STUDIES ON THE PRODUCTION OF TOXIC METABOLITES BY *Trichoconis padwickii* GANGULY IN CULTURE FILTRATE

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THESIS

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C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bonafide research work carried out by Sri K. Jayachandran Nair under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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A C K N O W L E D G E M E N T S

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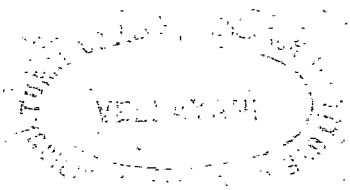

C O N T E N T S

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INTRODUCTION

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Increasing attention is being paid in recent times to studies on the physiology of plant pathogenic fungi in order that a better understanding of the mechanism of disease development in plants may be possible. In many cases fungal metabolites have been assigned key roles in these mechanisms. The metabolites so implicated include toxins produced by the organisms concerned. Eventhough their importance in plant diseases is now more or less widely recognised the information relating to their nature and role is still only of fragmentary character.

Parasitic diseases commonly arise as a result of an interchange of metabolites between the parasite and the invaded host. Several host-pathogen interactions are now believed to involve toxins. The concept of toxins in plant pathology emerged from the work of Hutchinson (1910) on a bacterial disease in India and by the time Gaumann introduced the term 'toxigenicity' in 1951, efforts were being made to find out a correlation between toxigencity and pathogenicity.

Many plant pathogenic fungi and bacteria are known to produce toxic metabolites in culture also. The presence of such substances in a metabolite solution does not, however, necessarily implicate them as causative agents in naturally occurring parasitic diseases. Nevertheless some of them have been shown experimentally to produce toxic effects on plants. While a few of the toxins, like the ones produced by Helminthosporium victoriae and Periconia circinata are specific to the hosts on which the organisms are pathogenic, others are less specific and can act on a variety of plants and on micro-organisms like bacteria and fungi. The present trend in thought seems to be that toxins are only one aspect of a more complex and closely knit chain of physiological phenomenon which is still poorly understood.

Current interest in learning more about the toxic metabolites produced by pathogenic organisms has prompted the present investigation. The rice stackburn fungus Trichoconis padwickii Ganguly was chosen for the study on account of the severe necrotic symptoms produced by this pathogen. The dark reddish brown patches often cover extensive areas of the leaf sheath. The involvement of some toxic principle in the manifestation of characteristic disease syndrome was, therefore, considered possible.

The present study is confined to the toxic metabolites produced by Trichoconis padwickii in culture. The suitability of different media as well as different carbon and nitrogen sources for the growth of the organism and production of toxic metabolites were determined. The culture filtrate was assayed for its toxic properties on paddy plants as well as on germinating seeds and fungal spores.

REVIEW OF LITERATURE

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A number of workers have found that plant pathogens can produce toxic metabolites. Thus, Talboys (1951) found the possible significance of toxic metabolites of Verticillium albo-atrum in the development of hop wilt symptoms. Gaumann (1957) described Fusaric acid as wilt toxin. Tamari and Kaji (1954) isolated two crystalline substances toxic to the growth of rice plants were isolated from the liquid cultures of rice blast fungus Piricularia oryzae. Paper chromatography revealed the presence of both these compounds in rice plants attacked by blast fungus. The two toxic substances are known as ∝-picolinic acid and piricularin.

Influence of media on the growth and toxin production of the test fungus.

The experiments of Rosen (1927) showed that the culture filtrate of Fusarium vasinfectum in Richards' solution was toxic to cotton plants, while that in culture media containing organic nitrogen was non-toxic. In very bright sunlight, it was noticed that the filtrate caused wilting more rapidly than in diffused light. It was concluded that the culture filtrates of Fusarium vasinfectum on Richards' solution possess at least two substances toxic to cotton plants,

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one a volatile compound with an alkaline reaction and the other an organic salt in the form of nitrate. The wilting was due to the toxic chemical substance produced bythe fungus.

Zentmeyer (1941) produced a substance toxic to elm by growing Ceratostomella ulmi on a liquid medium containing yeast extract along with other nutrients such as $MgSo_4$, $FeCl_3$, asparagine and dextrose, and found it was the best medium.

The findings of Brian et al. (1949) proved that Czapek's medium containing 10-15% sucrose was suitable to Alternaria solani for the greatest activity and production of toxic substance.

Carbon source for the growth and production of toxic substance by the test fungus.

White (1927) demonstrated that sucrose was better than glucose in Richards' solution for the growth and production of toxic substance by Fusarium lycopersici.

According to Ludwig (1957) the toxicity of the poisonous substance produced by Helminthosporium sativum increased with decrease in sugar content in the medium and hence greater activity was not associated with greater growth.

Brian, et al. (1951) showed that media containing high concentration (7.5% W/V or more) of sucrose proved to be best suited for the growth and activity of metabolic substances produced by the fungus Alternaria solani.

Nitrogen source for the growth and production of toxin by the test fungus.

Brian, et al. (1951) supplied nitrogen as nitrate or casein hycholysate for the production of alternaric acid by the test fungus Alternaria solani. They found that ammonia as nitrogen source was equally good when supplied in conjunction with a suitable organic acid 0.25% (W/v), acetic acid being particularly favourable.

Berry and Futrell (1961) studied the effect of different sources of nitrogen in the production of toxins by Helminthasporium victoriae. He used Almo for assay, and the primary toxin was produced in greater quantity in media containing ammonium nitrate, followed in descending order by ammonium sulphate, phenyl alanine, tryptophan, methionine, tyrosine and asparagine. When New Nortex seedlings were used for assay, the secondary toxin was produced in highest amount on methionine followed by phenylalanine, ammonium sulphate, tryptophan, asparagine and ammonium nitrate.

Variation of pH

Fahmy (1923) while conducting experiments with the fungus Fusarium solani for ascertaining the production of toxins found that the degree of alkalinity of the culture filtrate increased with the age of the culture but it was insufficient to explain as the cause of the wilt produced when stems of broad beans were inserted in test tubes containing the filtrate.

Clayton et al. (1944) showed that the stability of penicillin in metabolism solution was optimal at pH 7-8 and no activity was lost when such solutions were kept at 80°C for 2 hours. But a solution of pH 5 when heated at 60°C 4 hours lost all its activity. Solutions of pH 10 and above are stable. In aqueous solutions, the activity of crude penicillin is unchanged on standing for several days at pH 8.

While (1955) found that the production of maximum toxin titres was favoured in media with an initial pH of 3.4 to 4.2 in the case of oak wilt fungus Endoconidiophora fagacearum. It was inactivated totally at pH 8. The characteristic symptom of toxicity was noted in all the samples of culture filtrate after 42 hours independent of the pH values to which they had been adjusted. It was concluded that the toxicity symptoms are not explained as a direct effect of the pH and that the toxic substance is active throughout the pH range tested.

The study of Goodman (1960) showed that the pH of the culture filtrate of Colletotrichum fuscum rose from 4.6 to 7 at the 17th day of incubation when the production of toxin became intense.

Mathur and Mathur (1967) found that the ultimate severity of seedlings and vascular browning increased with increase in pH, especially at pH 8.5 and over, when the seedlings were placed on the culture filtrates of Fusurium oxysporium f. cumini.

Period of maximum growth and toxin production.

It was reported by Fahmy (1923) that the toxin production in the culture filtrate of Fusarium solani increased in concentration and produced rapid wilting of cut beans stem when placed in the culture filtrate, with the age of the culture, thereby showing that there was an increase in growth and toxin production by the fungus with an increase in the number of days.

Studies of Picado (1924) with the fungi Verticillium albo-atrum and Verticillium dubovs both parasitic on potatoes, a saprophytic Penicillium and Fusurium solani on melon, led to the conclusion that in general the toxic substance contained in the mycelium passed into the culture

medium only in old culture in which a large portion of the mycelium was plasmolysed. Clayton (1934) was of the opinion that the toxin of Bacterium tabacum was present even in young cultures.

The findings of Zentmeyer (1941) clearly showed that the maximum growth and optimum production of toxin, in the case of Ceratostomella ulmi, occurred after 12 days of incubation eventhough there was slight production of the poisonous metabolite on the 3rd day onwards.

According to Ludwig (1957) the greater activity of the culture filtrate was not associated with greater growth. The results suggested that toxins were produced until they limit further growth of the organism. He proved this with the test fungus Helminthosporium sativum.

The work of Goodman (1960) regarding the production of toxin by Colletotrichum fuscum proved that the initial evidence of toxin production appeared on the 11th day of incubation and the effect on tomato foliage became more intense through the 17th and 18th days.

Research works of Scheffer and Pringle (1961) with Periconia circinata the causal agent of milo disease of Sorghum vulgare showed that the peak of toxin content was on 21st day in resting culture, but cultures grown in

a shaker failed to produce toxin.

Sharma and Agnihotri (1967) who conducted experiments with Fusarium orthoceras f. lentis found that the longer the fungus was grown in the medium, the shorter was the time taken for wilt symptoms to be induced by the filtrate.

But the studies of Mathur and Mathur (1967) with cumin cuttings in culture filtrate of Fusarium oxysporum f. cumini showed that the age of the culture had no effect on the ultimate severity of the seedlings.

King (1967) found that 9 isolates of Alternaria solani differed in the quantity and effect of incubation time on production of toxin eventhough all of them produced toxin when grown in Czapeks-Dox broth for 2-6 weeks.

Sankhala (1967) isolated Gliotoxin and dehydrocarolic acid from culture filtrates of Penicillium restrictum after 18 days of incubation in Czapeks-Dox solution at 24°C in the dark.

Krishnaswamy, et al. (1969) incubated the cultures of different isolates of Piricularia oryzae in Czapeks-Dox medium for a period of 18 days to study the toxin production.

Specificity of the toxic substance produced by fungi in culture filtrate.

The toxic substance produced by Fusarium lycopersici when grown on a modified Richard's solution caused wilting of cut tomato plants. Besides this White (1927) found that wilting of cowpea, soybean and cabbage was obtained by placing them in the culture filtrate.

Experimenting with the culture filtrate of Alternaria solani, Brian et al. (1949) produced wilting of radish, cabbage and carrot seedlings and retardation of the growth of tomato seedlings.

Litzenberger (1949) found that the susceptible Victoria variety of oats rapidly developed chlorosis and necrosis accompanied by wilting when treated with the culture filtrate of Helminthosporium victoriae.

Zentmeyer (1941) reported that the tomato, elm, snapdragon and mapple cuttings placed in tubes containing culture filtrates of Ceratostomella ulmi wilted severely usually in one to four hours.

Demetriades (1950) demonstrated that Sclerotinia sclerotiorum grown in Richard's solution secreted a substance toxic to potato, tomato, chillies, fig and vegetable marrow.

It produced rapid irreversible wilting and in the case of fig leaf rolling.

The effect of culture filtrate of Periconia circinata was tested by Scheffer and Pringle (1961) against new hosts like rye, barley, wheat, oats, radish, tomato, cabbage etc. but no toxic effect was found on any of them.

Sharma and Agnihotri (1967) conducted experiments with Fusarium orthocearas f. lentis and proved that the metabolites of it as non-specific.

Pringle and Scheffer (1967) found that the toxin isolated from the culture filtrates of Helminithosporium carbonum (Cochliobolus carbonum) as a host specific one.

Inhibition of radicle and plumule elongation by the toxin.

Ludwig (1957) used seed inhibition as a method of bioassay for testing the toxicity of the culture filtrate of the Helminthosporium sativum.

The detailed study of the production of toxin as a metablite by Helminthosporium victoriae by Luke and Wheeler (1955) revealed that the susceptible varieties of oats treated with this toxic material produced a striking reduction in growth of both roots and shoots. Since tests

made it clear that roots were affected at lower concentrations of the toxic agent than shoots, root elongation was used to develop a method of bioassay of the toxin.

In the case of susceptible seedlings of Sorghum vulgare, the radicle growth was inhibited upto a dilution of 1 : 3200 of the culture filtrate of the fungus Periconia circinata, was demonstrated by Scheffer and Pringle (1961). The above workers in 1967 observed that the culture filtrates of Helminthosporium carbonum caused 50% inhibition of growth of roots of susceptible maize hybrid Pr x K61 at 0.5 µg/ml. The resistant hybraid Prl x K61 was affected to the same extent by 60 µg/ml. They named the above toxin as Helminthosporium carbonum toxin. Another toxin, carbtoxinine, equally toxic to both the hybrids causing 50% root inhibition at 25 µg/ml. was also crystallised by them.

Krishnaswamy et al (1969) tested the toxin production by different isolates of Piricularia oryzae by plumule and radicle inhibition bioassays.

Inhibition of germination or germ tube elongation by the toxin in fungus cultures

Characteristic stunting of developing germ tubes of spores of Botrytis alli and inhibition of germination of spores of Myrrothecium verrucaria , Absidia glauca

Stachybotrys altra were demonstrated by Brian et al (1949) using the culture filtrate and alternaric acid isolated from Alternaria solani in synthetic culture solution.

Ludwig (1957) used spore germination inhibition of Sclerotinia fructicola as an index for the dtermination of toxin production by Helminthosporium sativum.

Jefferys (1948) found out a new technique for rapid demonstration of the production of antifungal substances by the fungi or other microorganisms. In this method he examined the degree of germination of spores of Botrytis alli to detect the production ofthe toxic substance, by the action of which the germination was foundinhibited. There was a gradation of germination of the spores of Botrytis alli directly over the streak of the test fungus and those away from the streak.

Effect of temperature on the activity of the toxic substance.

The results of the experiments conducted by Fahmy (1923) indicated that the toxic substance produced by Fusarium solani in the culture solution was not destroyed by boiling. Piccado (1924) showed that the withering of plant treated with culture filtrates of Verticillium albo-

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atrum, Verticillium dubova, Fusarium solani and Penicillium were accentuated when the extracts were heated to 100°C. Clayton (1934) found that even steaming in the autoclave for 30 minutes at 15 lbs pressure did not inactivate the toxin, nor did boiling over a free flame for 3 hours destroyed the toxic effect of Bacterium tabacum.

Clayton, et al (1944) demonstrated that the activity of Penicillin was not lost when the metabolism solution was kept at 80°C for 2 hours at a pH 7-8. But a solution of pH 5 when heated at 60°C for 4 hours lost all its activity.

Irving, et al (1945) explained the fungistatic agent lycopersicin, from the tomato plant. In the crude preparation available lycopersicin was completely stable for at least one hour at 100°C and withstand autoclaving for at least 15 minutes at 15 lbs. pressure. Demetriades (1950) obtained a toxic substance from the culture filtrate of Sclerotinia sclerotiorum and found that the substance was thermostable. So also a heat withstanding toxic metabolite was observed by White (1955) in the case of the oak wilt fungus Endoconidiophora fagacearum.

Brian et al (1951) boiled solutions of alternaric acid in Czapek's solutions for 5 minutes in a range of pH 3-7.6 and observed no loss of activity. They found that it could even be autoclaved at 15 lbs. pressure for 20 minutes without any alterations in toxicity. But autoclaving at pH 3 caused a partial loss of activity.

Sharma and Agnihotri (1967) proved the toxic metablite obtained in the culture filtrate of Fusarium orthoceras f. lentis as thermoslable.

Effect of toxin on the different parts of the plants.

Hursh (1926) showed that the culture filtrate of Leptosphaeria herpotrichoides incapacitated the conducting vessels of the plants from functioning normally and stimulated the exeretion of the pentosans at the cut ends leading to immediate wilting. Smilar symptoms were produced with cabbages grown in the filtrate of Fusarium vasinfectum or Fusarium oxysporum. It was suggested that plant might attempt to get rid of the toxic substances by concentrating them in certain tissues such as leaves, which turn yellow dry up and fall off. White (1927) demonstrated the production of substances toxic to cut tomato plants, but not to rooted plants grown in water cultures of Fusarium lycopersici.

When the toxic product present in the culture solution of Bacterium tabacum was pricked into leaves of tobacco plants, Clayton (1934) observed the destruction of chlorophyll tissues and production of halo lesions.

Zentmeyer (1941) showed that injection of the culture filtrate of Ceratostomella ulmi into the healthy elm seedlings produced typical symptoms of the dutch elm disease.

Pound and Stathmann (1952) were able to produce chlorosis and necrosis by introduction of the sterile filtrate of Alternaria solani into a lower petiole of tomato plant, and the symptoms were indistinguishable from those caused by fungus lesions of the stem. When tomato cuttings were placed in a sterile filtrate of the pathogen immediate epinasty occured and wilting and necrosis developed in a few hours.

Production of necrosis in tomato and bean plants was demonstrated by Radha and Menon (1961) with the culture filtrate of the test fungus Gloesporium fructigenum.

Orsenigo (1956) found that Cochliobolin, the toxic principle of Helmintosporium oryzae, produced abnormalities in rice seedlings and it inhibited root and coleoptile growth.

Gayed (1961) could not produce the natural symptoms on two varieties of barely by dipping the cut shoots of them

in culture filtrate of Helminthosporum sativum eventhough they wilted without any necrosis.

Goodman (1960) reported that tomato foliage showed sunken or pitted areas in 24 hours after 6 hours exposure to the toxic substance produced by Colletotrichum fuscum in culture. Epidermal cells in this area remained normal but the pallisade cells were collapsed.

Mirocha, et al. (1961) found that rotting of the hulls (mesocarp) of almond fruit by Rhizopus stolonifer, R. circinas R. arrhizus, was accompanied by unilateral necrosis of the leaves on the same side of the twig as the infected fruit. Since mycelium of the fungus did not extend up to the twigs, a toxin originating in the rotted fruit was identified as the agent responsible for twig and leaf symptoms. They, using radioactive C14 isotopes, proved that fumaric acid produced in rotted hulls by Rhizopus is translocated to twigs and leaves, where it or some of its metabolic derivatives are highly toxic.

Gayed (1961) proved that the culture filtrate of Helminthosporium produced necrotic spots and wilting and twisting in cut shoots of all four varieties dipped in it,

the lesions being similar in each variety.

Samaddar and Scheffer (1968) described the effect of the specific toxin in Helminthosporium victoriae on host cell membrane and Hanchey et al. (1968) produced various changes in oat roots of susceptible variety with victorin.

MATERIALS AND METHODS

MATERIALS AND METHODS

1. Source and Maintenance of culture

The isolate of *Trichoconis padwickii* used in the investigation was obtained from infected paddy seed collected from Agricultural College and Research Institute, Vellayani. The isolate was purified by the single spore method. Stock cultures were maintained on oat agar medium with yeast extract.

2. Paddy seeds used

Annapurna variety of paddy was used for germination bioassay and for studies on the elongation of plumule and radicle.

3. Determination of dry weight

For determining the dry weight of the mycelium, the culture was vacuum filtered through previously weighed filter paper (Whatman no.1). Mycelial mats in the filter papers were washed with distilled water, dried in the hot air oven for 24 hours at 80°C along with the filter papers. After they have cooled down to room temperature, they were weighed to find out the dry weight of the mycelium, by deducting the weights of the filter paper from the final weights. With all replications 4 weighed filter papers without mycelium were also

dried to find out whether there was any loss in weight of the filter paper during drying. It was found that in no case the loss in weight of each filter paper exceeded one mg.

Media used

The following media were used for studying the growth and toxin production of the fungus. The pH of the media was adjusted to 6. For adjusting the pH of the media, required quantity of 6 N. Sodium hydroxide or 6 N hydrochloric acid solution was added to the medium. Determination of pH was done by using Universal Indicator (BDH) except in the case of pH study for which pH meter was employed.

LIQUID MEDIA

1. Potato dextrose solution

Sliced potato	200 g
Dextrose	20 g
Distilled water	1000 ml.

2. Richard's medium

KNO_3	10.0 g
KH_2PO_4	5.0 g
$MgSO_4$	2.5 g
$FeCl_3$	0.02 g
Canesugar	50.00 g
Distilled water	1000 ml.

3 Barne's medium

K_3PO_4	1.0 g
NH_4NO_3	1.0 g
KNO_3	1.0 g
Glucose	1.0 g
Distilled water	1000 ml.

4 Coones medium

Saccharose	7.20 g
Dextrose	3.60 g.
$MgSO_4$	1.23 g
Pot. acid phosphate	2.72 g
KNO_3	2.02 g
Distilled water	1000 ml

5 Czapek's Medium

$MgSO_4$	0.50 g
KH_2PO_4	1.00 g
KCl	0.50 g
$FeSO_4$	0.01 g
$NaNO_3$	2.00 g
Sucrose	30.00 g
Distilled water	1000 ml.

6 Czapek's-Dox medium

Sucrose	30.00 g
$NaNO_3$	2.00 g
KH_2PO_4	1.00 g
$MgSO_4$	0.50 g
$FeCl_3$	0.50 g
$FeSO_4$	0.01 g
Distilled water	1000 ml.

7 Medium used by Tamari and Kaji for growing Piricularia oryzae

Soluble starch	10.00 g
Glucose	5.00 g
Glutamic acid	0.01 g
$NaNO_3$	1.00 g
NH_4Cl	1.00 g
KH_2PO_4	0.50 g
$MgSO_4 7H_2O$	0.02 g
$MnSO_4 H_2O$	0.05 g
$CaCl_2\ 2H_2O$	0.07 g
$FeCl_3$	mo 1/20,000
$CuSO_4$	mo 1/60,000
Decoction of plant	100 ml
Distilled water	900 ml.

SOLID MEDIA

1 Potato Dextrose agar

Sliced peeled potato	200 g
Dextrose	20 g
Distilled water	1000 ml
Agar agar powder	15 g

2 Oat meal agar with yeast tablets

Oats	50 g
Agar agar	20 g
Distilled water	1000 ml
Yeast tablets	5 g

3 Host leaf extract

Rice leaves	200 g
Agar agar	15 g
Distilled water	1000 ml

Preparation of Host leaf extract

200 g paddy leaves were boiled in 500 ml. distilled water for one hour. The clear solution was decanted and filtered. Fifteen grams of agar agar powder was melted in 500 ml. of distilled water and mixed with the leaf extract. The volume was made upto 1000 ml.

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For studies on different carbon and nitrogen sources, Czapek's Dox medium was used as the basal medium changing the carbon and nitrogen sources in each treatment.

In the case of carbon sources mono, di and polysacharides were selected. The quantity of carbon sources used contained carbon equivalent to that in 30 g of sucrose per litre, the carbon source of standard Czapek's - Dox medium.

Carbon source	Wt. in g/litre
No carbon	00.000 g
Sucrose	30.000 g
Dextrose	31.575 g
Fructose	31.575 g
Soluble starch	14.360 g

The media were steam sterilized for 3 consecutive days, two hours each day.

The different sources of nitrogen were so selected to supply a ammoniacal, nitrite, nitrate and organic forms of nitrogen. The quantities of nitrogen sources were adjusted in all cases in such a way as to contain nitrogen equivalent to that in 2.0 g of sodium nitrate per litre, the nitrogen source of the standard Czepek's-Dox medium.

Nitrogen sources	Wt. in g/litre
No nitrogen	0.000
Sodium nitrate	2.000
Amm. sulphate	1.564
Sodium nitrite	1.150
Peptone	2.000

The pH of the media were adjusted at 6.0 in each case.

In the case of liquid media 100 ml of the medium was transferred into 250 ml Erlenmayer flasks, plugged with cotton wool and autoclaved.

Inoculation of liquid cultures was done by using culture discs of 5 mm diameter cut from actively growing zone of a 7 day old culture of the fungus. They were incubated at room temperature for the required period.

Germination Bioassay using paddy seeds

Paddy seeds were first soaked for 12 hours in the culture filtrate. They were then spread on sterile filter paper inside sterile petridishes wetted with 5 ml of the culture filtrate and incubated at room temperature. Two sets of control were maintained. One set was treated in the above manner with the culture solution and the other with distilled water. Germination percentage was observed after 72 hours.

Studies on radicle and plumule inhibition

The seeds were allowed to germinate in water for 3 days in germination tray and those with a plumule and radicle length of 4 mm. were selected and spread in sterile filter paper placed in sterile petridishes. The filter paper in each set was wetted with 5 ml of culture filtrate. Two sets of control were maintained. One set was wetted with uninoculated medium and the other set with distilled water. After 24, 48 and 72 hours, plumule and radicle lengths were measured.

Determination of the effect of culture filtrate on the germination of certain vegetable seeds.

Effect of culture filtrate on certain common vegetable seeds viz. Tomato, brinjal, cowpea, cucumber and bhindi was determined by soaking the seeds for 3 hours in the culture filtrate and planting them on sterile filter paper inside sterile petridishes. The filter paper was wetted with 5 ml of the culture filtrate and incubated for 3 days. Two sets of control were kept, one with culture solution and the other with water. Observations on germination were taken after 3 days.

The inhibition on the elongation of plumule and radicle of these seeds was determined as described in the case of paddy seeds.

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Effect of the culture filtrate on the germination of certain fungal spores.

Freshly collected spores of Piricularia oryzae, Pestalotia palmarum and Helminthosporium halodes were suspended in sterile water. The concentration of suspension was adjusted to 0.5 million per ml as was done by Brian and Hemming (1945) in the use of Botrytis alli.

The following dilutions were used.

1. Undiluted culture filtrate
2. 1 : 10 dilution
3. 1 : 100 dilution.

Uninoculated culture medium and distilled water were used as control.

One ml of the spore suspension was mixed with 5 ml of the liquid and shaken well to get a uniform distribution of spores. One drop of this was placed on a clean slide and kept in moist chamber at laboratory temperature (28-30°C). After incubation of 4 hours the number of spores of each fungus germinated were counted. At least four replications were made in all cases.

The germ tube elongation was measured after 4 and 6 hours. At least 200 spores were counted for this purpose, in each treatment to get the average germ tube elongation.

Effect of temperature on the activity of culture filtrate.

100 ml of the culture filtrate in 250 ml Erlenmayer flasks was placed in water bath for 10 minutes and 20 minutes at 100°C.

Another set was autoclaved at 15 lbs presure for 20 minutes. The treated culture filtrate was then tested for toxicity using the seed germination bioassay.

Effect of culture filtrate on paddy plants.

Undiluted culture filtrate and also culture filtrate diluted ten times and hundred times were used for this study. Four sets of experiments were done.

i) One month old paddy seedlings were placed in 10 ml specimen tubes containing 5 ml of the culture filtrate, with their roots completely immersed in the culture filtrate. The root system was made free of soil particles and other extraneous matter by carefully washing with distilled water before placing in the culture filtrate. They were kept under observations for a period of 4 days. Control plants were placed in distilled water, and also in the uninoculated medium.

ii) One month old paddy seedlings devoid of root system were planted in another set of specimen tubes containing culture filtrate as described above. For this a clean cut was made at the lower portion of the stem to separate the root system. The cut and was then dipped in the culture

filtrate.

iii) 10 cms. sterile petridishes with a thick layer of cotton wool in the lower dishes were moistened with distilled water. Paddy seeds were placed on the cotton wool and allowed to germinate. Five seeds were used in each dish. From the fourth day onwards nutrient solution was added to the dish 5 ml per dish per day for a period of 11 days. From the 15th day onwards the plants were irrigated with 5 ml of the culture filtrate. Three sets of plants were maintained. One set received undiluted culture filtrate while the second and third sets received culture filtrate diluted 1:10 and 1 : 100 respectively. Control plants were given uninoculated culture solution and also distilled water in place of culture filtrate. The plants were kept under observation for a week.

iv) Fine carborandum powder was gently applied on the leaves and leaf sheaths of 45 days old paddy seedlings using moist cotton wool. The culture filtrate was then sprayed on the plant using an atomizer. Undiluted culture filtrate and culture filtrate diluted 1 : 10 and 1 : 100 were tried. After spraying, the plants were covered with bell jar for 24 hours. In the control plants uninnoculated medium and distilled water were sprayed.

In another set of plants culture filtrate was applied without pretreating with carborandum powder.

Bacterial plate Bioassay.

The procedure adopted by Kalyanasundaram (1954) was followed with suitable modifications.

Bacillus subtilis was grown in nutrient agar plates for 10-15 days at room temperature when sporulation occurred. The bacterial growth in two agar slants was suspended in 20 ml of sterile water. The suspension, after filtering through absorbent cotton, was taken for seeding 200 ml of the medium. The bacterial suspension was heated in a water bath at 80°C for one minute to destory the vegetative forms. This was then added to the sterilized medium at room temperature and shaken well. 20 ml of the medium was uniformly poured in each petridish and kept in refrigerator for 30 minutes. In one set of such plates four cavities were bored with a sterile cork borer (6 mm diameter). The agar discs were removed with a sterile needle. The cavities were then filled with 0.01 ml of the culture filtrate. The plates were then incubated at room temperature.

In another set of plates the agar discs were not removed as in previous case, but sterile filter paper discs of 6 mm diameter dipped in culture filtrate were placed.

Four such discs were placed in each dish.

Suitable controls were kept with uninoculated medium and distilled water and observations were made for four days.

RESULTS

EXPERIMENTAL RESULTS

Growth of fungus in different liquid media

Trichoconis padwickii was able to grow in all the seven liquid media.

1. Potato dextrose solution
2. Richard's solution
3. Coon's medium
4. Barne's medium
5. Czapek's medium
6. Czapeck's-Dox medium, and
7. Medium formulated by Tamari and Kaji for growing *Piricularia oryzae*

Significant difference in the dry weights of the mycelium was, however, noticed in the different liquid media used. The maximum dry weight of 601 mg was obtained in Czapek's-Dox medium while minimum of 196 mg was obtained in the medium which was formulated by Tamari and Kaji. Satisfactory growth was observed in Potato-dextrose solution and also in Czapek's solution. Richard's, Barne's and coon's media gave only low dry weights (Table I).

TABLE I

Dryweight of the mycelium in different liquid media
(in milligrammes)

Period of growth	P.D.A.	Richards	Barnes	coons	Czapeks	Czapeks Dox	Tamari & Kajis medium
5 days	105	51	44	50	91	95	35
10 "	158	83	70	65	98	151	61
15 "	276	91	98	80	219	400	95
20 "	437	97	95	111	424	601	96
25 "	480	98	90	110	419	578	95
30 "	475	95	80	104	416	566	91

DRY WEIGHT OF THE MYCELIUM IN DIFFERENT LIQUID MEDIA

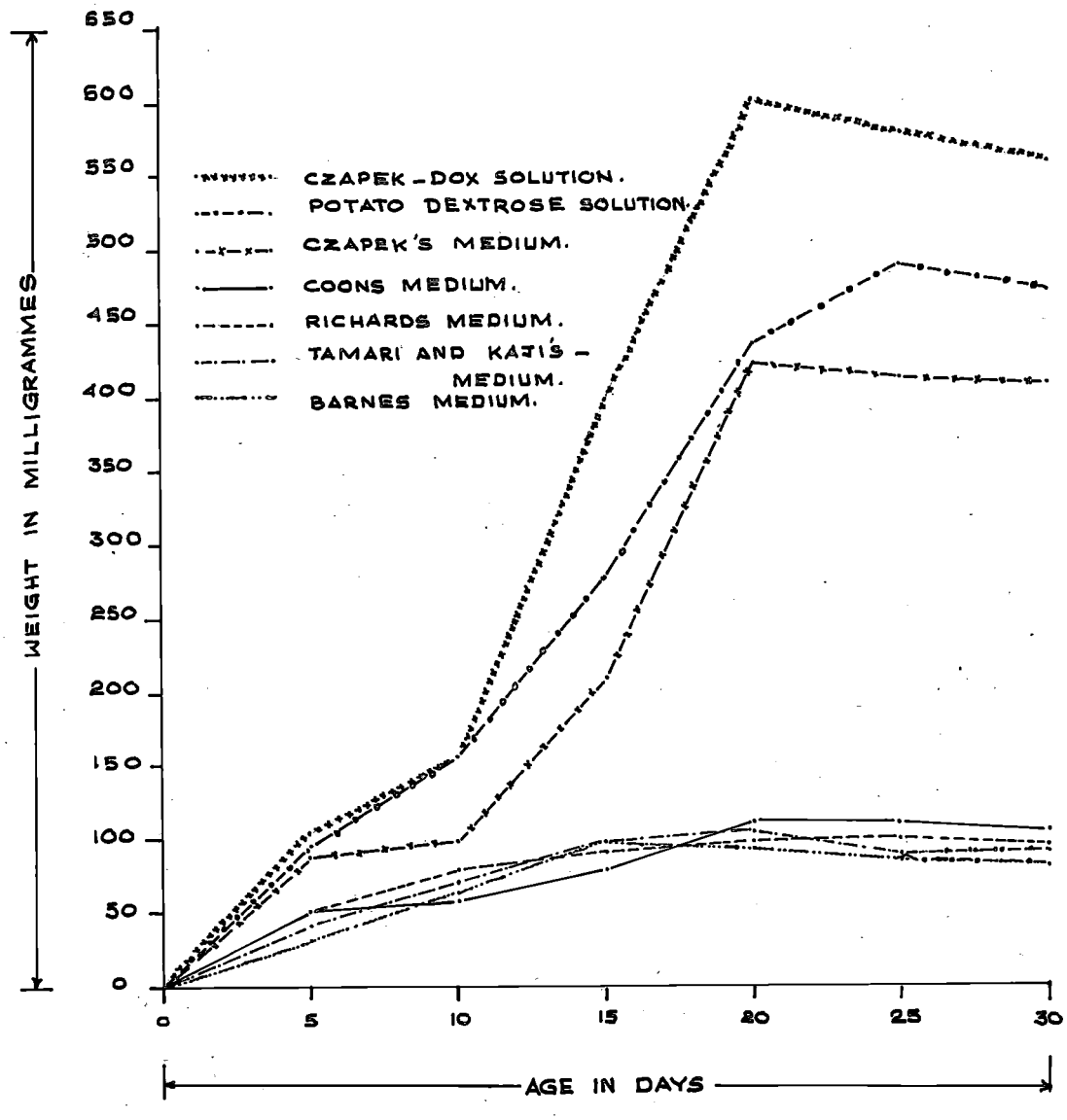


FIG 1

Production of toxic metabolite in different media

Maximum inhibition as determined by germination bioassay using paddy seeds was noted in Czapek's-Dox medium, being 63%. All the other media gave only 25-35% reduction in germination. The germination percentage was found to fall with increase in the age of the culture (Table II).

Growth of the fungus in different carbon sources

Of the different carbon sources tried, namely, sucrose, fractose, dextrose and soluble starch, dextrose was found to be the best for growth, closely followed by sucrose. Growth in fructose and soluble starch was comparatively poor. The dryweights of the mycelium in dextrose, sucrose, fructose and soluable starch were 652, 613, 314.6 and 246.4mg. respectively. (Table III).

Germination of paddy seeds was low in the culture filtrates irrespective of the carbon source used. However, greatest inhibition of germination was noted in the culture filtrate of the medium containing dextrose. The germination in this case was only 32%. This was followed by the culture filtrate of the medium containing sucrose and then by fructose and lastly by soluble starch being 43%, 45.5% and 62% respectively.

TABLE II

Average germination percentage of paddy seeds treated with the culture filtrate of *Trichoconis padwickii* grown on different liquid media.

Period of growth	P.D.A.	Richards	Barnes	Coons	Czapeks	Czapeks Dox	Tamari & Kajis medium	Control
5 days	100	93	100	99	98	96	100	100
10 "	100	99	98	97	98	96	98	100
15 "	82	89	83	80	68	66	82	100
20 "	83	83	79	73	69	37	76	100
25 "	81	77	75	74	71	37	72	100
30 "	80	77	76	69	65	37	71	100

TABLE III

Dryweight of mycelium of *Trichoconis padwickii* grown in Czapek Dox media with different carbon sources.
(in milligrammes)

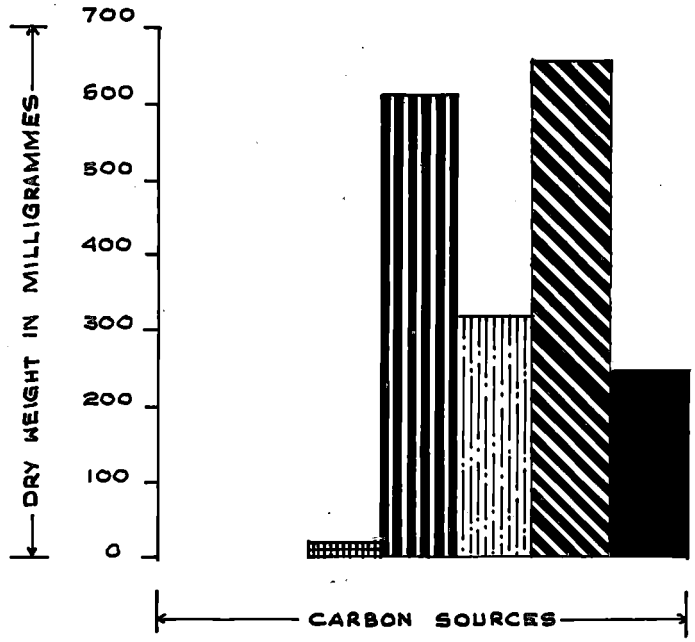
Replications	Carbon Sources				
	No Carbon	Surrose	Fructose	Dextrose	Soluble starch
1	25	598	282	648	242
2	28	602	306	652	231
3	20	612	332	650	260
4	21	621	346	641	256
5	17	628	307	659	243
Average	22.2	613	314.6	652	246.4

TABLE IV

Germination percentages of paddy seeds treated with culture filtrate of Trichoconis padwickii grown on Czapek Dox media with different Carbon Sources.

Carbon Source	Germination percentage				
	I	II	III	IV	Average % of germination
No carbon	96	100	100	98	98.5
Sucrose	48	46	38	40	43.0
Fructose	42	48	46	46	45.5
Dextrose	32	32	34	30	32
Soluble starch	60	62	58	68	62
Distilled Water	100	100	100	100	100

DRY WEIGHT OF MYCELIUM IN DIFFERENT CARBON SOURCES



PERCENTAGE OF INHIBITION OF GERMINATION OF PADDY SEEDS TREATED WITH CULTURE FILTRATE OF Trichoconis padwickii GROWN IN CZAPEKS-DOX MEDIUM WITH DIFFERENT CARBON SOURCES

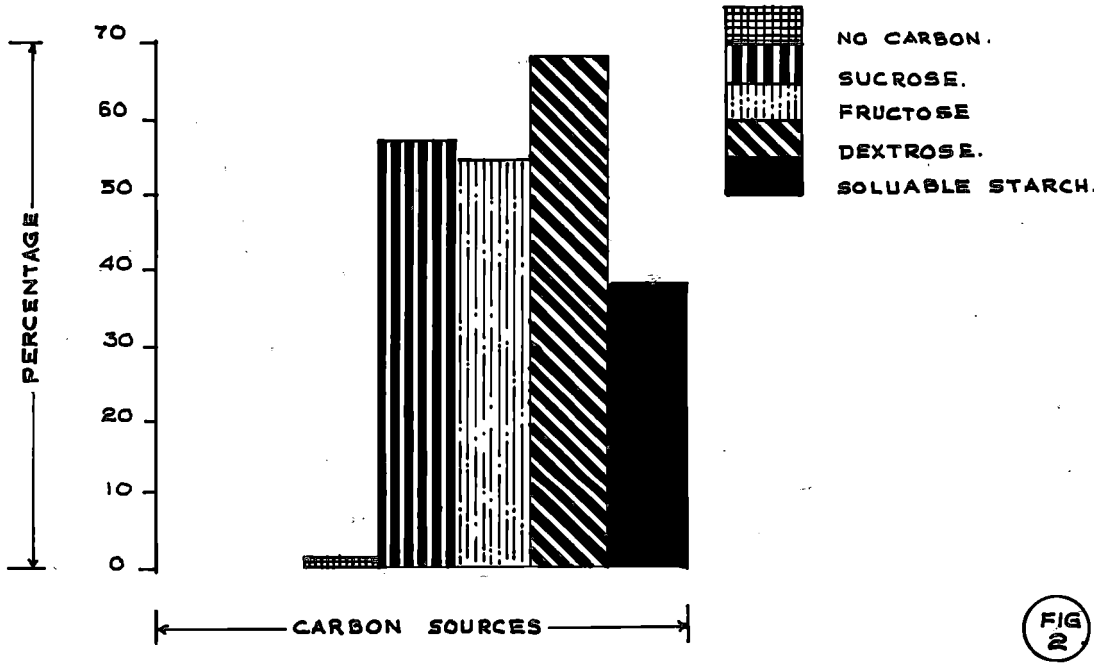


FIG 2

Growth of the fungus in different nitrogen sources

Sodium nitrate, the normal constituent of Czapek's-Dox medium was found to be the best nitrogen source. This was followed by peptone, sodium nitrate, ammonium sulphate in the descending order. Dry weight of the mycelium in the medium containing sodium nitrate was 625.4 mg, as compared to 284 mg, 217.8 mg. and 65 mg. for peptone, sodium nitrate, and ammonium sulphate respectively (Table V).

Definite inhibition of germination of paddy seeds was noticed in the culture filtrates irrespective of the nitrogen source. The inhibition was, however, greater in culture filtrate of medium containing sodium nitrate as the nitrogen source. This was followed by peptone and then sodium nitrite, and lastly by ammonium sulphate (Table VI).

Optimum incubation period for maximum growth and production of toxic metabolite.

Maximum growth of the organism was attained in 21 days. The dry weight of the mycelium on the 21st day was 668 mg. There was rapid growth during the period from ninth to fifteenth day after inoculation. Profuse growth of aerial mycelium appeared at this period. Practically no further growth was visible after the twentyfirst day (Table VII).

TABLE V

Dryweight of mycelium of Trichoconis padwickii grown in Czapeks Dox medium with different nitrogen sources (in milligrammes).

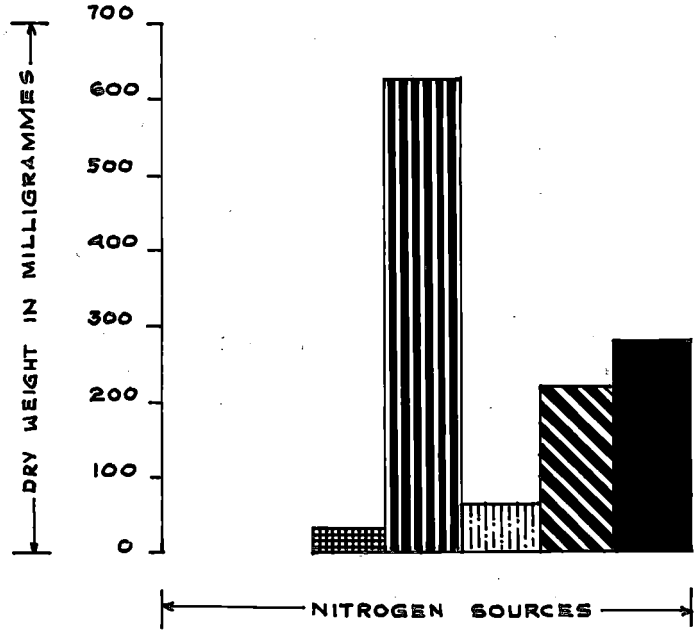
Replications	Nitrogen sources				
	No Nitrogen	Sodium nitrate	Ammonium sulphate	Sodium nitrate	Peptone
I	37	613	76	204	273
II	23	622	52	218	301
III	33	630	68	220	282
IV	27	627	66	232	285
V	34	635	63	215	279
Average	30.8	625.4	65	217.8	284

TABLE VI

Germination percentages of paddy seeds treated with culture filtrate of Trichoconis padwickii grown in Czapeks Dox medium with different nitrogen Sources.

Nitrogen source	Germination percentage Replications				
	I	II	III	IV	Average
No Nitrogen	98	94	94	92	94.5
Sodium nitrate	34	30	30	28	30.5
Ammonium sulphate	62	68	58	70	64.5
Sodium nitrite	58	52	56	52	54.5
Peptone	52	54	48	50	51.0
Distilled water	100	100	100	100	100

DRY WEIGHT OF MYCELIUM IN DIFFERENT NITROGEN SOURCES



PERCENTAGE OF INHIBITION OF GERMINATION OF PADDY SEEDS TREATED WITH CULTURE FILTRATE OF *Trichoconis padwickii* GROWN IN CZAPEKS - DOX MEDIUM WITH DIFFERENT NITROGEN SOURCES

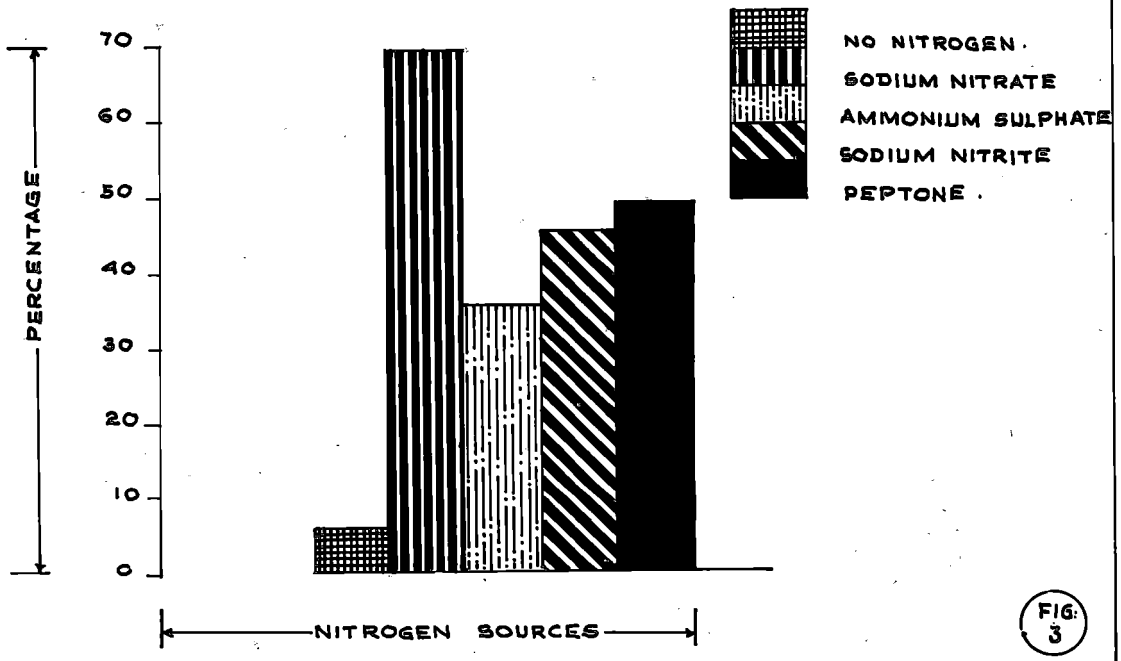


FIG: 3

TABLE VII

Dry weight of mycelium of Trichoconis padwickii at different periods of growth in modified Czapek's-Dox medium.

Period of growth	Average Dry weight of mycelium
5 days	93
7 "	112
9 "	146
11 "	238
13 "	336
15 "	487
17 "	516
19 "	548
21 "	668
23 "	650
25 "	640
27 "	627
30 "	625

The germination percentage of paddy seeds treated with the culture filtrate collected on the fifth day was as high as 98 while that on the twentyfirst day was as low as 32. (Table VIII). Thus the inhibitory property of the culture filtrate increased with the age of the culture attaining a maximum on 21st day. After that there was not much change.

A positive correlation existed between the age of the culture and toxicity of the culture filtrate.

Variation in pH

The pH of the culture filtrate at different periods of growth of the fungus in the modified Czapek's-Dox solution was recorded from fifth day after inoculation at two days interval upto thirtieth day. The initial pH of 6 gradually rose to 7.6 on the twentyfirst day and then fell to 6.9 on the thirtieth day. (Table IX).

Effect of culture filtrate on the elongation of plumule and radicle of germinating paddy seeds.

Culture filtrate was found to exert a marked inhibitory effect on the elongation of plumule and radicle of germinating paddy seeds (Tables X and XI).

TABLE VIII

Average germination percentage of paddy seeds, at different periods of growth of Trichoconis padwickii.

Period of growth	Average germination percentage		
	Culture filtrate	U.I.M.	Distilled water
5 days	98	91	100
7 "	96	90	100
9 "	92	91	100
11 "	85	89	100
13 "	82	89	100
15 "	77	89	100
17 "	59	89	100
19 "	49	88	100
21 "	32	88	100
23 "	32	86	100
25 "	32	88	100
27 "	33	88	100
30 "	34	88	100

TABLE IX

Hydrogen ion concentration of the culture filtrate of Trichoconis padwickii grown on modified Czapek's-Dox medium at different periods of growth.

Periods of Growth	pH
Initial pH	6.0
5 days	6.8
7 "	6.8
9 "	6.9
11 "	7.1
13 "	7.3
15 "	7.4
17 "	7.5
19 "	7.5
21 "	7.6
23 "	7.5
25 "	7.0
27 "	7.0
30 "	6.9

pH OF THE CULTURE FILTRATE OF *Trichoconis padwickii*
AT DIFFERENT PERIOD OF GROWTH

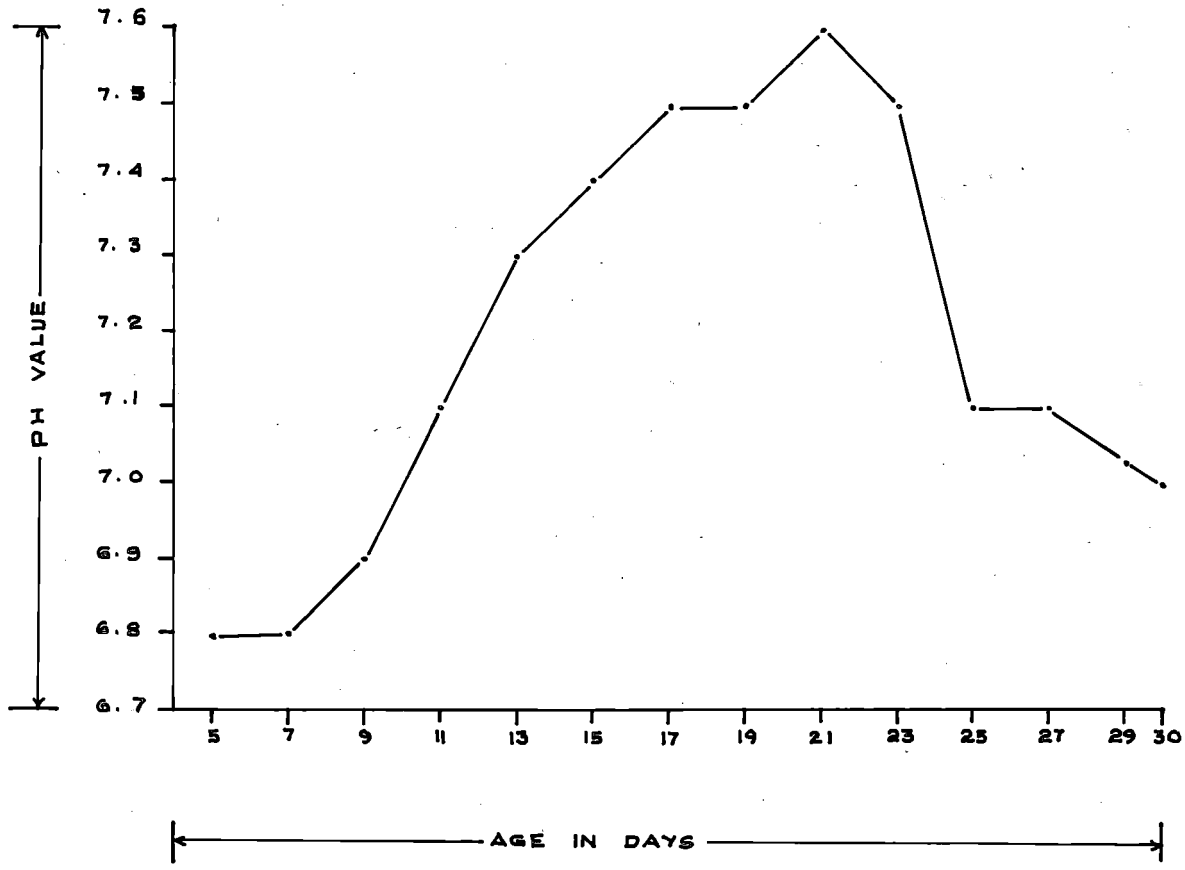


FIG: 4

TABLE X

Elongation of radicle of germinating paddy seeds in mm. in the culture filtrate of Trichoconis padwickii.

Sl No.	Treatments	24 hours						48 hours						72 hours					
		I	II	III	IV	Mean	Percentage of inhibition over U.IM.	I	II	III	IV	Mean	Percentage of inhibition over U.I.M.	I	II	III	IV	Mean	Percentage of inhibition over H. I. M.
1	Culture filtrate	6.5	5.0	5.5	6.0	5.8	41.2	8.0	7.0	7.0	7.5	7.4	41.7	12.0	7.3	7.3	8.2	8.7	59.9
2	Uninoculated medium	7.3	8.4	8.8	8.1	8.2		14.5	13.0	11.0	12.1	12.7		19 .1	23.7	21.6	22.4	21.7	
3	Distilled water	10.7	11.3	11.8	11.2	11.3		17.3	20.7	15.7	18.4	18.0		24.4	35.2	25.2	27.7	28.2	

TABLE XI

Elongation of plumule of germinating paddy seeds in mm. in the culture filtrate of Trichoconis padwickii.

Sl No.	Treatments	24 hours I	II	III	IV	Mean	% inhibition	48 hours I	II	III	IV	Mean	% inhibition	72hours I	II	III	IV	Mean	% inhibition
1	Culture filtrate	5.0	5.0	5.0	5.2	5.04	33.07	6.5	6.0	6.2	6.18	39.88	39.88	9.7	7.3	7.0	7.4	7.85	53.36
2	Uninoculated medium	7.0	7.3	8.0	7.8	7.53		10.4	10.1	10.4	10.2	10.28		16.0	17.3	16.8	17.2	16.83	
3	Distilled water	7.0	12.7	7.0	7.8	8.63		19.5	17.5	11.2	13.4	13.15		19.3	27.4	15.6	20.2	20.63	

Effect of culture filtrate on the germination of seeds other than paddy.

The culture filtrate of Trichoconis padwickii was found to exert a definite inhibitory effect on the germination of seeds of tomato, brinjal, cowpea, cucumber and bhindi. But the degree of inhibition differed with different seeds. The inhibition was highest in cucumber seeds, being 65.5 percent and lowest in tomato seeds, being 39.9, percent. An inhibition of 56.3% was noted in brinjal seeds, 54.2% in cowpea seeds and 42% in bhindinseeds. (Table XII).

Effect of culture filtrate on the elongation of radicle plumule of germinating seeds other than paddy.

The growth of the plumule and radicle of germinating seeds of tomato, brinjal, cowpea, cucumber and bhindi was retarded when they were treated with culture filtrate of Trichoconis padwickii.

Just as in the case of germinating seeds, the maximum inhibition of radicle and plumule was noted in the case of cucumber seeds and the minimum in the case of bhindi seeds.

The percentages of inhibition of radicle of cowpea seeds 24 hrs, 48 and 72 hours after treatment were 44.14%,

TABLE XII

Germination percentages of seeds other than paddy treated with culture filtrate of Trichoconis padwickii grown on modified Czapek's-Dox medium.

Sl No.	Treatments	Tomato						Brinjal						Cucumber						Cowpea						Bhindi					
		I	II	III	IV	Mean	% inhibition over U.I.M	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition.	I	II	III	IV	Mean	% inhibition
1	Culture-filtrate	40	42	54	48	51	39.9	32	30	30	32	31	56.3	32	30	28	28	25.5	65.5	36	34	42	42	38.5	54.2	42	46	52	58	49	42.0
2	Uninoculated medium	96	86	82	82	86.5		68	68	76	72	71		84	84	88	86	85.5		82	86	84	84	84.0		82	84	88	84	84.5	
3	Distilled water	100	98	98	100	99		86	88	84	80	84.5		100	100	100	100	100		100	100	100	100	100		100	100	100	100	100	

TABLE XIII

Elongation of radicle of germinating cucumber seeds in mm. in the culture filtrate of *Trichoconis padwickii*.

Sl. No.	Treatments	24 hours						48 hours						72 hours					
		I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition
1	Culture filtrate	5.0	5.3	5.2	5.0	5.13	60.9	7.5	7.5	7.1	6.7	7.20	69.8	16.5	15.0	15.0	14.3	14.95	74.2
2	Uninoculated medium	11.4	13.7	12.1	13.2	12.60		23.1	24.4	23.6	24.0	23.78		53.0	54.7	53.2	53.8	53.67	
3	Distilled water	19.3	18.5	19.1	18.8	18.93		32.8	28.3	31.2	30.1	30.85		58.8	55.9	57.9	56.3	5723	

TABLE XIV

Elongation of plumule of germinating cucumber seeds in mm. in the culture filtrate of Trichonis padwickii.

Sl. No.	Treatments	24 hours						48 hours						72 hours					
		I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition
1	Culture filtrate	5.1	5.2	5.3	5.3	5.23	16.9	7.1	7.2	8.1	7.7	7.52	23.1	9.4	9.2	10.4	10.1	9.78	69.9
2	Uninoculated medium	6.2	6.2	6.3	6.5	6.30		9.4	9.7	9.8	10.2	9.78		27.2	34.3	34.1	34.4	32.50	
3	Distilled water	8.5	8.5	8.2	8.6	8.45		14.1	15.3	14.1	15.6	14.78		34.1	35.8	34.0	36.6	35.15	

52.4% and 62.15% respectively while that of plumule were 22.1%, 39.03 and 56.1% respectively for the corresponding periods. (Tables XV and XVI).

The percentages of inhibition of radicle of brinjal seeds 24, 48, and 72 hours after treatment were 36.6, 43.2 and 35.7 respectively while those the plumule during the corresponding hours were 32.7, 28.7 and 32.4 (Tables XVII and XVIII).

In tomato seeds, the percentages of inhibition of radicle 24, 48 and 72 hours after treatment were 37.5, 43.6 and 54.9 respectively while those of plumule were 6.8, 27.2 and 27.5 respectively during the corresponding periods. (Tables XIX and XX).

In bhindi seeds, the percentages of germination of radicle 24, 48 and 72 hours after treatment were 10.6, 33.7, and 49.6 respectively while those of plumule were 27.8, 28 and 49 respectively (Tables XXI and XXII).

Effect of culture filtrate on the germination and elongation of germ tube of certain fungal spores:

Germination of spores.

Spores of Piricularia oryzae, Pestalotia palmarum and Helminthosporium halodes showed inhibition of germination

TABLE XV

Elongation of radicle of germinating cowpea seeds of in mm. in the culture filtrate of *Trichoconis padwickii*.

Sl. No.	Treatments	24 hours I	II	III	IV	Mean	% inhibition	48 hours I	II	III	IV	Mean	% inhibition	72 hours I	II	III	IV	Mean	% inhibition
1	Culture filtrate	12.7	11.8	11.2	11.9	11.90	44.14	17.7	16.0	17.1	19.5	17.58	52.4	23.9	23.4	24.3	29.7	25.33	62.15
2	Uninoculated medium	21.4	21.3	21.1	21.4	21.30		36.2	38.4	36.1	37.0	36.93		63.9	65.0	64.1	64.2	64.30	
3	Distilled water	23.2	23.5	23.0	22.0	22.93		49.3	46.6	46.1	45.0	46.75		78.1	77.4	74.2	74.1	76.20	

TABLE XVI

Elongation of plumule of germinating cowpea seeds in mm. in the culture filtrate of Trichoconis padwickii.

Sl. No.	Treatments	24 hours						48 hours						72 hours					
		I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition
1	Culture filtrate	5.7	6.0	5.9	5.9	5.88	22.1	6.9	8.6	7.9	9.2	8.15	39.03	10.7	13.4	11.8	14.7	12.40	56.1
2	Uninoculated medium	7.3	7.4	7.6	7.6	7.55		12.3	14.0	13.5	13.8	13.40		27.7	28.4	28.6	28.2	28.25	
3	Distilled water	9.4	9.4	9.2	10.1	9.53		15.3	14.8	14.0	19.6	15.93		31.7	25.1	30.0	35.2	30.50	

TABLE XVII

Elongation of plumule of germinating Brinjal seeds in mm. in the culture filtrate of Trichoconis padwickii.

Sl. No.	Treatments	24 hours I	II	III	IV	Mean	% inhibition	48 hours I	II	III	IV	Mean	% inhibition	72 hours I	II	III	IV	Mean	% inhibition
1	Culture filtrate	5.4	6.0	6.0	5.6	5.50	32.7	6.7	7.8	7.7	7.3	7.38	28.7	10.1	12.0	11.7	9.8	10.9	32.4
2	Uninoculated medium	7.0	7.0	7.6	7.2	7.20		8.9	10.8	11.1	10.6	10.35		14.7	16.0	17.1	16.8	16.15	
3	Distilled water	7.7	7.7	8.2	8.6	8.05		10.8	12.5	12.8	12.1	12.05		18.1	23.4	23.8	22.2	21.88	

TABLE XVIII

Elongation of radicle of germinating brinjal seeds in mm. in the culture filtrate of Trichoconis padwickii.

Sl. No.	Treatment	24 hours						48 hours						72 hours					
		I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition
1	Culture filtrate	7.4	6.2	6.8	6.7	6.78	36.6	11.1	9.4	10.5	11.8	10.70	43.2	17.4	16.8	17.2	19.7	17.78	35.7
2	Uninoculated medium	10.9	10.8	10.0	11.1	10.70		18.5	19.3	18.1	19.5	18.85		25.2	30.2	24.2	31.1	27.68	
3	Distilled water	11.1	11.2	10.8	11.4	11.15		19.8	19.0	18.2	20.0	19.25		33.3	34.6	33.5	36.1	34.38	

TABLE XIX

Elongation of radicle of germination of Tomato seed in mm. in the culture filtrade of Trichoconis padwickii.

Sl No.	Treatments	24 hours						48 hours						72 hours					
		I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition
1	Culture filtrate	7.5	7.0	7.7	7.6	7.45	37.5	10.0	9.2	9.0	9.4	9.40	43.6	12.7	12.5	11.6	12.2	12.25	54.9
2	Uninoculated medium	11.1	12.9	12.2	12.1	12.08		15.8	15.8	18.8	16.8	16.68		27.4	25.1	29.1	27.2	27.20	
3	Distilled water	15.3	14.4	14.0	14.6	14.58		25.1	22.0	22.8	22.6	23.13		44.3	37.1	35.0	38.0	38.60	

TABLE XX

Elongation of plumule of germinating Tomato seeds in mm.
in the culture filtrate of *Trichoconis padwickii*.

Sl. No.	Treatments	24 hours						48 hours						72 hours					
		I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition
1	Culture filtrate	6.5	8.2	6.2	7.6	7.13	6.8	11.5	9.3	7.8	9.6	9.55	27.2	16.8	17.0	14.8	16.1	16.18	275
2	Uninoculated medium	6.8	7.4	8.2	8.2	7.65		13.6	11.3	13.2	13.8	12.98		22.2	22.4	21.7	21.2	21.88	
3	Distilled water	8.6	8.5	8.5	9.2	8.70		16.4	15.9	15.3	14.8	15.60		25.1	21.1	22.7	23.1	23.00	

TABLE XXI

Elongation of radicle of germinating bhindi seeds in mm. in the culture filtrate of Trichoconis padwickii.

Sl. No.	Treatments	24 hours						48 hours						72 hours					
		I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition
1	Culture filtrate	12.4	12.6	14.5	11.3	12.70	10.6	15.8	18.7	20.2	15.6	17.58	33.7	30.5	23.8	31.5	25.7	27.8	49.6
2	Uninoculated medium	14.1	14.4	14.1	14.2	14.20		28.0	27.3	27.2	28.0	27.63		53.0	56.0	52.0	53.1	53.53	
3	Distilled water	18.6	16.9	17.2	17.1	17.45		35.6	36.3	34.1	34.0	35.00		61.3	61.8	59.2	56.1	59.35	

TABLE XXII

Elongation of plumule of germinating bhindi seeds in mm. in the culture filtrate of Trichoconis padwickii.

Sl. No.	Treatments	24 hours						48 hours						72 hours					
		I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition
1	Culture filtrate	5.4	5.6	5.5	6.0	5.63	27.8	6.8	7.3	7.4	8.5	7.50	28.0	9.2	9.5	9.1	10.5	9.33	49.0
2	Uninoculated medium	7.2	7.4	7.0	7.2	7.20		9.7	10.9	10.2	10.1	10.23		16.7	20.8	17.5	18.2	18.30	
3	Distilled water	7.8	7.9	7.2	7.6	7.68		11.9	14.3	13.2	14.1	13.38		27.6	30.2	28.7	28.2	28.68	

when treated with the culture filtrate of Trichoconis padwickii. Maximum inhibition was noted in the case of Pestalotia spores and minimum in the case of Helminthosporium spores. The inhibition was greater when undiluted culture filtrate was used. The inhibition of germination of spores was progressively reduced when the dilution of the culture filtrate was increased (Table XXIII).

The percentages of inhibition of Pestalotia spores in undiluted culture filtrate, and in culture filtrate diluted ten and hundred times were 96.7, 90.2 and 84.1 respectively.

In the case of Piricularia spores the percentage of inhibition in undiluted culture filtrate and in culture filtrate diluted 10 and 100 times were 62.4, 51.3 and 31.4 respectively.

The inhibition of germination of Helminthosporium halodes spores was comparatively low being 31.7, 25.0 and 26.1 respectively for undiluted culture filtrate and culture filtrate diluted 10 and 100 times.

Germ tube elongation

Inhibition of germ tube elongation was noted in all the three organisms. The inhibition was highest in

TABLE XXIII

Germination percentage of fungal spores treated with different dilutions of culture filtrate.

Treatments	Piricularia oryzae						Pestalotia palmarum						Helminthosporium halodes					
	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition	I	II	III	IV	Mean	% inhibition
Culture filtrate																		
original	44	32	30	41	36.75	62.4	3	2	5	4	3.75	96.7	58	67	56	60	60.25	31.7
1 : 10	45	47	51	47	47.50	51.3	9	7	10	9	8.75	90.2	72	67	72	72	70.75	25.0
1 :100	68	74	64	67	68.25	31.4	14	15	15	18	15.50	84.1	75	72	72	66	71.25	26.1
Uninoculated medium																		
original	99	98	97	99	98.25		85	85	80	83	83.25		87	92	84	90	88.25	
1 : 10	99	98	99	98	98.50		90	92	87	89	89.50		91	95	94	92	93.00	
1 :100	99	100	100	99	99.50		94	88	90	93	91.25		93	95	94	98	95.00	
Distilled Water	99	100	100	100	99.75		100	100	100	100	100.00		98	98	99	98	98.25	

Pestalotia spores and lowest in Helminthosporium spores. 69.3% and 66.3% inhibition was noted 4 and 6 hours after treatment with the culture filtrate in the case of Pestalotia spores. Piricularia spores exhibited 32.4% and 52.14% inhibition and Helminthosporium spores 50.1% and 37.7% inhibition during the corresponding periods.

Effect of temperature on the activity of the culture filtrate

No change in the activity of the culture filtrate was noted as a result of boiling for ten and twenty minutes. The germination percentages of paddy seeds in untreated culture filtrate and in culture filtrate boiled for 10 and 20 minutes were 32, 34 and 33 respectively. Autoclaving reduced the toxicity of the culture filtrate only to a very limited extent. Paddy seeds gave 41.25% germination in autoclaved culture filtrate.

Effect of culture filtrate on rice seedlings and grown up plants

No visible effect was noted when one month old paddy seedlings were planted in culture filtrate in such a manner that the root system was completely immersed in the culture filtrate.

TABLE XXIV

Average germ tube length of different fungal spores treated with culture filtrate of Trichoconis padwickii.
(in microns)

Pestalotia palmarum

Treatments	4 hours I	II	III	IV	Mean	% inhibition	6 hours I	II	III	IV	Mean	% inhibition
Culture filtrate	9.2	9.1	9.4	9.6	9.33	69.3	13.1	14.0	13.6	13.7	13.60	66.3
Uninoculated medium	28.9	30.4	31.9	30.3	30.38		42.5	41.1	38.0	40.6	40.55	
Distilled water	42.6	41.0	47.1	41.4	43.03		72.9	68.4	66.8	68.2	69.08	

Pirricularia oryzae

Culture filtrate	15.2	16.1	15.1	15.1	15.13	32.4	19.8	21.3	22.8	20.6	21.13	52.1
Uninoculated medium	21.3	21.2	24.3	22.6	22.35		42.5	42.5	47.1	44.4	44.15	
Distilled water	41.0	42.3	41.1	40.6	41.25		76.0	73.0	72.9	72.6	73.61	

Helminthosporium halodes

Culture filtrate	12.2	12.2	13.7	12.6	12.68	50.1	30.4	31.9	32.1	31.6	31.60	37.7
Uninoculated medium	24.3	25.8	25.8	24.9	25.20		48.6	50.2	52.3	51.2	50.58	
Distilled water	36.5	36.5	41.1	38.7	38.20		63.8	66.9	64.2	65.4	65.08	

TABLE XXV

Germination percentages of paddy seeds treated with culture filtrate, boiled and autoclaved.

Replications	Culture filtrate			Uninoculated medium			Distilled water		
	Boiled for 10 minutes	Boiled for 20 minutes	Auto-claved	Boiled for 10 minutes	Boiled for 20 minutes	Auto-claved	Boiled for10 minutes	Boiled for 20 minutes	Auto-claved
I	35	35	43	89	88	87	99	100	100
II	33	30	43	89	89	87	99	100	100
III	34	34	41	83	83	87	100	100	100
IV	34	34	38	89	85	86	99	100	99

But when seedlings devoid of roots were placed in the culture filtrate, rolling of leaves was produced after 48 hours. This effect was, however, reversed when such seedlings were subsequently placed in distilled water for 4 hours.

Effect on the grown up seedlings irrigated with culture filtrate

Paddy seeds were placed on moist absorbent cotton wool in sterile petridishes of 10 cm size. The seeds were allowed to germinate and grow for 15 days. During this period each dish was wetted with 5 ml of nutrient solution every day. After fifteenth day the seedlings were irrigated with 5 ml of culture filtrate for five days. The plants treated exhibited a reduction in the height. The older leaves showed yellowing from the 3rd day onwards. This was followed by necrosis from the tip downwards. The leaves turned brown and finally died. The innermost leaves, however, did not show any marked yellowing eventhough it was paler than the healthy leaves of the control plants kept in uninoculated medium and distilled water.

Effect of the culture filtrate on the leaf lamina and leaf sheath

Clear necrobic spots were produced when the sterile culture filtrate was placed on leaf lamina and leaf sheaths

of 45 days old paddy plants, after making slight injury with the help of carborandum powder using moist absorbent cotton, under aseptic conditions. The spots were similar to that produced by the organism on the paddy plants in nature. The spots were straw coloured. They did not increase in size even after several days. No such necrotic spots could be seen on leaves treated with uninoculated medium or distilled water under the same set of conditions. The effect was noticeable in the case of undiluted as well as diluted culture filtrate.

When the sterile culture filtrate of the organism was applied on leaves and leaf sheaths without making injury with abrassive no clear symptom could be noticed eventhough the point of application became slightly yellowish in colour.

Bacterial plate bioassay

No inhibition zone was formed when the culture filtrate was applied in plate cultures of Bacillus subtilis either by the cavity method or by the filter paper method.

DISCUSSION

DISCUSSION

It is already known from earlier work that certain pathogenic microorganisms can produce metabolic substances in culture which are toxic to plants. For instance Clausen Kass and Netal (1944) detected and isolated lycomarasmin from the culture filtrate of the tomato wilt fungus Fusarium oxysporum f., lycopersici while Gaumann (1952) isolated fusaric acid from the same organism. Similarly, pireularin and ∝-picolinic acid were isolated from the culture filtrate of Piricularia oryzae by Tamari and Kaji (1954); alternaric acid from Alternaria solan by Brian et al (1951) and victoxinine from Helminthosprium victoriae by Litzenberger (1949). All these substances, when applied on the host, are known to be capable of inducing symptoms more or less similar to those seen in nature.

In the present studies, the culture filtrate of Trichoconis padwickii when applied on paddy leaves was found to produce necrotic spots which in colouration and other aspects closely resembled those produced by the fungus under natural infection. This indicates that the culture filtrate contained some toxic principle which in all probability, is similar to that produced by the organism

in the host. The necrosis was evident even at a dilution of 1: 100. Tamari and Kaji (1954) obtained similar necrotic lessions on paddy using the toxin of Piricularia oryzae.

The fungus was grown in a number of culture solutions to find out a medium which is best suited for its growth and toxin production. Maximum growth as determined by dry weight of the mycelium was obtained in Czapek's-Dox medium. The superiority of this medium over the others was evident at all stages of growth of the fungus. This medium was also found to be best for the production of toxic metabolites in so far as the culture filtrate of this medium produced maximum inhibition of germination as well as radicle and plumule elongation of rice grains. Czapek's medium was almost as good as Czapek's-Dox medium. Different fungi are known to prefer different media for their growth and toxin production. Thus, while growth and toxin production of the present fungus was found to be very poor in Richards Solution, Rosen (1927) found this medium to be the best for Fusarium vasinfectum. Similarly the medium which was found by Tamari and Kaji (1954) to be good for the toxin production of Hricularia oryzae was found to be poor for Trichoconis padwickii. However, Czapek's medium and Czapek's-Dox medium were found to be good for the growth

and toxin production of certain fungi by earlier workers also. Thus Brian (1949) found the former medium to be the best for Alternaria solani while Krishnaswamy et al (1969) used the latter medium satisfactorily while studying the toxin production of certain isolates of Piricularia oryzae.

The use of dextrose in the place of sucrose in Czapek's-Dox medium was found to improve the growth and toxin production of Trichoconis padwickii to a certain extent. The growth of the organism in the medium containing fructose was only less than half of that in the medium containing dextrose. But with regard to the production of toxic metabolites fructose was not so poor. The percentage of germination of paddy seeds in the culture filtrate of media containing dextrose, sucrose and fructose was found to be 32, 43 and 45.8 respectively. Soluble starch was invariably poor with regard to the growth as well as the production of toxic metabolites.

The nitrogen source was also found to be equally important. Eventhough the fungus could utilize sodium nitrite and peptone to a certain extent, growth as well as toxin production was poor in media containing these substances. Ammonium sulphate was practically unfit for the growth of the

organism. Sodium nitrate was undoubtedly the best for growth and production of toxic metabolites.

The carbon and nitrogen sources in the medium, therefore, seem to exert considerable influence on the growth as well as toxin production of the organism, the best results being obtained when dextrose was given along with sodium nitrate. While the production of maximum toxic metabolites was noted in the medium which was best suited for growth, the results obtained in the other media go to show that no relationship exists between the extent of growth and the amount of toxic metabolites produced by the organism. Eventhough the growth of the organism in certain media was poor, the quantity of toxic metabolites produced, determined on the basis of seed germination bioassay, does not seem to be proportionately low.

The toxin production was found to increase with age of the culture. Maximum growth of the fungus and production of toxin was attained when the culture was three weeks old. These findings are more or less similar to those of earlier works like, that of Fahmy (1923) Zentmeyer (1941) and Scheffer and Pringle (1961) who worked on other pathogens. Inhibition of germination of paddy seeds was comparatively low in culture filtrates drawn from younger cultures.

Keeping the culture beyond three weeks did not increase the toxicity of the culture filtrate. This finding is similar to those of Goodman (1960), Sharma and Agnihotri (1967) and King (1967).

The inhibitory property of the toxic metabolites produced by Trichoconis padwickii is not limited to rice seeds only. The culture filtrate inhibited not only the germination of seeds of five other species of plants viz. cucumber, brinjal, cowpea, bhindi and tomato, used in the present studies, but also their radicle and plumule elongation. In the case of cucumber seeds the inhibition of germination and also that of radicle and plumule elongation was higher than that in paddy seeds.

The culture filtrate was found to possess antifungal properties in so far as it inhibited the germination and germ tube elongation of certain fungal spores viz. Pestalotia palmarum, Piricularia oryzae and Helminthosporium halodes. The inhibition of germination was higher in the case of Pestalotia spores. But in all cases the germination percentage increased with a corresponding increase in the dilution. Similar inhibition of spore germination by the toxin produced by other fungi has been noted by earlier workers also. Thus Brian et al (1951) noted a reduction in

germination of spores of a number of fungi including those of Botrytis alli when treated with alternaric acid. In a like manner Ludwig (1957) found a reduction in germination percentage of Sclerotinia fructicola spores when treated with the toxin produced by Helminthosporium sativum.

The germ tube elongation was also differently affected the maxium inhibition being noted in the case of Pestalotia. This finding is similar to those of Brian et al (1951) who found that the extension of germ tubes of spores of Botrytis alli, Myrrothecium verrucariae, Mucor mucedo etc. were differently inhibited by the culture filtrate of Alternaria solani, Myrrothecium verrucariae was extremely susceptible, germination being completely inhibited, and Fusarium was resistant while Botrytis alli showed stunted germ tubes.

The toxic principle in the culture filtrate was found to be thermostable. The activity was not diminished by exposing it to 100°C. for twenty minutes. Even autoclaving at 15 lbs pressure for twenty minutes brought about only a slight decrease in the activity. Similar thermostable toxic principles of fungal origin have been noted by earlier workers also. Thus Fahmy (1923) noted that the toxic substances produced by Fusarium solani, Verticillium albo-atrum,

Verticillium dubouys were not destroyed by boiling. Irwing(1945) found that even autoclaving did not change the activity of lycopersicin. Other thermostable metabolic principles have been described by Clayton (1934) Demetriades (1950) and Sharma and Agnihotri (1967).

With the experimental evidences now available, it cannot be said with certainity whether the toxic principle is absorbed through the root system eventhough seedlings irrigated with the culture filtrate exhibited a certain amount of stunting. This aspect, therefore,needs further study.

The culture filtrate was not inhibitory to Bacillus subtilis. Whether it could cause inhibition in other species of bacteria can be known only by further studies.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The ability of Trichoconis padwickii to produce toxic metabolites in culture was determined by growing the organism in different liquid media and by testing the culture filtrate for its effect on paddy plants and on germinating seeds and spores of fungi.

The culture filtrate of Trichoconis padwickii, when applied on paddy leaves, was found to produce necrotic spots which in colouration and other aspects closely resembled those produced by the fungus under natural infection. This indicates that the culture filtrate contained some toxic principle which, in all probability, is similar to that produced by the organism in the host. Necrosis was evident even at a dilution of 1: 100.

Best growth of the fungus as determined by dry weight of the mycelium was obtained in Czapek's-Dox medium. The superiority of this medium over the others was noted at all stages of growth of the fungus. For the production of toxic metabolites also this medium was found to be the best.

Sucrose was found to be a good carbon source for the growth of Trichoconis padwickii. The use of dextrose in place of sucrose in Czapek's-Dox medium improved the growth and toxin production of the organism to a certain extent. The best nitrogen source was found to be sodium nitrate, the normal constitutent of Czapek's-Dox Medium.

The toxin production was found to increase with the age of the culture, maximum growth and toxin production was attained when the culture was three weeks old.

The inhibitory property of the culture filtrate was not confined to rice seeds only. Germination as well as radicle and plumule elongation of seeds of five other species of plants viz. cucumber, brinjal, cowpea, bhindi and tomato were inhibited by the culture filtrate. The effect of the culture filtrate on cucumber seeds was considerably greater than that on other seeds.

The culture filtrate was found to possess anti-fungal properties in so far as it inhibited the germination and germ tube elongation of certain fungal spores viz., Pestalotia palmarum, Piricularia oryzae, and Helminthosporium halodes. The inhibition was highest in the case of Pestalotia spores.

The toxic principle in the culture filtrate was found to be thermostable. With the experimental evidences now available, it cannot be said with certainty whether the toxic principle is absorbed through the root system even-though seedlings irrigated with the culture filtrate exhibited a certain amount of stunting.

The culture filtrate was not inhibitory to *Bacillus subtilis*.

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