MATERIALS AND METHODS

1. Pathological Studies

1.1 Collection of diseased materials, isolation, purification and maintenance of culture

The infected leaves of Costus speciosus showing typical Notch symptoms were collected from experimental farm of CIMAP, Lucknow. Isolations from diseased leaves were made by tissue segment method on potato-dextrose agar medium supplemented with 200 ppm Vancomycin hydrochloride (a gift from Eli Lilly & Co., Indianapolis, Ind., U.S.A.). The isolates were purified by single hyphal tip segment and maintained on oat-meal agar medium at 25°C. Pathogenicity was tested on healthy potted plants. The leaves of the plants were sprayed with the suspension of mycelium and spores of the fungus and the plants were kept in humidity chamber for 48 hrs. The plant sprayed with distilled water served as control.

1.2 Host-range studies

Host-range of the pathogen was tested on detached leaves of Nicotiana rustica, N. tabaccum var. samsun, N. tabaccum var. white burly, Datura innoxia, D. metel,
Lycopersicon esculentus, solanum tuberosum, S. melongena, piper betle and Zingiber officinalis.

2. Studies on the cultural and morphological characters of the pathogen

Cultural characters of the pathogen, Phytophthora nicotianae var. nicotianae were studied by visual observations. The morphological characters of the mycelium, sporangium and sex organs were examined by using compound microscope and scanning electron microscope. For light microscopy, observations and photography were made with Zeiss Photomicroscope. Scanning electron microscopic examinations were carried out by using JEOL-35C scanning electron microscope. A small piece of mycelium was carefully teased apart and spread out in a drop of distilled water placed on a double stick cellophane on SEM copper alloy specimen stub and dried in a critical point dryer. The specimens were coated uniformly with gold vapour using 'Fine Coat' sputtering device. Samples were viewed and photographed at a beam voltage of 10 KV.

3. Studies on the effect of nutrients and factors affecting on the growth of pathogen

The fungus was grown on both solid and liquid media. Each medium was sterilized by autoclaving at 15 psi for 15 minutes. The sterilized media were inoculated
with 5 mm discs of actively growing mycelium. Growth was determined by radial spread of mycelium in solid medium while in liquid media it was found out in terms of biomass production.

P-1 broth (Hohl, 1975) with inorganic nitrogen was used as basal medium to study the effect of various nutritional factors viz., hydrogen-ion concentration (pH), carbon and nitrogen on the growth of fungus. For pH studies buffered basal medium was used at pH range 3-10.

Various carbon sources used in the studies were L-arabinose, L-rhamnose, D-fructose, D-glucose, D-galactose, D-mannose, D-xylose, maltose, lactose, sorbose, raffinose, dextrin, starch, inulin, CM-cellulose, mannitol, sorbitol, citric acid, tartaric acid, lactic acid, maleic acid, and succinic acid. They were substituted for sucrose in basal medium. The concentration of carbon adjusted to 8 g/lit of carbon in basal medium except polysaccharides which were added in same quantity as sucrose. Chloride, nitrate, sulphate, tartrate, oxalate, succinate and phosphate salts of ammonium, sodium nitrite, calcium nitrate, potassium nitrate, yeast ext., peptone, casein, glycine, L-asparagine, aspartic acid, L-alanine, glutamic acid and urea were substituted for sodium nitrate as nitrogen sources for the growth of fungus. The concentration of nitrogen adjusted to 0.509 g/lit of nitrogen in basal medium. Each of the
factors were added to 25 ml basal medium, poured in 150 ml Erlenmeyer flasks, sterilized at 15 psi and inoculated with actively growing mycelium discs of equal size. After incubation period of 12 days at 28 ± 2°C, the mycelium was filtered off, dried at 60°C and average dry weight was recorded. Five replicates were maintained in each experiment.

4. **Production of toxic metabolite/s in vitro**

The pathogen was grown in different synthetic and semi-synthetic liquid media at 25°C. The mycelium was separated by filtration and culture filtrates were heated for 10 min at 10 psi to inactivate enzyme activity. The filtrates thus obtained, were used as toxin samples in subsequent studies.

4.1 **Toxicity test of culture filtrate**

4.1.1 **Leaf spot test**

Toxicity of culture filtrates were carried out by placing 30-50 μl of filtrate on the healthy leaves of Costus plants and an injury was made with a sterile needle, gently. The development of lesion around the prick was observed. Uninoculated media were used as control.
4.1.2 **Wilting test**

5-10 days old tomato and *Datura innoxia* seedlings were placed in 15 ml glass vial filled with 8 ml of culture filtrate, and wilting symptoms were observed at different intervals. Uninoculated medium and distilled water were used as control.

4.1.3 **Studies on the factors affecting the production of toxic metabolite/s in vitro**

Richard's modified synthetic medium containing 3% sucrose was used to study the effect of various factors viz., incubation period, pH, carbon and nitrogen sources on the production of toxic metabolite/s.

For studying the effect of age of culture, the synthetic medium was inoculated with *P. nicotianae* var. *nicotianae A2* and incubated for 5 to 30 days. The culture filtrate of each incubation period was tested for leaf spot. For pH studies, medium was adjusted at different pH levels ranging from 3 to 10 with N/2 HCl or N NaOH. For studying the effect of various carbon and nitrogen sources, sucrose and potassium nitrate were replaced by different carbon and nitrogen compounds. L-arabinose, L-rhamnose, D-fructose, D-glucose, D-mannose, D-xylose, maltose, dextrin, starch and mannitol were substituted to sucrose in synthetic medium on equivalent carbon basis.
Similarly, different nitrogen compounds viz., ammonium chloride, ammonium nitrate, ammonium sulphate, sodium nitrate, L-asparagine, glycine, aspartic acid, glutamic acid and L-alanine were substituted to potassium nitrate. The concentration of the each compound was adjusted to the amount of nitrogen present in basal medium.

In all these studies, 25 ml aliquot medium was filled in 150 ml Erlenmeyer flasks, sterilized at 15 psi and inoculated with 7 days old culture and incubated for 15 days at 25°C. After incubation period, mycelia were filtered off and the filtrates were used as toxin samples for test.

5. Studies on the production of pectolytic and cellulolytic enzymes in vitro

5.1 Preparation of enzyme sample

The pathogen, P. nicotianae var. nicotianae was grown on P-1 medium (Hohl, 1975). The various carbon compounds used for the study were 1% sucrose, 1% pectin (NBC, Cleveland, USA) 1% sodium polypectate (a gift from Schuylkill Chem. Co., R.I., USA), 1% filter paper pulp and 1% carboxy methyl cellulose (CMC 30, Hercules Power Co, Delware, USA). Twentyfive ml of each medium substituted with one of the above carbon sources separately in 150 ml Erlenmeyer flasks were inoculated with 7 days old
culture and incubated for 10, 15 and 20 days at 25°C. The mycelium was separated by filtering through double layer of cheese cloth and the clear culture filtrates, obtained were passed through bacteria proof sintered glass funnel to remove the spores. The filtrates, thus, obtained were used for enzyme assay.

5.2 Enzyme assay

5.2.1 Pectin methyl esterase (PME)

The PME activity was assayed by the continuous titration method of Kertesz (1955). Twenty ml of 1% citrus pectin (Sigma Chem. Co. USA) in 0.1 M NaCl was adjusted to 7.5 pH with 0.1N NaOH and placed on a constant temperature bath at 30°C. Two ml of enzyme sample (culture filtrate) was added, readjusted to pH 7.5 using pH meter with glass electrode and the zero time was noted. The change in pH was noted and corrected periodically by adding known volume of 0.1N NaOH. The enzyme activity was determined according to the following formula.

\[ PME = \frac{\text{Total volume of 0.1N NaOH} \times 3.1}{\text{unit time}} \]

volume of enzyme solution

5.2.2 Endo-polygalacturonase

Endo-polygalacturonase (endo-PG) activity was determined by viscometric method and the reaction product was tested by thiobarbutaric acid (TBA).
a. Viscometric method

The loss of viscosity of the substrate was determined according to Bell et al. (1955) using Fenske-Ostwald viscometers (minimum efflux time 20 sec for water). Twenty ml of 1% sodium polypectate (Sigma, USA) in citrate buffer pH 5.5 was used as substrate. 2 ml of enzyme sample was added to it and flow time at 0, 5, 15, 30, 45, 60, 75 and 90 min was recorded. Temperature was maintained at 30°C throughout the experiment. The percent loss in viscosity was calculated by the following formula:

$$\frac{To-Tt}{To-Tw} \times 100 = \text{percent loss in viscosity}$$

where $To$ = is the flow time immediately after the addition of the enzyme solution

$Tt$ = Flow time after specified time interval

$Tw$ = Flow time for water

Relative activity (RA) of the enzyme was calculated as the reciprocal of time in minutes for 50% loss of viscosity multiplied by 1000.

$$RA = \frac{1}{\text{Time for 50% loss of viscosity}} \times 10^3$$

b. TBA Method

Reaction product of Endo-PG was tested for thio­barbutaric acid (TBA) test by the method described by
Neukom (1960) and modified by Sherwood (1966). Two ml of the enzyme sample was mixed with 4 ml of 1% sodium polypectate in citrate buffer at pH 5.5 and incubated for 4 hrs at 30°C. After incubation 1 ml of the reaction mixture was added to acidified TBA solution (5 ml 0.5N HCl and 10 ml 0.01N TBA) and kept in boiling water bath for 1 hr to develop colour. The absorption spectrum was noted between 480 to 600 nm using Bausch & Lomb spectronic-20.

5.2.3 **Endo-polymethyl galacturonase (endo-PMG)**

**Endo-PMG** was assayed by viscometric method of (Bell et al., 1955) using 1% citrus pectin in citrate buffer at pH 5.5 as substrate. The reaction product was tested for TBA reaction as described earlier.

5.2.4 **Exo-polygalacturonase (exo-PG)**

**Exo-PG** activity was determined by measuring the increase of galacturonic acid content in enzyme-substrate reaction by dinitrosalicylic (DNS) method of Hancock and Miller (1965). Reaction mixture containing 2 ml enzyme sample, 2 ml 0.25% polygalacturonic acid (a gift from Schuylkill Chem. Co., R.I., USA) in citrate buffer at pH 5.5 and 6 ml distilled water was incubated at 30°C. After reaction time of 0, 30, 45 and 60 minutes, 3 ml
sample was removed from reaction mixture after each specific reaction time, 0.2 ml of M \( \text{Na}_2\text{CO}_3 \) was added followed by 3 ml DNS reagent (Miller, 1959) in test tubes which were held in boiling water bath for 10 min to develop colour. The test tubes were cooled to room temperature and OD was measured at 575 nm against reagent blank using Bausch & Lomb Spectronic-20. The activity of the enzyme was expressed as the rate of increase in galacturonic acid concentration in the enzyme-substrate reaction mixture after specified time.

5.2.5 Exo-polymethylgalacturonase (Exo-PMG)

Exo-PMG in culture filtrate of the pathogen was also assayed by DNS method as described earlier using 0.25% pectin as substrate.

5.2.6 Trans-eliminases (PGTE and PMTE)

Polygalacturonase trans-eliminase (PGTE) and pectin methyl trans-eliminase (PMTE) were assayed by viscometric method of Nagel and Vaughn (1962) and reaction product was tested for TBA reaction as described by Neukom (1960) and modified by Sherwood (1966). One percent pectin or sodium polypectate in Tris-HCl buffer at pH 8.5 used as substrate.
5.2.7 **Cellulase (CX)**

The cellulase enzyme activity was assayed by viscometric method of Bell et al. (1955) using 0.5% carboxymethyl cellulose in citrate buffer at pH 5.5 as substrate.

6. **Estimation and assay of the production of extracellular and intracellular amylase and invertase enzymes**

Culture filtrate obtained after different incubation periods were used for the studies. For intracellular enzyme assay, known amount of fungal mycelium of different age was extracted in chilled 50 mM acetate buffer pH 5.0 and centrifuged at 15,000 rpm using T-24 refrigerated centrifuge at 4°C. The supernatant was decanted and assayed for enzyme activity.

6.1 **Assay of invertase**

The activity of invertase enzyme was determined according to Luthra (1982). The composition of reaction mixture was, 0.2 ml of 50 mM sucrose (AR/BDH, England); 0.7 ml of 0.1 M acetate buffer pH 5.0 and 0.1 ml of enzyme sample. The reagent blank contained 0.9 ml buffer and 0.1 ml enzyme extract without substrate. The reaction mixture was incubated at 37°C in a temperature controlled
water bath for 30 min. After incubation period, the Nelson-Somogyi alkaline copper solution was added to the reaction mixture. The amount of reducing sugars was determined with the colorimetric procedure of Nelson (1944) as modified by Somogyi (1952) with glucose as reference standard at 620 nm wave length using Pye-Unicam SP 8-100 UV spectrophotometer. The amount of corresponding sucrose hydrolysed was calculated by multiplying the invert sugars using a factor 0.95.

6.2 Assay of amylases

The enzyme was assayed according to Luthra (1982). The composition of reaction mixture was 1.0 ml starch solution (0.6% starch in 0.06 M KH₂PO₄), 0.9 ml of 0.1 M acetate buffer pH 5.0 and 0.1 ml of enzyme sample. In the case of blank the reaction mixture contained 0.1 ml of buffer instead of enzyme. After incubation in a temperature controlled water bath at 25°C for 30 min, 1 ml of iodine-HCl solution (60 mg KI and 6 mg I₂ in 100 ml of 0.5 N HCl) was added to reaction mixture. Intensity of blue colour developed was recorded at 620 nm with starch as reference standard using Pye-Unicam SP8-100 UV spectrophotometer. The amount of starch hydrolysed by alfa and beta amylases was calculated as the value of the concentration of starch in blank minus the concentration of starch in test reaction mixture.
β-amylase was inactivated by heating the enzyme solution at 70°C for 5 min (Dure, 1960). The α-amylase which is stable at this temperature was assayed following the assay method described above. The starch hydrolysis index on solid starch medium was calculated according to Ho and Foster (1972).

7. Extraction and estimation of bioconstituents of healthy and infected leaves of *C. speciosus* inoculated with *P. nicotianae* var. *nicotianae* 2

7.01 Estimation of chlorophylls

Known amount of infected and healthy *Costus* leaves were repeatedly extracted separately in 80% cold acetone and centrifuged at 5000 rpm for 30 min. The colourless residues were discarded and the supernatants were pooled and diluted with 80% acetone to a known volume. Chlorophyll contents were estimated according to the procedure of Arnon (1949) using Pye-Unicam Sp 8-100 UV spectrophotometer at 645 nm and 663 nm against acetone as blank. The amounts of chlorophyll 'a' & 'b' (mg/g) were calculated according to the formula given below. Total chlorophyll was calculated by adding Chlorophyll 'a' and chlorophyll 'b'.
Chlorophyll a = \frac{12.7 \lambda_{663} - 2.69 \lambda_{645}}{1000 \times W} \times V

Chlorophyll b = \frac{22.9 \lambda_{645} - 4.68 \lambda_{663}}{1000 \times W} \times V

whereas \( \lambda \) = absorbance

\( V \) = volume of extract in ml

\( W \) = weight of sample in g

7.1 Extraction of plant tissues in alcohol

The leaf samples were chopped and plunged them into hot methanol and allowed to boil for 5-10 min. The contents were cooled and thoroughly ground in a mortar with pestle, filtered through two layers of cheese cloth and the residue was re-extracted in 80% methanol for 3-5 min and filtered. The extracts were pooled and filtered through Whatman No. 1 filter paper and diluted to a known volume. This methanolic extract was used for the estimation of phenolic compounds and carbohydrates (Mahadevan and Sridhar, 1982).

7.2 Estimation of carbohydrates

7.2.1 Total sugar

Alcohol soluble sugars were estimated using Phenol-Sulphuric acid reagent (Dubois et al., 1951). A known aliquot of the alcohol extract (7.1) was reduced
to dryness in vacuum at 50°C and dissolved in distilled water. To this, 1 ml of 5% phenol solution followed by 5 ml of 96% H₂SO₄ was added and the contents were mixed gently and cooled. Absorbance was measured at 490 nm using Bausch & Lomb spectronic-20 with glucose as standard.

7.2.2 Reducing sugar

The reducing sugars were estimated with dinitro-salicylic acid (DNS) reagent (Miller, 1959). One ml of alcohol extract (7.1) was reduced to dryness in vacuum and dissolved in known amount of distilled water. To 1 ml of this extract 3 ml DNS reagent was added and heated for 10 min in a boiling water bath and allowed the colour to develop. To this, 1 ml of 40% Rochelle salt was added and absorbance was recorded at 575 nm in Bausch & Lomb spectronic-20 using glucose as standard.

7.2.3 Non-reducing sugar

A known volume of alcohol extract (7.1) was dried under vacuum and 1 ml of distilled water and 1 ml of N H₂SO₄ were added to the residue and hydrolysed at 50°C for 30 min on a water bath. The solution was neutralized with N NaOH and amount of sugar was estimated with DNS reagent as described earlier.
7.3 **Estimation of phenolic compounds**

7.3.1 **Estimation of total phenol**

The quantities of total phenol were estimated according to Swain and Hillis (1959) using Folin-Denis reagent (Folin and Denis, 1912). To an aliquot of 0.5 ml alcohol extract (7.1), 0.5 ml Folin-Denis reagent was added and thoroughly mixed by vigorous shaking for 3-5 min, diluted to 7 ml. To this, 1 ml of saturated Na$_2$CO$_3$ solution was added. The solution was thoroughly mixed and volume made up to 10 ml with distilled water. After one hour of incubation the absorbance was recorded in Pye-Unicam SP 8-100 UV spectrophotometer at 725 nm using chlorogenic acid as standard.

7.3.2 **Estimation of glycosidic, free, acid and basic phenols**

The glycosidic phenols were determined according to the method of Seevors and Daly (1970). An aliquot of alcohol extract (7.1) was reduced to dryness. Glycosidic phenols were extracted by acid hydrolysis of dried residue with 5 ml of 2N HCl for one hour at 100°C followed by alkaline hydrolysis with 5 N NaOH at room temperature for 2 hr. Free phenols, acid phenols and basic phenols were extracted according to Chattopadhyay and Samaddar (1980). Amount of phenols in all cases were determined with Folin-Denis reagent described elsewhere.
7.3.3 **Estimation of wall bound phenols**

The wall bound phenols were extracted and estimated by the method of Chattopadhyay and Samaddar (1980) and Mahadevan and Sridhar (1982). A known amount of leaves chopped into small pieces, thoroughly ground in mortar with pestle with the addition of 3% sodium dodecyl sulphate (SDS). The ground tissues were centrifuged at 10,000 rpm for 20 min, discarded the supernatant and washed the residue successively, once with 3% SDS, twice with water, twice with ethanol, twice with diethyl ether and once with ethanol and diethyl ether in 3:1 ratio. After each washing the material was centrifuged and the supernatant was discarded. Dried residue was re-suspended in 0.5 M NaOH for 12 hrs and centrifuged. Supernatant was used for the estimation of phenols with Polin-Denis reagent mentioned elsewhere.

7.3.4 **Estimation of Ortho-dihydroxy (O.D.) phenols**

The O.D. phenols were estimated with Arnow's reagent (Arnow, 1937). To 0.5 ml of alcohol extract (7.1), 0.5 ml of 0.05 N HCl, 0.5 ml of Arnow's reagent, 5 ml of distilled water and 1 ml of N NaOH were added. The absorbance of pink colour developed was recorded using a Bausch & Lomb spectronic-20 at 515 nm. Catechol was used as standard.
7.4 Estimation and extraction of oxidative enzymes

A known amount of infected and healthy leaf samples were extracted separately in chilled 0.1 M phosphate buffer pH 6.0 using prechilled pestle and mortar. The homogenate was centrifuged at 15000 rpm in T-24 refrigerated centrifuge at 4°C for 15 min and supernatant was used for assay of polyphenol oxidase and peroxidase activity.

7.4.1 Assay of polyphenol oxidase

The activity of enzyme was determined according to Mahadevan and Sridhar (1982). Two ml of plant extract in a cuvette was diluted with 3 ml of 0.1 M phosphate buffer pH 6.0, mixed, placed in a colorimeter set at 495 nm and adjusted the absorbance to zero. One ml of 0.01 M catechol in 0.1 M phosphate buffer pH 6.0 was added into the cuvette and changes in absorbance at every 30 sec intervals upto 3 min were recorded. The enzyme activity was calculated as changes in absorbance between first 30 sec and 150 sec of incubation. Heated enzyme extract was used as control.

7.4.2 Assay of peroxidase

The enzyme activity was assayed according to Mahadevan and Sridhar (1982). The reaction mixture contained 3 ml of 0.05 M pyrogallol in 0.1 M phosphate buffer pH 6.0, mixed, placed in a colorimeter set at 495 nm and adjusted the absorbance to zero. One ml of 0.01 M catechol in 0.1 M phosphate buffer pH 6.0 was added into the cuvette and changes in absorbance at every 30 sec intervals upto 3 min were recorded. The enzyme activity was calculated as changes in absorbance between first 30 sec and 150 sec of incubation. Heated enzyme extract was used as control.
buffer pH 6.0. To this 0.1 ml of plant extract was thoroughly mixed and the mixture was taken into a cuvette. After the absorbance was set to zero at 420 nm in the colorimeter, 0.5 ml of 1% H₂O₂ was added to the mixture and changes in absorbance at 20 sec intervals for 3 min were recorded. The activity of enzyme was calculated as changes in the absorbance between 40 to 160 sec. Heated extract was used as control.

7.5 Estimation of buffer soluble leaf proteins

7.5.1 Extraction

The leaf samples were extracted with cold 100 mM phosphate buffer at pH 7.0 in prechilled mortar with pestle. The homogenate was filtered and centrifuged at 40,000 g in Beckman L-50 Ultracentrifuge at 4°C. The supernatant thus obtained containing soluble proteins was extensively dialysed against 200 volumes of distilled water for 48 hrs with several changes of water. Protein samples were stored at -20°C.

7.5.2 Studies on soluble leaf proteins using Disc, Electrophoresis

Concentration of the protein was measured according to the method of Lowry et al. (1951) with BSA as standard. Electrophoretic pattern of soluble leaf
protein was studied following the method of Davis (1964) and Maurer (1971) using Pharmacia, Sweden electrophoretic apparatus. Polyacrylamide gels were prepared in glass tubes (10 X 0.8 mm) with 7.5% separating and 2.5% spacer gels and were carefully loaded with 100 μg of protein. Electrophoresis was carried out at 25°C using tris-glycine as electrophoretic buffer at pH 8.5 with a current of 3 mA per tube until the tracking dye (Bromophenol blue) had moved to the lower end of separating gel. The gels, then were removed from the tubes, stained with 0.2% Coomassie blue for 1 hr and destained with several changes of 7.5% acetic acid and 5.0% methanol in water. Gels were scanned on Shimadzu (Model, CS-910) densitometer at 600 nm wave length.

7.6 Estimation of nucleic acids

7.6.1 Extraction

The leaf samples were extracted in 80% hot methanol, centrifuged and supernatant was discarded. The residue was suspended in 10% cold trichloroacetic acid (TCA) for 1 hr to precipitate the proteins and nucleic acids and centrifuged for 30 min at 10,000 rpm. Thus, precipitate obtained, contains proteins and nucleic acids (Mahadevan and Sridhar, 1982).
7.6.2 **Estimation of nucleic acids**

To separate the nucleic acids present in TCA precipitate (Osborne, 1962), the precipitate was suspended in 0.3 N KOH for 16-18 hr at 37°C, centrifuged at 15000 rpm for 20 min and the supernatant was collected. Residue was washed twice with distilled water, centrifuged and the supernatant was collected. The supernatants were pooled and made up to a known volume. This extract contains the nucleic acids. Before estimation, RNA and DNA were separated according to Mahadevan and Sridhar (1982).

7.6.3 **Estimation of RNA**

The RNA contents were determined according to the method of Markham (1955) using orcinol reagent. To 1 ml of RNA preparation, 2 ml of orcinol reagent was added and heated in boiling water bath for 10 min. Absorbance of green colour developed, was measured in Pye-unicam SP 8-100 UV spectrophotometer at 665 nm using yeast-RNA as standard.

7.6.4 **Estimation of DNA**

DNA concentration was determined with diphenylamine reagent (Burton, 1968). To 1 ml of DNA preparation 4 ml of diphenylamine reagent was added and incubated the mixture for 20 hr at 27°C to develop the blue colour.
Absorbance of contents was measured in Bausch & Lomb spectronic-20 at 600 nm using calf-thymus RNA as standard.

All the data obtained from host-pathogen interaction studies were subjected for statistical analysis except results of oxidative enzymes.

8. In vitro screening of fungicides against \( P. \) nicotianae var. nicotianae \( \lambda_2 \).

The efficacy of different fungicides were tested on growth inhibition of the pathogen in vitro by food poison technique. The fungicides used for this study were i) Bavistin (2-[Methoxy-carbamoyl] benzimidazole); ii) Benlate (Methyl-1 [butylcarbamoyl]-2-benzimidazole-carbamate); iii) Blitox-50 (Copper oxychloride, 50% copper); iv) Captan (N-[trichloroethylthio]-4-cyclohexane-1-2-dicarboximide); v) Cuman L (Zinc-dimethyl-dithiocarbamate); vi) Lifolatan or Folcid (N-[tetrachloroethyl] sulphenyl cis-4-cyclohexane-1, 2-dicarboximide); vii) Dithane M-45 (coordination product of zinc and manganese ethylene bisdithiocarbamate); viii) Dithane Z-78 or Zineb (Zinc ethylenebisdithiocarbamate, 75%); ix) Ridomil or Metalaxyl or CGA-48988 (N-[2,6-dimethylphenyl]-N-[2-methoxyacetyl] alanine methyl ester) (a gift from Ciba-Geigy, Switzerland); x) Thiram (tetramethyl thiuramdisulphide) and xi) Vitavax (5,6-dihydro-2-methyl-1,4-oxathin-3-carboxanilide). The
fungicides were mixed with sterilized potato-dextrose agar medium in 10, 50, 100, 250, 500, 750 and 1000 ppm concentration except Ridomil that was added in 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ppm concentration. The fungicide incorporated medium was poured in sterilized petriplates and inoculated with 5 mm disc of 7 days old fungus culture. Radial growth of the fungus was measured after different intervals. Five replicates were maintained in the experiment for each treatment.