at the concentration of 0.05, 0.1 and 0.15 per cent and five non-systemic fungicides at the concentration of 0.1, 0.2 and 0.25 per cent were analyzed. The fungicides used were given here under.

Sl.No.	Common Name	Trade Name
Systemic Fungicides		
1	Propiconazole	Tilt 25% EC
2	Hexaconozole	Contaf 5% EC
3	Carbendazim	Bavistin 50WP
4	Thiophanate methyl	Topsin-M 70WP
5	Penconazole	Topas 25EC
6	Difenconazole	Score 25%EC
7	Benomyl	Benlate 50WP
Non-systemic Fungicides		
1	Mancozeb	Dithane M-45 75WP
2	Chlorothalonil	Kavach 75%WP
3	Propineb	Antracol 70%WP
4	Carbendazim (12%) + Mancozeb (63%)	Saaf
5	Copper Oxychloride	Blitox-50WP

Required quantity of individual fungicides was added separately into molten and cooled potato dextrose agar so as to get the different concentration of the fungicides. Later, 20 ml of the poisoned medium was poured into sterile Petri plates. Mycelial disc of 5 mm size from 15 day old culture was placed at the centre of each agar plate. Control was maintained without adding any fungicide to the medium. Each treatment was replicated thrice. Then, such plates were incubated at 25^0C for 19 days at the end of which redial colony growths were measured. The efficacy of fungicides was expressed as per cent inhibition of mycelial growth over control that was calculated by using the following formula [Vincent, 1947].

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

Where,

I = Per cent inhibition

C = Radial growth in Control

T = Radial growth in treatment

3.7 *In vitro* evaluation of bio-agents

Bioagents such as *Trichoderma harzianum* Ritai, *Trichoderma viride* Persex Fr., *Trichoderma koningii* Oudem*, Trichoderma virens* Miller, *Pseudomonas fluorescens* Migula and *Bacillus subtilis* Cohn. were tested *in vitro* against *Cercospora nicotianae*. Both bioagents and test fungus were cultured on Potato dextrose agar in order to get fresh and active growth.

About 20 ml of sterile cooled Potato dextrose agar was poured into sterilized Petri plates and allowed to solidify. In the evaluation of fungal bioagents, mycelial discs of test fungus were inoculated at one end of the Petri plate and that of the antagonistic fungus opposite to it at the other end. In case of evaluation of bacterial antagonist, the bacterium was streaked at the center of the Petri plate and two mycelial discs of the fungus were placed at opposite ends. The antagonistic bacteria and fungi were inoculated five days after inoculation with the test fungus and incubated at 25^0C for a further period of five days. Each treatment was replicated thrice and appropriate control was maintained. The colony diameter of the test fungus in control and in all the test plates was recorded. The per cent inhibition of the growth of pathogen was calculated by following the formula given by Vincent (1947).

3.8 Management of frog eye leaf spot of bidi tobacco

A field experiment was laid out at the Agricultural Research Station (ARS), Nipani, University of Agricultural Sciences, Dharwad during 2006. The efficacy of four systemic fungicides, two non-systemic fungicides, two bioagents and a botanical was evaluated. The experiment was conducted in randomized block design with ten treatments and three replications with cultivar A-119. The details of the treatments are given here under.

Treatment No.	Treatment details
T_1	Hexaconazole 5% EC (Contaf) @ 0.1%
T_2	Propiconazole 25% EC (Tilt) @ 0.1%
T_3	Chlorothalonil (Kavach) @ 0.2%
T_4	Carbendazim-12% + Mancozeb-63% @ 0.2% (Saaf)
T_5	Thiophanate methyl 70% WP (Topsin-M) @ 0.2%

T_6	Carbendazim (Bavistin) @ 0.05%
T_7	*Pseudomonas fluorescens* @ 2%
T_8	*Trichoderma harzianum* @ 2%
T_9	Parthenium leaf extract spray @ 10%
T_{10}	Control

Plot size of 4 x 7.5 m was maintained per treatment. The transplanting of tobacco was taken up on 26.08.2006. First spray was taken up immediately after disease appearance followed by another spray at 10-12 days interval.

The observations on per cent disease index was recorded by following scale of 0- 5. The rating is described here under.

0 = No symptoms observed (Highly resistant)

1 = 1- 20% leaf infection (Resistant)

2 = 21- 40% leaf infection (Moderately resistant)

3 = 41- 60% leaf infection (Moderately susceptible)

4 = 61- 80% leaf infection (Susceptible)

5 = 81- 100% leaf infection (Highly susceptible)

Per cent disease index (PDI) was calculated by using the following formula (Wheeler, 1969).

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves examined.}} \times \frac{100}{\text{Maximum grade value}}$$

The yield per plot, height of the plant, leaf length, leaf breadth and other quality parameters were recorded at 80days after planting (DAP) and at harvest. The economic analysis was made and cost to benefit ratio was worked out for each treatment to ascertain economic feasibility of treatments. The data was statistically analyzed by following statistical procedures of Sukhatme and Amble (1985).

3.9 Quality parameter study

The quality parameters such as Nicotine per cent, Reducing sugars per cent and Chlorides per cent were analyzed at Quality parameter assessment Laboratory, CTRI, Rajhmundry.

3.10 *In vivo* screening of tobacco genotypes/entries

Tobacco varieties/entries/genotypes were screened against *Cercospora nicotianae* under field condition at Agricultural Research Station (ARS) Nipani. Different trial material belonging to Advanced varietal trial (AVT), Advanced Hybrid trial (AHT), Initial varietal trial (IVT), Initial Hybrid trial (IHT), Station trial-I (ST-I), Station trial-II (ST-II) and germplasm lines were screened for frog eye reaction under naturally unprotected conditions.

4. RESULTS

The results of the investigation on frog-eye leaf spot of bidi tobacco, caused by *Cercospora nicotianae* Ell. & Eve. are presented hereunder.

4.1 Survey and surveillance of frog eye leaf spot in Nipani area.

Rowing survey was taken up during 2006-07 in Nipani area. Over 21 locations were surveyed during four month cropping period and data are presented in Table 1.

The mean incidence ranged from 8.38 per cent to 13.05 per cent irrespective of location surveyed. The disease pressure was high initially in the month of September, October, November and later decreased in the month of December. Maximum incidence of 16.9 per cent was recorded in Aadi area followed by 16.2 and 16.1 per cent in Galataga and Sankeshwar area in September, 2006. Interestingly, there was no incidence of frog eye leaf spot in Hunnargi area during September, 2006. Mean incidence of 13.05 per cent was noticed in September, 2006. Minimum incidence of 10.2 per cent was noticed in Yadanwadi.

In the month of October, 2006 mean incidence was 12.06 per cent. The incidence ranged from 8.1 to 15.4 per cent. The Aadi area again recorded maximum incidence of 15.4 per cent followed by 14.8 per cent in Akkol, Jatrat and Khadakalat area. Minimum incidence of 8.1 per cent was noticed in Yadanwadi. In the month of November, Akkol recorded maximum incidence of 13.9 per cent followed by 13.2 per cent in Aadi and 13.1 per cent in Galataga area. Minimum incidence of 7.3 per cent was noticed in Yadanwadi. The mean incidence during November, 2006 was 10.42 per cent.

In the month of December, 2006 the disease incidence showed decreasing trend. The incidence ranged from 6.1 to 12.2 per cent. The mean incidence during the month was 8.38 per cent. Akkol area recorded maximum incidence of 12.2 per cent followed by 11.9 per cent in Aadi and 10.8 per cent in Walki. The locations which recorded more than 10 per cent incidence were Akkol, Aadi, Walki, Galataga and Kodni. Minimum incidence of 6.1 per cent was recorded in three locations such as Nagnur, Pattankudi and Yadanwadi.

Among the different locations mean maximum incidence of 14.35 per cent was recorded in Aadi followed by 14.05, 13.57, 13.25 and 12.85 per cent in Akkol, Galataga, Walki and Kodni areas. The mean incidence in all areas was 10.98 per cent and ranged from 8.15 to 14.35 per cent.

4.2 Isolation of the pathogen

The causal organism was isolated from frog-eye infected tobacco leaves showing typical symptoms of frog-eye leaf spot of the cultivar A-119 by following the tissue isolation technique as described in 'Material and Methods'.

4.2.1 Indentification of the pathogen

The pathogen formed uniformly dense colonies on potato dextrose agar. The colonies generally appeared olivaceous brown colour at the edges with light brown center. The medium beneath the colony became dark purple in colour. Hyphae of the fungus were hyaline and septate when observed under compound microscope. The fungus grew very slowly on potato dextrose agar medium reaching a maximum growth of 60-75 mm. On the basis of these characters the pathogen was identified as *Cercospora nicotianae* Ell. and Eve. Further, the Agharkar Research Institute, Pune, which is an autonomous grant in aid institute under the Department of Science and Technology, Government of India, has also confirmed the identity of the fungus.

4.2.2 Pathogenicity studies

Artificial inoculation of the tobacco plants with the pathogen was carried out as explained in 'Material and Methods'.

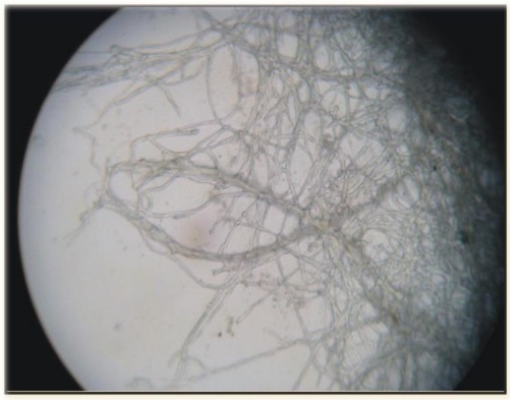
Table- 1. Survey and Surveillance of frog eye leaf spot disease in Nipani area

Sl. No.	Village	Disease Incidence (%) /Month				
		September	October	November	December	Location mean
1	Akkol	15.3	14.8	13.9	12.2	14.05
2	Galataga	16.2	14.5	13.1	10.5	13.57
3	Sadalga	11.6	9.2	8.1	7.0	8.97
4	Kodni	15.6	14.0	12.0	10.1	12.85
5	ARS, Nipani	14.2	13.6	12.0	9.8	12.40
6	Sirguppi	15.6	11.6	11.2	9.8	12.05
7	Pangeri A	12.1	10.6	8.8	6.9	9.60
8	Nagnur	11.0	10.8	8.8	6.1	9.17
9	Jatrat	15.6	14.8	10.6	6.9	12.72
10	Khadaklat	15.2	14.8	10.9	9.6	12.62
11	Aadi	16.9	15.4	13.2	11.9	14.35
12	Benadi	15.4	12.1	11.4	6.9	11.45
13	Shirpewadi	10.5	9.6	8.2	6.5	8.7
14	Hunnargi	--	12.6	10.8	9.2	8.15
15	Pattankudi	10.6	9.2	7.5	6.1	8.35
16	Appachiwade	12.6	10.9	9.8	7.5	10.2
17	Yadanwadi	10.2	8.1	7.3	6.1	7.92
18	Walki	15.2	14.1	12.9	10.8	13.25
19	Tavandi	11.5	10.1	8.6	7.2	9.35
20	Sankeshwar	16.1	12.3	10.1	6.9	11.35
21	Giragan	12.8	10.3	9.6	7.9	10.15
Monthly Mean		13.05	12.06	10.42	8.38	10.98

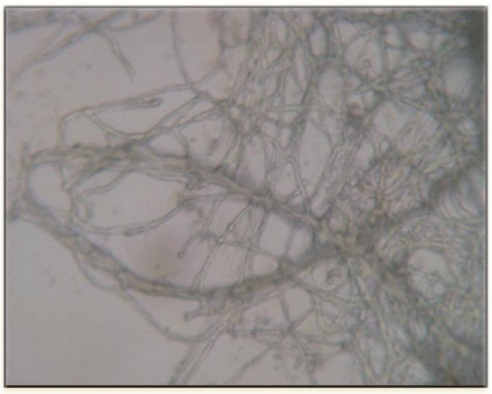
Inoculated

Uninoculated

a. Proving pathogenicity of C.nicotianae



Abundant production of mycelial mat at a Magnification of 400x



Mycelium of C. nicotianae at a magnification of 400x

Plate1: Proving pathogenicity and microphotographs of C. nicotianae

Initial stage of frog eye leaf spot

Severe stage of frog eye leaf spot

Plate 2: Symptoms of frog eye leaf spot of bidi tobacco

Symptoms started developing on inoculated plants 6 to 7 days after their removal from the growth chambers. The lower and matured leaves are first one to be often affected by the pathogen. The spots are brown with ash grey centers. Later the centers turned white and dried up. The typical spot has a whitish center, surrounded in succession by grey and brown portions, with dark brown to black margin. The spot size varied from about 5 to 10 mm in diameter, one to over hundred spots appeared on each leaf blade and later several spots coalesced towards the leaf tip and margin. The affected leaves withered prematurely. The test fungus was reisolated from such symptoms and compared with original culture. Thus, proved the pathogenicity of *Cercospora nicotianae* on bidi tobacco. There was yellowing, drying and dropping of the leaves at the later stage of disease (Plate-1a and b).

4.2.3 Symptomatology

The symptoms of the disease are exemplified by initial production of small dark brown spots with whitish center. As the disease advanced these spots coalesced to form bigger pathes resembling eyes of frog. There were varied symptoms due to *Cercospora* infection on bidi tobacco (Plate-2). One to over hundred spots appeared on each leaf blade and several spots coalesced towards the leaf tip and margin causing the leaf to dry up from the margin and withered prematurely.

4.3 Cultural studies

4.3.1 Growth on Solid media

The growth characters of the fungus were studied on eleven different solid media as described in 'Material and Methods'. The results are presented in Table-2a, Table-2b, Fig.-1 and Plate- 3.

The studies revealed that host extract dextrose agar supported maximum radial growth (87.33 mm), which was on par with Czapek (dox) agar (85.3 mm). Growth supported by Carrot leaf decoction agar (84.0 mm), Richard's agar (83.6 mm), Malt agar (83.0 mm) were on par with each other. Minimum radial growth was observed on V-8 juice agar (42.0 mm). All the synthetic media supported abundant mycelial growth.

The maximum growth of the fungus was noticed after 19 days of incubation showed a considerable difference in growth on different media. However, sporulation was not observed in any of media tested. V-8 juice agar and Saboaurd's agar did not support good growth of the fungus.

4.3.2 Growth studies in liquid media

4.3.2.1 Growth phase

The experiment was conducted as detailed in 'Material and Methods' to ascertain length of period required for maximum growth of the pathogen. The data are presented in Table-3 and Fig.-2.

Growth studies revealed that the fungus took five days for appearance of mycelium on medium. The fungus showed a positive increasing trend up to 19 days after inoculation. Maximum growth of the fungus (7.47 g) was observed on 19^{th} day of incubation and later the growth decreased significantly. As maximum growth was observed at 19 days after inoculation this period was taken as a maximum growth period for further studies.

4.3.2.2 Selection of basal medium

Growth of the fungus was studied in eleven different liquid media to select a medium that would support maximum growth as explained in 'Material and Methods'. The results are presented in Table-4, Fig.-3 and Plate-3.

Among the eleven liquid media tested, maximum growth of the fungus was observed in Host extract dextrose broth (8.23 g) followed by Czapek (dox) broth (8.14 g) and Carrot leaf decoction broth (7.97 g), which were on par with each other. Least growth of the fungus was observed in Saboaurd's broth (4.22g), which was on par with that of Asthana and Hawker's

Table- 2a.: Growth studies of *C. nicotianae* on different solid media

Sl. No	Medium	Mean colony diameter (mm)
1	Asthana and Hawker's agar	76.00
2	Carrot leaf decoction agar	84.00
3	Czapek (dox) agar	85.33
4	Host extract agar	70.00
5	Host extract dextrose agar	87.33
6	Malt agar	83.00
7	Oat meal agar	60.66
8	Potato dextrose agar	80.00
9	Richard's agar	83.66
10	Sabouard's agar	54.00
11	V-8 Juice agar	42.00
S.Em±		1.12
C D at 1%		4.47
C. V. (%)		2.65

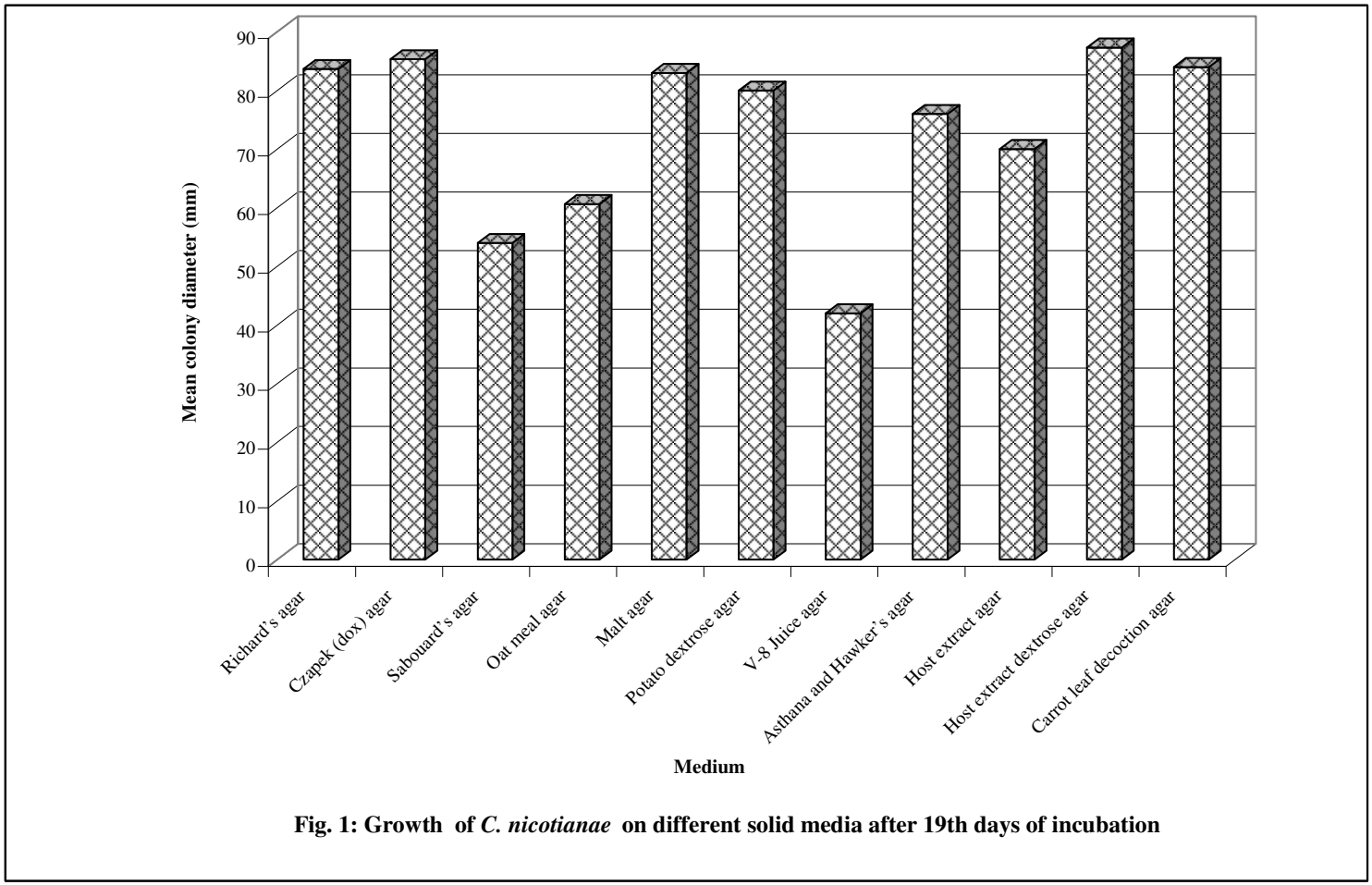


Fig. 1: Growth of *C. nicotianae* on different solid media after 19th days of incubation

Fig.1: Growth of C. nicotianae on different solid media after 19th days of incubation

Table- 2b. Cultural characteristics of *C. nicotianae* on different solid media after 19 days of incubation

Sl. No	Medium	Growth characters
1	Richard's agar	Abundant mycelial growth, smooth margin, white with brown center and pink margin. Dense center and flat edges, concentric rings of growth visible
2	Czapek (dox) agar	Abundant mycelial growth, white with brown center and dull white margin, uniformly dense, concentric rings clearly visible, smooth margin,
3	Sabouard's agar	Moderate mycelial growth, light pink with brown center and dull pink margin, uniformly dense and flat edges, radial growth is clearly visible, smooth margin
4	Oat meal agar	Moderate mycelial growth, pinkish white with black center and dull white margin, dense and flat edges, concentric rings of growth
5	Malt agar	Moderate mycelial growth, grey with black center and white margin, sparse center and flat edges
6	Potato dextrose agar	Abundant mycelial growth, dull white with brown center and white margin, uniformly flat growth, dense margin
7	V-8 Juice agar	Poor mycelial growth, light white with light green center and dull white margin, uniformly flat growth, smooth margin
8	Asthana and Hawker's agar	Abundant mycelial growth, pinkish white with brown center and dull white margin, flat center and flat edges, raised in between concentric rings of growth clearly visible, smooth margin
9	Host extract agar	Moderate mycelial growth, bright white center and margin, raised at center, smooth margin and flat edges
10	Host extract dextrose agar	Abundant mycelial growth, white with light green center and dull white margin, dense center and flat edges, uniformly mycelial growth, smooth margin
11	Carrot leaf decoction agar	Abundant mycelial growth, pink with yellowish center and dark margin, dense center and brown flat edges, concentric rings sparsely visible, smooth margin

Table- 3. Growth studies of *C. nicotianae* in potato dextrose broth

Sl. No	Days after inoculation	Mean dry mycelial weight (gm)
1	5	0.78
2	7	1.14
3	9	3.95
4	11	5.28
5	13	6.05
6	15	6.87
7	17	7.21
8	19	7.47
9	21	7.10
10	23	6.85
11	25	6.38
12	27	6.08
13	29	5.87
S.Em±		0.04
C D at 1%		0.18
C. V. (%)		1.45

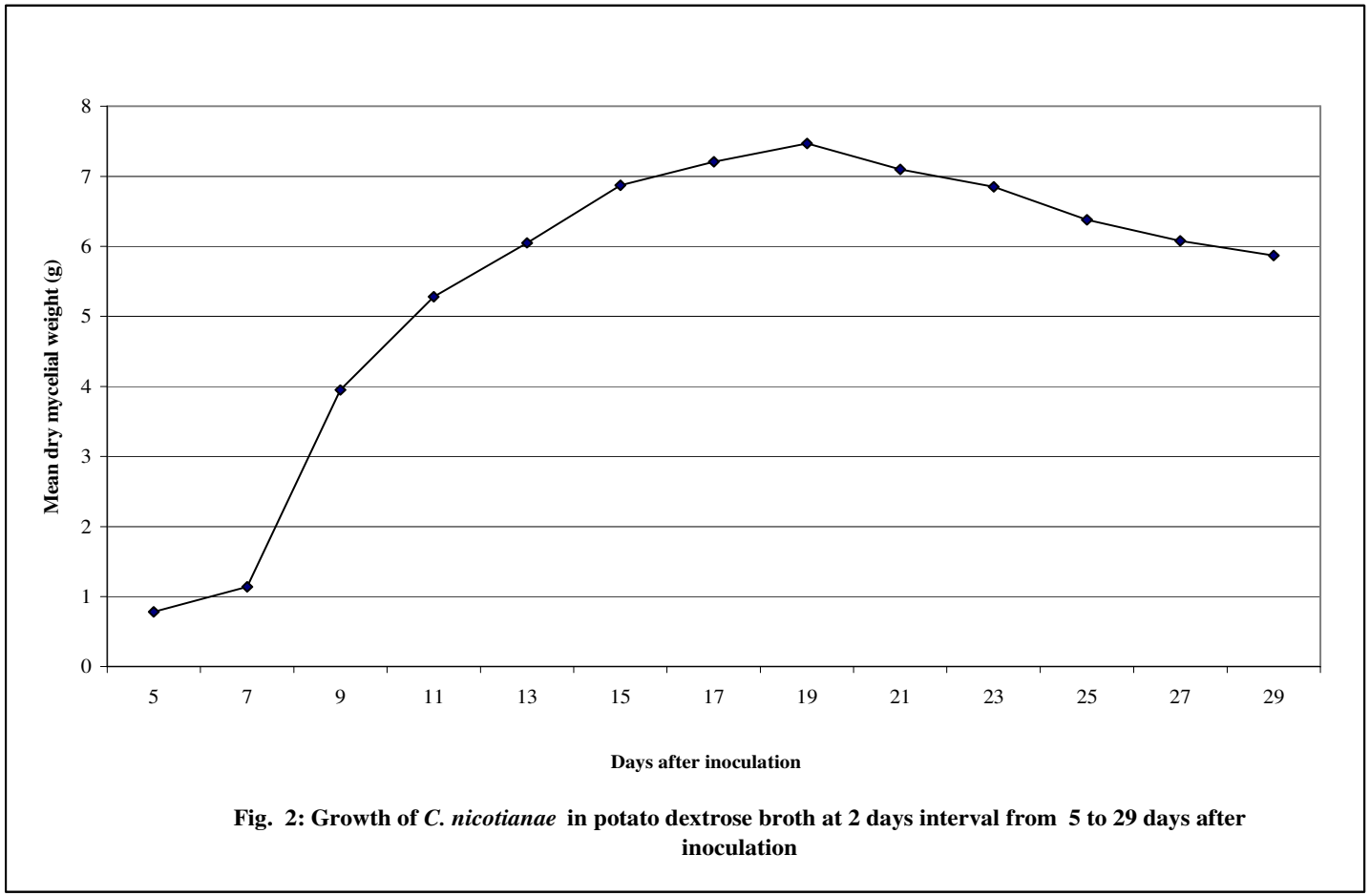


Fig. 2: Growth of *C. nicotianae* in potato dextrose broth at 2 days interval from 5 to 29 days after inoculation

Fig2: Growth of incotianae in potato dextrose broth at 2 days interval from 5 to 29 days inoculation

Table- 4. Dry mycelial weight of *C. nicotianae* on different liquid media

Sl. No	Medium	Mean dry mycelial weight (gm)
1	Asthana and Hawker's broth	4.29
2	Carrot leaf decoction broth	7.97
3	Czapek (dox) broth	8.14
4	Host extract broth	7.24
5	Host extract dextrose broth	8.23
6	Malt broth	6.64
7	Oat meal broth	5.12
8	Potato dextrose broth	7.35
9	Richard's broth	7.16
10	Sabouard's broth	4.22
11	V-8 Juice broth	5.04
S.Em±		0.09
C D at 1%		0.35
C. V. (%)		2.31

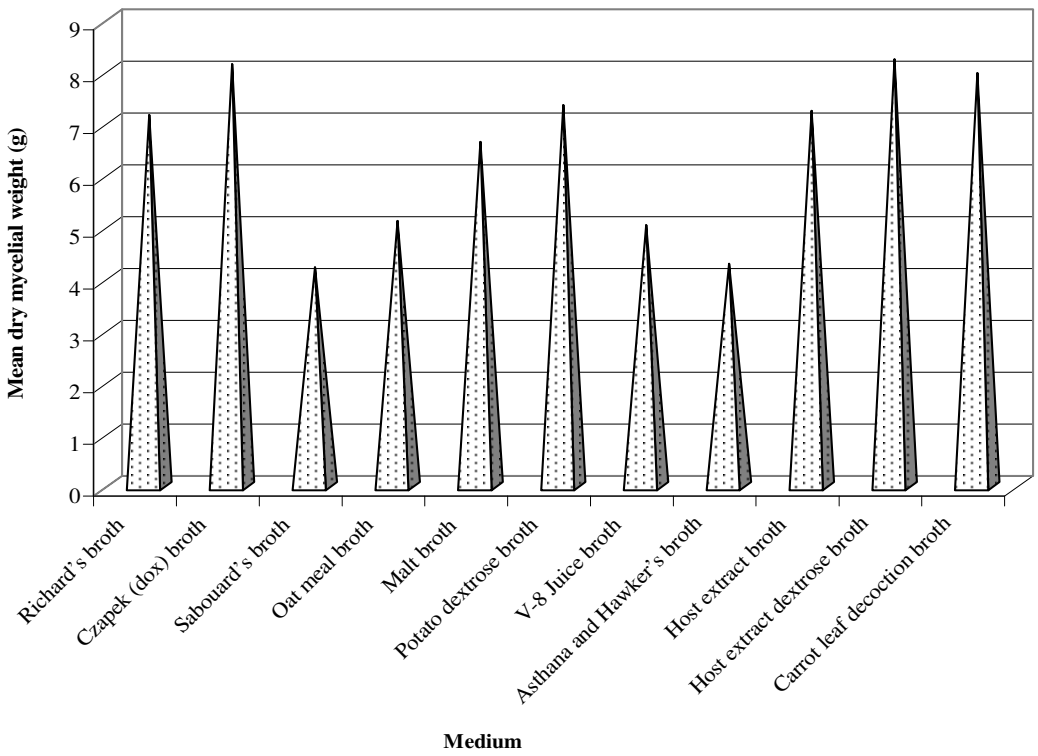


Fig. 3: Growth of *C. nicotianae* in different liquid media at 19th days of incubation

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Fig.3: Growth of C. nicotianae in different liquid media at 19th days of incubation

broth (4.29 g) and Oat meal broth (5.12 g). Czapek (dox) broth (8.14 g) supported better growth than Richard's broth (7.16 g). Hence, Czapek (dox) broth was used as synthetic medium for nutritional studies and for physiological studies, Potato dextrose broth was selected which supported the maximum growth of the fungus.

4.4 Nutritional studies

4.4.1 Carbon utilization

This experiment was carried out to study the utilization of various carbon sources by the fungus. Seven carbon sources were used in the study as given in 'Material and Methods', and the results are presented in Table-5, Fig.- 4 and Plate-4a.

The results of study revealed that among the different carbon sources, Fructose supported mean maximum dry mycelial weight (4.67 g) of the fungus followed by Lactose (3.87 g), Sucrose (3.12 g) and Dextrose (2.31 g). Minimum growth of the fungus (1.50 g) was observed in case of Cellulose, which were on par with Starch (1.60 g) and Glucose (1.68 g).

4.4.2 Nitrogen utilization

The utilization of different nitrogen sources by the fungus was tested as detailed in 'Material and Methods'. The results of the experiment are presented in Table-6, Fig.-5 and Plate-4b.

Mean maximum dry mycelial weight (3.83 g) of the fungus was recorded when Ammonium Orthophosphate was used as a source of nitrogen followed by Asparagine (3.50 g) and Sodium nitrate (2.95 g). Least mean dry mycelial weight (0.84 g) was observed in case of urea compared to control (1.05 g).

4.5 Physiological studies

4.5.1 Temperature

Effect of different temperatures on growth of the fungus was studied as explained in 'Material and Methods' and the results are presented in Table 7, Fig.-6 and Plate-5a.

The maximum growth of the fungus (7.04 g) was observed at a temperature of 25^0C, which was found on par with room temperature (26 ± 1^0C,) (6.96 g) and 30^0C (6.83 g). Growth at 40^0C (1.19 g) was on par with that of at 10^0C (1.18 g). The growth of fungus was minimum at 5^0C (0.83 g).

4.5.2 Hydrogen-ion Concentration (pH)

The growth of the fungus was studied at different pH levels as described in 'Material and Methods' and the results are presented in Table 8, Fig.-7 and Plate-5b.

The fungus grew at all pH levels tested. Maximum growth was recorded at a pH of 5.0 (6.98 g), which was on par with that of pH 4.5 (6.86 g) and 5.5 (6.80 g). The growth decreased as the pH moved away from 5.0 on either side. Lowest growth was observed at pH 3.0 (3.95). Growth at pH 3.5 (4.23 g) was found to be on par with that of at pH 8.0 (4.53 g).

4.6 *In vitro* evaluation of fungicides against *C. nicotianae*

Seven systemic and five non-systemic fungicides were tested at three concentrations in the laboratory for their efficacy against the pathogen as described in 'Material and Methods'. The results are presented in the Table 9a and 9b, Fig.-8 and Plate-6.

a. Growth of C. nicotianae on solid media

1.Richard's agar 2. Czapek (dox) agar 3. Sabouard's agar

4.Oat meal agar 5.V-8 Juice agar 6.Potato dextrose agar

7.Asthana and. Hawker agar 8.Carrot leaf decoction agar 9.Host extract agar

10.Host extract dextrose agar 11.Malt agar

b. Growth of C.nicotianae on liquid media

1. Richard's broth 2. Czapek (dox) broth 3. sabouard's broth

4.Oat meal broth 5.Malt broth 6.Potato dextrose broth

7.V-8 broth 8.Asthana and Hawker's broth 9.Host extract broth

10.Host extract dextrose broth 11. Carrot leaf decoction broth

Plate 3: Cultural studies

Table – 5. Effect of Carbon sources on the growth of *C. nicotianae*

Sl. No	Carbon source	Mean dry mycelial weight (gm)
1	Dextrose	2.31
2	Fructose	4.67
3	Lactose	3.87
4	Sucrose	3.12
5	Starch	1.60
6	Cellulose	1.50
7	Glucose	1.68
8	Control	1.92
S.Em±		0.04
C D at 1%		0.17
C.V. (%)		2.79

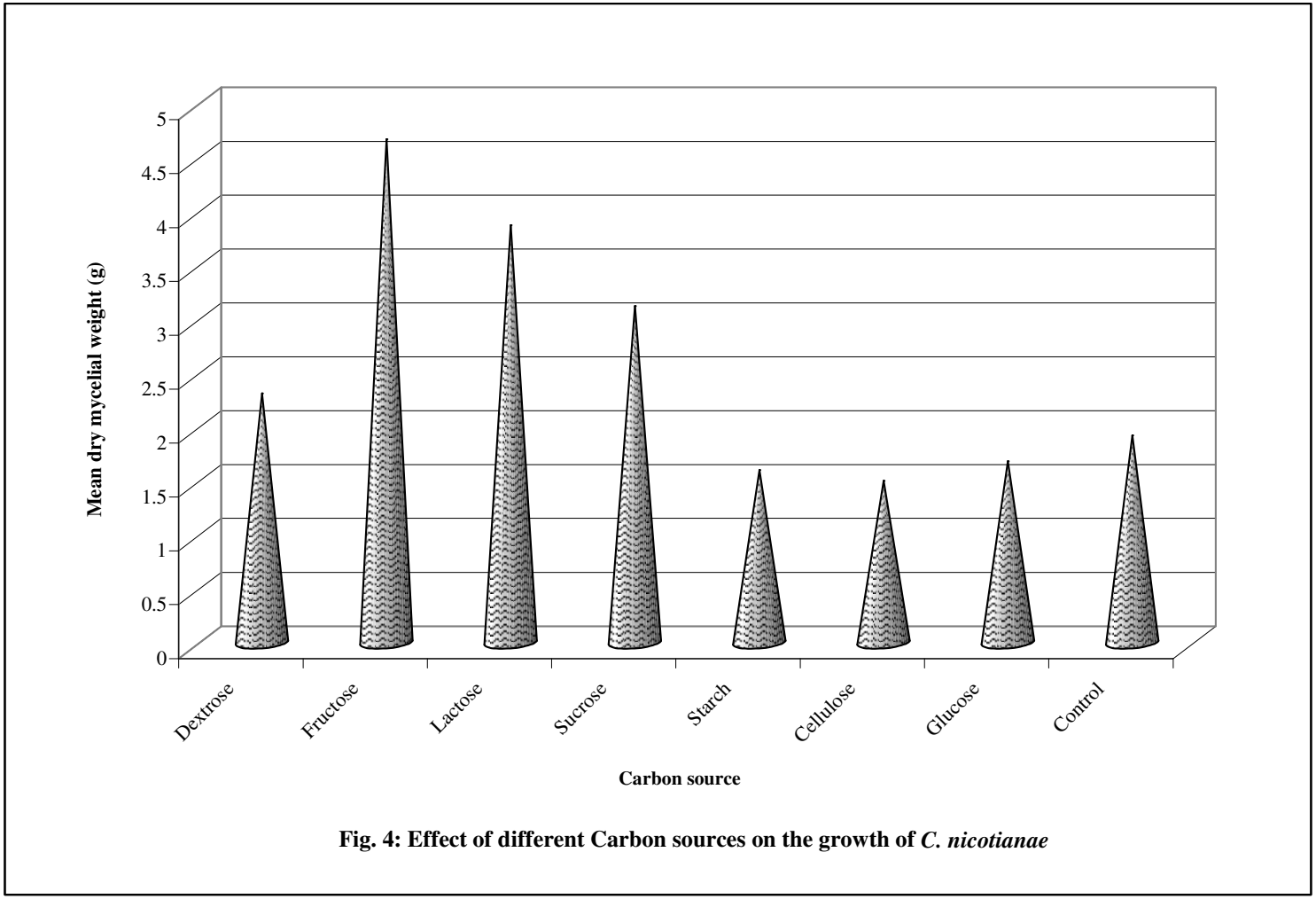


Fig. 4: Effect of different Carbon sources on the growth of *C. nicotianae*

Fig.4: Effect of different Carbon Sources on the growth of C.nicotianae

Table – 6. Effect of Nitrogen sources on the growth of *C. nicotianae*

Sl. No	Nitrogen source	Mean dry mycelial weight (gm)
1	Ammonium chloride	1.71
2	Ammonium orthophosphate	3.83
3	Ammonium sulphate	2.04
4	Asparagine	3.50
5	Sodium nitrate	2.95
6	Urea	0.84
7	Control	1.05
S.Em±		0.04
C D at 1%		0.16
C.V. (%)		2.84

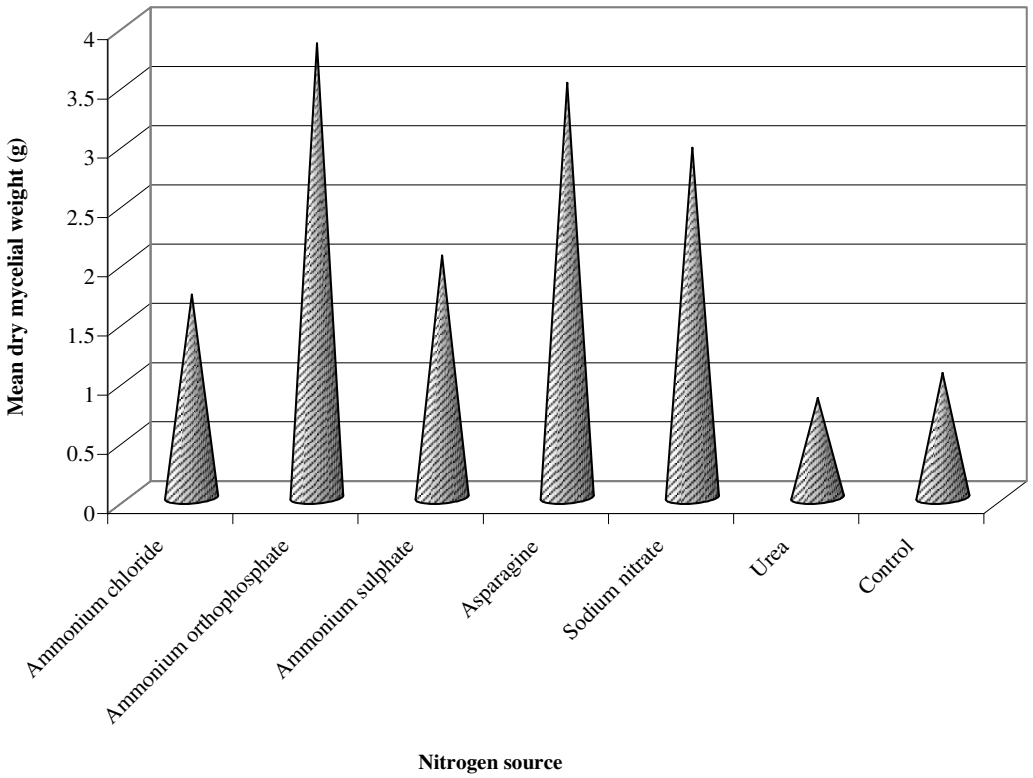


Fig. 5: Effect of different nitrogen sources on the growth of *C. nicotianae*

Fig.5: Effect of different nitrogen sources on the growth of C.nicotianae

a. Carbon utilization by C. nicotianae

1. Dextrose 2.Fructose 3.Lactose 4.Sucrose

5.Starch 6.Cellulose 7.Glucose 8.Control

b. nitrogen utilization by C. nicotianea

1. Ammonium Chloride 2.Ammonium orthophosphate

3.Ammonium sulphate 4.Asparagine

5.Sodium nitrate 6.Urea 7.Control

4.6.1 *In vitro* evaluation of systemic fungicides against *C. nicotianae*

The results indicated that Penconazole (T_5), Benomyl (T_7), Hexaconazole (T_2), recorded 100 per cent inhibition at all the tested concentrations followed by 94.04 per cent inhibition in Propiconazole (T_1) and 92.40 per cent in Carbendazim (T_3).

Hexaconazole (T_2), Penconazole (T_5) and Benomyl (T_7) recorded 100 per cent inhibition at 0.05 per cent concentration followed by 89.28 per cent inhibition in Propiconazole (T_1).

At 0.1 per cent concentration of T_2, T_5 and T_7 recorded 100 per cent inhibition over control followed by 92.8 per cent in T_1 and 90.62 per cent in T_3. At 0.15 per cent concentration all the treatments recorded 100 per cent inhibition over control except T_4 and T_6.

4.6.2 *In vitro* evaluation of non-systemic fungicides against *C. nicotianae*

The non-systemic fungicides were not effective at all the concentrations tested. At 0.1 per cent concentration maximum per cent inhibition (66.14%) was recorded in Carbendazim + Mancozeb (T_4) followed by 59.18 per cent in Propiconazole (T_3). The minimum per cent inhibition was 8.94 per cent in T_5.

At 0.2 per cent concentration, maximum per cent inhibition was noticed in T_4 (71.84%) followed by 62.86 per cent in T_3 and 57.74 per cent in T_1. Minimum per cent inhibition of 15.09 was recorded in T_5. At 0.25 per cent concentration maximum per cent inhibition of 100 was noticed in T_4 followed by 70.19 in T_2 and 68.55 per cent in T_3. The mean per cent inhibition was 79.33 per cent in T_4 followed by 63.53 in T_3 and 59.45 in T_1. However, minimum per cent inhibition was 15.63 per cent in T_5.

4.7 *In vitro* evaluation of bioagents

The competitive ability of antagonists against *C. nicotianae* was studied by dual culture method as described in 'Material and Methods'. The results obtained are presented in Table-10, Fig.-9 and Plate-7.

Six bioagents were screened against *C. nicotianae* in dual plate technique. *Trichoderma koningii* recorded maximum inhibition over control (69.10%) followed by 57.73 and 50.74 per cent over control in T_1 and T_2 respectively. *Pseudomonas fluorescens* (T_6) recorded least per cent (25.29) inhibition over control. The per cent inhibition ranged from 25.29 to 69.10 irrespective of bioagents screened.

4.8 Management of frog eye leaf spot of bidi tobacco

4.8.1 Influence of different treatments on Percent Disease Index (PDI) during 2006-07

Ten treatments including positive check and untreated check were evaluated against frog-eye leaf spot and the data are presented in Table-11, Plate-8.

Spraying with Carbendazim (T_6) @ 0.05 per cent immediately after appearance of the disease followed by another spray at 10-12 days interval recorded minimum per cent disease index (PDI) of 24.5 which was on par with Propiconazole 5% EC (T_2) @ 0.1 per cent (27.1 PDI) and Hexaconazole 5% EC (T_1) @ 0.1 per cent (28.2 PDI). Among the fungicides Chlorothalonil (32.9 PDI), Carbendazim and Mancozeb (T_4) (33.4 PDI) and Thiophanate methyl (T_5) (37.3 PDI) did not perform better in checking the incidence of the disease. Among bioagents tested *Trichoderma harzianum* (T_7) recorded minimum incidence of 39.8 PDI followed by *Pseudomonas fluorescens* (T_8) (48.5 PDI) indicating poor performance in managing the disease. Parthenium leaf extract (T_9) recorded incidence of 42.8 PDI. Maximum disease incidence was noticed in untreated control (55.9 PDI).

Table- 7. Effect of temperature on the growth of *C. nicotianae*

Sl. No	Temperature (°C)	Mean dry mycelial weight (gm)
1	5	0.83
2	10	1.18
3	15	4.27
4	20	6.59
5	25	7.04
6	30	6.83
7	35	3.59
8	40	1.19
9	Room temperature (26±1ºC)	6.96
S.Em±		0.05
C. D at 1%		0.24
C.V. (%)		2.34

Table-8. Effect of hydrogen ion concentration on the growth of *C. nicotianae*

Sl.No	pH of the medium	Mean dry mycelial weight (gm)
1	3.0	3.95
2	3.5	4.23
3	4.0	5.34
4	4.5	6.86
5	5.0	6.98
6	5.5	6.80
7	6.0	6.40
8	6.5	6.12
9	7.0	5.97
10	7.5	5.07
11	8.0	4.53
12	8.5	4.06
S.Em±		0.09
C. D at 1%		0.34
C. V. (%)		2.66

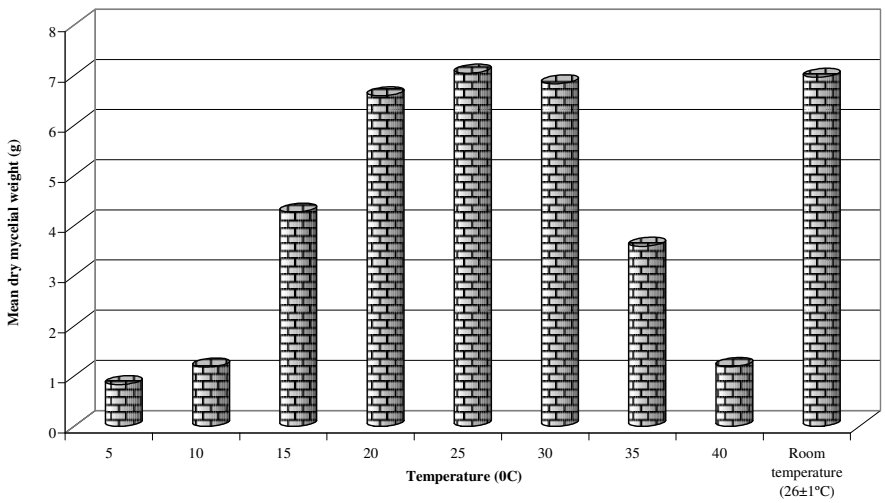


Fig. 6: Effect of temperature on the growth of C. *nicotianae*

Fig.6: Effect of temperature on growth of C. nicotianae

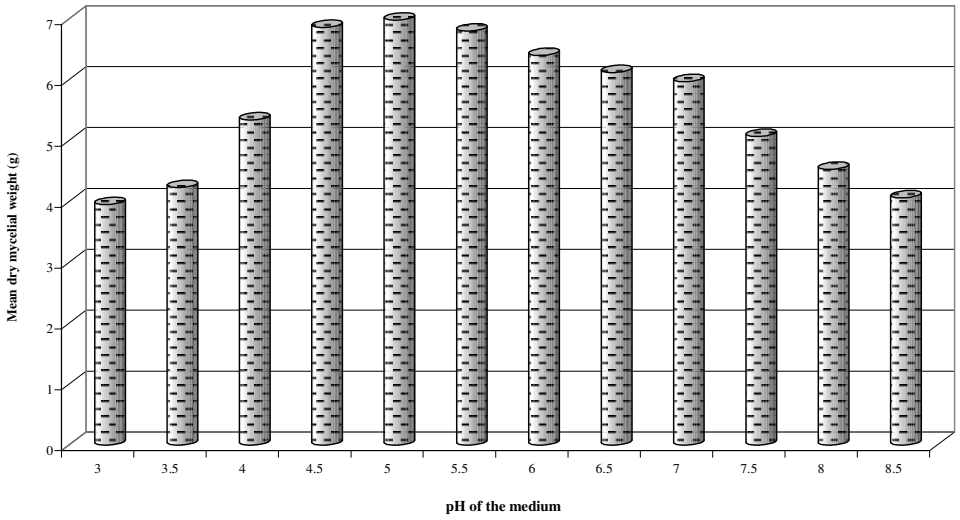
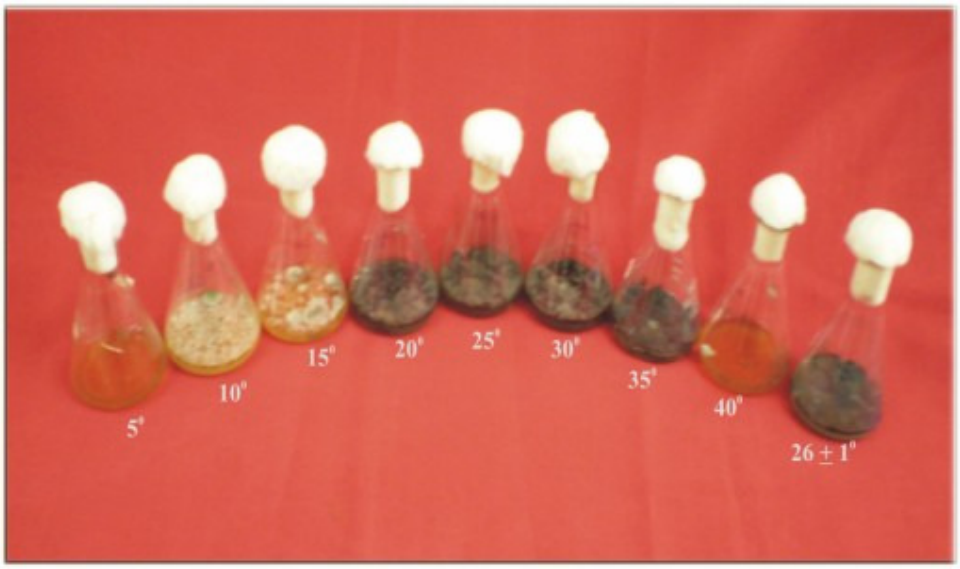
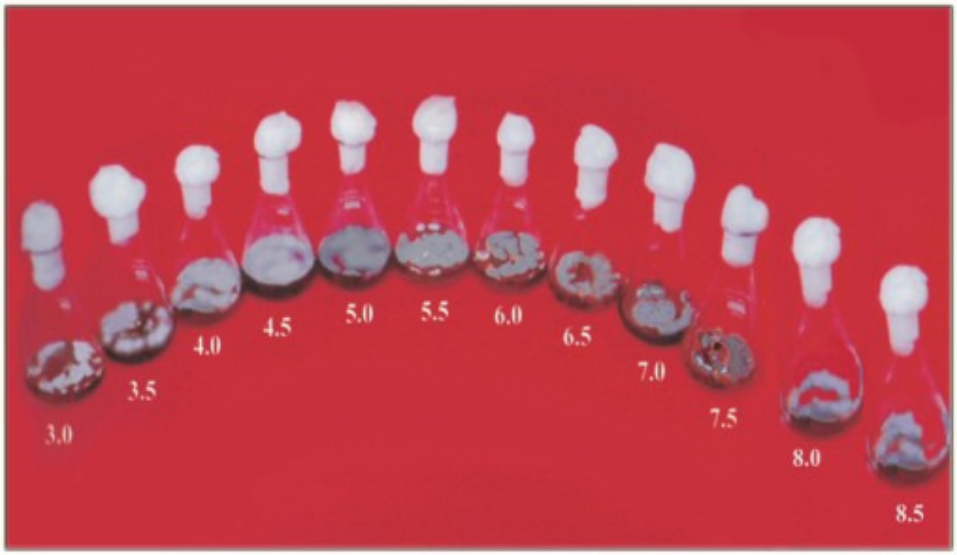


Fig. 7: Effect of hydrogen ion concentration on the growth of *C. nicotianae*

Fig.7: Effect of hydrogen ion concentration on the growth of C. nicotianae



a. Effect of temperature on growth of C. nicotianae



b. Effect of hydrogen-ion concentration on growth of C.nicotianae

Plate5: Physiological studies

Table-9a. *In-vitro* evaluation of different systemic fungicides against *C. nicotianae*

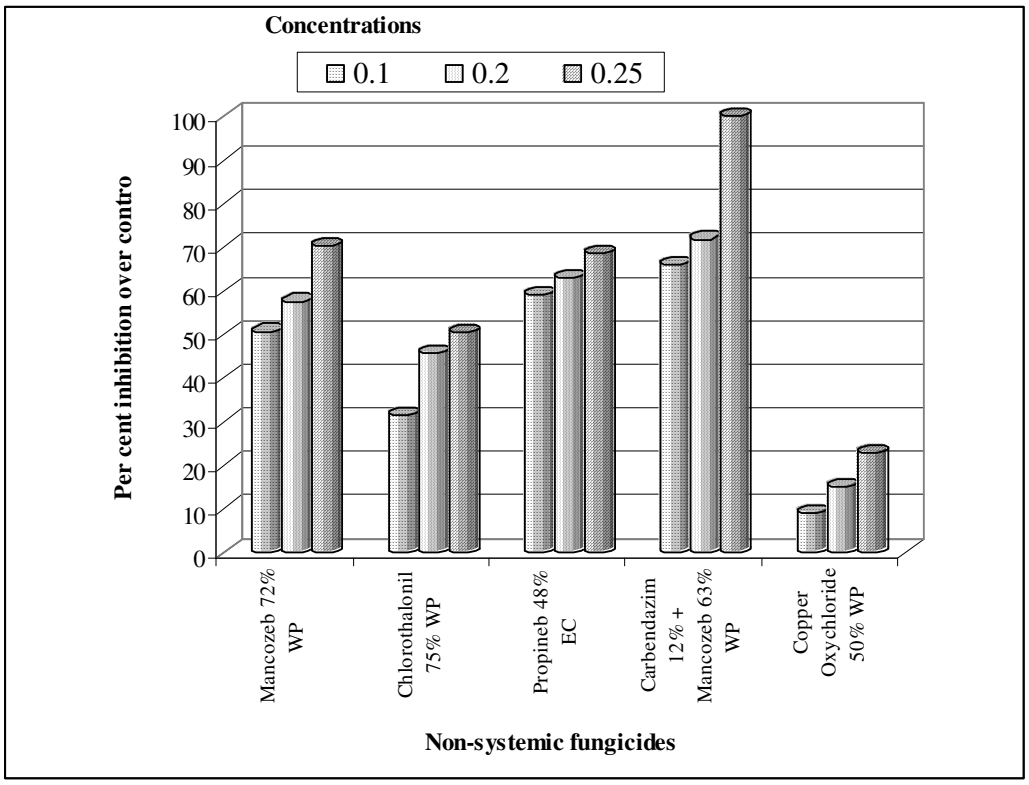
Sl.No	Fungicide	Per cent inhibition over control /Concentrations (%)			Mean
		0.05	0.10	0.15	
T_1	Propiconazole 5% EC	89.28 (70.85)*	92.85 (74.45)	100 (90.00)	94.04 (78.43)
T_2	Hexaconazole 25% EC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_3	Carbendazim 50% WP	86.57 (68.50)	90.62 (72.15)	100 (90.00)	92.40 (76.88)
T_4	Thiophanate methyl 70% WP	74.50 (59.90)	81.22 (64.30)	91.50 (73.03)	82.40 (65.74)
T_5	Penconazole 5% EC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T_6	Difenconazole 25% EC	85.67 (67.77)	89.71 (71.26)	91.96 (73.47)	89.11 (70.83)
T_7	Benomyl 50% WP	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
Mean		90.86 (76.72)	93.49 (78.90)	97.64 (85.21)	93.99 (80.27)
		Fungicides (F)	Conc. (C)	F x C	
S.Em±		0.28	0.18	0.48	
C. D at 1%		2.01	1.63	2.65	
C. V. (%)		1.04			

*Figures in parentheses are angular transformations

Table-9b. *In vitro* evaluation of different non-systemic fungicides against *C. nicotianae*

Sl.No	Fungicide	Per cent inhibition over control /Concentrations (%)			Mean
		0.1	**0.2**	**0.25**	
T_1	Mancozeb 72% WP	50.62 (45.34)*	57.54 (49.31)	70.19 (56.85)	59.45 (50.50)
T_2	Chlorothalonil 75% WP	31.36 (33.96)	45.71 (42.51)	50.59 (45.32)	42.55 (40.60)
T_3	Propineb 48% EC	59.18 (50.39)	62.86 (52.41)	68.55 (55.86)	63.53 (52.89)
T_4	Carbendazim 12% + Mancozeb 63% WP	66.14 (54.39)	71.84 (57.92	100 (90.00)	79.33 (67.44)
T_5	Copper Oxychloride 50% WP	8.94 (17.13)	15.09 (22.82)	22.86 (28.50)	15.63 (22.82)
Mean		43.25 (40.24)	50.61 (45.00)	62.44 (55.31)	52.10 (46.85)
		Fungicides (F)	Conc. (C)	F x C	
S.Em±		0.54	0.42	0.94	
C D at 1%		2.86	2.51	3.76	
C. V. (%)		3.49			

*Figures in parentheses are angular transformations



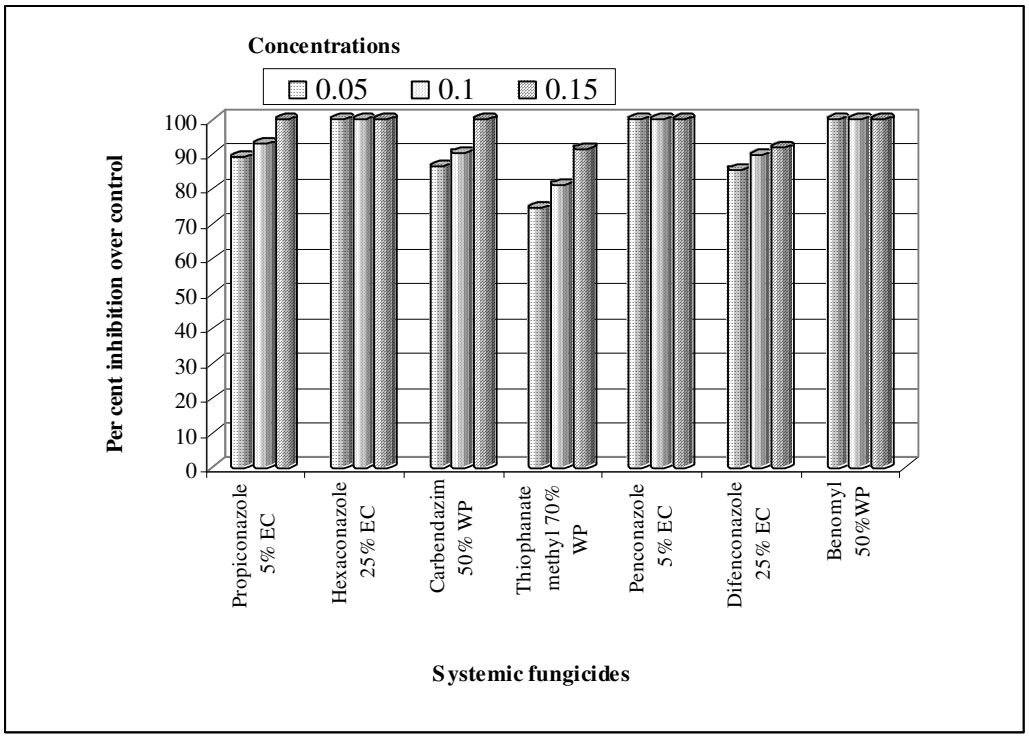


Fig8: percent inhibition of C. nicotianae by difsferent systematic and non-systematic fungicides

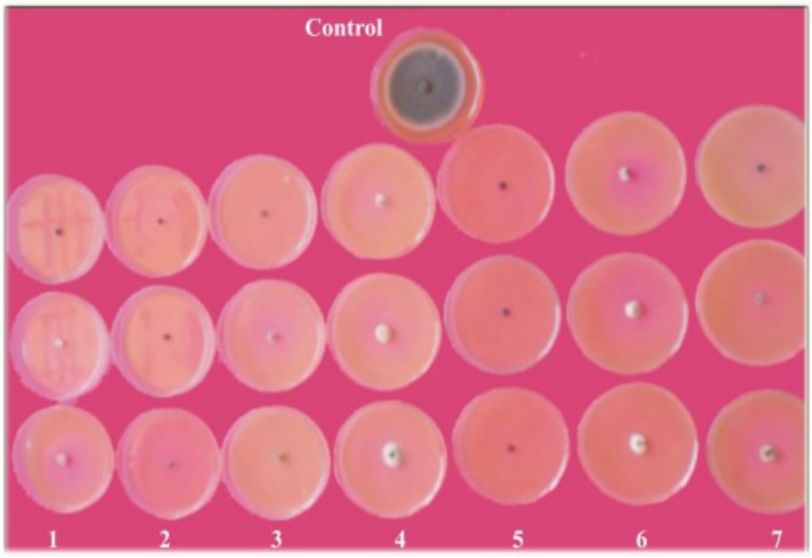
Legend

Systematic fungicides

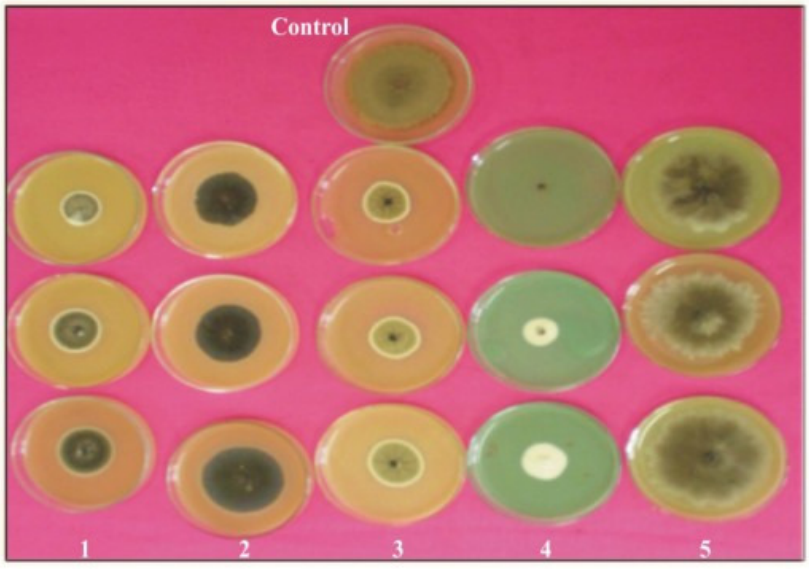
1. Propiconazole (Tilt 25% EC)
2. Hexaconozole (Contaf 5%EC)
3. Carbendazim (Bavistin 50 WP)
4. Thiophanate methyl (Topsin-M70WP)
5. Penconazole (Topas 25EC)
6. Difenconazole (Score25%EC)
7. Benomyl (Benlate 50WP)

Non-systematic fungicides

1. Mancozeb (Dithane M-45 75 Wp
2. Chlorothalonil (Kavach 75%WP)
3. Propineb (Antracol 70%WP)
4. Carbendazim (12%) +Mancozeb (63%) (Saaf)
5. Copper Oxychloride (Blitox-50WP)



a. Systematic fungicides



b. Non-systematic fungicides

Plate6: In viro evaluation of fungicides

Table-10. *In-vitro* evaluation of bioagents against *C. nicotianae*

Sl. No	Bioagents	Per cent inhibition over control
T_1	*Trichoderma viride*	57.73 (49.44)*
T_2	*T. harzianum*	50.74 (45.40)
T_3	*T. koningii*	69.10 (56.24)
T_4	*T. virens*	44.19 (41.62)
T_5	*Pseudomonas fluorescens*	25.29 (30.11)
T_6	*Bacillus subtilis*	45.31 (42.28)
S.Em±		0.72
C. D at 1%		3.12
C. V. (%)		3.30

*Figures in parentheses are angular transformations

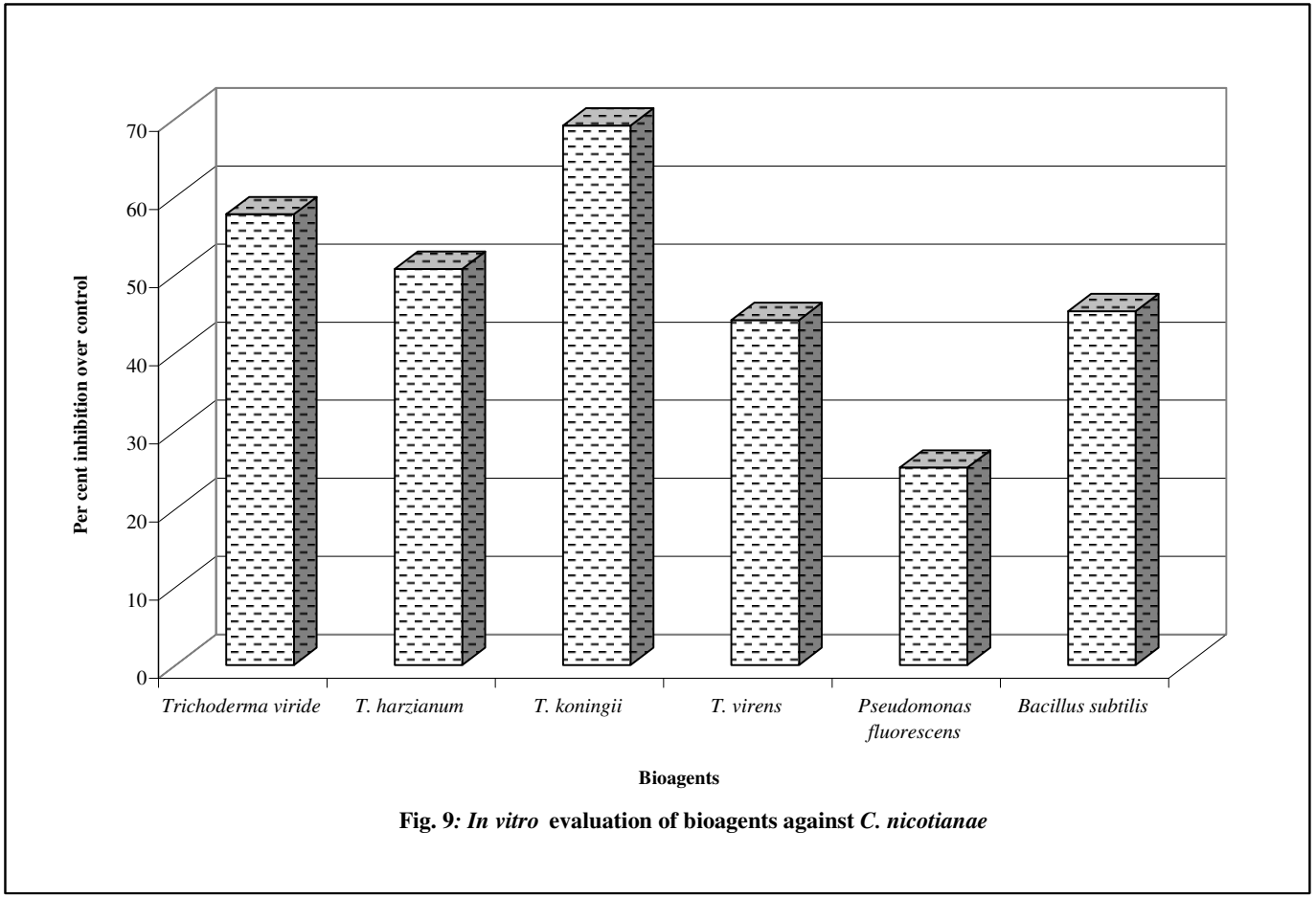
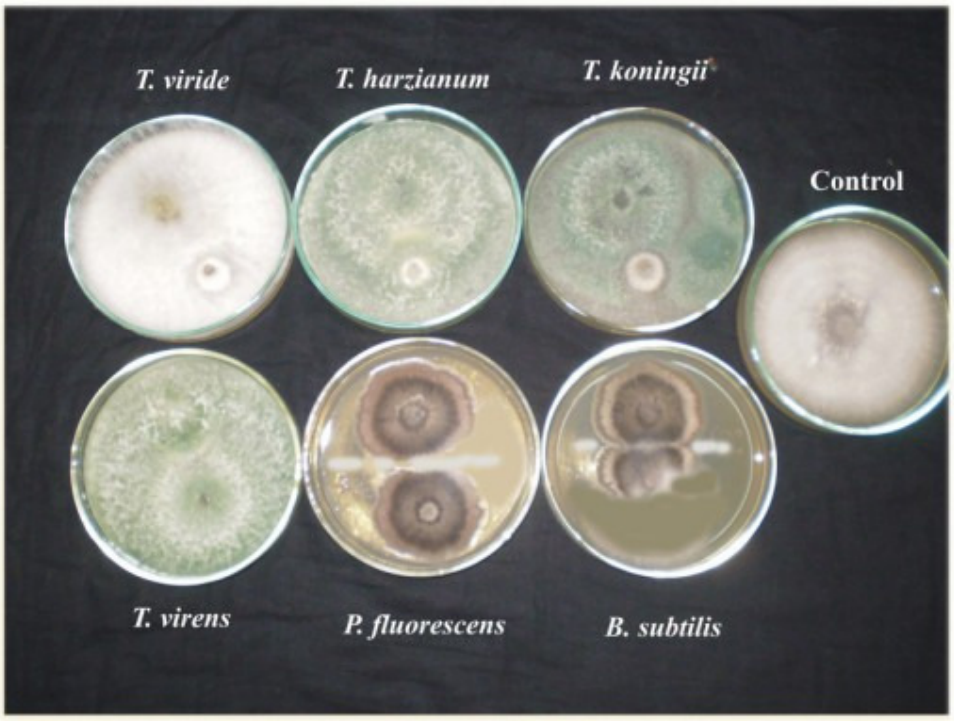


Fig. 9*: In vitro* evaluation of bioagents against *C. nicotianae*

Fig.9: In vitro evaluation of bioagents against C. nicotianae



a: In vitro evaluation of bioagents

b. General field view of experiment along with spore trap.

Plate7: In vitro evaluation of bioagents and general field view of Experimental site.

4.8.2 Influence of different treatments on cured leaf yield

The maximum cured leaf yield of 1380 kg/ha was recorded in plots which received the spray of Propiconazole (T_2) @ 0.1 per cent followed by 1300 kg/ha in case of Carbendazim @ 0.05 per cent (T_6) and Hexaconazole @ 0.1 per cent (1292 kg/ha). There was no significant difference with respect to cured leaf yield among different treatments tested. The application of *T. harzianum* (T_7) recorded cured leaf yield of 1217 kg/ha, while *Pseudomonas fluorescens* (T_8) (1245 kg/ha) spraying with Parthenium leaf extract (T_9) recorded minimum cured leaf yield of 1020 kg/ha when compared to control (1100 kg/ha).

4.8.3 Influence of different treatments on growth parameters of bidi tobacco

The observations on growth parameters such as plant height, leaf length and leaf breadth were recorded at two stages (80 days after transplanting (DAP) and at harvest) are presented in Table-12.

4.8.3.1 Influence of different treatments on plant height

Maximum plant height of 69.0 cm was recorded in T_8 followed by 67.5 cm in T_4 and 66.0 cm in T_5 at 80 days after transplanting. At the time of harvest, maximum plant height of 74.0 cm was recorded in T_2 and T_8 followed by 71.7 cm in T_5 and 70.1 cm in T_4. However, the difference were non significant among different treatments in respect to plant height.

4.8.3.2 Influence of different treatments on leaf length

The leaf length was recorded at 80 DAP and at harvest. Maximum length of 43.3 cm was recorded in Thiophanate methyl (T_5) followed by 42.3 cm in Carbendazim (T_6) and 40.89 cm in *Trichoderma harzianum* (T_7). Minimum leaf length of 35.0 cm was recorded in Parthenium leaf extract (T_9). There was significant difference with respect to leaf length at 80 DAP. AT harvest, maximum leaf length of 46.4 cm was recorded in Chlorothalonil (T_3) followed by 44.7 in Saaf (T_4) and 43.9 cm in Thiophanate methyl (T_5). The bio-agents also recorded leaf length of 43.4 cm and 41.2 cm in *Pseudomonas fluorescens* (T_8) and *Trichoderma harzianum* (T_7) respectively. Leaf length was 40.9 cm in Parthenium leaf extract (T_9). Leaf length of 42.03 cm was recorded in untreated control (T_{10}). There was no significant difference with respect to leaf length at harvest.

4.8.3.3 Influence of different treatments on leaf breadth

At 80 DAP, the highest leaf breadth of 21.2 cm was recorded in Saaf (T_4) followed by 20.7 in Chlorothalonil (T_3) and 20.6 cm in *Pseudomonas fluorescens* (T_8). Propiconozole (T_2) recorded minimum leaf breadth of 16.2 cm. There was significant difference with respect to leaf breadth at 80 DAP. At harvest, maximum leaf breadth of 21.6 cm was recorded in Saaf (T_4) followed by 21.0 cm in *Pseudomonas fluorescens* (T_8) and 20.7 cm in Thiophanate methyl (T_5). Minimum leaf breadth of 18.6 cm was noticed in Parthenium leaf extract (T_9) and untreated control recorded leaf breadth of 19.6 cm. There was no significant difference among different treatments with respect to leaf breadth.

4.9 Economic analysis

The economics was worked for each of the treatments employed in the present investigation. The data on economic parameters are presented in Table-13.

The highest gross return of Rs. 48,300 was worked out in Propiconazole at 0.1 per cent (T_2) followed Rs. 45,500 in Carbendazim (T_6) and Rs. 45,220 in Hexaconazole (T_1). The bioagents recorded gross income of Rs. 42,595 and Rs. 43,575 in *Trichoderma harzianum* (T_7) and *Pseudomonas fluorescens* (T_8) respectively. Minimum gross income of Rs. 35,700 was worked out in Parthenium leaf extract (T_9) and untreated check recorded a gross income of Rs. 38,500/-.

The net income was maximum (Rs.28,880) in Propiconozole (T_2) followed by Rs. 27,220/- in Carbendazim (T_6) and Rs. 26,520/- in Hexaconazole (T_1). The net income was

Table –11. Management of frog eye leaf spot of bidi tobacco during 2006-07.

Tr. No	Treatment Details	PDI	Yield kg/ha
T_1	Hexaconazole 5% EC. (Contaf) at 0.1%	28.2 (32.1) *	1292
T_2	Propiconazole at 25% EC. (Tilt) at 0.1%	27.1 (31.4)	1380
T_3	Chlorothalonil (Kavach) 75% WP at 0.2%	32.9 (35.0)	1164
T_4	Carbendazim – 12% + Mancozeb – 63% (Saaf) at 0.2%	33.4 (35.3)	1289
T_5	Thiophanate methyl 70% WP (Hilnate) at 0.2%	37.3 (37.6)	1242
T_6	Carbendazim (Bavistin) 50% WP at 0.05%	24.5 (29.7)	1300
T_7	*Trichoderma harzianum* @50g/plot and spray @ 2g/lit.	39.8 (39.1)	1217
T_8	*Pseudomonas fluorescens* @ 50g/plot and spray @ 2g/lit.	48.5 (44.2)	1245
T_9	Parthenium leaf extract spray @ 5% spray	42.8 (40.8)	1020
T_{10}	Control	55.9 (48.4)	1100
	S.Em±	1.62	63.79
	C.D. 5%	4.82	NS
	C.V. %	7.76	21.81

Note: PDI= Percent Disease Index

*Figures in parentheses are angular transformations

Hexaconazole@ 0.1%

Propiconazole @ 0.1%

Carbendazim@0.1%

Chlorothalonil@ 0.1%

Thiophanate methyl @ 0.1%

Control

Plate 8: In vivo evaluation of fungicides

Table–12. Influence of different treatments on growth parameters of bidi tobacco

Tr. No	Treatment Details	Plant height (cm)		Leaf Length (cm)		Leaf Breadth (cm)	
		80 DAP	At harvest	80 DAP	At harvest	80 DAP	At harvest
T_1	Hexaconazole 5% E.C. (Contaf) at 0.1%	62.60	68.3	40.6	42.1	16.6	18.9
T_2	Propiconazole at 25% E.C. (Tilt) at 0.1%	62.73	74.0	38.6	42.6	16.2	19.8
T_3	Chlorothalonil 75%WP (Kavach) at 0.2%	61.3	66.6	39.9	46.4	20.7	20.5
T_4	Carbendazim – 12% + Mancozeb – 63% (Saaf) at 0.2%	67.5	70.1	42.3	44.7	21.2	21.6
T_5	Thiophanate methyl 70% WP (Hilnate) at 0.2%	66.0	71.7	43.3	43.9	19.6	20.7
T_6	Carbendazim (Bavistin) 50%WP at 0.05%	59.07	68.0	42.3	42.8	19.2	19.3
T_7	*Trichoderma harzianum* @ 50g/plot and spray @ 2g/lit.	59.07	68.3	40.89	41.2	19.1	19.3
T_8	*Pseudomonas fluorescens* @ 50g/plot and spray @ 2g/lit.	69.03	74.0	39.8	43.4	20.6	21.0
T_9	Parthenium leaf extract spray @ 5% spray	60.53	67.8	35.0	40.9	18.3	18.6
T_{10}	Control	59.6	63.4	40.33	42.03	18.68	19.6
	S.Em±	6.53	5.52	2.29	2.13	1.26	0.56
	C.D. 5%	NS	NS	6.74	NS	3.70	NS
	C.V. %	17.64	14.16	9.27	8.97	10.98	12.96

Table- 13. Economic analysis in the management of frog eye leaf spot of bidi tobacco.

Sl.No	Treatment Details	Yield Kg/ha	Gross income Rs/ha	Cost of cultivation Rs/ha	Net Income Rs/ha	B: C
T_1	Hexaconazole 5% E.C. @ 0.1%	1292	45220	18700	26520	1:1.41
T_2	Propiconazole at 25% E.C. @ 0.1%	1380	48300	19420	28880	1:1.48
T_3	Chlorothalonil @ 0.2%	1164	40740	19828	20912	1:0.15
T_4	Carbendazim – 12% + Mancozeb – 63% @ 0.2%	1289	45115	19204	25911	1:1.35
T_5	Thiophanate methyl 70% WP @ 0.2%	1242	43470	20020	23450	1:1.17
T_6	Carbendazim 50 WP @ 0.5%	1300	45500	18280	27220	1:1.49
T_7	*Trichoderma harzianum* @ 50g/plot and spray @ 2g/lit	1217	42595	25500	17095	1:0.67
T_8	*Psuedomonas fluorescens* @ 50g/plot and spray @ 2g/lit	1245	43575	25500	18075	1:0.70
T_9	Parthenium leaf extract spray @ 10%	1020	35700	18100	17600	1:0.97
T_{10}	Control	1100	38500	18000	20500	1:1.14

least (Rs. 17,095/-) in *Trichoderma harzianum* (T_7) and untreated check recorded net income of Rs. 20,500/-.

The highest B: C (1:1.49) ratio was recorded in Carbendazim (T_6) followed by 1:1.48 in Propiconazole (T_2) and 1:1.41 in Hexaconazole (T_1). The minimum B: C ratio of 1:0.15 was obtained in Chlorothalonil (T_3).

4.10 Influence of different treatments on quality parameters

The data on different quality parameters such as Nicotine percentage, reducing sugars percentage and Chlorides percentage are presented in Table -14.

4.10.1 Influence of different treatments on Nicotine percentage

The nicotine percentage in different treatments ranged from 2.64-4.06. Maximum nicotine percentage (4.06) was recorded in *Pseudomonas fluorescens* (T_9) followed by 3.56 % in Hexaconazole (T_1) and 3.547% in *Trichoderma harzianum* (T_7). Minimum nicotine percentage of 2.64 was recorded in Thiophanate methyl (T_5). The untreated check recorded nicotine percentage of 2.70.

4.10.2 Influence of different treatments on reducing sugars percentage

The reducing sugars in different treatments ranged from 6.58 to 7.88%. Maximum reducing sugars percentage was recorded in Carbendazim (T_6) (7.88) followed by *Trichoderma harzianum* (T_7) (7.79) and Thiophanate methyl (T_5) (7.36). Minimum reducing sugars percentage of 6.58 was recorded in *Pseudomonas fluorescens* (T_8).

4.10.3 Influence of different treatments on Chloride percentage

The chloride percentage in different treatments ranged from 0.39 to 0.67%. Maximum chloride percentage was in Thiophanate methyl (T_5) (0.67%) followed by 0.66% in Propiconazole (T_2) and 0.47% in Saaf (T_4). The bioagent treatments recorded chloride percentage of 0.39 and 0.43 in *Trichoderma harzianum* (T_7) and *Pseudomonas fluorescens* (T_8) respectively. The untreated check recorded chloride percentage of 0.40.

4.11 Screening of different entries, germplasm lines and station material against frog eye leaf spot

A total 117 entries belonging to advanced, initial hybrid and varietal trial material and 190 germplasm lines were evaluated for frog eye leaf spot under naturally unprotected condition. The promising entries in each of trial along with maximum disease pressure are presented in Table-15, Plate-9.

4.11.1 Screening of advanced lines and other trial entries for frog eye leaf spot disease

The advanced and other trial entries in AVT-I, AVT-II, IVT and IVHT were screened for frog eye leaf spot reaction. In AVT-I trial, the entries NBD-154, 155, 159, ABD-99, 100, NBD-43 and NPN-22 recorded minimum disease grade of 2.0. The popular cultivar A-119 recorded disease grade 3.0 and ABD-96 showed susceptible reaction for frog eye leaf spot recording disease grade of 5.0.

In AVT-II trial, the entries which recorded minimum disease grade of 2.0 are ABD-94, ABD-95, ABD-138, 139, 146, 147 and checks NPN-22 and PL-5. The entries which recorded disease grade of 3.0 are ABD-93, NBD-134, 136, 43 and popular check A-119. Maximum disease grade 4.0 was recorded in ABD-92. In IVT trial, the entries ABD-106, 107 and NPN-22 recorded disease grade of 2.0, while ABD-105, 108 along with checks A-119, GT-5 and NBD-43 recorded disease grade of 3.0. In IVHT trial, six entries recorded minimum disease grade of 2.0 *Viz.,* ABD-13, 94, 100, 103 along with checks A-119, BTH-126 and NBD-22. The entries which recorded disease grade of 3.0 are ABD-92, 95, 96, 99, 101, 102, 104, BTH-127, 128, GTH-1, NBD-43 and A-119.

Table-14: Influence of different treatments on quality parameters

Tr. No	Treatment Details	Nicotine %	Reducing sugars %	Chlorides %
T_1	Hexaconazole 5% EC. (Contaf) at 0.1%	3.56	7.19	0.41
T_2	Propiconazole at 25% EC. (Tilt) at 0.1%	3.46	7.17	0.66
T_3	Chlorothalonil (Kavach) at 0.2%	3.32	7.28	0.42
T_4	Carbendazim – 12% + Mancozeb – 63% (Saaf) at 0.2%	3.03	7.51	0.47
T_5	Thiophanate methyl 70% WP (Hilnate) at 0.2%	2.64	7.36	0.67
T_6	Carbendazim (Bavistin) 50 WP at 0.05%	3.17	7.88	0.46
T_7	*Trichoderma harzianum* @ 50 g/plot and spray @ 2g/lit	3.47	7.79	0.39
T_8	*Pseudomonas fluorescens* @ 50 g/plot and spray @ 2g/lit	3.44	6.58	0.43
T_9	Parthenium leaf extract spray @ 10%	4.06	6.89	0.48
T_{10}	Control	2.70	7.35	0.40

4.11.2 Screening of station trial material against frog eye leaf spot

In Station Trial-I, the entries NBD-190 recorded disease of grade of 1.0 along with check NPN-22. Eighteen entries and popular check A-119 recorded disease grade of 2.0. The entries belonging to NBD series 192, 193, 197, 199, 208, 213, 214 and NBD-43 recorded disease grade of 3.0. NBD-196 recorded susceptible reaction with disease grade 4.0. In Station Trial-II (ST-II) material, the entries NBD-185 and 186 recorded disease grade of 2.0. The NBD-entries 180, 181, 182, 183, 184, 187, along with checks NBD-43, NPN-22 and A-119 recorded disease grade of 3.0. In Station Hybrid Trial, NBTH-105, 41 recorded disease grade 2.0 along with check A-119. The NBTH series 652, 42, 309, 801, 653, 43, 325, 44, 326, 802,803, 28, 602, 118, 824, 43 recorded disease grade of 3.0 along with check NBD-43.

4.11.3 Screening of genetic stock against frog eye leaf spot disease

Total of 190 germplasm lines were screened for frog eye leaf spot disease under naturally unprotected condition. Thirty three lines were found promising for frog-eye leaf spot recording a disease grade of 2.0. The popular checks A-119, NPN-22 and recently released variety NBD-43 recorded disease grade of 3.0.

Table-15: Screening of different genotypes and germplasm lines against frog-eye leaf spot of bidi tobacco during 2006-07

Sl. No	Name of the trial	Disease grade (0-5) scale	Promising entries
1	AVT-I	2	NBD-154, NBD-155, NBD-159, ABD-99, ABD-100, NBD-43 and NPN-22
		3	A-119
		5	ABD-96
2	AVT-II	2	ABD-94, ABD-95, NBD-138, NBD-139, NBD-146, NBD-147, NPN-22 and PL-5
		3	ABD-93, NBD-134, NBD-136, NBD-43 and A-119
		4	ABD-92
3	IVT	2	ABD-106, ABD-107 and NPN-22
		3	ABD-105, ABD-108, A-119, GT-5 and NBD-43
4	IVHT	2	ABD-93, ABD-94, ABD-100, ABD-103, BTH-126 and NPN-22
		3	ABD-92, ABD-95, ABD-96, ABD-99, ABD-101, ABD-102, ABD-104, BTH-127, BTH-128, GTH-1, NBD-43 and A-119
5	ST-I	1	NBD-190 and NPN-22
		2	NBD-191, NBD-194, NBD-195, NBD-198, NBD-200, NBD-201, NBD-202, NBD-203, NBD-204, NBD-205, NBD-206, NBD-207, NBD-209, NBD-210, NBD-211, NBD-212 and A-119
		3	NBD-192, NBD-193, NBD-197, NBD-199, NBD-208, NBD-213, NBD-214 and NBD-43
		4	NBD-196
6	ST-II	2	NBD-185 and NBD-186
		3	NBD-180, NBD-181, NBD-182, NBD-183, NBD-184, NBD-187, NBD-189, NBD-43, NPN-22 and A-119

Continued

7	SHT-I	1	NPN-22
		2	NBTH-105, NBTH-41 and A-119
		3	NBTH-652, NBTH-42, NBTH-309, NBTH-801, NBTH-653, NBTH-43, NBTH-325, NBTH-44, NBTH-326, NBTH-802, NBTH-803, NBTH-28, NBTH-602, NBTH-118, NBTH-824, NBTH-43 and NBD-43
8	Genetic stock	2	169-119-15 (88-47 x Sokha),169-119-16(88-47xSokha), 169-119-17(88-47xSokha), 169-119-9(8847xSokha),169-119(Medium through A-23),K-20(Pinle leaves),BSP(Black spangle),BL-2-1,RPK 1-2,RPK-2,20-49-36-36(A-2xcolor),K-20(Pinle leaves),655-27-38-39(A-2xolor)SBR-1, 428-32 103-38 (70-6-6 x olor), 6-2-18-37-15 (108-15 x olor), 46-11-29-23, 428-32-103-38 (70-6-6 x olor), 378-4-40-38 (A-23 x olor), 626-33-23-32 (A-23 x olor), 626-11-21-26 (A-23 x olor),G.P,NP 15,NP-37,NP-23,NP-43, Gandiu-6,Keliu-49,Keliu-20,Anand-3,Anad 23,Sokhada(Zones),Sokhada(Spread),Akol,Kukummatri.

a. Screeing of germplasm lines against frog eye leaf spot

b. Field screening of advanced trail material against frog eye leaf spot: A Promising entry:ABD96

c. nstallation of spore trap in management trail

Plate9: Screening of bidi tobacco entries C. nicotianae

5. DISCUSSION

Frog-eye leaf spot disease of bidi tobacco caused by *C. nicotianae* is a serious disease wherever bidi tobacco is grown and is one of the limiting factor in bidi tobacco production. There was no systematic work on various aspects of pathogen and its management in this crop. Hence, in this context, the present investigation on disease survey, isolation and identification of the pathogen, cultural and physiological studies, *in vitro* evaluation of bioagents and chemicals, management of frog eye leaf spot through chemicals, bioagents and plant extracts and identification of resistance sources were studied.

5.1 Survey and surveillance for frog eye leaf spot in Nipani area

Survey was taken up in Nipani area during 2006-07. Totally 21 villages were surveyed for about four months during September, October, November and December 2006-07 by rowing survey method. The average incidences recorded in each month are presented. The investigation revealed that September and October months are favorable period for frog-eye leaf spot, recording a mean incidence of 13.05 per cent and 12.06 per cent irrespective of locations respectively. The villages Akkol and Aadi are considered as most severe zone and hot spots for frog eye leaf spot in Nipani. The other areas such as Galataga, Kodni, Nipani, Siraguppi, Jatrat, Khadaklat and Walki are considered as other moderate to severe zones for frog-eye leaf spot. The areas where relatively less severity of frog eye leaf spot noticed are Sadalaga, Pangeri-A, Nagnur, Shirpewadi, Hunnurgi, Pattanakudi and Tavandi. Irrespective of areas surveyed September, October and November months are major cropping period of bidi tobacco identified as critical months for frog eye leaf spot management. Generally the intermittent rainfalls, cloudy weather and high relative humidity prevailed during these three months are the major environmental factors that helped in development and spread of the disease. Similar reports of severe incidence of frog eye leaf spot were reported (Anon, 2002 and 2003). Frog eye leaf spot as the major disease in Nipani area affecting both yield and quality of the crop (Anon., 2005).

5.1.1 Symptomatology

The pathogen *C. nicotianae* upon infection to bidi tobacco produced varied types of symptoms. The symptoms of disease are seen initially on lower leaves as small spots either circular or irregular with brown colour and whitish center. The size of spots varied with different genotypes and time of infection. The early infected plants showed relatively smaller spots than late infection. As the disease progressed these spots coalesced to form dots resembling “eyes of frog”. Hence, it is called as “frog eye leaf spot”. These spots later joined together and formed bigger patches and blighted appearance. The infection under unprotected conditions progressed towards the topmost leaves. It was observed interestingly the mixed infection of both brown leaf spot and frog-eye spot, showing type of synergestic effect among leaf spot diseases. The symptoms of frog-eye spot are most pronounced in irrigated bidi tobacco with more severity. Later, there was drying up of leaves and leaf remained intact with minute contact with stem of plant. The symptoms variability and degrees of infection suggest possible presence of different strains of *Cercospora nicotianae* affecting the crop in Nipani area. This has markedly affected both yield and quality of bidi tobacco. Similar reports of symptom expression were reported by Rangaswamy (1972) in FCV tobacco and Patel *et al.* (2001), Mulder and Holliday (1974) also reported type of spots, severity and even latent infection leading to severe stages seen only later infections in FCV tobacco. Tsay and Chen (1974) findings of the disease on older leaves infection initially followed by young leaves supported the present findings. The rainfall as key factor in spread of the disease was reported by Murthy and Elias (1975). The occurrence of mixed infection of *C. nicotianae* and *Alternaria longipes* was reported by Norse, (1972) in FCV tobacco which supported present findings in bidi tobacco.

5.2 Isolation, identification and pathogenicity studies

The isolation of the pathogen from infected leaves was taken up by standard tissue isolation method and it was purified by hyphal tip method. The culture on Potato dextrose agar produced olivaceous brown colour at edges with light brown center. The medium

beneath the colony was initially pink, later changing to dark colour suggesting possible production of Cercosporin toxin by the pathogen initially. However, it was reported that pathogen produces toxin under stress condition if it is grown especially on minimal media. Otherwise, the initial growth exhibited toxin production, later pathogen grows normally in nutrient rich medium. Hyphae of fungus were hyaline and septate. Growth of the fungus was slow taking 19 days to attain maximum growth of 87.33 cm. On basis of all these characters, the isolated culture was identified as *Cercospora nicotianae* Ell. and Eve. Later, the culture was sent to Mycology laboratory of Agharkar Institute, Pune and confirmed the identity of the fungus as *C. nicotianae* Ell. and Eve. Hortill (1976) identified the *C. nicotianae* based on physical and colony characters. Vasudeva (1963), Lanetskii *et al.*(1976) and Pululu and Corbaz (1989) also reported identification of C*. nicotianae* based on microsopic, physical and cultural observations as and supported present findings. The pathogenicity study was taken up upon artificial inoculation to the tobacco seedlings grown in pot culture. The 60 days old seedlings were inoculated with culture by pin prick method and sprayed the culture on both lower and upper leaves. The typical symptoms of *Cercospora* infection developed on lower leaves after six to seven days of inoculation. The spots initially were brown with grayish center, later turned white and dried up. The symptoms were also noticed on upper leaves. The fungus was reisolated from such infected plants and compared with original culture to prove pathogenicity of fungus. The studies also suggested requirement to prove pathogenicity by “rapid analysis” method if a tissue culture plant of bidi tobacco is being employed. These studies were supported by the reports of Pululu and Corbaz (1989).

5.3 Cultural studies

Eleven different media were screened for growth of *C. nicotianae*. Host extract agar, Czapek (dox) agar, Carrot leaf decoction agar, Richard’s agar, Malt agar and Potato dextrose agar supported more than 80 cm colony growth and abundant production of mycelial mat, V-8 juice agar and Sabouard’s agar are only two media which didn’t support maximum growth of the fungus. The present investigation with respect to synthetic media revealed that the maximum growth of fungus was governed by composition of media and host specificity of fungus for its growth on Host extract dextrose agar. The studies identified dextrose as major – component in all the synthetic media which supported maximum growth of the fungus. Similar results were also reported by Mandelson (1933) Hell (1936), Verma and Agnihotri (1952). Alasoadura and Fajola (1970) observed abundant production of conidia on tobacco decoction agar and Schneider *et al.* (1974) reported maximum growth of fungus in Potato dextrose carrot agar medium. In the present investigation all the synthetic media failed to support sporulation of the fungus, in spite of subjecting the treatment for 12 hrs light and dark. These investigations are in contrary to earlier reports of maximum conidial production by *C. nicotianae* by Carrot leaf decoction agar and antibiotic potato dextrose carrot agar medium reported by Stavely and Nimmo (1968) and Schneider *et al.* (1973) respectively.

In the present investigation, the duration for maximum growth of the fungus has been optimized. The Potato dextrose broth supported maximum growth of the fungus (7.47 g) after 19 days of inoculation. Initially up to nine days the growth of the fungus was slow. Later, increased gradually. The growth again showed a declining trend after 19 days of inoculation. The growth of fungus decreased and attained stable phase around 29 days of inoculation. Lily and Barnett (1951) categorized different phases of fungus growth and present investigations followed similar trends. Kanti (1975) reported maximum growth *C. moricola* at 20 days on Potato dextrose broth, Lakshminarayana (1981) obtained maximum of *C. solani-melongenae* and Dinesha (1984) harvested maximum growth of the fungus *C. sorghi* Ell. and Eve. on 16 days, which supported present findings of 19 days taken for maximum growth of the fungus *C. nicotianae*. This is the first systematic investigation report on *C. nicotianae* growth phase studies in bidi tobacco in India and standardized 19 days as incubation period to ascertain maximum growth *C. nicotianae* Indian isolate. In present investigation 11 different liquid media were used for determining the growth of *C. nicotianae*. Host extract dextrose broth and Czapek (dox) broth supported maximum growth of fungus followed by Carrot leaf decoction broth, Host extract broth and Potato dextrose broth. The present investigation results were supported by the similar work of Dange and Patel (1968) in case of *C. beticola*, Chen *et al.* (1979) in case of *C. kikuchii* and Raghunathan (1969).

5.4 Nutritional studies

The investigation on utilization of seven carbon sources by the fungus revealed that Fructose (4.67 g), Lactose (3.87 g), Sucrose (3.12 g) and Dextrose (2.31 g) supported maximum growth of the fungus after 19 days of incubation. Starch, Cellulose and Glucose supported comparatively less growth of *C. nicotianae.* Similar findings were reported by Rangaswamy and Chandrasekharan (1962) in case of *Cercospora* infections on Cucurbits, Verma and Agnihotri (1972) in *C. cruenta* and Lakshminarayana (1981) in case of *C. solani-melongenae*. In the present investigation glucose did not support as carbon sources for growth of fungus. However, in other species of *Cercospora beticola* and *C. canescens,* glucose supported as best source of carbon as reported by Dange and Patel (1968) and Khandar *et al*. (1985) respectively.

Six different nitrogen sources were tried as to ascertain best nitrogen source for growth of the fungus. Ammonium orthophosphate (3.83 g) supported maximum growth of fungus followed by Asparagine (3.50 g). Urea and ammonium chloride showed inhibitory effect on growth of *C. nicotianae* after 19 days of incubation. Similar results of supporting maximum growth of fungus by asparagine, glutamic acid were reported by Berger and Hanson (1963) in *C. zebrins* and Siddaramaiah (1986) in *C. moricola*. Similar findings were also reported by Dayal and Ram (1968) and Lakshminarayana (1981).

5.5 Physiological studies

Eight temperature regimes were studied for growth of *C. nicotianae*. The temperature of 25^0C, 30^0C and room temperature of 26 ± 1^0C supported maximum growth of fungus and thus, studies identified these temperature regimes as ideal for growth. The temperature up to 15^0C and $>35^0$C did not support maximum growth of the fungus. Similar studies were reported by Makadia *et al*. (1979) in *C. nicotianae* on tobacco supporting maximum growth of fungus at 27^0C. Similar findings were also reported by Dayal and Ram (1968) in *C. jasminicola*, Dinesha (1984) in *C. sorghi*, Khandar *et al.* (1985) in *C. canescens* and Jenns *et al.* (1989) in *Cercospora* species.

Different pH regimes ranging from 3.0 to 8.5 pH were studied in the present investigation. The results revealed that pH in the range of 5.0 to 5.5 supported maximum growth of the fungus. The growth of fungus showed positive trend but relatively negative increase till pH 6.5. The alkaline pH regimes of 7.0 to 8.5 did not support maximum growth of the fungus. Similar reports of acidic pH of 4.5 supporting maximum growth of the fungus was reported by Makadia *et al.* (1979) in *C. nicotianae*, Chandrasekhar and Rangaswamy (1960) in *C. cruenta* and Dange and Patel (1968), Raghunathan (1969) in *C. canascens* and *C. dolichi* respectively. The studies identified pH range of 4.5 to 5.5 as the best range for maximum growth of fungus. However, Verma and Agnihotri (1972) reported the alkaline pH of 6.8 and pH 7.0 supported better growth of *C. cruenta* and *C. beticola* respectively.

5.6 Management

5.6.1 *In vitro* evaluation of systemic and Non-systemic fungicides and bioagents

Out of five non systemic fungicides tested, Carbendazim + Mancozeb performed better in recording maximum (79.33%) inhibition over control followed by Propineb (63.5 %) and Mancozeb (59.45%). Copper oxychloride could not inhibit the pathogen at all the concentrations tested. The performance of non systemic fungicides even at 0.25 per cent was not satisfactory except combi-product Carbendazim + Mancozeb. Similar findings were reported by Anjeneyulu *et al.* (1988), and Fajola and Alasoadura (1973). In the *in vitro* studies, all the seven systemic fungicides tested were found effective at all the concentrations tested. Hexaconazole, Propiconazole, Carbendazim, Penconazole and Benomyl were found to be effective even at 0.05 per cent concentration recording 100 per cent inhibition over control. Among these systemic fungicides Thiophanate methyl was least effective. Similar findings were also reported in management of *C. nicotianae* by Rosa (1963), Peptines (1960), Anjeneyulu *et al.* (1988), Chandwani and Lal (1979).

In laboratory evaluation of bioagents *Trichoderma koningii*, *T. harzianum* and *T. viride* were found to be effective against *C. nicotianae* recording more than 50 per cent inhibition over control. The fungal bioagents performed better than bacterial bioagents such as *P. fluorescens* and *B. subtilis*. This is the first report of utilization of bioagents in the management of *C. nicotianae* in India. However, similar reports on bioefficacy of *T. harzianum* on *C. moricola* by Siddaramaiah (1986), *C. kikuchii* causing purple seed stain disease of soybean (Satyaprashant, 2004). The effectiveness of *Bacillus subtilis* against *C. moricola* and *C. kikuchii* was reported by Siddaramaiah (1986) and Satyaprasanth (2004) against *C. kikuchii*. The present investigation also identified effectiveness of *B. subtilis* against *C. nicotianae*.

5.6.2 Management of frog eye leaf spot through chemicals, bioagents and plant extracts

In the investigations on field evaluation of chemicals, bioagents and plant extracts, application of Carbendazim at 0.05 per cent, Propiconazole at 0.1 per cent and Hexaconazole at 0.1 per cent found effective in checking disease incidence, increasing yield and affecting other growth and quality parameters of bidi tobacco. The bioagents *Trichoderma harzianum* and *P. fluorescens* could not manage the disease. There was a significant difference with respect to checking the disease incidence among the different treatments. However, there was no significant difference with respect to yield of bidi tobacco. Though there was numerical superiority in all the treatments except Parthenium leaf extract over control. Similar findings on field and nursery investigation in *C. nicotianae* was reported by Reddy (1978), Wajid *et al.* (1986) in *C. nicotianae* of FCV tobacco, Peptines (1960) reported efficacy of five fungicides against frog-eye leaf spot in FCV tobacco, Patel *et al.* (1987 and 1991) reported in *Cercospora* leaf spot of bidi tobacco in nursery. Reddy *et al.* (1992) reported effectiveness of Carbendazim at 0.04 per cent or 0.025 per cent against frog eye leaf spot of burley tobacco, while Hundekar *et al.*(2005) reported effectiveness of Carbendazim and Hexaconazole against frog-eye leaf spot of bidi tobacco in Karnataka.

In the present investigation, the effectiveness of different treatments on growth parameters such as plant height, leaf length and leaf breadth revealed that there was no significant difference among these parameters at harvest. However, there was a significant increase in treatments at 80 DAP. The bioagents application as soil and spray during cropping season supported plant height, leaf length and leaf breadth indicate the possible operation of Induced Systemic Resistance (ISR) approach in host system against the pathogen and might stabilize these characters if used continuously for minimum of four to five years. This concept will found effective in reducing use of fungicides in management of tobacco diseases in future, though, these treatments failed to perform with respect to bioefficacy on par with chemical treatments. Different quality parameters were also employed to assess the influence of different treatments on bidi tobacco quality. The nicotine percentage ranged from 2.64 to 4.06 Among, fungicides samples treated with Thiophanate methyl at 0.2 per cent recorded minimum nicotine percentage (2.64%) followed by Control (2.70 %). Interestingly, though Parthenium leaf extract did not perform better in bioefficacy and yield parameters recorded relatively higher nicotine percentage of 4.06. The reducing sugars were in the range of 6.58 to 7.88. The highest reducing sugars was noticed in Carbendazim (7.88 %) followed by *Trichoderma harzianum*. The chloride per cent was well within the range of 1.0 . Similar, results were reported by Stavely and Chaplin (1972) and Patel *et al*. (2001). However, all these quality parameters are well within the acceptable range.

5.6.3 Host plant resistance

The advanced lines of ICAR trials, station trials and genetic stock were screened for frog eye leaf spot resistance. Around 25 lines from advanced breeding trials, 17 lines from station varietal trials and 10 hybrids from station trial were identified as resistant /moderately resistance for frog eye leaf spot and around 30 germplasm lines maintained at ARS, Nipani were characterized for frog-eye leaf spot resistance. These lines of advanced trial will definitely serve as good source of contemporary stable and durable resistance breeding programme. The germplasm lines identified help us in identifying the plant type required for incorporation of resistant gene under high yielding background of a genotype. Similar reports

on resistant sources were reported by Stavely (1971), Nagarajan *et al*. (1978), Nagarajan and Reddy (1982), Anon. (1984 and 1987), Goy *et al*. (1992), Narayanswamy *et al*. (2000) and Shamarao Jahagirdar *et al*.,(2005).

5.7 Economic analysis

The economic analysis of field investigation was made by working out net return and B: C ratio. Application of Propiconazole (T_2) recorded highest net return of Rs. 28,880 followed by Carbendazim (T_6) (27,220) and Hexaconazole (T_1) (26,520). The application of Chlorothalonil, *Trichoderma harzianum* recorded minimum net return of Rs. 20,912 and 17,095 respectively. The Benefit: Cost ratio analysis revealed that the maximum B: C ratio (1.49) was obtained with Carbendazim treatment (T_6) followed by 1.48 in Propiconazole (T_2) and Hexaconazole (T_1) (1.41). There was negative B: C ratio/net loss with respect to application Chlorothalonil (T_3), *Trichoderma harzianum* (T_7), *P. fluorescens* (T_8) and Parthenium leaf extract (T_9). Thus, application of bioagents and leaf extract though helped marginally in managing the disease ultimately realized net loss in those treatment in bidi tobacco in one year investigation trial.

5.8 Quality parameters

Different quality parameters were also employed to assess the influence of different treatments on bidi tobacco quality. The nicotine percentage ranged from 2.64 to 4.06 Among, fungicides samples treated with Hexaconazole at 0.1 per cent recorded minimum nicotine percentage (3.56) followed by Propiconazole (3.46 %). The bioagents *T. harzianum* and *P. fluorescens* the nicotine percentage was on par with chemical treatment. Interestingly, though Parthenium leaf extract did not perform better in bioefficacy and yield parameters recorded highest nicotine percentage of 4.06. The reducing sugars were in the range of 6.58 to 7.88. The highest reducing sugars per cent was noticed in Carbendazim (7.88 %) followed by *Trichoderma harzianum* (7.79%). The chloride per cent was well within the range of <1.0 in all the treatments.

Future line of work

1. To identify the variability in *C. nicotianae*.
2. To work out in detail about epidemiology of disease in bidi tobacco growing environments.
3. To initiate work on Cercosporin mediated resistance in Indian bidi tobacco/tobacco in general.
4. To screen the botanicals in the management of frog eye leaf spot of bidi tobacco.

6. SUMMARY AND CONCLUSIONS

Rowing survey conducted in Nipani area for frog eye leaf spot incidence during 2006-07 revealed that the incidence ranged from 8.38 to 13.05 per cent. September and October months favored the frog-eye leaf spot infection, recording mean incidence of 13.05 and 12.06 per cent respectively. Based on data on incidence and severity over months, Akkol and Aadi are considered as most severe zones for frog-eye leaf spot. The areas such as Galataga, Kodni, Nipani, Siraguppi, Jatrat, Kadakhat and Walki are considered as moderate to severe zones. The areas where relatively minimum frog-eye leaf spot was noticed are Sadalaga, Pangeri-A, Naganur, Shirpewadi, Hunneragi and Pattankudi.

The pathogen *Cercospora nicotianae* upon infection to bidi tobacco produced typical symptom of small brown spots with greyish center, later turned white and dried up. Symptoms are seen initially on lower leaves and later spread to upper leaves. In some cases there was mixed infection of brown leaf spot and frog-eye leaf spot. The pathogen was isolated from such infected leaves on Potato dextrose agar by employing standard tissue isolation method. The culture on Potato dextrose agar produced olivaceous brown colour at edges with light brown center. The hyphae of the fungus was hyaline and septate and slow growing. The pathogen was identified as *C. nicotianae* based on these characters and also by Agharkar Institute, Pune. Pathogenicity studies were conducted on 60 days old plant and proved Koch's postulates on tobacco seedlings of Cv. A-119 in glass house studies.

In the cultural studies, out of eleven different media screened, Host extract dextrose agar, Czapek (dox) agar, Carrot leaf decoction agar, Richard's agar, Malt agar and Potato dextrose agar were identified as the best media for growth of *C. nicotianae.* The present investigation optimized 19 days required for maximum growth of the fungus. The investigation revealed that out of eleven different liquid media screened, Host extract dextrose broth and Czapek (dox) broth as the best media for mass culturing of the pathogen.

The studies also identified fructose, lactose and sucrose as the best carbon sources and ammonium orthophosphate and Asperagine as better nitrogen sources to obtain maximum growth of *C. nicotianae.* The investigation on temperature and pH studies revealed that temperature of 25ºC, 30ºC and room temperature (26±1ºC) with optimum pH of 4.5 to 5.5 favored maximum growth of *C. nicotianae.*

In field management studies, application of Carbendazim at 0.05% (24.5 PDI) or Hexaconazole at 0.1% (28.2 PDI) or Propiconazole at 0.1% (27.1 PDI), two sprays, first one immediately after appearance of the disease followed by another spray at 10-12 days interval helped effectively in managing the disease. These treatments not only decreased the disease incidence but also increased cured leaf yield compared to control. Maximum cured leaf yield of 1300 Kg/ha was recorded in Propiconazole at 0.1% (T2) when compared to minimum cured leaf yield of 1020 Kg/ha in Parthenium leaf extract (T_9). In present investigation bioagents *Trichoderma harzianum* and *Psuedomonas fluorescens* and Parthenium leaf extract did not show significant change in checking the disease and influencing the cured leaf yield.

Economic analysis was also worked out for the different treatments in management trial. Highest net return (Rs. 28,880) was obtained from Propiconazole treatment (T_2) followed by Carbendazim (Rs.27,220) (T_6). Application of *Trichoderma harzianum* (Rs. 17,095) recorded minimum net return. The Cost: Benefit ratio analysis revealed that the maximum B:C ratio of 1:1.49 was obtained with Carbendazim (T_6) followed by 1:1.48 in Propiconazole (T_2) and Hexaconazole (T_1) respectively. Application of bioagents resulted in net loss recording negative B: C cost ratio in case of Chlorothalonil (T_3), *Trichoderma harzianum* (T_7) and *Psuedomonas fluorescens* (T_8)

In quality parameter assessment, the nicotine percentage was found maximum in case of Parthenium leaf extract sprayed plots where as least nicotine percentage was observed in case of Thiophanate methyl sprayed plot. Reducing sugars were found highest at Carbendazim treatment and least at *Pseudomonas fluorescens* spray. Chlorides percentage was highest in Propiconazole but less in *Trichoderma harzianum* treatment. However, they are in acceptable range.

In host plant resistance investigation, the studies identified 25 lines from advanced trial, 17 lines and 10 hybrid entries from station trial and 30 germplasm lines identified as resistant sources, these lines can be further used in contempory resistant breeding programme.

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* Original not seen

STUDIES ON FROG EYE LEAF SPOT OF BIDI TOBACCO CAUSED BY *Cercospora nicotianae* Ell. & Eve.

MALLAPPA PAIRASHI 2007 Dr. SHAMARAO JAHAGIRDAR
CHAIRMAN

ABSTRACT

Investigations comprised of laboratory and field experiments conducted at UAS, Dharwad during 2006-07. Studies on survey of Nipani area revealed that frog eye leaf spot incidence ranged between 8.38-13.05 per cent. September and October months favoured the disease, Akkol and Aadi area considered as most severe zones.

In cultural studies, out of eleven media screened, Host extract dextrose agar, Czapek (dox) agar, Carrot leaf decoction agar, Richard's agar, Malt agar and Potato dextrose agar supported better growth of *C. nicotianae.* Nineteen days of incubation optimum for maximum growth of fungus. Among liquid media, Host extract dextrose broth and Czapek (dox) broth were identified for mass culturing of the pathogen. In nutritional studies fructose, lactose and sucrose proved as best carbon sources. Ammonium orthophosphate and asperagine were better nitrogen sources for maximum growth of pathogen. In physiological studies, temperature of 25ºC, 30ºC and room temperature (26±1ºC) with optimum pH of 4.5-5.5 favored maximum growth of fungus.

In field management studies, Carbendazim at 0.05% or Hexaconazole at 0.1% or Propiconazole at 0.1%, (two sprays) were effective in managing the disease. Maximum cured leaf yield of 1300 Kg/ha was recorded in Propiconazole at 0.1%. In economic analysis highest net return (Rs. 28,880) was obtained from Propiconazole treatment followed by Carbendazim (Rs.27,220). In quality parameter assessment, highest nicotine percentage was found in case of Parthenium leaf extract sprayed plots. Reducing sugars and Chlorides percentage were found highest in Carbendazim and Propiconazole treatments respectively. In host plant resistance 25 lines from advanced trial, 17 lines and 10 hybrid entries from station trial and 30 germplasm lines were identified as resistant sources, these lines can be further used in resistant breeding programme.