IN VITRO STUDIES ON THE INTERACTION OF ANTAGONISTIC MYCOFLORA WITH *THIELAVIOPSIS PARADOXA*, THE CAUSAL AGENT OF COCONUT STEM BLEEDING DISEASE.

DISSERTATION
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To

My Parents

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Dated 21-12- 1987

(P.Venkatramana Gowda)

CERTIFICATE FROM THE GUIDE

This is to certify that the dissertation entitled 'In vitro studies on the interaction of antagonistic mycoflora with Thielaviopsis paradoxa, the causal agent of coconut stem bleeding disease' submitted in partial fulfilment for the award of the degree of 'Master of Philosophy' in Bioscience (Plant Protection) of Mangalore University is a record of bona fide research work carried out by Mr.P.Venkatramana Gowda under my guidance and supervision. No part of the dissertation has been submitted for any other degree or diploma.

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1. INTRODUCTION

The coconut palm, Cocos nucifera Linn. is one of the most beautiful trees in the world. It is grown in all the tropical countries. Harries (1979) rightly called it as 'the milk bottle on the door step of mankind'. Each and every part of coconut palm is useful to man oneway or another. It is no wonder then that the palm is looked upon with reverence and affection by the inhabitants of the coconut producing countries and given such eulogistic epithets such as "Kalpa Vriksha (Tree of Heaven)", "Tree of Life" etc.

Like all other crop plants, the coconut palm, in spite of its hardy nature is affected by a number of diseases which are responsible for enormous economic losses in the different coconut growing regions. 'Stem bleeding disease' is one such disease of coconut reported from all the tropical countries where coconut is grown. The disease is popularly known as "Chennir Olikkal" or "Kara Olikkal" in Malayalam, "Raktha Kariroga" in Kannada, "Saruozhughalnoi" in Tamil and "Lakkaragadum" in Telugu. The disease is found in all soil types and through out the year. All varieties of coconut are susceptible to the disease.

The disease was till recently grouped under diseases of uncertain etiology. Thielaviopsis paradoxa (de Seynes) von Hohnel has been a suscepted pathogen till now (Ohler, 1984). Recently Nambiar et al.(1986) could establish pathogenicity with the above fungus.

The damages due to the disease are both direct and indirect. The palm will not die suddenly due to the disease, but gradually the vigour of the palm will be reduced with a gradual reduction in yield. After a few years the palm will succumb to the disease. Hence it becomes difficult to assess the crop loss due to this disease especially in the early and middle stages of the disease. The general recommendation for the control of the disease is to apply coal tar to the affected bark region after chiselling the affected bark tissues. This temporarily arrests the progression of the disease in the diseased palm. Root feeding with systemic fungicides like Bavistin, Calixin etc. is being tested at Central Plantation Crops Research Institute, Kasaragod with a view to controlling the disease. But this has its own implications since such frequent administrations of systemic chemicals may lead to residue problems in the nut water and kernel used for human consumption. The disease can be managed to an extent by phytosanitation, providing proper drainage in the garden, alleviating soil moisture stress in the basins of coconut palm, supplying adequate and balanced fertilizers along with neem-oil

cake to the palm basin etc. However, the above measures do not help in completely curing the disease.

T. paradoxa is a slow and a weak pathogen. Earlier workers (Anon, 1976) had reported the presence of other fungi viz. Aspergillus sp., Diplodia sp., Fusarium sp., Mucor sp., Penicillium sp., Rhizoctonia sp., Trichoderma sp. etc. from bark and/or soil samples of stem bleeding affected palms. There is no report on the effect of these associated fungi on the pathogen viz. T. paradoxa. Hence in vitro studies on the interaction of the fungi isolated from tissues and soils with T. paradoxa was taken up, with a view to mainly isolating mycoflora antagonistic to the pathogen. Such antagonistic fungi may be useful in evolving biological control measures against the stem bleeding pathogen. The studies envisage in isolating the strainal variations in the pathogen and finding out the reaction of the associated fungi against these pathogenic isolates. No work has been done on these aspects and to fill in this lacuna this work was taken up.

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2. REVIEW OF LITERATURE

2.1 Disease occurrence

Stem bleeding disease of coconut was first reported from Sri Lanka (Petch, 1906). There are many types of stem bleeding disease in coconut. Ill-drainage in the garden, soil moisture stress, hard pan formation in soil, imbalanced nutrition leading to physiological disorders, excessive salinity of soil, stem injury, lightning injury etc. may lead to stem bleeding disease of coconut or predispose the palms to infection by pathogens. The present study is related to stem bleeding disease caused by Thielaviopsis paradoxa and as such the review of literature deals mainly on this aspect only.

Sundararaman (1922) reported stem bleeding disease of coconut from India. Now the disease is known to occur in nearly all coconut growing countries. The disease has now been reported from the Philippines (Lee, 1922), Malaysia (Sharples, 1923), Andaman Islands (Mitra, 1929) Trinidad (Briton-Jones, 1940), Papua New Guinea (Dwyer, 1953), Fiji (Mc.Paul, 1962) and Indonesia (Renard et al. 1984). Menon and Pandalai (1960) considered T. paradoxa as a suspected pathogen of the disease.

The disease is found in all soil types from coastal sands to laterite. It may occur sporadically in isolated palms or gardens or endemically in certain areas. Although mainly occurring in coastal sandy loams and reclaimed clay soils, stem bleeding has also been found to occur in shallow laterite and loamy inland soils where a hard pan of clay or granite has been noticed. The extent of damage varies from reduction in yield to complete death of the palms (Radha, 1962). Gapasin (1983) reported that the disease could decrease the yield and kill the trees in the Philippines.

2.2. Symptomatology:

The symptoms have been described by various workers (Petch, 1908 , Sundararaman, 1922; Lee, 1922; Sharples, 1923; Mitra, 1929; Briton-Jones, 1940; Salgado, 1942; Dwyer, 1953; Menon and Pandalai, 1960; Goberdhan, 1961; Radha, 1962; Mc Paul, 1962; Thampan, 1984; Ohler, 1984; Renard et al., 1984). The typical symptom of the disease is the exudation of a reddish brown liquid through cracks on the trunk, but more towards the base. In the early stage, bleeding occurs only through one or two longitudinal growth cracks at the base. Later it appears higher up on the stem. In very severe cases, the exudation may extend upto the crown. On drying, the liquid turns black. The irregular streaks of exudation may coalesce to form larger lesions. The tissues inside the lesions show discolouration and decay. The external symptoms need not reflect the extent of internal damage (Nambiar et al., 1985). The internal decay often extends beyond

the external lesion size. With age, the decayed tissues become fibrous and cavities are formed, especially in young palms. A clear liquid gushes out when the bark is pressed or punctured with a knife, especially after the monsoon season (Radha, 1962).

On chiselling the affected portion, it is seen that the decay is confined to the bark and underlying tissues, but seldom enters deep inside the core. However, in cooler conditions and also in young palms, it has been noticed that the decay can reach the deep lying tissues (Nambiar et al.,1985). Infestation of stem borers like Diocalandra and Xyleborus has been found to accentuate the damage to vasculature causing rapid deterioration of the palm(Radha, 1962).

As a result of stem decay, the crown nature also changes. The leaves in the outer whorl become yellow rather prematurely, droop and dry up. The production of bunches is affected followed by nutfall especially in palms exposed to drought conditions. The trunk gradually tapers at the apex and crown size reduces. The production of nuts dwindles and ultimately the palm dies (Ohler, 1984). The crown symptoms are more pronounced during summer season when moisture stress is experienced while in rainy season as well as in well managed irrigated gardens, crown symptoms are not conspicuous always(Nambiar, personal communication).

.3 Etiology:

Petch (1908) reported Thielaviopsis paradoxa (de Seynes) von Hohnel as a weak pathogen entering the stem tissues through wounds or growth cracks occurring naturally on the coconut trunk. The perfect stage of the fungus, Ceratocystis paradoxa has been found to occur on the decaying tissues (Nambiar, personal communication). Though this fungus has till now been a suspect pathogen (Menon and Pandalai, 1960, Ohler, 1984), it was only recently that symptoms could be reproduced by inoculating healthy trees with the fungus, thus establishing its pathogenicity (Nambiar et al., 1986). At Kayangulam, in addition to T. paradoxa, Phomopsis cocoina and Schizophyllum commune also produced stem bleeding symptoms on inoculation to healthy palms.(Anon, 1987).

There are other types of stem bleeding, reported to be caused either by nutrient imbalance or deficiency (Potty and Radhakrishnan 1978; Renard et al., 1984; von Uexkull, 1985) or excessive salinity of soil (Nagarajan, 1985), or trash burning (Goberdhan, 1961; La Broma, 1973), or lightning (Nambiar, personal communication) or Ganoderma lucidum infection (Vijayan and Natarajan, 1972) etc. However, stem bleeding disease due to T. paradoxa is prevalent in most of the coconut growing areas.

.4 Disease cycle:

T. paradoxa entering the stem tissues through the growth cracks (Petch, 1906) multiplies in the infected stem tissues and produces two types of spores viz. phialospores (endoconidia)

and chlamydospores. The chlamydospores are able to survive in soil to tide over unfavourable conditions. They again germinate producing the mycelium which is capable of infecting the host. The fungus has a variety of plant species as hosts viz. coconut, arecanut, sugarcane, pineapple etc.

2.5. Predisposing factors:

Various factors like development of growth cracks, ill-drainage, soil-moisture stress, hard pan formation in soil, imbalanced nutrition leading to physiological disorders, excessive salinity of soil, stem injury etc. have been reported as the predisposing factors responsible for the incidence of stem bleeding disease in coconut. Longitudinal growth cracks seen on the stem may be the sites through which the weak pathogen, T. paradoxa enters the stem tissue(Petch, 1908). Heavy rains following an extensive dry period cause the development of more growth cracks. Sudden heavy manuring could cause more growth cracks in coconut palms leading to stem bleeding (Briton-Jones, 1940). Undue fluctuations in soil reaction and moisture or ill-drained soil conditions could cause severe stem bleeding and death of palms (Radha, 1962). Salgado (1942) reported that ill-drainage and hard lateritic pan formation led to crippled root growth, imbalanced mineral nutrition or other physiological causes in the palms. Goberdhan (1961) and La Broma(1973) reported that trash burning at the palm base damaged the stem, paving way for infection.

Based on the results of a fertilizer trial, Potty and Radhakrishnan (1978) reported that application of nitrogen at the rate of 0.35 kg/palm reduced the infection by 44 percent, though further increase in the dose did not have any impact on the incidence. They also found that increased levels of Phosphorus and Potassium tended to increase the incidence of the disease though the effect was of very low magnitude in case of Potassium. A ratio of 1:4 between N and P and 1:2 between N and K was found to minimize the occurrence of the disease. It was concluded that an increase in the phosphorus content without a concomitant increase in other nutrients, particularly nitrogen, proved to be the primary cause of the disease, the imbalanced nutrient situation disrupting the physiological system of the palm. However, Mathew and Ramanand (1980) could not find any significant difference in major nutrient contents between healthy and diseased palm nor in soil pH and electrical conductivity of soil which is an indirect measure of water soluble salts.

Renard et al. (1984) reported that the disease incidence could be prevented if Chlorine deficiency was corrected. von Uexkull (1985) found that the deficiency of Chlorine was a predisposing factor for stem bleeding disease since it played an important role in the water relation of the coconut palms. It was reported that Chlorine had a role in the stomatal mechanism of transpiration. Nagarajan (1985) reported that

excessive salinity of soil with high sodium during summer paved way for the bleeding disease of coconut.

Stem injury due to stem borers, red palm weevil or any other damage to the tree during tilling the garden etc. may also aid in hastening infection process by the fungi resulting in decaying of the affected tissues, characteristic of stem bleeding disease. Lightning injury also caused stem bleeding of the affected palms. Later pathogen enters the trunk through the weakened tissues (Menon and Pandalai, 1960).

2.6. Management of the disease.

The control measures now recommended are very ad hoc. This involves phytosanitation, balanced nutrient supply, maintaining the soil moisture status, fungicide treatment etc. When the disease is diagnosed in the early stages, the infected lesions are chiselled off to remove all decayed tissues. The chiselled portion is then protected by application of hot coal tar or 10 percent Bordeaux paste. The chiselled tissues should be destroyed by burning to prevent the spread of the disease to other healthy palms(Radha, 1962).

When stem bleeding occurs in water logged areas, the important cultural practices to be adopted is the provision for good drainage in the garden. In areas where drought is prevalent, appropriate agronomic practices like intercultivation,

mulching etc. are found helpful. Thus growing of leguminous cover crops which when allowed to trail along the ground reduces soil temperature to a great extent and prevents soil moisture stress thereby helping the coconut palm to tide over the drought season. Incorporation of green manure is found beneficial in improving soil texture in addition to its manurial value (Radha 1962).

Root feeding of the diseased palms in the early to middle stages, with systemic fungicides like Bavistin has been found effective in reducing the disease (Nambiar, personal communication

7. Biological control of plant diseases:

Biological control through induction of antagonists to the soil was first attempted by Hartley in 1921 against damping off of coniferous seedlings. Later Millard and Taylor (1927) used this technique against common scab of potato, and Sanford and Broadfoot (1931) against take-all of wheat. Since then there are many reports of biological control of plant diseases using antagonistic microorganisms.

7.1 Biological control by bacteria:

Common scab of potato caused by *Streptomyces scabies* was found to be controlled by *Bacillus subtilis* (Labruyere, 1971). Labruyere (1971) found that when green soybean plants were incorporated into soil before planting potato, it favoured antibiotic production by *Bacillus subtilis* which inturn inhibited multiplication of the potatoscab pathogen. *Bacillus subtilis* was also reported for the biological control of

Rhizoctonia solani causing damping off of chilly seedlings (Broadbent et al., 1971). Sinha and Basu (1977) reported the control of sorghum anthracnose due to Colletotricum graminicola by Streptomyces gaumycicus. Spraying the spore suspension of S. gaumycicus on sorghum plants at the time of inoculation with C. graminicola or one week earlier reduced disease development by 64.44 percent and 82.24 percent respectively. The corresponding reductions were 70 percent and 87.77 percent when culture filtrate of the antagonist was used. Applications one week after inoculation gave only 33.32 percent reduction. Gupta and Dixit (1982) reported the seasonal variations in the populations of Colletotricum capsici and its antagonist, Streptomyces griseus on the surface of chilli (Capsicum sp.). When S. griseus populations were high, C. capsici fruit rot did not develop in the field. Agrobacterium radiobacter pathovar radiobacter was found to be effective in controlling Agrobacterium radiobacter pathovar tumefaciens causing crown gall of a wide range of woody and herbaceous plants (Kerr, 1980; Cooksey and Moore, 1982). Bare-root nursery stock or seed was inoculated with A. radiobacter pathovar radiobacter to control the pathogen. (Kerr, 1980, Cooksey and Moore, 1982).

2.7.2 Biological control by fungi:

2.7.2.1 Penicillium

Lee and Wu (1979) studied the in vitro effects of Penicillium sp. and reported the production of some antibiotic substances inhibitory to the growth of

Sclerotinia sclerotiorum. Utkhede and Rahe (1980) reported the production of antibiotics by Penicillium nigricans in culture, antagonistic to the growth of Sclerotium cepivorum causing white rot of onion. But when evaluated as seed treatment in the field, this did not protect the plant from infection. Melgarejo and Sagasta (1986) observed the degeneration of the hyphae of Monilinia laxa by Penicillium purpurogenum in dual culture. The interaction was discussed in relation to the possible biological control of M. laxa. Webber and Hedger (1986) reported the in vitro antagonistic effect of Penicillium sp against Ceratocystis ulmi.

Kharchenko et al. (1977) studied the effect of culture liquids of Penicillium implicatum and other species of Penicillium on infection of barley by Erysiphe graminis. Powdery mildew of barley by E. graminis was reduced by the treatment of barley plants with the culture liquids of Penicillium sp. without affecting plant growth. Nikitina et al. (1977) investigated the effect of culture liquids of Penicillium sp. on the growth development and infection of maize by Ustilago maydis. Antibiotics from these fungi caused degeneration and disintegration of the pathogen. Fedoseeva et al. (1979) reported that presowing treatment of oats, millet, barley and wheat with Penicillium sp.. viz. P. bilai, P. cyclopium, P. implicatum and P. martensii had a favourable

effect on the growth, development and disease resistance. Two applications of P. implicatum to barley roots reduced the incidence of Erysiphe graminis f. sp. hordii 2-5 times. Frias Trevino and Garcia Espinosa (1981) reported Penicillium sp. as antagonistic to Phytophthora palmivora (Butl.) causing black rot of cacao pods. Application of conidial suspension of Penicillium sp. around the tree base and on the stump upto 1.8 meters delayed the initiation of the disease by 30 days. Pod rot incidence was reduced by upto 84 percent due to this treatment. Melgarejo et al.(1985) studied the variation of fungal population on peach twigs and flowers at different times of the year. They found Penicillium chrysogenus, P. frequentans and P. purpurogenum inhibiting the growth of Monilinia laxa on twigs and flowers.

2.7.2.2 Aspergillus sp.

Bora (1977) tested in vitro effects of Aspergillus niger isolated from soil against Fusarium solani, Alternaria alternata and Rhizoctonia solani from egg plant. A. niger showed the greatest antagonism which was confirmed with in vivo tests on egg plant seedlings. Gokulapalam and Nair (1984) studied the antagonistic effect of A. niger on Rhizoctonia solani causing rice sheath blight and found that A. niger was antagonistic to the pathogen in vitro. Venkatasubbaiah and Safeeulla (1984) studied the interaction of A. niger with Rhizoctonia solani, the incitant of collar rot of coffee seedlings. A. niger

hypherparasitised R. solani completely in dual culture. Seed treatment with A. niger significantly reduced disease incidence.

Bailey and Garcia (1978) reported the inhibition of sporulation of Phytophthora palmivora from cacao pods by Aspergillus flavipes, A. flavus-oryzae and A. terreus. Frias Trevinoo and Garcia Epsinosa (1981) reported that application of conidial suspension of A. terreus around the tree base and on the stump upto 1.8 meters delayed the initiation of black rot of cacao pods due to Phytophthora palmivora by 30 days. Melgarejo et al. (1985) found A. flavus inhibiting the growth of Monilinia laxa on peach twigs and flowers.

2.7.2.3 Trichoderma sp.

Mirzabaev (1977) reported that the application of Trichoderma sp. with glauconite sand to cotton fields after three and four year monoculture decreased infection by Verticillium dahliae and increased yield of raw cotton. Tronsmo (1981) obtained biological control of Botrytis cinerea on strawberry and apple by spraying flowers with a conidial suspension of Trichoderma sp. in field experiments but satisfactory control was not obtained every year. Elad et al. (1982) reported the control of Rhizoctonia solani

in cotton by seed coating with Trichoderma sp. spores. In the field experiments reduction in disease severity of cotton was 47-60% equalling that obtained with PCNB (Quintazene) fungicide.

Trichoderma hamatum:

Trichoderma hamatum was reported to control Pythium ultimum causing damping off of seedlings of different crops (Leach, 1947; Baker, 1957; Olsen, 1964; Waterhouse, 1968; Chet and Baker, 1980; Harman et al.,1980). Inoculation of pea and radish seed or infestation of soil with T. hamatum helped in protection against P. ultimum. Bisiach et al. (1985) reported the reduction of incidence of Botrytis cinerea on grape in the field by 4 to 6 applications of conidial suspension of T. hamatum from flowering time. The effect varied greatly with weather conditions, cultivar and vineyard.

Trichoderma harzianum:

Lee and Wu (1979) reported plasmolysis of hyphae of Sclerotinia sclerotiorum by T. harzianum in vitro. Trichoderma harzianum grew more rapidly and covered colonies of S. sclerotiorum in culture. Venkatasubbaiah et al. (1984) studied the interaction of T. harzianum with Rhizoctonia solani, the incitant of collar rot of coffee seedlings. Seed

treatment with T. harzianum significantly reduced disease incidence. Soil incorporation of T. harzianum gave even better results.

Pal and Chaudhuary (1975) observed a necrotrophic type of mycoparasitism between T. harzianum and Rhizoctonia solani in Crotalaria juncea plants. Pottle et al. (1977) reported the biological control of wound hymenomycetes in Acer rubrum by T. harzianum. Agrawal et al. (1978) studied the antagonistic effect of T. harzianum against Sclerotium rolfsii causing collar rot of lentil. In pot experiments the antagonist controlled seedling death due to S. rolfsii. Cultures were more effective when applied to seed rather than soil. Abd-Ed-Moity and Shatla (1981) reported biological control of white rot disease of onion due to Sclerotium cepivorum by T. harzianum. Application of mycelium and spores of T. harzianum decreased infection of onions in pots, glass house pots and in the field. Elad et al. (1981) reported the biological control of Rhizoctonia solani in strawberry fields by T. harzianum. The disease severity in daughtef plants was reduced by 18-46% in nursery plots treated with preparations of T. harzianum. Plants treated with T. harzianum and transferred to the commercial field gave a 21-37% increase in early yield. Marois et al. (1981) reported a decreased incidence of crown rot of tomato caused by Fusarium sp. under field conditions

by applying conidial suspension of T. harzianum to the roots and crown of tomato plants at the time of planting. Schulz (1981) investigated the biological control of Cytospora persoonii and C. cineta causing cankers on peach, plum and cherry trees. Good inhibition of the pathogen was obtained by the prophylactic treatment of trees with T. harzianum. Treatment of established cankers was unsuccessful. Smith et al. (1981) postulated the mechanism of biological control of Fomes connatus in red maple wounds treated with T. harzianum. It was postulated that T. harzianum increased phenol content of the plant that inhibited the decay fungus, F. connatus. Bisiach et al. (1985) reported the reduction of incidence of Botrytis cinerea on grape in the field by 4-6 applications of conidial suspension of T. harzianum from flowering time. The effect varied greatly with weather conditions, cultivar and vineyard. Strashnov et al. (1985) reported 43% and 85% reduction of fruit rot of tomato due to Rhizoctonia solani by the application of T. harzianum to soil and by coating tomato fruits respectively under lab conditions. When mixed with naturally infested soil, T. harzianum reduced the fruit rot by 27-51% in tomato.

Trichoderma viride:

Sychev and Shaposhnik (1982) found the inhibition of growth of Rhizoctonia solani, the pathogen of Cucumis sativum, on Czapek-Dox medium, by Trichoderma viride. The culture filtrate less than 10% was found enough for the biocontrol

of the pathogen. Gokulapalan and Nair (1984) studied the antagonistic effect of T. viride on Rhizoctonia solani causing rice sheath blight and found that T. viride was antagonistic to the pathogen in vitro. Webber and Hedger (1986) reported the antagonistic effect of T. viride on Ceratocystic ulmi both in vitro and in vivo.

There are a number of reports where Trichoderma viride was used for the biological control of plant pathogens. T. viride was reported to act as a biocontrol agent against Armillaria root rot (Armillaria mellea) of shade trees, fruit trees etc.(Heald, 1933; Munnecke et al. 1976; Rishbeth, 1979). Musatova and Kirik (1977) reported that the introduction of T. viride into the soil limited infection of winter wheat by Fusarium sp. increasing yield. Nikitina et al. (1977) investigated the effect of culture liquids of T. viride on the growth development and infection of maize by Ustilago maydis. Antibiotics from the fungus caused degeneration and disentegration of the pathogen. Dubos et al. (1978) studied the effect of T. viride on Botrytis cinerea and Phomopsis viticola. In vineyard trials at Bordeaux, a suspension of T. viride culture on oat agar, diluted to 10^8 spores/ml had an efficacy of 70% against B. cinerea and 80% against P. viticola. Morshchatskii (1978) reported that dusting winter wheat seeds with a preparation of T. viride decreased infection by common bunt (Tilletia caries) by 3.31 to 16.65%. Antagonistic nature of T. viride against

Helminthosporium sativum infecting wheat seedlings was reported by Prasad et al. (1978). There was only 14.3% infection of seedlings when wheat seeds were treated with suspension of T. viride as compared with 96.7% in water controls. Yuldashev (1978) reported decreased Verticillium wilt of cotton due to Verticillium dahliae by the introduction of T. viride into soil after maize crop followed by another intermediate crop. Dumitras and Fratilescu-Sesan (1979) reported the in vivo protection of beet and cotton seedlings against Pythium debaryanum by T. viride as effectively as fungicidal products. Rebenko et al. (1979) reported that treatment of Odessa 46 winter barley with T. viride increased resistance and reduced incidence of the disease in trials using inoculation of plants with Fusarium oxysporum, Helminthosporium sativum and Ustilago hordii. Dumitras and Sesan (1980) studied the antagonistic effect of 39 isolates of T. viride against Rhizoctonia solani on sugar beet, bean (Phaseolus vulgaris) and cotton seedlings. In biological treatments of seeds and soils in the glasshouse, the results were similar to those obtained by chemical method. Ubaidullaev(1980) reported the use of T. viride with fertilizers for the biological control of Verticillium dahliae infecting cotton. In field trials, the yield was the highest when spore preparation of T. viride at 450 mg/m^2 was introduced into the soil with superphosphate after ploughing in Autumn or before sowing in spring. The effects of the soil preparations remained noticeable in the third year.

Schulz (1981) investigated the biological control of Cytospora persoonii and C. cincta causing cankers on peach, plums and cherry trees. Good inhibition of the pathogen was obtained by the prophylactic treatment of trees with T. viride. T. viride showed the best effect four days after application. Treatment of established cankers was unsuccessful. Dubos et al. (1982) also reported that homogenized cultures of a strain of T. viride sprayed on clusters of grape vine were nearly as effective as dichlofluanid against Botrytis cinerea, early treatments around flowering being particularly important. Application of T. viride at the start of flowering prevented saprophytic establishment of B. cinerea. Bisiach et al.(1985) reported the reduction of incidence of Botrytis cinerea on grape in the field by 4-6 applications of conidial suspensions of T. viride from flowering time. The effect varied greatly with weather conditions cultivar and vineyard.

2.7.2.4 Other fungi:

Rishbeth (1963), Artman (1972) and Greig(1984) reported the biological control of Heterobasidion annosum, causing root rot of pine by Peniophora gigantea. Freshly cut, nearly sterile stump surfaces were inoculated with the antagonist P. gigantea which inhibited the growth of H. annosum. Poleshchuk and Yakimov (1986) reported Peniophora gigantea and Pleurotus ostreatus as the most promising fungi

for the biological control of H. annosum infecting pine stands. The cut stumps of pines were treated with a paste of mycelium of the antagonists.

Liang and Li (1982) found that in field plots of Pinus massoniana, prior occupation of the wound surfaces of new shoots by Pestalotia cryptomeriae could prevent subsequent infection by Diplodia pinea and spraying a culture suspension on slightly infected trees gave significant control.

7.3 Biological control of Ceratocystis sp.

Biological control by bacteria:

Myers and Strobel (1983) reported Pseudomonas syringae as a microbial antagonist of Ceratocystis ulmi in the apoplast of American elm when specific isolates of P. syringae were introduced into the apoplast of green house grown elm seedlings, they suppressed the development of vascular discolouration due to C. ulmi. Schuffer (1983) studied the antagonistic effect of Pseudomonas sp. against C. ulmi causing Dutch elm disease. Elms treated with bacteria by low pressure injection into trees remained healthy throughout two growing seasons.

Biological control by fungi:

Gibbs (1980) reported that if Ceratocystis piceae was introduced with C. fagacearum to wounds on healthy Quercus ellipsoidalis, it had no influence upon subsequent development

of oak wilt, but if it was introduced 24 hrs. before C. fagacearum, it prevented infection. Webber and Gibbs (1984) reported Phomopsis oblonga as the more active colonizer of the inner bark of Wych elm (Ulmus glabra) showing Dutch elm disease than in other elms and thus as the potential disease controlling agent against C. ulmi in North and West England and Scotland. Gemma et al. (1984) tested fourteen isolates of entomogenous fungi in laboratory against two strains of C. ulmi, the causal agent of Dutch elm disease. Of the fourteen, eleven inhibited one or both the strains of C. ulmi. Metarrhizium anisopliae was found as the most inhibitory fungus to C. ulmi. Ricard (1973) treated 15 elms with T. viride pellets by inserting the pellets into the trunks of the diseased tree, every 10 cm. around the circumference. There was no progress of the disease due to C. ulmi during three growing seasons.

Webber and Hedger (1986) compared the interactions between Ceratocystis ulmi and elm bark saprobes in vitro and in vivo. Of the nineteen fungal spp. isolated from the elm bark, sixteen fungi viz. Fusarium lateritium, F. sambucinum, F. solani, Gliocladium roseum, Penicillium putterillii Phomopsis oblonga, Trichoderma polysporum, T. viride etc. significantly reduced the growth of C. ulmi during competitive interactions in dual culture. Though these saprobes inhibited

the growth of the pathogen to varying degrees *in vitro*, only *Glocladium roseum*, *Trichoderma polysporum* and *T. viride* were found as antagonistic to *C. ulmi in vivo*.

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3. MATERIALS AND METHODS

3.1. Isolation of fungi from the diseased coconut palm and soil from palm basin and identification:

3.1.1 Isolation of fungi from the bark tissues of diseased palms:

The affected bark tissues were collected separately from each coconut palm from different places of Kerala State and one place in Karnataka State. From Kerala, tissue samples were collected from Kayangulam, Pilicode, Uduma, Kotikulam, Kallangai, Kudlu, Madhur, Shiria and Central Plantation Crops Research Institute, Kasaragod garden (Two locations - Campus garden and Hill Block garden). From Karnataka, samples were collected only from one garden in Puttur Taluk. Depending upon the availability, bark samples were collected from both young and old lesions on the trunk. Bark samples from lesions of palms treated with coal tar and neem-oil cake were also collected separately and suitably labelled. In each case, locality, age of the palm, type of lesion etc. were recorded.

3.1.1.1 Method of sample collection:

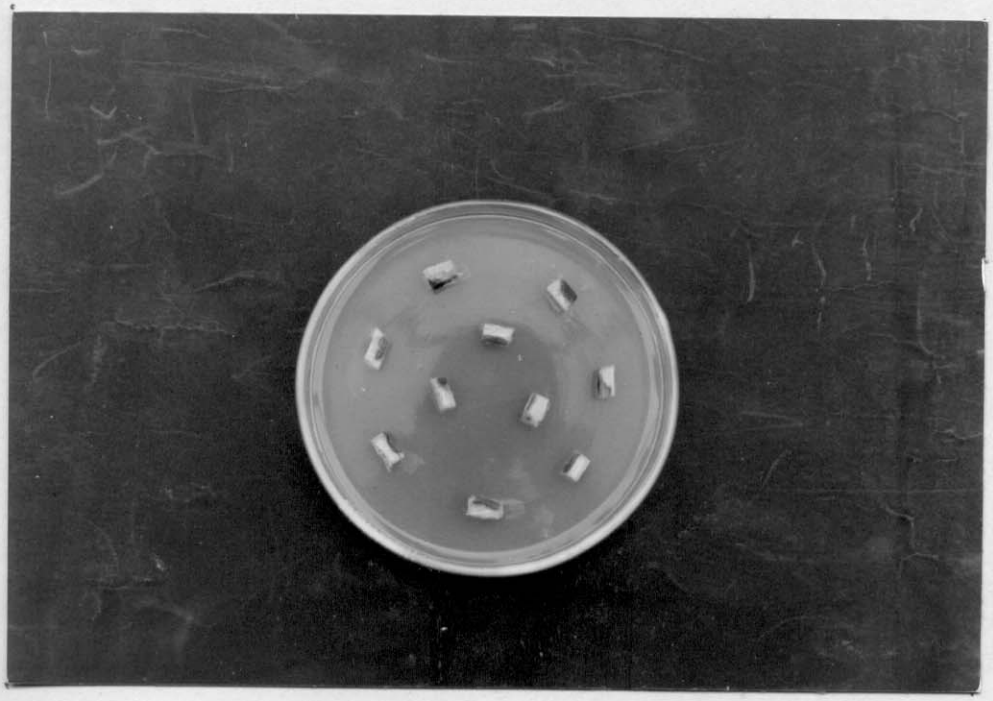
Bark samples were collected by chiselling the bark at the region of the lesion as identified by the presence

of bleeding symptoms. These bark samples were collected in field separately in fresh polythene bags, suitably labelled and brought to the laboratory. From each site five samples were collected.

1.1.2. Isolation of fungi:

From the margins of lesions small rectangular pieces of bark tissue (10 mm x 2mm x 2mm) were cut out with a scalpel. These were used as source of inoculum for isolation of different fungi. These pieces were surface sterilised with 0.1 percent Mercuric Chloride solution for one minute and rinsed in three changes of sterile distilled water. These bits were plated on Potato Dextrose Agar(PDA) medium in sterilised petridishes of 9 cm. diameter at 10 pieces per plate. (Plate-1). 10 mg of Dicrystisin was added to each plate before pouring the medium into the petridish to avoid bacterial growth. The plates were incubated at 23°C for 3-7 days as found necessary. The growth of different fungi from the inoculum source was observed at an interval of 24 hrs. Isolation and identification of each fungus from the bark samples collected from different localities were done. Pure cultures of each isolate of the fungi were maintained on PDA slants with appropriate labels for further use.

Plate-1. Diseased coconut bark tissues kept for isolation



3.1.1.3 Preparation of Potato Dextrose Agar(PDA) medium:

Chemical composition (HiMedia)

The dehydrated PDA medium (HiMedia) used for the preparation of 1000 ml of medium contains the following ingrediants:

Evaporated potato infusion	: 4.0 g
Dextrose	: 20.0 g
Agar	: 15.0 g

Dehydrated Potato Dextrose Agar (HiMedia) was used for preparing the medium. The medium consisting of 39g of dehydrated PDA powder was dissolved in thousand ml of distilled water and boiled for sometime till the powder completely dissolved in water. 100 ml of this medium was added to 250 ml conical flasks and plugged with nonabsorbent cotton. The medium was sterilised in an autoclave at 6 kg (15 lb) pressure for 15 minutes. The molten medium was poured into sterilised petridishes of 9 cm diameter after the addition of 10 mg of Dicrystisin.

3.1.2 Isolation of fungi from the soil in the palm basin:

Soil samples were collected from the basins of stem bleeding affected coconut palms from all the localities from which bark samples were also collected. They were individually labelled. Soil samples were collected under strictly aseptic conditions. The sample was taken from the top 15 cm of the soil after scraping off

of the surface layer of undecomposed organic matter and debris. Five samples were taken at random from each of the palm basin and each sample was put in a clean fresh polythene bag. In the laboratory they were mixed thoroughly so as to obtain a composite sample and air dried for 24 hours. These samples were used for isolating fungi adopting 'soil plate method' of Warcup (1950).

3.1.2.1. Soil plate method (Warcup, 1950):

In this method 50 mg of the soil sample was weighed and transferred into a sterile petridish of 9 cm diameter containing 10 mg of Dicrystisin. 20 ml of PDA medium was poured to the petridish when it is warm and shaken gently to disperse the soil particles and Dicrystisin uniformly throughout the medium. Five replications were maintained for each soil sample. The plates were incubated at 23°C for 2-5 days. For every 24 hours, fungal growth was observed and fungal colony was isolated and the cultures maintained on PDA slants with appropriate labels for further use during *in vitro* studies. Wherever possible identification was also simultaneously done.

3.1.3 Isolation of fungi from root samples:

Root samples showing brown lesions and decay were collected from diseased palms. Root samples were collected only from one place in Kerala viz. Shiria where the roots showed extensive decay. Such roots were brought to the laboratory in fresh polythene bags. Root bits of one cm length were cut and placed

in petridishes lined with moistened sterile filter paper to serve as a humid chamber. Ten pieces of root sample were kept in each petridish and incubated at 23°C for 3-7 days. Growth of fungus, if any, from root bits was watched at 24 hrs. interval

3.1.4 Identification of fungi:

Wherever possible, identification of fungi was done simultaneously with isolation, using standard books and keys like 'Illustrated genera of Imperfect fungi' by Barnett(1960); 'A manual of soil fungi' by Gilman (1967); 'The fungi - an adva treatise' by Ainsworth, Sparrow and Sussman (1973) etc. Some unidentified cultures were got identified at Indian Agricultura Research Institute, New Delhi. Some cultures identified by me were sent for confirmation to Indian Agricultural Research Institute, New Delhi.

3.2 Purification of T. paradoxa and other fungi and maintenance of pure cultures on slants:

All the isolates of T. paradoxa and other fungi isolated from bark, root and soil samples were purified by 'single hyphal tip isolation method' and maintained in pure state on PDA slants with appropriate label.

3.3 Multiplication of T. paradoxa on host tissue:

Multiplication of each isolate of T. paradoxa was done on sterilised coconut inflorescence rachilla bits as described

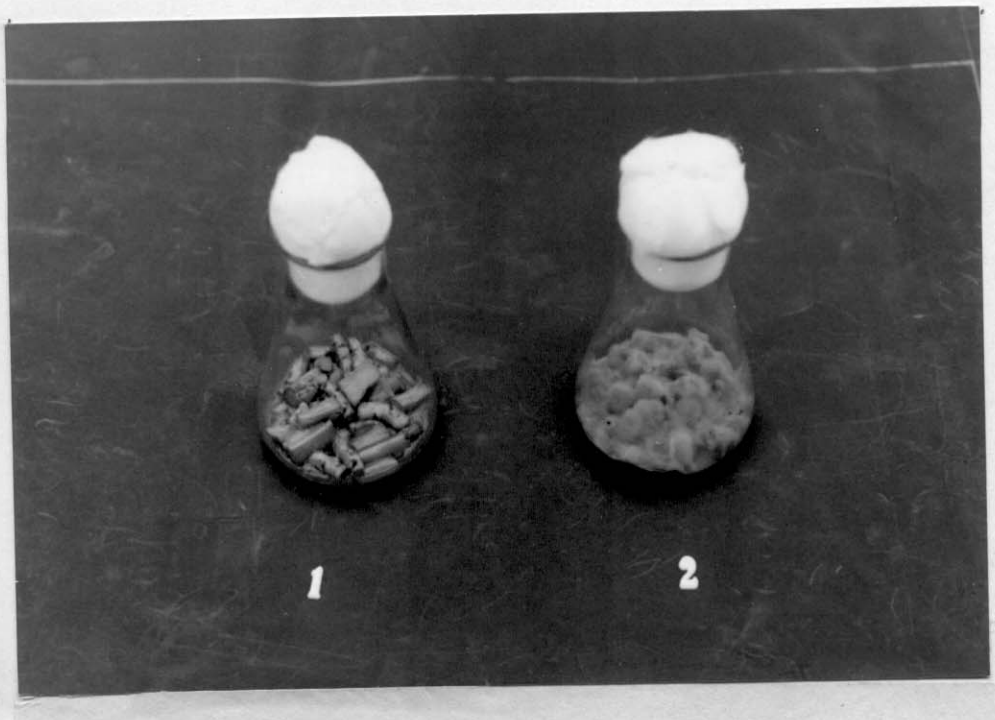
by Nambiar et al.(1986) with suitable modification. About twenty five rachilla bits of 1.5 cm length were taken in 250 ml conical flask along with five ml of two percent dextrose solution and plugged with nonabsorbent cotton. Sterilisation was done by autoclaving at 6 kg (15 lb) pressure for 15 minutes. A few hyphae of T. paradoxa picked up from the pure culture of the isolate was inoculated on the sterilised rachilla bits and incubated at 23°C for 2-5 days. Each isolate of T. paradoxa was multiplied in this method (Plate 2) and later maintained in pure state on PDA slants for further in vitro studies.

3.4 Cultural and morphological characters of isolates of T. paradoxa.

Before doing the in vitro study of the interaction of the antagonistic mycoflora with T. paradoxa, cultural and morphological characters of different isolates of T. paradoxa were studied with a view to determining whether the different isolates vary in their morphological and cultural characters from one another. To determine the best media in which the different isolates of T. paradoxa gave maximum growth, eight different growth media were used. These media were the following:

i) Potato Dextrose Agar
ii) Corn Meal Agar
iii) Oat Meal Agar
iv) Coconut Tissue Extract Agar

Plate-2. Multiplication of T. paradoxa inoculum on coconut rachille



1. Sterilised coconut rachillae bits.
2. Growth of T. paradoxa on sterile coconut rachillae bits (Three days after inoculation)

v) Oak Wilt Agar-A

vi) Oak Wilt Fungus Agar-C

vii) Dextrose Asparagine Phosphate Agar.

viii) Kirchoff's Agar.

3.4.1 Preparation of different media.

3.4.1.1 Potato Dextrose Agar (PDA) medium.

The preparation of PDA has been explained elsewhere (Please refer. 3.1.1.3)

3.4.1.2 Corn Meal Agar (C M A) medium

Chemical composition (HiMedia)

The dehydrated C M A medium (HiMedia) used for preparation of 1000 ml of medium contains the following ingredients.

Evaporated Corn Meal infusion	:	2.0 g
Agar	:	15.0 g

17 g of Corn Meal Agar dehydrated powder (HiMedia) was dissolved in 1000 ml of distilled water and boiled for sometime till the powder completely dissolved in water. 100 ml of the medium was poured into 250 ml conical flasks and plugged it with nonabsordent cotton. Sterilisation was done by autoclaving at 6 kg (15 lb) pressure for 15 minutes and then plated into sterilised petridishes of 9 cm diameter

3.4.1.3 Oat Meal Agar (O M A) medium:

Chemical composition (HiMedia)

The dehydrated O M A medium (HiMedia) used for the preparation of 1000 ml of medium contains the following ingredients:

Oat malt	: 20.0 g
Agar	: 18.0 g
$Fe\ So_4$	: 1 mg
$Mn\ Cl_2$	: 1 mg
$Zn\ So_4$	: 1 mg

38 g of dehydrated power of Oat Meal Agar (HiMedia) was dissolved in 1000 ml of distilled water and boiled till the powder completely dissolved in water. 100 ml of medium was poured to 250 ml conical flasks and sterilised in an autoclave as done earlier. Then the medium was plated into sterile petridishes of 9 cm diameter.

3.4.1.4 Coconut Tissue Extract Agar (CTEA) medium:

Chemical composition

Coconut bark tissue	: 250.0 g
Dixtrose sugar	: 20.0 g
Agar	: 20.0 g
Distilled water	: 1000 ml

About 250 g of coconut bark tissue collected from healthy palms were cut into small pieces. These pieces were boiled with 250 ml distilled water and crushed in

mixer-grinder. The extract was prepared by filtering the crushed tissue through muslin cloth. To this extract 20 g of Dextrose sugar and 20 g of agar powder were added and the volume was made to 1000 ml with distilled water. The medium was sterilised by autoclaving at 6 kg (15 lb) pressure for 15 minutes and then plated to sterile petridishes of 9 cm diameter.

1.5 Oak Wilt Agar-A (O W A-A) medium:

Chemical composition (HiMedia)

The dehydrated O W A-A medium (HiMedia) used for the preparation of 1000 ml of medium contains the following ingredients:

Yeast Extract	: 0.5 g	$MgSO_4$	: 0.5 g
Casamino acids	: 1.0 g	$ZnSO_4$	: 0.2 mg
Maltose	: 5.0 g	$FeSO_4$	: 0.2 mg
Inositol	: 5.0 mg	$MnSO_4$	: 0.1 mg
Pyridoxine	: 0.1 mg	Agar	: 20.0 g
Monopotassium phosphate	: 1.0 g		

28 g of Oak Wilt Agar-A dehydrated powder (HiMedia) was dissolved in 1000 ml of distilled water and boiled to dissolve the powder completely. Autoclaving was done at 6 kg (15 lb) pressure for 15 minutes and the medium was plated to sterile petridishes of 9.0 cm diameter.

3.4.1.6. Oak Wilt Fungus Agar-C(OWA-C) medium:

Chemical composition (HiMedia)

The dehydrated OWA-C medium (HiMedia) used for the preparation of 1000 ml of medium contains the following ingredients:

Malt Extract	: 17.0 g
Mycological peptone	: 3.0 g
Ox gall	:15.0 g
Agar	:15.0 g

50 g of dehydrated powder of Oak Wilt Fungus Agar-C (HiMedia) medium was dissolved in 1000 ml of distilled water and boiled for complete dissolution. The medium was sterilised by autoclaving at 6 kg (15 lb) pressure for 15 minutes and plated into sterile petridishes of 9.0 cm diameter.

3.4.1.7 Dextrose Asparagine Phosphate Agar (DAPA) medium:

Chemical composition

Dextrose Glucose	: 30.0 g
Asparagine	: 1.0 g
$Mg\ So_4\ 7\ H_2O$	: 0.5 g
$KH_2\ PO_4$	: 1.5 g
Agar	: 20.0 g
Distilled water	: 1000 ml

The contents were dissolved in 1000 ml of distilled water and boiled for complete dissolution of the contents. Sterilisation was done by autoclaving at 6 kg (15 lb) pressure for 15 minutes and the medium was poured into sterile petridishes of 9.0 cm diameter.

3.4.1.8 Kirchoff's Agar (KA) medium

Chemical composition

Sucrose	: 100.0 g
Asparagine	: 1.0 g
Mg SO_4 $7H_2O$	: 0.25 g
KH_2PO_4	: 1.0 g
Agar	: 20.0 g
Distilled water	: 1000 ml

The contents were dissolved in 1000 ml of distilled water and boiled for complete dissolution. Sterilisation was done by autoclaving at 6 kg (15 lb) pressure for 15 minutes and then poured to sterile petridishes of 9.0 cm diameter.

3.4.2 Effect of different culture media on different isolates of T. paradoxa.

The above mentioned eight different growth media were used for this study. A total of six isolates of T. paradoxa were studied. Each isolate of T. paradoxa was grown first on PDA medium to prepare the fungus inoculum. Discs of the fungus

from the margin of the growing colony (5 days old colony in slow growing isolates and 2 days old colony in fast growing isolates of T. paradoxa) were removed using sterile cork borer of 5 mm diameter and one disc each was placed at the centre of each growth medium in petridishes. Four replicates of each growth medium were maintained and incubated at 23°C. For every 24 hrs. time interval, colony and morphological characters were recorded. This study was carried out mainly to find outwhether there was any variability in the different isolates and how much they differed in the different colony and morphological characters.

3.4.2.1 Colony characters:

Colony characters like colony diameter, colony margin, colony colour, colony reverse colour, elavations of colony and odour of colony were recorded at regular intervals. The colony diameters at two planes at right angles to each other were measured and the mean diameter of the colony was recorded in all the four replicates and presented as mean values of the four replicates. The observations were recorded for each growth medium. The medium in which each of the fungal isolates first filled the entire petridish was observed. This medium was selected for further in vitro studies of interaction of other fungi with that particular T. paradoxa isolate.

3.4.2.2 Morphological characters:

Study of mycelial characters (colour of hyphae, diameter of hypha, presence of hyphal rings etc.), conidia and chlamydospore of each isolate of T. paradoxa in all the growth media was done. Occurrence, shape, size, colour, position and time of formation of conidia and chlamydospores in each isolate of T. paradoxa in each of the growth medium were observed and the results were recorded. The cultures were kept till spores were produced and recorded. The culture which did not form spores initially was kept for two months for observation of spore formation if any.

3.4.2.3 Variability of different isolates of T. paradoxa in each culture medium:

Variability in culture characters and rate of growth of isolates of T. paradoxa in each growth medium was studied in the above experiment and the results were compared.

3.5 In vitro studies on the interaction of antagonistic mycoflora with T. paradoxa isolates:

3.5.1 Interaction of associated mycoflora with T. paradoxa isolates:

The fungi isolated from the lesions of stem bleeding affected palms and/or from the palm basin soil were used for the in vitro studies for their interaction with the

different isolates of T. paradoxa. Among the various fungi isolated, eighteen isolates of fungi viz. Acremonium sp (two isolates), Aspergillus niger, A. terreus, Chalaropsis sp. Paecilomyces varioti, Penicillium citrinum, P. diverseum, P. janthinellum (two isolates), Pestalotiopsis palmarum, Trichoderma harzianum (two isolates), T. viride (three isolates) and two isolates of unidentified sterile fungi were used for the interaction studies with T. paradoxa isolates. Among tne six isolates of T. paradoxa, only four were used for this study, the other two being shy growers in culture.

Each fungus used for antagonistic study, was grown on PDA plates in sterile petridishes. T. paradoxa isolate No.2 was grown on Kirchoff's Agar (KA) medium in which it was found to grow faster than in other growth media. T. paradoxa isolate Nos. 3 and 5 were grown on Dextrose Asparagine Phosphate Agar (DAPA) and isolate No.6 on PDA plate in which they grew faster than in other media.

To investigate the in vitro interaction of these antagonistic fungi with the isolates of T. paradoxa, 'Direct opposition method' used by Webber and Hedger (1986) was adopted as detailed below.

5.1.1 Direct Opposition Method (Webber and Hedger, 1986)

Dual cultures of T. paradoxa isolate No.2 and the respective saprobe were established at 3.5 cm apart from each other on Kirchoff's Agar (KA) medium in sterile petridishes of 9 cm diameter. Using sterile cork borer of 5 mm diameter, the fungus inoculum discs were cut separately from the edge of two days old cultures of T. paradoxa isolate No.2 and other test fungi.

The fungal disc (T. paradoxa isolate-2) and the saprobe (test fungus) disc were placed at 3.5 cm distance from each other at the centre of the petridish containing KA medium (Plate 3.). The discs were placed in such a way that the fungus side of the disc was touching the surface of the growth medium. Five replicates of each T. paradoxa - saprobe duel culture were maintained and incubated at 23°C temp. for seven days in darkness. Radial growth was measured in two opposite directions at 24 hr. intervals so that the growth of the fungus towards the saprobe vis a vis the opposite direction (control) could be determined. From these measurement the percentage of inhibition of T. paradoxa isolate No.2 caused by each saprobe was found out.

The same 'Direct Opposition Method' was also followed in case of other three isolates of T. paradoxa viz. isolate nos. 3, 5 and 6. For getting the fungal disc of these isolates, two days old cultures were used.

Interactions between the opposing colonies of T. paradoxa and the saprobes were also visually assessed after a further seven days incubation using a key based on the observations of Webber and Hedger (1986). The key consists of the following characters:-

1. Intermingling of the colonies with no macroscopic or microscopic signs of interaction(I)
2. Overgrowth of T. paradoxa colony by opposing fungi, usually accompanied by inhibition of T. paradoxa on or shortly after contact (O)

Plate-3. Testing of antagonistic fungi against *T. paradoxa* for growth inhibition.

(Note: In petridish *T. paradoxa* is on left side and test fungus is on right side.)

3. Mutual inhibition of the growth of the opposing colony with eventual contact with no apparent intermingling, a band of pigmented mycelium (zone) often developing at the junctions of the colonies.(M.P.).

4. Mutual inhibition of both the colonies with no apparent intermingling, a clear zone remaining between the colonies.(MC).

5. Extreme inhibition of T. paradoxa colony at a distance of 5 mm or more, little or no reciprocal inhibition.(E).

3.5.2 Interaction of antagonistic mycoflora with T. paradoxa isolated:

From the studies on interaction of associated mycoflora with the isolates of T. paradoxa, fungi, antagonistic to T. paradoxa were found out. The interaction of each of these antagonistic fungi, with each isolate of T. paradoxa was further studied in three different conditions namely-

1. When T. paradoxa was inoculated 12 hrs. before the antagonistic fungus.
2. When both T. paradoxa and the antagonistic fungus were simultaneously inoculated and
3. When T. paradoxa was inoculated 12 hrs. after the inoculation of antagonistic fungus.

For this study also 'Direct Opposition Method' (Webber and Hedger 1986) was used. The dual cultures were kept for 15 days after inoculation and the results were tabulated.

3.6. In vitro studies on the effect of neem-oil and neem-oil cake on T. paradoxa.

3.6.1 Effect of neem oil cake on T. paradoxa.

To find out the effect of neem oil cake on T. paradoxa, T. paradoxa isolate No.5 was used. The fungus was grown on PDA plates to serve as the inoculam source. To three separate conical flasks containing 100 ml PDA medium, 1000 mg., 100 mg and 10 mg respectively of neem oil cake powder were added, and autoclaved as mentioned before. Conical flask containing PDA without neem oil cake served as control. After autoclaving, the media were dispersed into separate sterile petridishes and suitably labelled. Using a sterile cork borer of 5 mm diameter, the fungus discs from the margin of growing colony of T. paradoxa were taken and one disc is placed at the centre of each plate with the fungus side of the disc touching the growth medium. The plates without the neem oil content were taken as control. Four replicates of each set were maintained and the plates were incubated at 23°C for four days. Colony dimensions in each case were measured at two planes for every 24 hrs. and the readings were recorded.

3.6.1.1 Effect of neem oil cake extract on T. paradoxa.

T. paradoxa isolate No.5 was used for this experiment. 50g of neem oil cake was dissolved in 100 ml of distilled water. Different aliquots of the extract were mixed with distilled water to obtain different concentrations as follows:

1. 10 ml of extract + 90 ml of distilled water = 50,000 ppm (on oil cake basis).

2. 5 ml of extract + 95 ml of distilled water = 25,000 ppm

3. 1 ml of extract + 99 ml of distilled water = 5,000 ppm.

The above extract was used to prepare PDA medium with 3.9 g of dehydrated PDA powder (HiMedia) added to each flask containing the above extract of 100 ml. All the flasks were autoclaved at 6 kg (15 lb) pressure for 15 minutes and the sterilised medium was poured into separate sterile petridishes of 9 cm diameter and appropriately labelled. As mentioned in para 3.6.1, fungal discs were similarly inoculated here also. Four replicates were taken for each set and incubated at 23°C for four days. The plate without neem oil cake extract served as control. The colony diameters were measured at 24 hrs intervals and growth of T. paradoxa isolate in each case was recorded.

3.6.2 Effect of neem oil on T. paradoxa.

For the study of effect of neem oil on T. paradoxa, isolate No.5 was used. T. paradoxa isolate no.5 was grown on PDA in petriplates of 9 cm diameter. Using sterile cork borer of 5 mm diameter, a disc of the fungus from the growing margin of the colony (2 days old) grown on PDA plate was cut and removed and inoculated at the centre of a second PDA plate of same diameter. Using the above sterile cork borer, four wells were made at four corners of the plate, equidistant (3 cm) from the fungal disc inoculated already.

These wells were fully filled with neem oil (Plate 4) using a sterile pipette and the plates were incubated at 23°C. Suitable control plates with the wells filled with PDA medium were maintained. There were five replicates. The colony diameters in two planes (perpendecular to each other) were measured in each case at 24 hour intervals and and average colony diameter was found.

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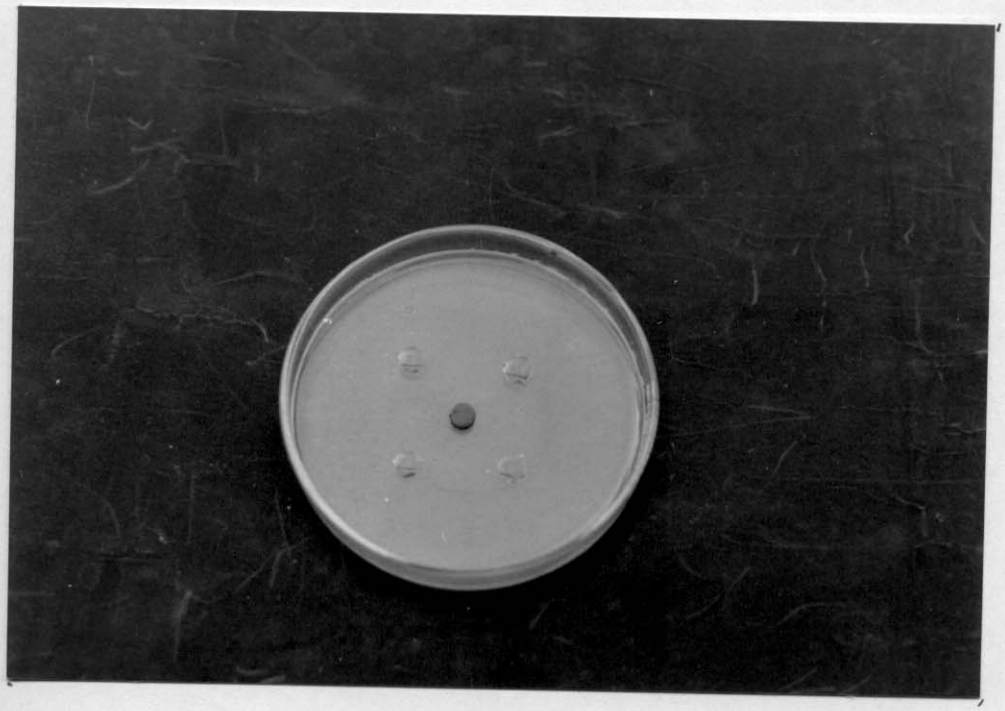
Plate-4. Testing the effect of neem oil on the growth of T. paradoxa.



4. RESULTS

4.1 Isolation of fungi and identification.

4.1.2 Isolation from affected bark tissues:

Tissue samples from the affected bark were collected separately from different localities in Kerala and Karnataka as detailed in 'Materials and Methods', for the isolation of the fungi from palms with stem bleeding disease. A total of sixty three samples were collected from as much stem bleeding palms. The samples comprised young and/or old lesions on the trunk. The various fungi isolated from the bark tissue were identified using standard keys mentioned in 'Materials and Methods'. Some cultures were got identified at Indian Agricultural Research Institute, New Delhi. The different fungi isolated from the bark tissue are enumerated in Table-1.

From Table-1, it can be seen that *Thielaviopsis paradoxa*, the causal agent of stem bleeding disease of coconut, could be isolated only from young fresh lesions with the frequency of isolation ranging from 10-100%. (Plate-5).

Plate-5. Fungal growth on the bark tissues kept for isolation.

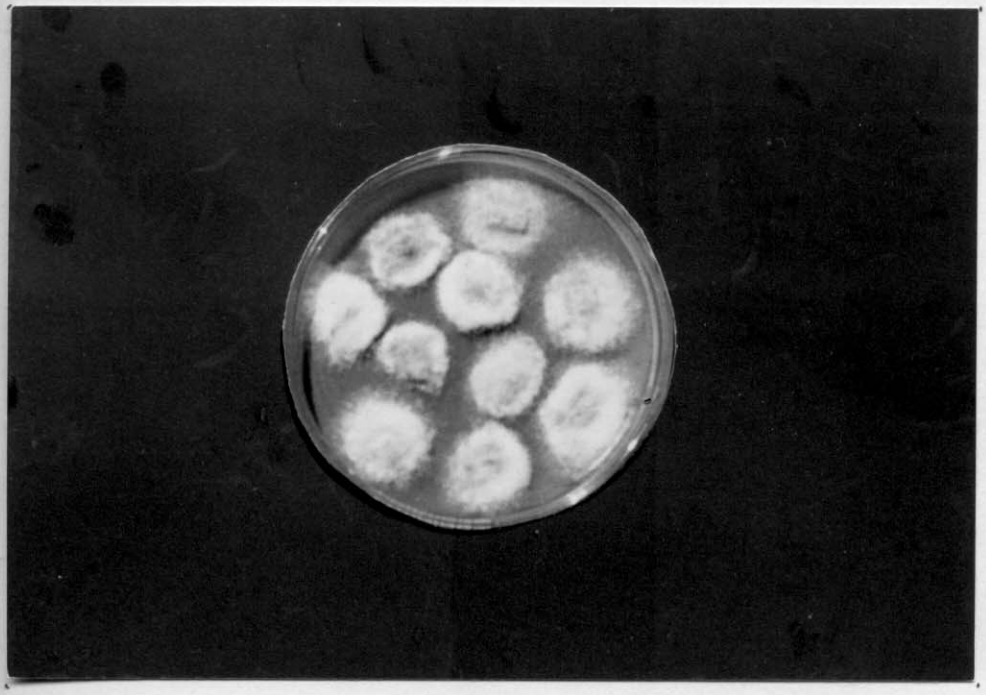


Table 1. Isolation of fungi from lesions of stem bleeding affected coconut palms.

Sample Number	Date of sample collection	Place of sample collection	Age of palm (in years)	Management practices given			Type of lesion	Fungi isolated	Percentage of isolation of T. paradoxa
				CTA	NCA	FA			
1	2	3	4	5	6	7	8	9	10
1.	20.10.86	C:P:C:R. I., C.P.C.R.I., Kasaragod Hill block garden, (Kerala)	30	No	No	Yes	Fresh	Mucor sp., Penicillium citrinum, Thielaviopsis paradoxa.	70
2.	20.10.86	"	"	"	"	"	"	Penicillium citrinum, T. paradoxa.	50
3.	"	"	"	"	"	"	"	Mucor sp., P. diverseum T. paradoxa.	60
4.	"	"	"	"	"	"	"	Aspergillus niger,, Mucor sp. P. citrinum, T. paradoxa.	40
	"	"	35	"	"	"	"	" "	30
	27.10.86	Kudlu (Kerala)	20	Yes	No	No	Old	Acremonium sp.-1., P. janthinellium-1	
	"	"	"	"	"	"	Fresh	P. citrinum	0
	"	"	"	"	"	"	"	Mucor sp., P. citrinum.	0

contd...

1	2	3	4	5	6	7	8	9	10
9.	27.10.86	Kudlu, (Kerala)	20	Yes	No	No	Old	Acremonium sp.-1., Mucor sp. P. citrinum Trichoderma harzianum.	0
10.	29.10.86	Kallangai (Kerala)	15	No	No	No	Old	Acremonium sp.-1., Chalaropsis sp., Mucor sp. P. diverseum	0
11.	"	"	30	"	"	"	Fresh	Mucor sp. T. paradoxa	70
12.	"	"	"	"	"	"	Old	Acremonium sp.-1 Chalaropsis sp., T. harzianum	0
13.	9.12.86	Kayangulam (Kerala)	35	No	No	No	Old	Acremonium sp.-2., Trichoderma viride - 1	0
14.	"	"	"	"	"	"	Fresh	P. janthinellum-2., T. paradoxa	50
15.	"	"	"	"	"	"	"	P. janthinellum-2., T. paradoxa, T. viride -1	20
16.	"	"	50	"	"	"	"	" "	30

contd....

1	2	3	4	5	6	7	8	9	10
17.	9.12.86	Kayangulam (Kerala)	50	No	No	No	Old	Chalaropsis sp. P. diverseum	0
18.	"	"	25	"	"	"	Fresh	Mucor sp., P. diverseum, T. paradoxa	10
19.	"	"	"	"	"	"	Old	P. diverseum, T. viride-1	0
20.	"	"	35	"	"	"	"	P. janthine-llum-2	0
21.	"	"	"	"	"	"	Fresh	P. janthine-llum-2, T. paradoxa.	10
22.	"	"	40	"	"	"	Old	P. diverseum T. viride-1	0
23.	"	"	35	"	"	"	Fresh	P. diverseum T. paradoxa	10
24.	17.12.86	Kallangai (Kerala)	35	No	No	No	Fresh	Mucor sp. P. citrinum Rhizopus sp- T. paradoxa	3 40
25.	"	"	"	"	"	"	Fresh	Mucor sp. P. citrinum Pestalotiopsis palmarum, T. paradoxa.	50
26.	19.12.86	C.P.C.R.I., Kasaragod campus garden (Kerala)	25	No	No	Yes	Fresh	T. paradoxa	100
27.	"	"	"	"	"	"	"	P. diverseum P. palmarum T. paradoxa	

contd....

1	2	3	4	5	6	7	8	9	10
28.	23.12.86	Pilicode, (Kerala)	30	No	No	No	Fresh	P. diverseum P. palmarum T. paradoxa	30
29.	"	"	50	"	"	"	Old	Mucor sp. P. diverseum P. palmarum	0
30.	"	"	"	"	"	"	Fresh	P. diverseum T. paradoxa	10
31.	"	"	35	"	"	"	"	" "	70
32.	"	"	"	"	"	"	"	Acremonium sp-1 P. diverseum T. paradoxa	10
33.	12.5.87	Pilicode, (Kerala)	25	No	No	No	Fresh	Mucor sp. P. citrinum T. paradoxa T. harzianum	10
34.	"	"	"	"	"	"	Old	A. niger, Mucor sp. P. citrinum T. harzianum	0
	"	"	"	"	"	"	Fresh	P. citrinum T. paradoxa	10

contd....

1	2	3	4	5	6	7	8	9	10
36.	15.5.87	Madhur (Kerala)	30	Yes	No	No	Fresh	Acremonium sp-2 A. niger Mycor sp. T. harzianum-1	0
37.	"	"	"	No	"	"	Old	T. harzianum-1	0
38.	"	"	25	"	"	"	"	A. niger T. harzianum-1	0
39.	"	"	"	"	"	"	"	" "	0
40.	"	"	30	"	"	Yes	Fresh	A. niger, A. terreus P. janthinellum-1	0
41.	"	"	"	"	"	No	Old	A. niger, Mucor sp. P. janthinellum-1 T. harzianum-1	0
42.	24.5.87	Puttur (Karnataka)	25	Yes	No	Yes	Old	Acremonium sp.-1 Mucor sp. P. janthinellum-1 T. viride-2	0
43.	"	"	30	No	No	Yes	Old	A. niger, Mucur sp. T. viride-2	
44.	"	"	25	No	No	Yes	Old	Acremonium sp-1 A. niger, P. janthinellum-1	0
45.	"	"	40	"	"	"	"	Acremonium sp-1 A. niger Mucor sp. P. palmarum T. viride-2	0

contd.....

1	2	3	4	5	6	7	8	9	10
46.	30.5.87	Kottikulam (Kerala)	30	Yes	No	No	Old	A. niger P. citrinum	0
47.	"	"	20	"	"	"	"	" "	0
48.	"	"	35	"	"	"	Fresh	" "	0
49.	"	"	25	"	"	"	Old	" "	0
50.	1-6-87	Shiria (Kerala)	25	No	No	Yes	Fresh	T. paradoxa T. viride-3	100
51.	"	"	30	"	"	"	"	A. niger, Mucor sp. T. paradoxa T. viride-3	70
52.	"	"	"	"	"	"	"	A. terreus P. diverseum T. paradoxa T. viride-3	60
53.	"	"	40	"	"	"	"	Mucor sp. T. paradoxa T. viride-3	90
54.	15.6.87	C.P.C.R.I., Kasaragod, Hillblock garden. (Kerala)	30	No	Yes	Yes	Fresh	A. niger, P. citrinum	0
55.	"	"	"	"	"	"	"	T. harzianum-2 A. niger, Mucor sp., P. citrinum	0
56.	"	"	30	"	"	"	"	T. harzianum-2 A. niger, Paecilomyces varioti, T. harzianum-2	0

contd...

1	2	3	4	5	6	7	8	9	10
57.	15.6.87	C.P.C.R.I., Kasaragod Hillblock garden (Kerala)	40	No	Yes	Yes	Fresh	A. niger Mucor sp. P. citrinum T. harzianum -2	0
58.	"	"	35	"	"	"	"	" "	0
59.	18.6.87	Uduma (Kerala)	20	No	No	No	Old	A. niger, P. diverseum	0
60.	"	"	30	Yes	No	No	Fresh	P. diverseum	0
61.	21.7.87	Kudlu (Kerala)	30	No	No	No	Old	A. niger, P. citrinum Rhizopus sp. T. harzianum	0
62.	21.7.87	C.P.C.R.I., Kasaragod Hillblock garden (Kerala)	30	No	Yes	Yes	Fresh	A. niger T. harzianum-2	0
63.	"	"	35	"	"	"	"	" "	0

CTA = Coal tar applied
NCA = Neem cake applied
FA = Fertilizer applied

From old lesions T. paradoxa was not isolated. Only other saprobes viz. Acremonium sp, Aspergillus niger, Chalaropsis sp. Mucor sp. Penicillium citrinum, P. diversum, P. janthinellum Pestalotiopsis palmarum, Rhizopus sp, Trichoderma harzianum T. viride etc. were isolated from old lesions on the bark. Aspergillus niger, Penicillum sp. Trichoderma harzianum and T. viride were predominantly isolated from old lesions. The frequency of isolation of T. paradoxa was more in wet and cool months rather than in summer months.(Table-1). But in irrigated gardens, as in Shiria, there was no such difference. Even in June month very high percentage of isolation (Average 80%) was obtained from fresh lesions.

T. paradoxa was not isolated from the bark samples collected from the palms treated with coal tar or ammended with neem oil cake application, eventhough the lesions were young and fresh. Age of the palm did not show any significant role as far as the isolation of T. paradoxa was concerned. From the fertilizer applied palms, more number of saprobes were isolated (Table-1).

4.1.2 Isolation from palm basin soil:

Isolation of fungi from soil in the palm basin showed that various fungi could be isolated from the soil samples, collected from all the localities (Table-2). The fungi which were isolated from soil were identified by mounting

Table 2. Isolation of fungi from basin soil of stem bleeding affected coconut palms.

Sample Numbers	Date of sample collection	Place of sample collection	Management Practices given		Fungi isolated
			NCA	F.A.	
1	2	3	4	5	6
1 to 5	20.10.86	C.P.C.R.I. Kasaragod Hillblock garden (Kerala)	No	Yes	Aspergillus niger, Mucor sp. Penicillium citrinum Trichoderma harzianum-2
6 to 9	27.10.86	Kudlu (Kerala)	No	No	Acremonium sp-1 Mucor sp., P. citrinum T. harzianum
10 to 12	29.10.86	Kallangai (Kerala)	No	No	Acremonium sp-1 A. niger. Mucor sp. P. diverseum T. harzianum
13 to 23	9.12.86	Kayangulam (Kerala)	No	No	Acremonium sp-2 P. jenthinellum-2 T. viride- 1
24 to 25	17.12.86	Kallangai (Kerala)	No	No	A. niger., Mucur sp P. citrinum
26. to 27	19.12.86	C.P.C.R.I, Kasaragod campus (Kerala)	No	Yes	Mucor sp., Rhizopus sp. P. diverseum T. harzianum

contd....

1	2	3	4	5	6
28 to 32	23.12.86	Pilicode (Kerala)	No	No	Acremonium sp-1 Mucor sp., P. diverseum
33 to 35	12-5-87	Pilicode (Kerala)	No	No	A. niger., Mucor sp., P. citrinum P. diverseum Rhizopus sp. T. harzianum
36 to 41	15-5-87	Madhur (Kerala)	No	No	A. niger Mucor sp., T. harzianum-1.
42 to 45	24-5-87	Puttur (Karnataka)	No	Yes	A. niger. Mucor sp. Paecilomyces sp varioti, T. viride-2
46 to 49	30-5-87	Kottikulam (Kerala)	No	No	A. niger., Mucor sp., P. citrinum T. harzianum
52 to 53	1-6-87	Shiria (Kerala)	No	Yes	Mucor sp. Paecilomyces varioti, P. diverseum T. viride-3
54 to 58	15-6-87	C.P.C.R.I., Kasaragod Hillblock garden (Kerala)	Yes	Yes	A. niger., Mucor sp., P. varioti Rhizopus sp T. harzianum -2

contd.....

1	2	3	4	5	6
59 to 60	18-6-87	Uduma (Kerala)	No	No	P. diverseum P. diverseum T. harzianum
61	21-1-87	Kudlu (Kerala)	No	No	A. niger., P. citrinum Rhizopus sp. T. harzianum
62 to 63	21-7-87	C.P.C.R.I., Kasaragod Hillblock garden (Kerala)	Yes	Yes	A. niger., Mucor sp., P. varioti T. harzianum -2

NCA -= Neem cake applied

FA = Fertilizer applied

them in lactophenol stain and observing under the microscope. For identification standard text books and keys mentioned in 'Materials and Methods' were used. Some cultures were got identified from Indian Agricultural Research Institute, New Delhi.

T. paradoxa could not be isolated from any of the soil sample. Various other fungi isolated from soil are Acremonium sp. Aspergillus niger, Mucor sp, Paecilomyces varioti, Penicillium citrinum, P. diverseum, P. janthinellum, Rhizospus sp Trichoderma harzianum, and T. viride.

From more than 90% of the soil samples, species of Trichoderma (T. harzianum and T. viride) were predominantly isolated and identified. A.niger was also isolated from many soil samples. The occurrence of A. niger and Trichoderma sp. was found more in the soil samples collected from the basin soil of diseased coconut palms which were ammended with neem oil cake and fertilizer application.(Table-2).

4.1.3 Isolation from root samples.

Root samples were collected from one locality namely Shiria, where the roots showed extensive decaying symptoms. When these samples were kept for fungal isolation in petri-dishes lined with moistened sterile filter paper for 5 days at 23°C, fungal growth was noticed emerging from the decayed pieces of root samples. The fungus was identified as T. paradoxa.

Table 3: Isolation of fungi from root samples of stem bleeding affected coconut palm.

Sample Number	Date of sample collection	Place of sample collection	Age of palm (in years)	Management practices given			Fungi isolated	% of isolation of T. paradoxa
				CTA	NCA	FA		
1	1-6-87	Shiria (Kerala)	25	No	No	Yes	T. paradoxa	10
2	"	"	30	"	"	"	"	50
3	"	"	30	"	"	"	"	10
4	"	"	40	"	"	"	"	20

CTA = Coal tar applied
NCA = Neem cake applied
FA = Fertilizer applied

From all the four root samples, T. paradoxa could be isolated (Table-3). No other fungi was found to grow from the root samples.

4.2 Purification of T. paradoxa and other fungi:

T. paradoxa and other fungi were purified by the 'Single hyphal tip isolation method' as described in 'Materials and Methods' and maintained on PDA slants.

4.3 Multiplication of T. paradoxa and other fungi.

T. paradoxa was alone multiplied on coconut rachillae, (Plate-2) (vide Materials and Methods) and other fungi on PDA.

4.4 Cultural and Morphological characters of isolates of T. paradoxa.

Before doing the in vitro study of the interaction of saprophytic mycoflora with T. paradoxa, cultural and morphological characters of different isolates of T. paradoxa were studied with a view to understanding the wide variability prevalent among T. paradoxa isolates for these characters. A total of six isolates of T. paradoxa from six different localities of Kerala namely Kayangulam, Pilicode, Kallangai, Central Plantation Crops Research Institute, Kasaragod Campus Garden and C.P.C.R.I., Kasaragod-Hill Block Garden, and Shiria, were taken for this type of study and they were named as T. paradoxa isolate No.1, 2, 3, 4, 5 and 6 respectively.

4.4.1. Effect of different culture media on the isolates of T. paradoxa.

The rate of growth and other morphological characters of six isolates of T. paradoxa on eight culture media was studied in petridishes. The six isolates were the following

i) T. paradoxa isolate No.1(from Kayangulam)

ii) T. paradoxa isolate No.2(from Pilicode)

iii) T. paradoxa isolate No.3(from Kallangai)

iv) T. paradoxa isolate No.4(from C.P.C.R.I. Kasaragod Campus garden)

v) T. paradoxa isolate No.5(from C.P.C.R.I. Kasaragod Hill-Block Garden)

vi) T. paradoxa isolate No.6(from Shiria)

4.4.1.1 Colony characters:

Isolate No.1: When colony dimensions were measured on each culture medium, T. paradoxa isolate-1 was found to grow very slowly on all the culture media tried. (Plate 6.) Even on the fifth day, the colony diameter had not crossed one cm in any growth medium, (Table-4).(Fig.1). On Oak Wilt Fungus Agar-C medium, T. paradoxa did not show any sign of growth. The colony was circular with entire margin (Table-5). There was no luxuriant growth of the mycelium on any culture media. The colour of the colony was grey on the top surface with dark grey colour on the reverse side in most of the culture media (Table-5). There was no odour to the colony.

Plate-6. Effect of culture media on growth of *T. paradoxa* isolate No.1.

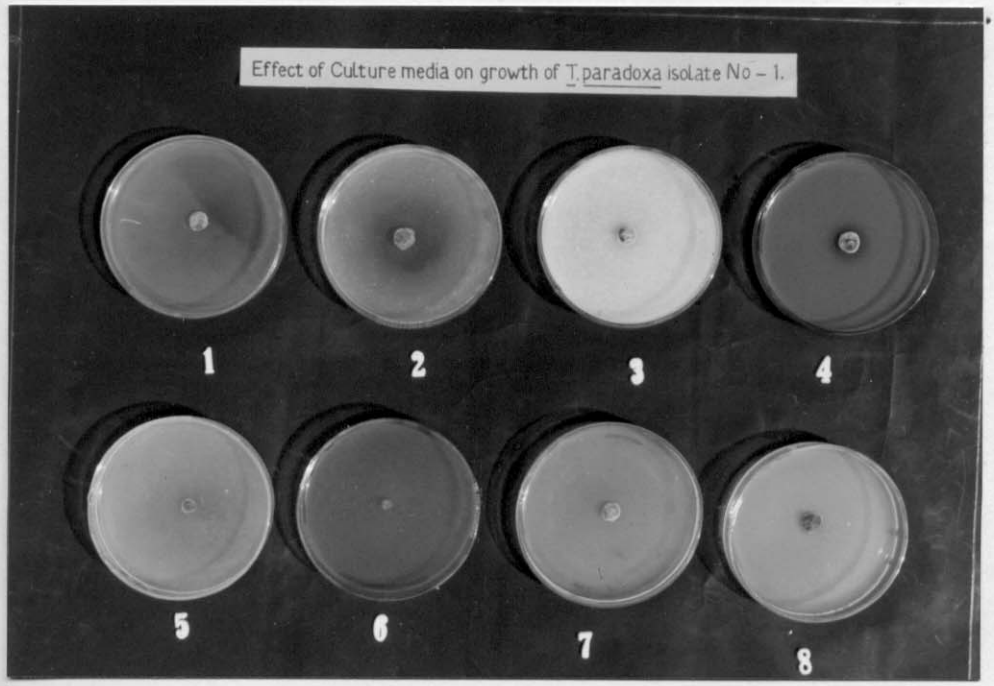


Plate-7. Effect of culture media on growth of *T. paradoxa* isolate No.2.

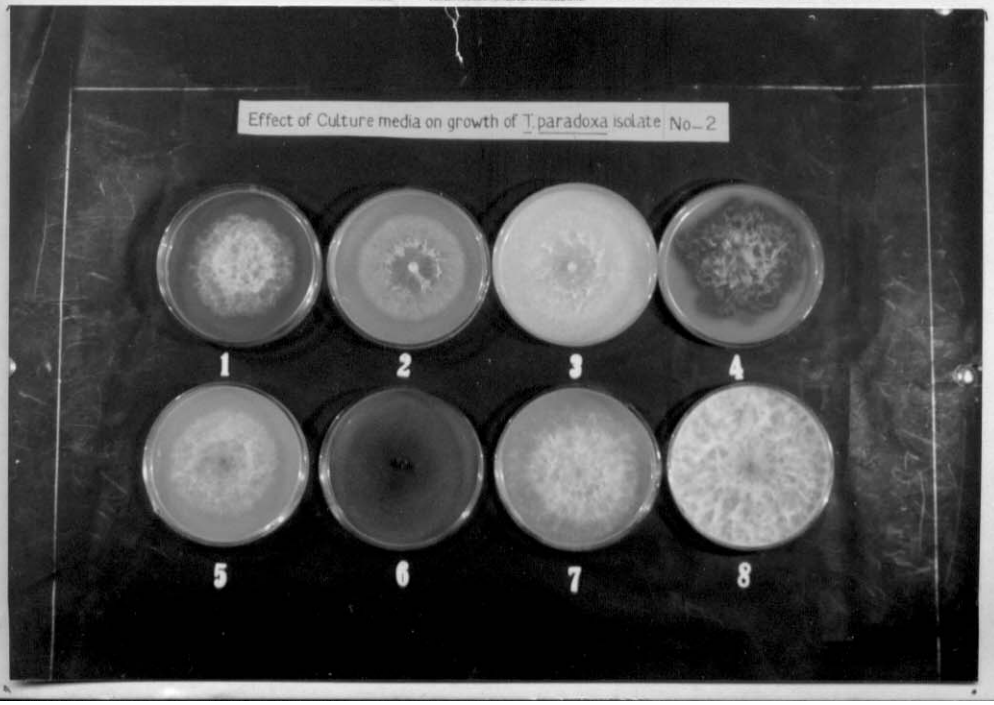


Table 4: Colony dimensions of T. paradoxa isolate No.1 on different culture media on different days

(Mean of four replicates in cms)

Time of observation after inoculation in days.	Colony diameter of T. paradoxa isolate No.1							
	PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	0.6	0.6	0.5	0.6	0.6	0.5	0.6	0.6
3	0.6	0.6	0.5	0.5	0.6	0.5	0.6	0.6
4	0.7	0.7	0.6	0.6	0.6	0.5	0.7	0.7
5	1.0	1.0	0.6	1.0	1.0	0.5	1.0	1.0

PDA = Potato Dextrose Agar
CMA = Corn Meal Agar
OMA = Oat Meal Agar
CTEA= Coconut Tissue Extract Agar
OWA-A = Oak Wilt Agar-A
OWA-C = Oak Wilt Fungus Agar-C
DAPA = Dextrose Asparagine Phosphate Agar
KA = Kirchoff's Agar

Fig.1 Growth rate of T. paradaxa isolate - 1 on different culture media

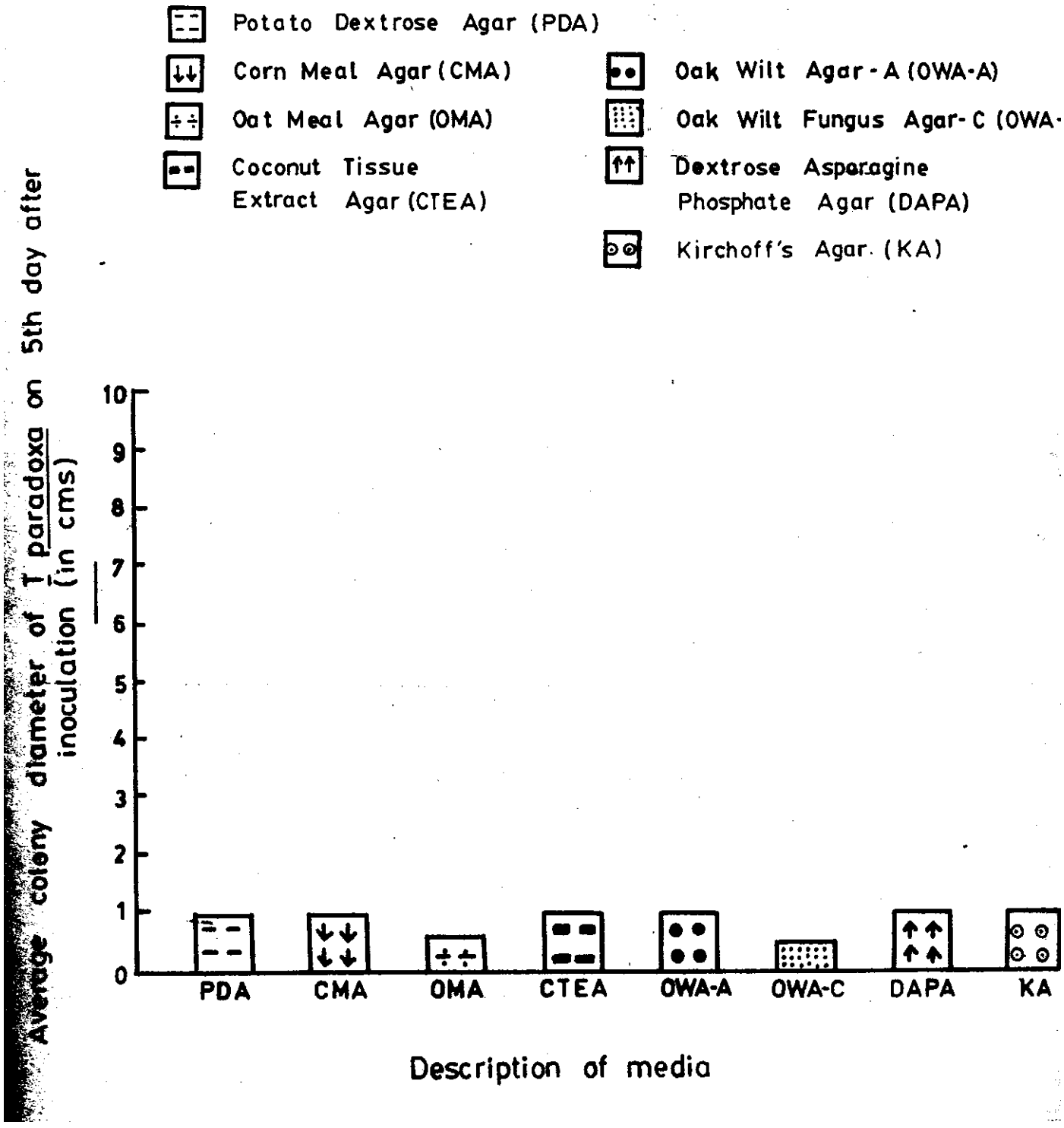


Table-5: Colony characters of T. paradoxa isolate No.1 on different culture media on different days.

Time of observation after inoculation in days	Colony characters		Culture media used							
			PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1	2		3	4	5	6	7	8	9	10
1	Margin									
	Elevation									
	Colour	Top surface	NG	NG	NG	NG	NG	NG	.NG	N
		Riverse side								
	Odour									
2	Margin		C+E	C+E	NG	C+E	C+E	NG	C+E	C+E
	Elevation		-	-	-	-	-	-	-	-
	Colour	Top surface	G	G	NG	G	G	NG	G	G
		Riverse side	G	G	NG	B	G	NG	G	G
	Odour		-	-	-	-	-	-	-	-
3	Margin		C+E	C+E	NG	C+E	C+E	NG	C+E	C+E
	Elevation		-	-	-	-	-	-	-	-
	Colour	Top surface	G	G	NG	G	G	NG	G	G
		Riverse side	DG	DG	NG	B	DG	NG	DG	DG
	Odour		-	-	-	-	-	-	-	-
4	Margin		C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation		-	-	-	-	-	-	-	-
	Colour	Top surface	G	G	G	G	G	NG	G	G
		Riverse side	DG	DG	NV	RB	DG	NG	DG	DG
	Odour		-	-	-	-	-	-	-	-

contd.....

Table 5 contd.

1	2	3	4	5	6	7	8	9	10
5.	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation	+	-	-	-	+	NG	+	+
	Colour Top surface	G	G	G	G	G	NG	G	G
	Riverse side	DG	DG	DG	RB	DG	NG	DG	DG
	Odour	-	-	-	-	-	-	-	-

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar.
OMA = Oat Meal Agar.
CTEA = Coconut Tissue Extract Agar.
OWA-A = Oak WiltAgar-A.
OWA-C = Oak Wilt Fungus Agar-C.
DAPA = Dextrose Asparagine Phosphate Agar.
KA = Kirchoff's Agar.
B = Brown
C+E = Circular and Entire
DG = Dark Grey
G = Grey
NG = No Growth
NV = Not visible
RB = Reddish Brown
\+ = 1/4th height of Petridish.
\- = Absent

Isolate No.2: T. paradoxa isolate-2 was found as the fastest growing isolate among all the isolates of T. paradoxa. The fungus showed very good growth on seven culture media. On Oak Wilt Fungus Agar-C medium it showed negligible growth. The fungus grew very fast(9 cm diameter on second day after inoculation) on Kirchoff's Agar medium (Plate 7), (Fig.2), containing L(-) Asparagine monohydrate as one of the constituents of the medium (Table-6). 48 hr. after inoculation, the colony was found circular with entire margin in all the culture media except on OWA-C medium wherethe fungus did not show any sign of growth.(Table-7). Elevation of the colony was maximum on OMA, OWA-A, DAPA and KA medium. The colony colour was white both on the top surface and reverse side. There was no odour to this colony in any media.

Isolate No.3: T. paradoxa isolate-3 grew faster on DAPA and KA media, both containing L(-) Asparagine monohydrate as one of the ingredients of the culture medium. Among these two culture media the growth was found faster (9 cm diameter on the third day after inoculation) on DAPA medium.(Plate 8) (Fig.3). The fungus did not show any sign of growth on OWA-C medium even when the fungus filled the entire petriplates in all other seven media on the fifth day after inoculation.(Table-8). The colony was found circular with entire margin on all the other seven culture media (PDA, CMA, OMA, CTEA, OWA-A DAPA, and KA) on the third day after inoculation. Among DAPA and KA media, elevation of the mycelium was maximum on DAPA medium (Table-9). The top surface colour of the colony was pure white. The colony did not give any odour in any of the culture media tried. (Table-9).

Table 6: Colony dimensions of T. paradoxa isolate No.2 on different culture media on different days

(Mean of four replicates in cms)

Time of observation after inoculation in days	Colony diameter of T. paradoxa isolate No.2							
	PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1	1.7	3.1	2.7	2.8	2.7	0.5	3.6	4.2
2	4.6	6.6	7.1	6.3	6.2	0.5	7.4	9.0
3	9.0	9.0	9.0	9.0	9.0	0.6	9.0	9.0
4	9.0	9.0	9.0	9.0	9.0	0.9	9.0	9.0
5	9.0	9.0	9.0	9.0	9.0	1.1	9.0	9.0

PDA = Potato Dextrose Agar
CMA = Corn Meal Agar
OMA = Oat Meal Agar
CTEA= Coconut Tissue Extract Agar
OWA-A = Oak Wilt Agar-A
OWA-C = Oak Wilt Fungus Agar-C
DAPA = Dextrose Asparagine Phosphate Agar
KA = Kirchoff's Agar

Fig .2 Growth rate of T. paradoxa isolate - 2 on different culture media

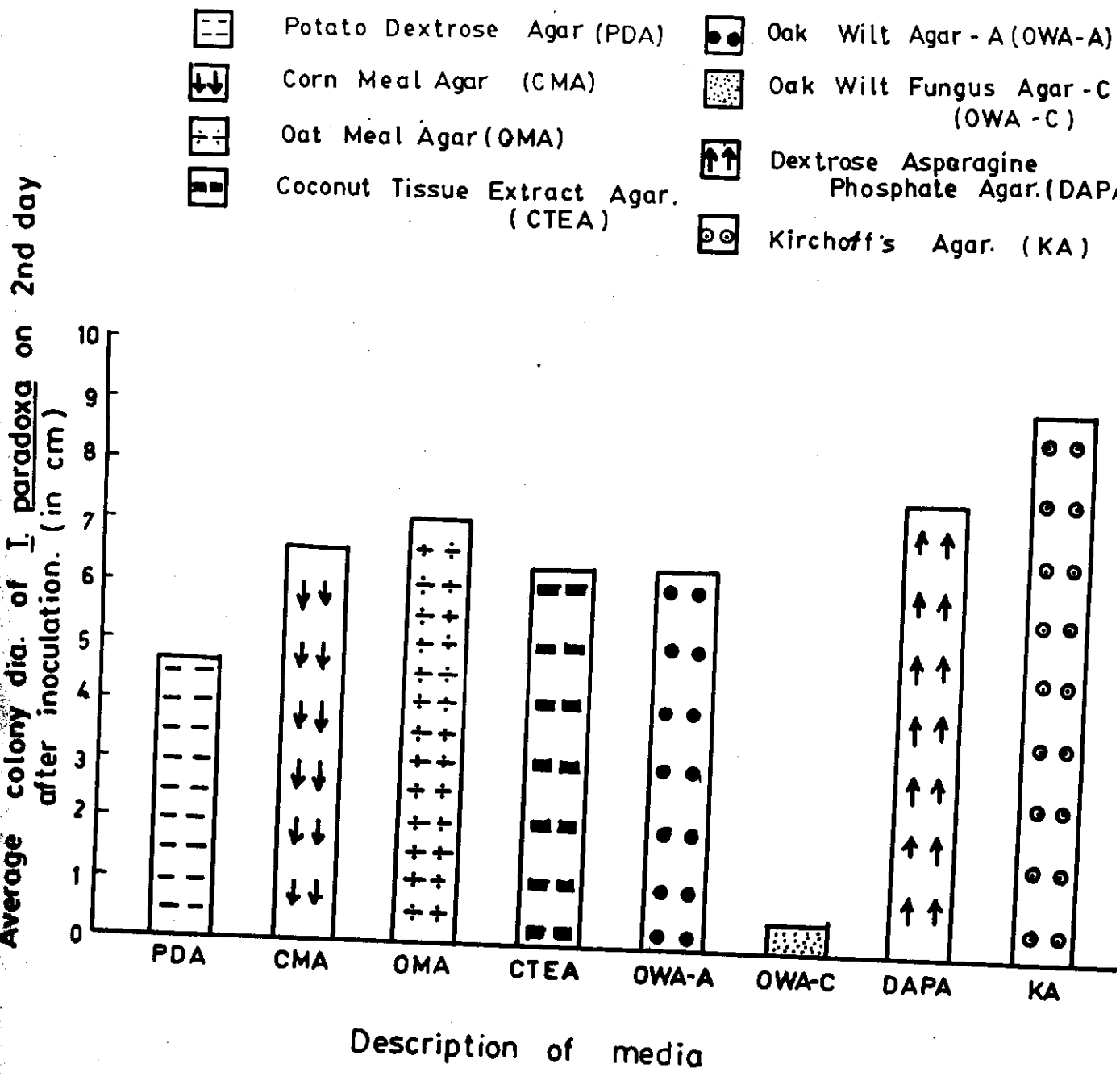


Plate-8. Effect of culture media on growth of *T. paradoxa* isolate No.3.

Table-7: Colony characters of T. paradoxa isolate No.2 on different culture media on different days.

Time of observation after inoculation in days	Colony characters	Culture media used							
		PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1	2	3	4	5	6	7	8	9	10
1	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+S	C+
	Elevation	-	-	-	+	+	NG	++	++
	Colour: Top surface	W	W	W	W	W	NG	W	W
	Riverse side	W	W	NV	Cr	W	NG	W	W
	Odour	-	-	-	-	-	-	-	-
2	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+E	C+
	Elevation	+++	+	++++	++	++++	NG	++++	++
	Colour Top surface	W	W	W	W	W	NG	W	W
	Riverse side	W	W	NV	NV	W	NG	W	W
	Odour	-	-	-	-	-	-	-	-
3	Margin	C+E	C+E	C+E	C+E	C+E	C+E	C+E	C+E
	Elevation	++++	+	++++	+++	++++	-	++++	++++
	Colour: Topsurface	G	W	W	W	W	G	W	W
	Riverse side	G	W	NV	NV	W	G	G	G
	Odour	-	-	-	-	-	-	-	-
4	Margin	C+E	C+E	C+E	C+E	C+E	C+E	C+E	C+E
	Elevation	+++	+	+++	+++	++++	-	++++	++
	Colour: Top surface	G	W	G	G	W	G	G	G
	Riverse side	G	W	G	NV	Cr	DG	DG	G
	Odour	-	-	-	-	-	-	-	-

contd....

Table 7 contd

1	2	3	4	5	6	7	8	9	10
5. Margin		C+E	C+E	C+E	C+E	C+E	C+E	C+E	C+E
Elevation		++	+	+++	+++	++++	-	++++	++
Colour	Top surface	G	G	G	G	W	G	G	G
	Riverse side	DG	G	DG	GB	Cr	DG	DG	G
Odour		-	-	-	-	-	-	-	-

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar.
OMA = Oat Meal Agar.
CTEA = Coconut Tissue Extract Agar.
OWA-A = Oak Wilt Agar-A.
OWA-C = Oak Wilt Fungus Agar-C.
DAPA = Dextrose Asparagine Phosphate Agar.
KA = Kirchoff's Agar.
C+E = Circular and Entire
C+S = Circular and Serrate
Cr = Cream
D+G = Dark Green
G = Grey
GB = Greyish Brown
NV = Not visible
W = White
- = Absent
+ = 1/4th height of Petridish.
++ = 1/2nd "
+++ = 3/4th "
++++ = Full "

Table-8: Colony dimensions of T. paradoxa isolate No.3 on different culture media on different days

(Mean of four replicates in cms)

Time of observation after inoculation in days.	Colony diameter of T. paradoxa isolate No.3							
	PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1	1.4	2.1	1.8	2.5	1.9	0.5	2.4	2.1
2	3.5	4.9	4.8	5.8	5.1	0.5	7.1	6.8
3	3.6	6.5	6.4	7.4	7.3	0.5	9.0	8.7
4	8.0	7.5	7.9	9.0	8.0	0.5	9.0	9.0
5	9.0	9.0	9.0	9.0	9.0	0.5	9.0	9.0

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar.
OMA = Oat Meal Agar
CTEA= Coconut Tissue Extract Agar
OWA-A = Oak WiltAgar-A
OWA-C = Oak Wilt Fungus Agar-C
DAPA = Dextrose Asparagine Phosphate Agar.
KA = Kirchoff's Agar

Fig. 3 Growth rate of T. paradoxa isolate- 3 on different culture media

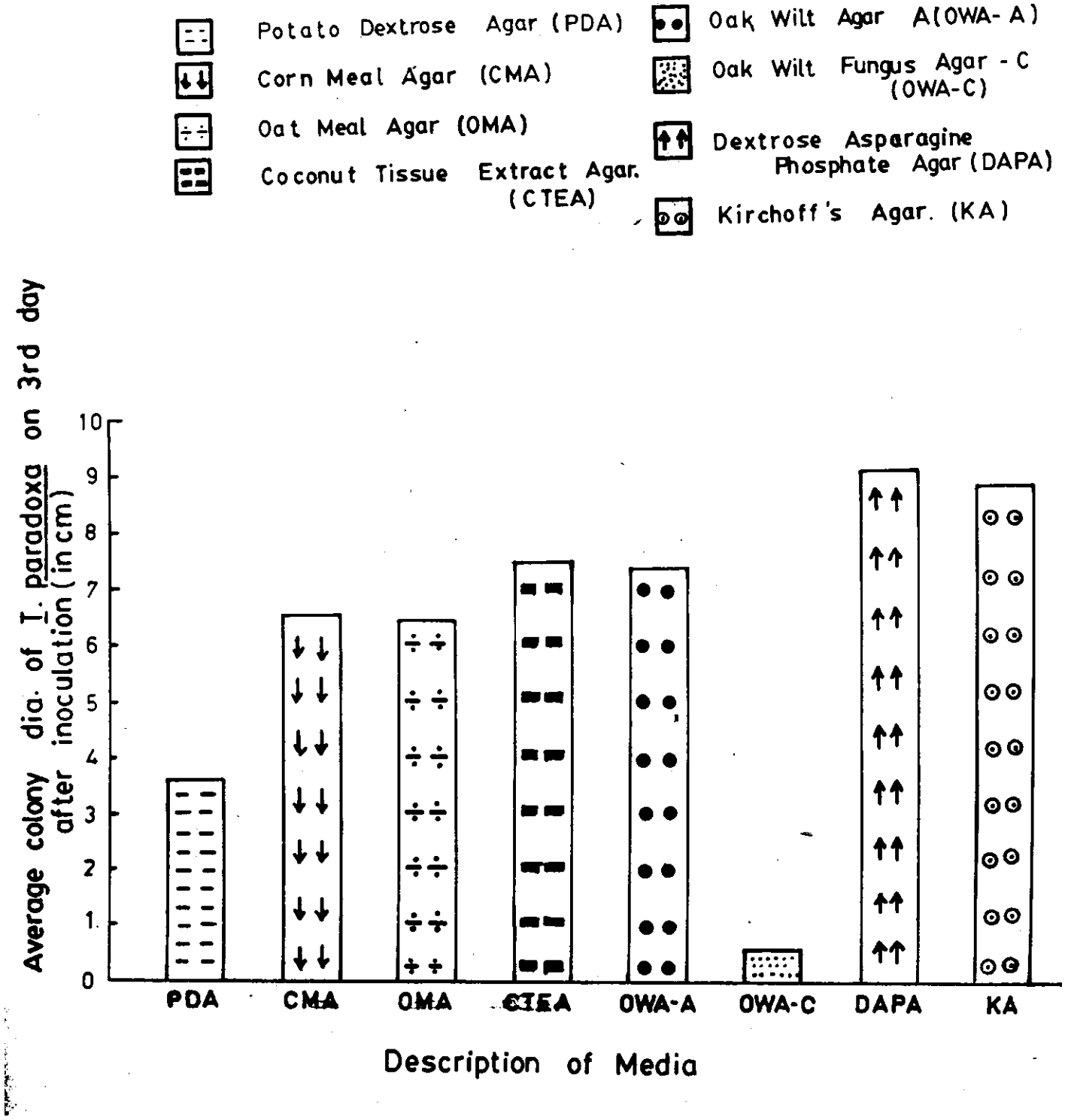


Table 9: Colony characters of T. paradoxa isolate No.3 on different culture media on different days.

Time of observation after inoculation in days	Colony characters	Culture media used							
		PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1	2	3	4	5	6	7	8	9	10
1	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+S	C+S
	Elevation	+	-	-	-	+	NG	+	++
	Colour: Top surface	PW	PW	PW	PW	PW	NG	PW	PW
	Riverse side	PW	PW	NV	NV	PW	NG	PW	PW
	Odour	-	-	-	-	-	-	-	-
2	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+S	C+S
	Elevation	++++	+	++++	+	++++	NG	++++	++++
	Colour: Top surface	PW	PW	PW	PW	PW	NG	PW	PW
	Riverse side	PW	PW	NV	NV	W	NG	PW	PW
	Odour	-	-	-	-	-	-	-	-
3	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation	++++	+	++++	++	+++	NG	++++	+++
	Colour: Top surface	PW	PW	PW	PW	PW	NG	PW	PW
	Riverse side	LY	W	NV	NV	W	NG	PW	PW
	Odour	-	-	-	-	-	-	-	-
4	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation	++++	-	++++	++++	+++	NG	++++	+++
	Colour : Top surface	PW	PW	PW	PW	PW	NG	PW	PW
	Riverse side	LY	PW	LY	NV	PW	NG	PW	PW
	Odour	-	-	-	-	-	-	-	-

contd....

Table 9 contd.

1	2	3	4	5	6	7	8	9	10
5.	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation	++++	-	++++	++++	+++	NG	+++	+++
	Colour: Top surface	PW	PW	PW	PW	PW	NG	PW	PW
	Riverse side	LY	PW	LY	NV	PW	NG	G	G
	Odour	-	-	-	-	-	-	-	-

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar.
OMA = Oat Meal Agar.
CTEA = Coconut Tissue Extract Agar.
OWA-A = Oak Wilt Agar-A.
OWA-C = Oak Wilt Fungus Agar-C.
DAPA = Dextrose Asparagine Phosphate Agar.
KA = Kirchoff's Agar.
C+E = Circular and Entire
C+S = Circular and Serrate.
G = Grey
LY = Light Yellow
NV = Not visible
PW = Pure white
\- = Absent
\+ = 1/4th height of petridish.
++ = 1/2nd "
+++ = 3/4th "
++++ = Full "

Isolate No.4: T. paradoxa isolate-4 did not show good growth on any of the culture media (Plate 9). The colony diameter did not exceed 1.5 cm on fifth day on any culture media tried.(Table 10) (Fig.4). It showed negligible growth on OWA-C medium. The colony was found circular with entire margin in all the media where growth was observed (Table-11). Even on the third day after inoculation, the colony was flat. The top surface of the colony was grey in colour. The colony did not give any odour in any media used.

Isolate No.5: T. paradoxa isolate No.5 showed maximum rate of growth (9 cm. diameter on fifthday after inoculation) on DAPA (Table-12) with an insignificant rate of growth on OWA-C medium (Plate-10) (Fig.5). The colony was found to grow with an irregular wavy margin on PDA whereas in other culture media, the colony was found circular with entire margin (Table-13). Maximum elevation of the colony (++++) was found on DAPA medium when compared to other media. The colour of the top surface of the colony varied in different culture media. The colony was white on PDA after 72 hr. of growth, grey on CMA and OMA, and dark grey on CTEA medium. In OWA-A, DAPA and KA media, the colony was found grey at the centre with a peripheral white coloured margin. The reverse side of the colony was greyish white on PDA and CTEA; and dark grey on CMA and OMA media on the third day after inoculation. There was no difference in colour on the top surface and reverse side of the colony in OWA-A, DAPA and KA medium. The colony did not give any

Plate-9. Effect of culture media on growth of *T. paradoxa* isolate No.4.

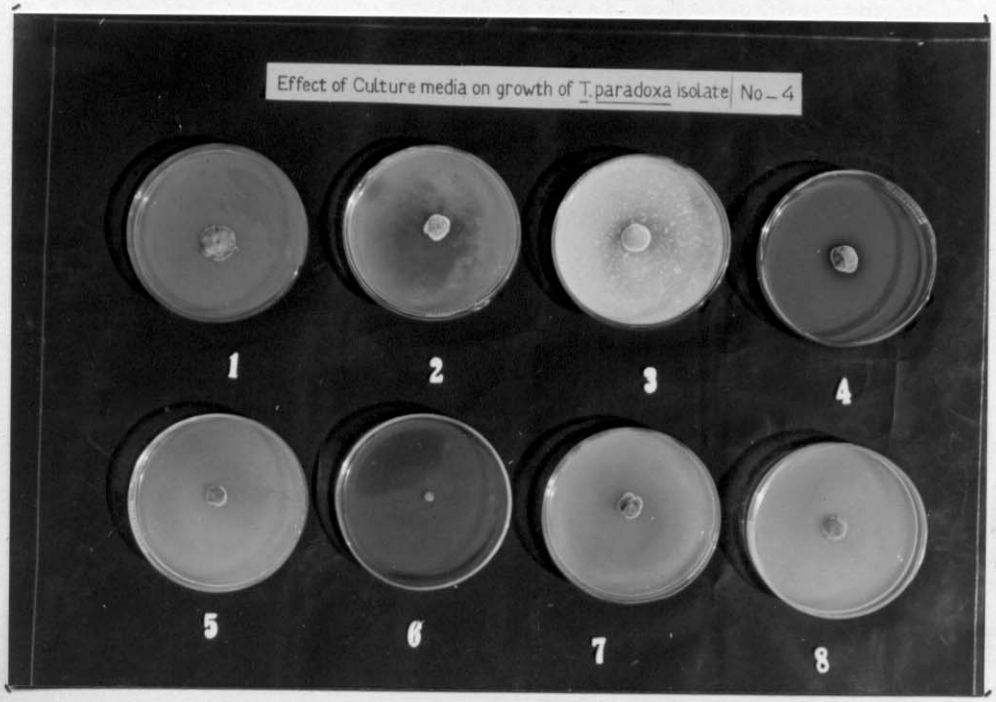


Plate-10. Effect of culture media on growth of *T. paradoxa* isolate No.5.

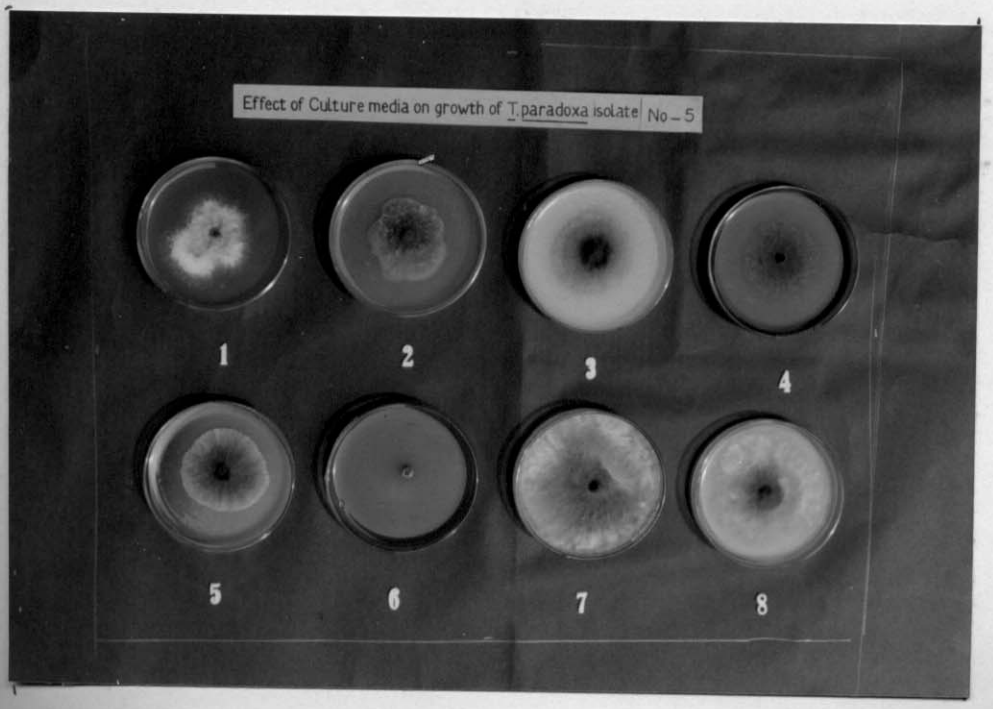


Table 10: Colony dimensions of T. paradoxa isolate No.4 on different culture media on different days

(Mean of four replicates in cms)

Time of observation after inoculation in days	Colony diameter of T. paradoxa isolate No.4							
	PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	0.6	0.7	0.6	0.6	0.6	0.5	0.6	0.6
3	1.0	1.2	0.9	1.2	1.1	0.5	1.0	0.9
4	1.3	1.3	1.1	1.2	1.1	0.5	1.2	1.2
5	1.5	1.4	1.3	1.4	1.3	0.6	1.1	1.3

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar
OMA = Oat Meal Agar
CTEA =Coconut Tissue Extract Agar.
OWA-A=Oak Wilt Agar-A
OWA-C=Oak Wilt Fungus Agar-C
DAPA =Dextrose Asparagine Phosphate Agar
KA =Kirchoff's Agar

Fig. 4 Growth rate of T. paradoxa isolate- 4 on different culture media

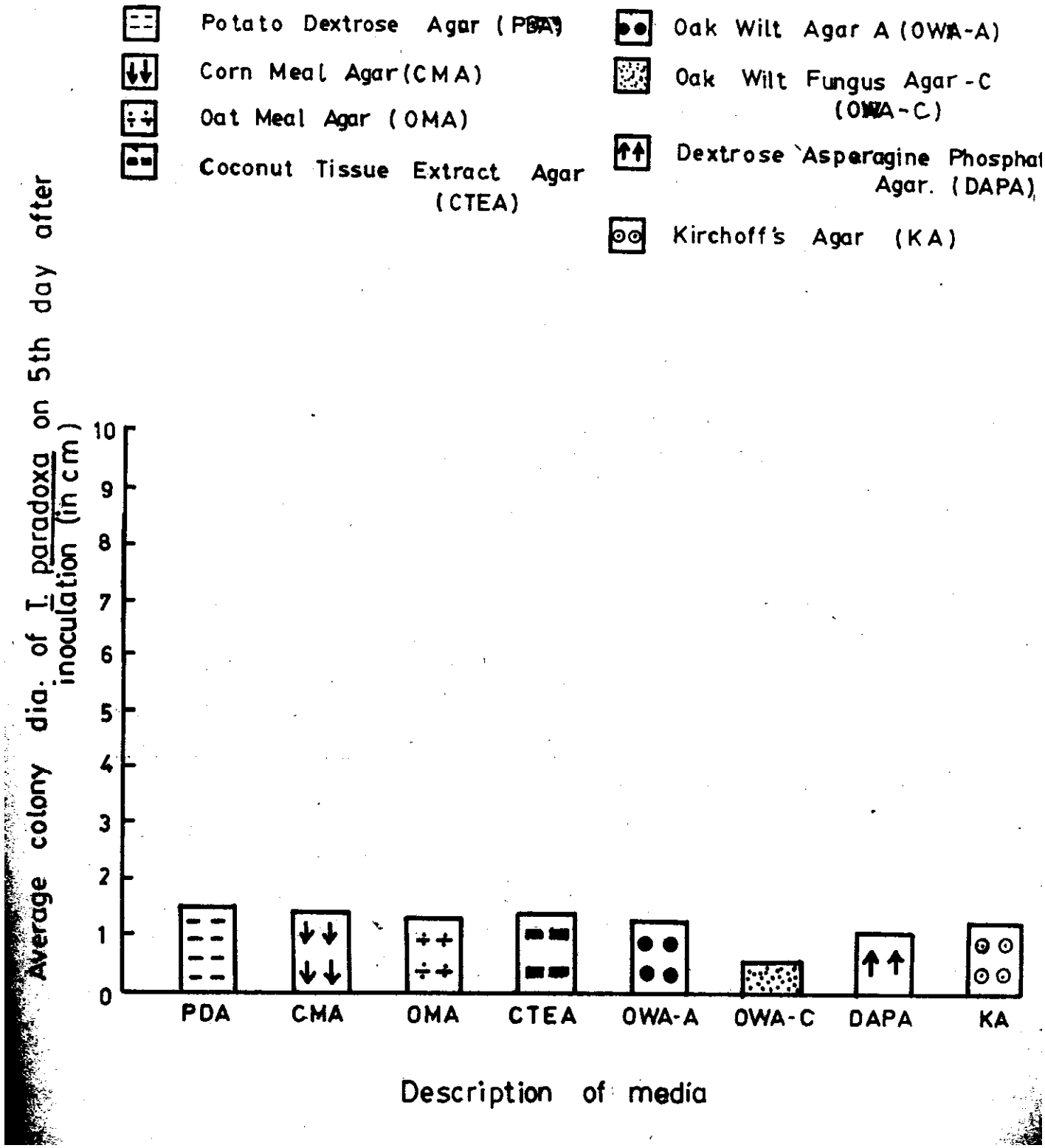


Table 11: Colony characters of T. paradoxa isolate No.4 on different culture media on different days.

Time of observation after inoculation in days	Colony characters		Culture media used							
			PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1	2		3	4	5	6	7	8	9	10
1	Margin		NG	NG	NG	NG	NG	NG	NG	NG
	Elevation									
	Colour	Top surface								
		Riverseside								
	Odour									
2	Margin		C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation		-	-	-	-	-	NG	-	-
	Colour:	Top surface	G	G	G	G	G	NG	G	G
		Riverseside	G	G	G	RB	G	NG	DG	DG
	Odour		-	-	-	-	-	NG	-	-
3	Margin		C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation		-	-	-	-	-	NG	-	-
	Colour	Top surface	G	G	G	G	G	NG	G	G
		Riverseside	G	G	G	RB	G	NG	DG	DG
	Odour		-	-	-	-	-	-	-	-
4	Margin		C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation		+	-	-	+	-	NG	-	-
	Colour:	Top surface	G	G	G	G	G	NG	G	G
		Riverseside	DG	G	G	RB	G	NG	DG	DG
	Odour		-	-	-	-	-	-	-	-

contd.....

Table 11 contd.

1	2	3	4	5	6	7	8	9	10
5.	Margin	C+E	C+E	C+E	C+E	C+E	C+E	C+E	C+E
	Elevation	+	+	-	+	+	-	+	+
	Colour Top surface	G	G	G	G	G	G	G	G
	Colour Riverseside	DG	DG	G	RB	G	G	B	B
	Odour	-	-	-	-	-	-	-	-

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar.
OMA = Oat Meal Agar.
CTEA = Coconut Tissue Extract Agar.
OWA-A = Oak WiltAgar-A.
OWA-C = Oak Wilt Fungus Agar-C.
DAPA = Dextrose Asparagine Phosphate Agar.
KA = Kirchoff's Agar.
C+E = Circular and Entire.
DG = Dark Grey.
G = Grey.
RB = Reddish Brown
- = Absent
+ = 1/4th height of petridish.

Table 12: Colony dimensions of T. paradoxa isolate No.5 on different culture media on different days

(Mean of four replicates in cms)

Time of observation after inoculation in days	Colony diameter of T. paradoxa isolate No.5							
	PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1	1.3	1.3	1.2	1.2	1.5	0.5	1.2	0.9
2	2.5	2.7	2.6	3.3	3.1	0.5	3.9	4.4
3	3.4	3.7	4.1	4.0	4.1	0.5	6.4	5.5
4	5.1	4.5	5.0	4.6	5.5	0.6	9.0	7.1
5	6.9	5.4	7.1	9.0	7.8	0.8	9.0	9.0

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar.
OMA = Oat Meal Agar.
CTEA = Coconut Tissue Extratt Agar.
OWA-A = Oak WiltAgar-A.
OWA-C = Oak Wilt Fungus Agar-C.
DAPA = Dextrose Asparagine Phosphate Agar.
KA = Kirchoff's Agar.

Fig. 5 Growth rate of T. paradoxa isolate - on different culture media

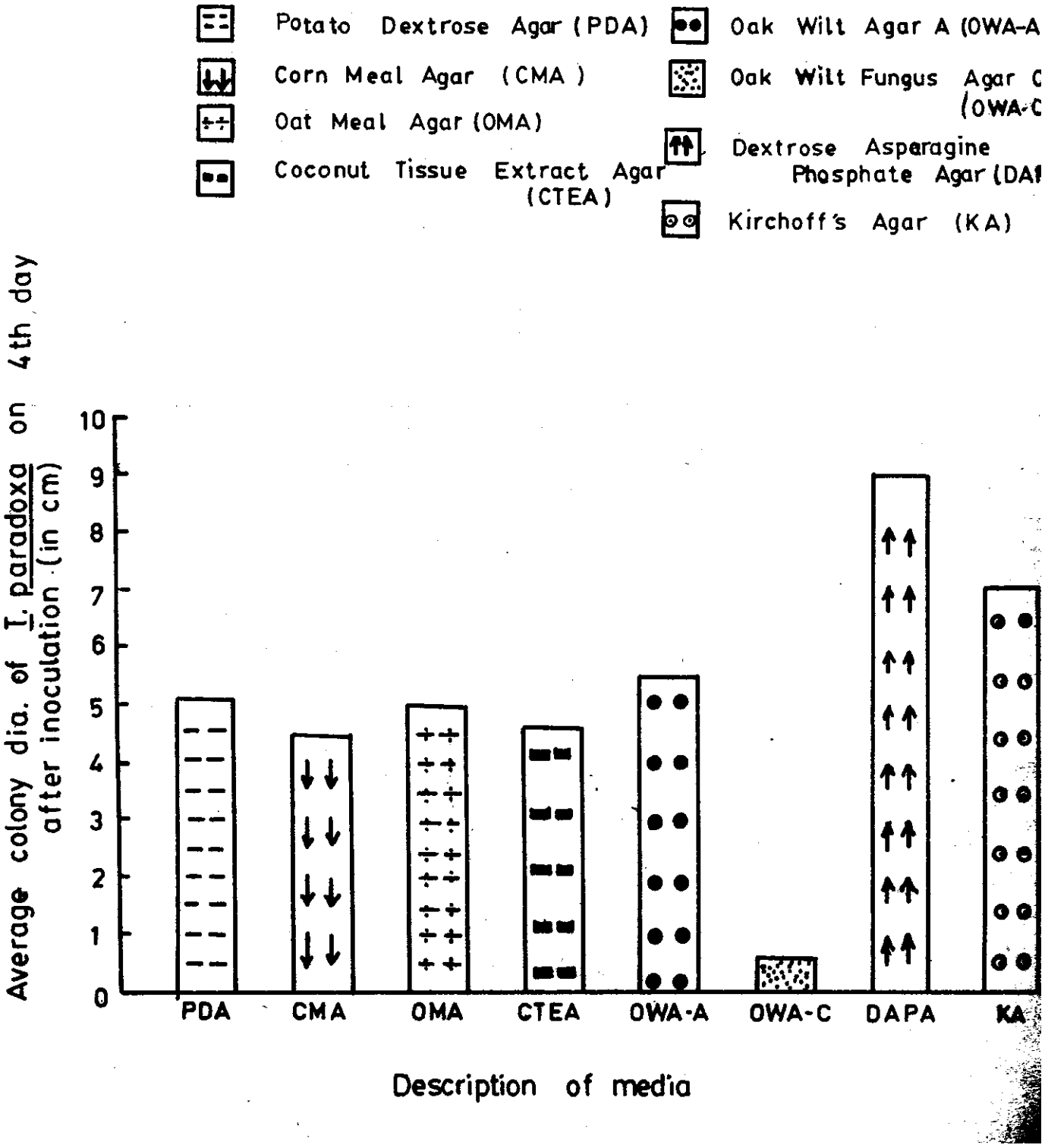


Table 13: Colony characters of T. paradoxa isolate No.5 on different culture media on different days.

Time of observation after inoculation in days	Colony characters	Culture media used							
		PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1	2	3	4	5	6	7	8	9	10
1	Margin	I	C+E	C+E	C+E	C+E	NG	I	C+E
	Elevation	+	+	+	+	+	NG	+	+
	Colour: Top surface	W	W	W	GW	W	NG	W	W
	Colour: Riverseside	LY	W	LY	RB	W	NG	W	W
	Odour	-	-	-	-	-	-	-	-
2	Margin	I	C+E	C+E	C+E	C+E	NG	C+	C+
	Elevation	+	+	++	++	+	NG	++	++
	Colour: Top surface	W	CGPW	W	G	CGPW	NG	CGPW	CGPW
	Colour: Riverseside	W	CGPW	LG	GB	CGPW	NG	CGPW	CGPW
	Odour	-	-	-	-	-	-	-	-
3	Margin	I	C+E	C+E	C+E	C+W	NG	C+E	C+E
	Elevation	+	+	++	++	+	NG	+++	++
	Colour: Top surface	W	G	G	DG	CGPW	NG	CGPW	CGPW
	Colour: Riverseside	GW	DG	DG	GB	CGPW	NG	CGPW	CGPW
	Odour	-	-	-	-	-	-	-	-
4	Margin	I	C+E	C+E	C+W	C+W	C+E	C+E	C+E
	Elevation	+	+	++	++	+	-	++++	++
	Colour: Top surface	W	GW	G	G	G	GW	WG	WG
	Colour: Riverseside	GW	GW	DG	DB	G	DG	G	G
	Odour	-	-	-	-	-	-	-	-

contd.....

Table 13 contd.

1	2	3	4	5	6	7	8	9	10
5.	Margin	I	C+E	C+E	C+W	C+W	C+E	C+E	C+E
	Elevation	+	+	++	+++	+	-	++++	++
	Colour: Top surface	G	G	G	G	G	GW	G	G
	Colour: Riverseside	G	DG	DG	DB	G	DB	DG	DG
	Odour	-	-	-	-	-	-	-	-

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar.
OMA = Oat Meal Agar.
CTEA = Coconut Tissue ExtractAgar.
OWA-A = Oak Wilt Agar-A.
OWA-C = Oak Wilt fungus Agar-C.
DAPA = Dextrose Aspargine Phosphate Agar.
KA = Kirchoff's Agar.
C+E = Circular and Entire.
C+S = Circular and Surrate.
C+W = Circular and Wavy
CGPW = Centre Grey and Perifery white.
DG = Dark Grey
GB = Greish Brown
GW = Greyish White
I = Irregular.
LG = Light Grey
LY = Light Yellow
RB = Reddish Brown
W = White
~ = Absent
\+ = 1/4th height of petridish.
++ = 1/2nd "
+++ = 3/4th "
++++ = Full height of petridish.

odour in any of the media used.

Isolate No.6: T. paradoxa isolate-6 showed maximum rate of growth (9 cm. diameter on third day after inoculation) on PDA (Plate 11) (Fig.6). Among the six isolates of T. paradoxa, this was the only one isolate which showed maximum rate of growth on PDA medium. On the fifth day of inoculation itself, the fungus filled the entire petridish in all the seven media except OWA-C, in which it did not show any sign of growth at all.(Table-14). The colony was circular with entire margin on the third day of inoculation on all the seven culture media where growth was noticed.(Table 15). Slight elevation of the colony (++) was found only on PDA and CMA where as the colony was flat in all other culture media. The top surface of the colony was found white in colour on CTEA medium on the third day after inoculation. The colour of the top surface of the colony was found grey on CMA and OWA-A media, and dark grey on CMA medium. On PDA, DAPA and KA media, the colour of the top surface and reverse side of the colony was alike with grey centre and whitish periphery on the third day after inoculation. There was no significant difference in the colour of the top surface and reverse side of the colony on CMA medium, on the third day after inoculation. The reverse side of the colony was black in CMA and OWA-A media on the third day after inoculation. In CTEA medium the colony colour on the reverse side was not visible due to opacity of the medium. A characteristic ripe

Plate-11. Effect of culture media on growth at *T. paradoxa* isolate No.6.

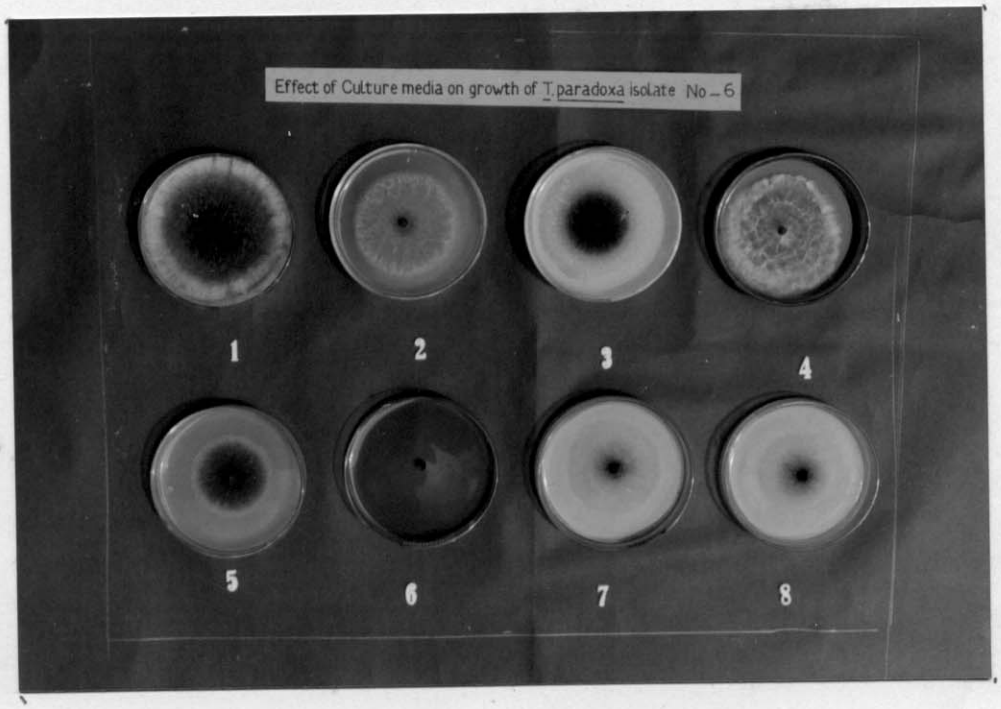


Plate-12. Effect of PDA on the growth of different isolates of *T. paradoxa*.

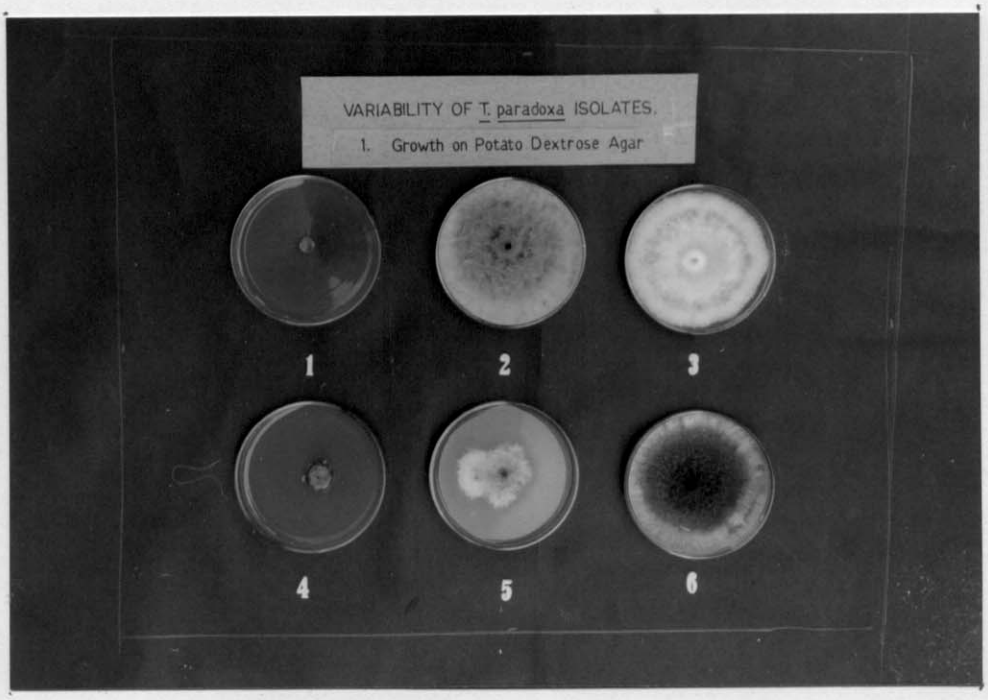


Table 14: Colony dimensions of T. paradoxa isolate No.6 on different culture media on different days (Mean of four replicates in cms)

Time of observation after inoculation in days	Colony diameter of T. paradoxa isolate No.6							
	PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1	3.0	2.6	1.5	1.8	2.7	0.5	2.6	2.5
2	7.0	4.6	2.2	4.5	4.1	0.5	4.6	4.7
3	9.0	7.3	5.2	8.0	6.0	0.5	7.1	7.0
4	9.0	8.0	9.0	8.5	7.0	0.5	7.5	7.5
5	9.0	9.0	9.0	9.0	9.0	0.5	9.0	9.0

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar.
OMA = Oat Meal Agar.
CTEA= Coconut Tissue Extract Agar.
OWA-A = Oak Wilt Agar-A.
OWA-C = Oak Wilt Fungus Agar-C.
DAPA = Dextrose Asparagine Phosphate Agar.
KA = Kirchoff's Agar.

Fig. 6 Growth rate of T. paradoxa isolate - 6 on different culture media

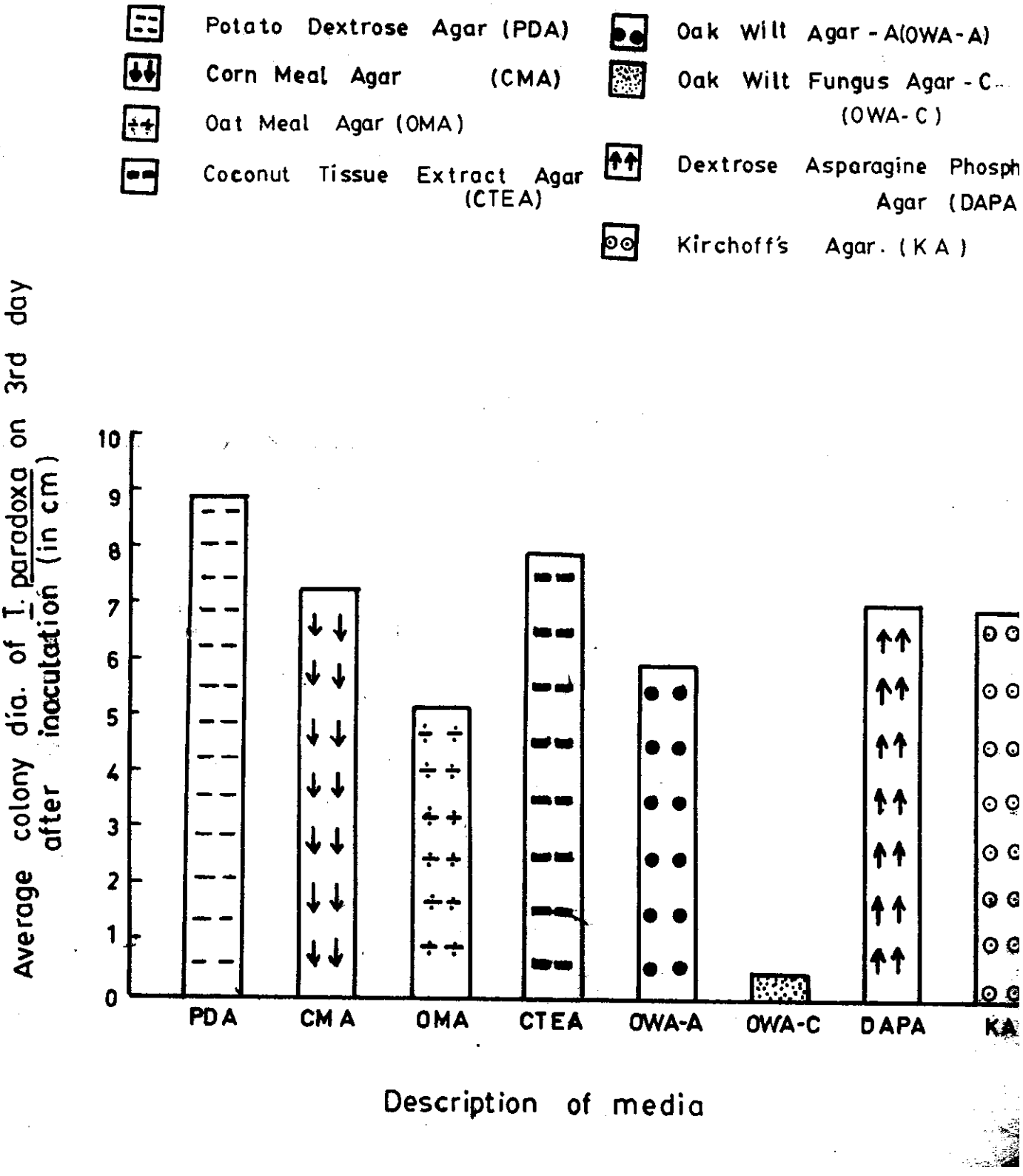


Table 15: Colony characters of T. paradoxa isolate No.6 on different culture media on different days.

Time of observation after inoculation in days	Colony characters	Culture media used							
		PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
1	2	3	4	5	6	7	8	9	10
1	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation	-	-	-	-	-	NG	-	-
	Colour : Top surface	W	W	ST	W	WG	NG	WG	WG
	Riverseside	W	W	MNV	NV	WG	NG	WG	WG
	Odour	FS	FS	FS	FS	FS	NG	FS	FS
2	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation	+	+	-	-	-	NG	-	-
	Colour : Top surface	CGPW	W	W	W	G	NG	CGPW	CG
	Riverseside	"	W	W	NV	G	NG	"	PW "
	Odour	FS	FS	FS	FS	FS	NG	FS	FS
3	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation	+	+	-	-	-	NG	-	-
	Colour : Top surface	CGPW	G	DG	W	G	NG	CGPW	CG
	Riverseside	"	"	B	NV	B	NG	"	PW "
	Odour	FS	FS	FS	FS	FS	NG	FS	FS
4	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation	+	+	-	-	-	NG	-	-
	Colour : Top surface	G	WG	G	G	G	NG	CGPW	CG
	Riverseside	DG	WG	B	NV	B	NG	"	PW "
	Odour	FS	FS	FS	FS	FS	NG	FS	FS

contd.....

Table 15 contd.

1	2	3	4	5	6	7	8	9	10
5	Margin	C+E	C+E	C+E	C+E	C+E	NG	C+E	C+E
	Elevation	+	+	-	-	-	NG	-	-
	Colour Top surface	DG	WG	DG	DG	G	NG	CGPW	CGPW
	Colour Riverseside	DG	WG	B	NV	B	NG	"	"
	Odour	FS	FS	FS	FS	FS	NG	FS	FS

PDA = Potato Dextrose Agar.

CMA = Corn Meal Agar.
OMA = Oat Meal Agar.
CTEA = Oat Meal Agar.

OWA-A = Oak Wilt Agar-M.

OWA-C = Oak Wilt Fungus Agar-C.

DAPA = Dextrose Asparagine Phosphate Agar.

KA = Dirchoff's Agar.

B = Black
C+E = Circular and Entire
CGPW = Centre Grey and Perifery white.
DG = Dark Grey
G = Grey
NV = Not visible
ST = Semi transparent.
W = White
WG = Whitish Grey.
- = Absent.
+ = 1/4th of height of petridish.
FS = Fruity Smell.

pineapple smell was found to emit from the colony in all the eight culture media(Table 15). Perhaps due to this fruity smell, flies like Drosophila sp. were attracted to the cultures. Such a characteristic fruity smell was not given by any other isolates of T. paradoxa in any growth media tried.

4.4.1.2 Comparision of growth rate of different isolates in different media:

When the growth rate of all the six isolates of T. paradoxa was compared on different media tested, a lot of variations was observed in different isolates in their growth rate in each culture medium.(Table-16)(Plates 12, 13 & 14) (Fig.7). T. paradoxa isolate Nos.2,3,5 and 6 were found to grow faster in culture media whereas first and fourth isolate of T. paradoxa were found as slow growers in all the eight media tried. Fruity smell was discernible only in isolate No.6 in all media, whereas no other isolate produced this smell.

4.4.2 Morphological characters:

Study of mycelial characters (Colour of hyphae, diameter of hypha, presence of hyphal rings in the mycelium etc.), conidia and chlamydospore of each isolate of T. paradoxa in all the eight growth media was done.

4.4.2.1 Mycelial characters:

The colour of hyphae under the microscope using tungston illumination and blue filter was found to be brown in

Plate-13. Effect of OWA-A medium on the growth of different isolates of T. paradoxa.

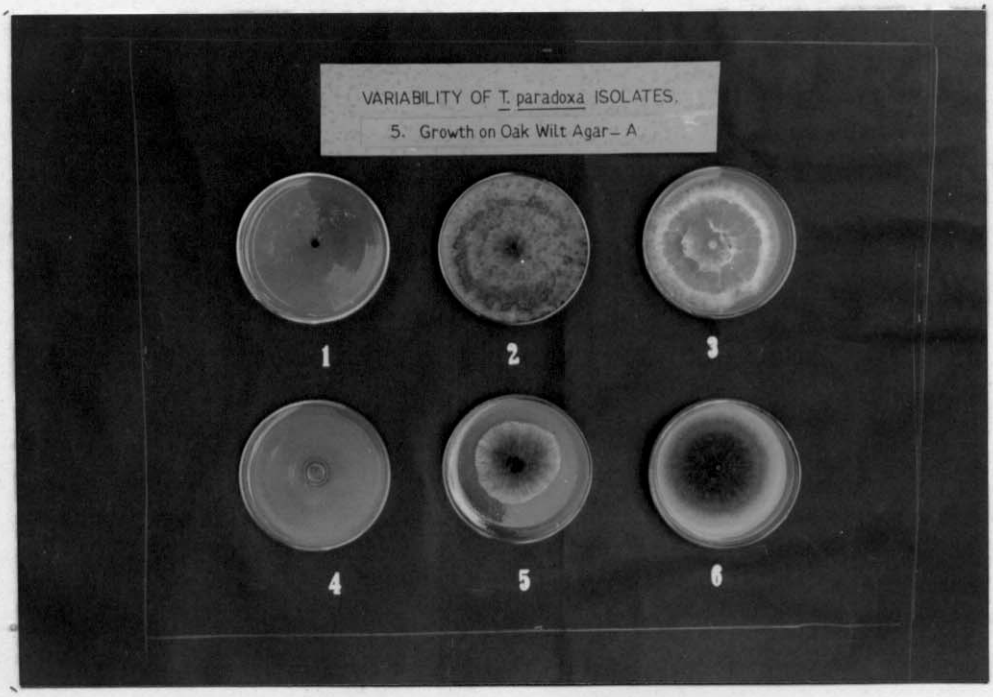


Plate-14. Effect of DAPA medium on the growth of different isolates of T. paradoxa.

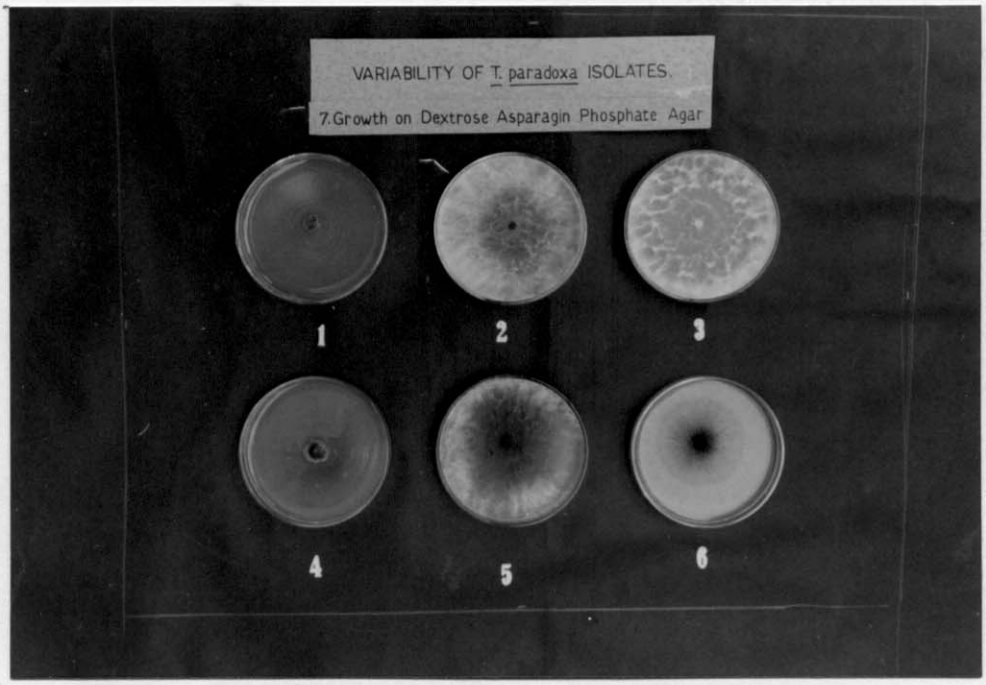
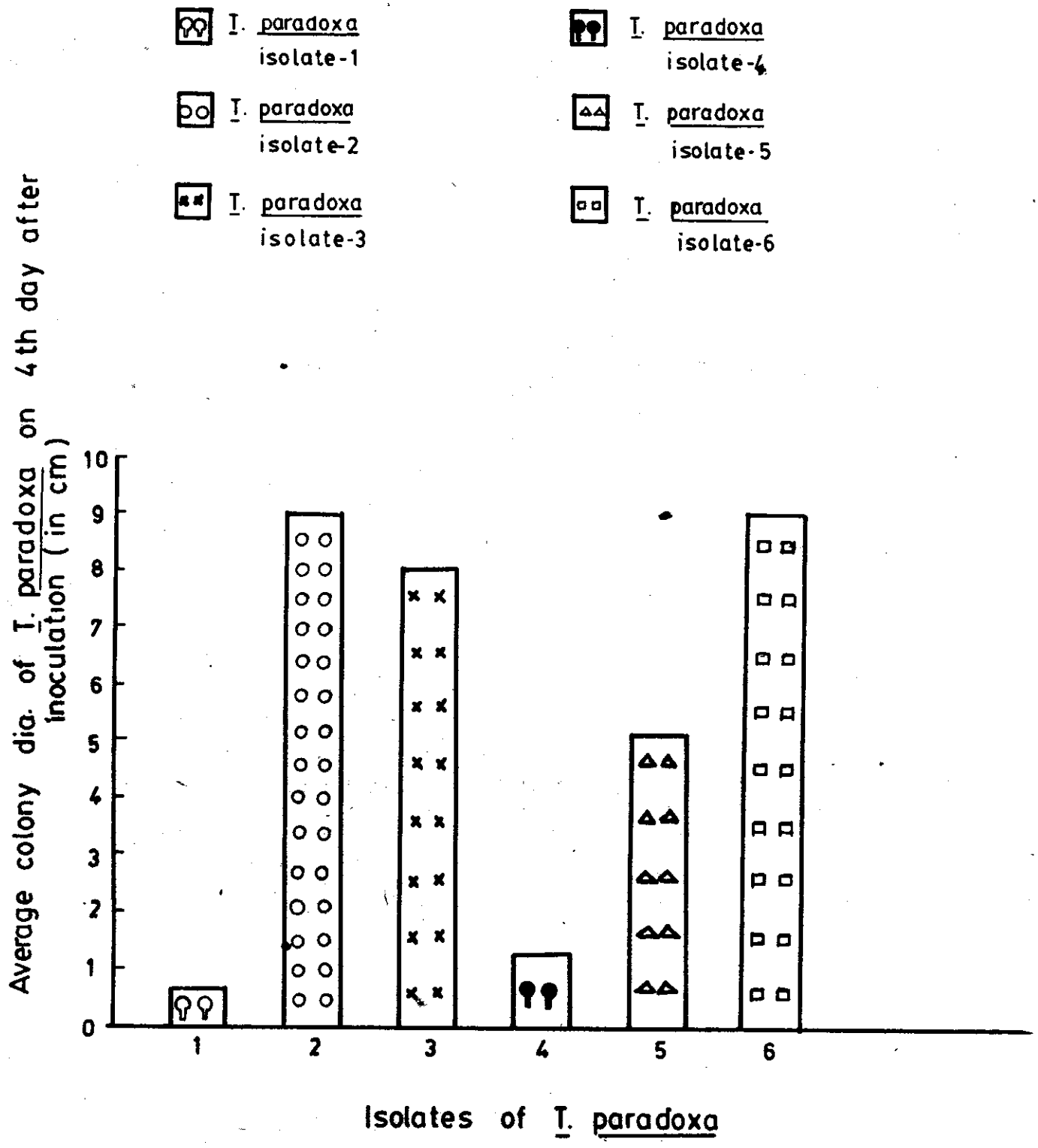


Table 16: Growth of different isolates of T. paradoxa on eight culture media on the fourth day after inoculation

Culture medium used	Colony diameter of T. paradoxa isolates (Mean of four replications in cm.)					
	1	2	3	4	5	6
PDA	0.7	9.0	8.0	1.3	5.1	9.0
CMA	0.7	9.0	7.5	1.3	4.5	8.0
OMA	0.6	9.0	7.9	1.1	5.0	9.0
CTEA	0.6	9.0	9.0	1.2	4.6	5.5
OWA-A	0.6	9.0	8.0	1.1	5.5	7.0
OWA-C	0.5	0.9	0.5	0.5	0.6	0.5
DAPA	0.7	9.0	9.0	1.2	9.0	7.5
KA	0.7	9.0	9.0	1.2	7.1	7.5

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar.
OMA = Oat Meal Agar.
CTEA = Coconut Tissue Extract Agar.
OWA-A = Oak Wilt Agar-A
OWA-C = Oak Wilt Fungus Agar-C.
DAPA = Dextrose Asparagine Phosphate Agar.
KA = Kirchoff's Agar.

Fig. 7. Variability in growth rate among isolates of T. paradoxa on Potato Dextrose Agar medium



in T. paradoxa isolate Nos.1 and 5; light grey in T. paradoxa isolate Nos.2 & 4 and hyaline in T. paradoxa isolate Nos. 3 and 6 respectively (Table-17). The hyphal diameter was found to vary from 1.8µto 3.6µ in isolate Nos.1, 2, 4 and 6 and from 1.5µ to 3.0µ in isolate Nos.3 and 4. A characteristic hyphal ring like structure was observed in the mycelium of T. paradoxa isolate-1, which was not seen in other isolates(Table 17) Plate15). A special dark brown, thickwalled, coiled structure was observed predominantly in the old cultures of T. paradoxa isolate-1 (Plate 16) which was lacking in other five isolates of T. paradoxa.

4.4.2.2 Conidial formation in different isolates of T. paradoxa:

The endoconidial formation was studied in all the six isolates of T. paradoxa in eight different culture media. viz. PDA, CMA, OMA, CTEA, OWA-A, OWA-C, DAPA, and KA media. Observation was done for ten days after inoculation. Only isolate No.6 of T. paradoxa produced endoconida. However none of the other five isolates produced endoconidia in any of the media tested (Table 18).

When formation of conidia in isolate no.6 of T. paradoxa in eight different culture media was studied, it was found that the fungus isolate produced endoconidia in seven culture media viz. PDA, CMA, OMA, CTEA, OWA-A, DAPA and KA media on the third day after inoculation. The fungus did not show growth on OWA-C medium upto seventhday after inoculation. On the eight day after inoculation, the fungus showed growth and on the nineth day it produced endoconidia in OWA-C medium also.

Table 17: Mycelial characters of different isolates of T. paradoxa.

Mycelial character	Isolates of T. paradoxa					
	1	2	3	4	5	6
Colour	Brown	Light Grey	Hyaline	Light Grey	Brown	Hyaline
Hyphal diameter (in µ)	1.8 µ to 3.6 µ	1.8 µ to 3.6 µ	1.5 µ to 3.0 µ	1.5 µ to 3.0 µ	1.8 µ to 3.6 µ	1.8 µ to 3.6 µ
Presence of hyphal rings in the mycelium	P	A	A	A	A	A
Presence of a special hyphal coiled structure	P	A	A	A	A	A

P = Present
A = Absent

Plate-15. Fungal mycellium of T. paradoxa isolate No.1 showing ring formation.

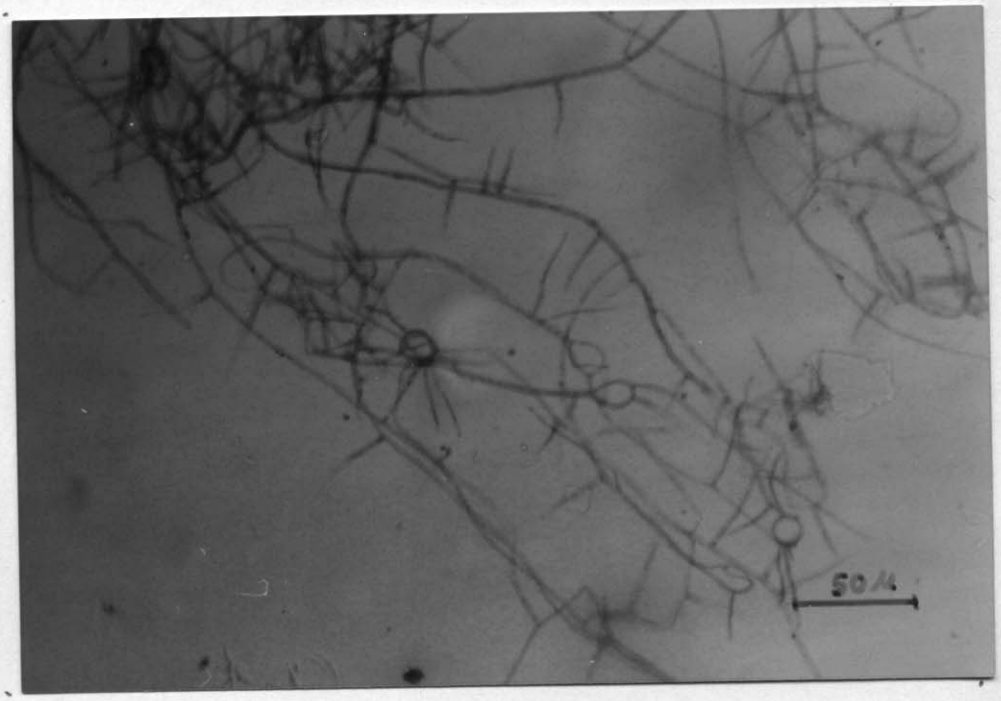


Plate-16. Fungal mycellium of T. paradoxa isolate No.1 showing thick coiled structures.

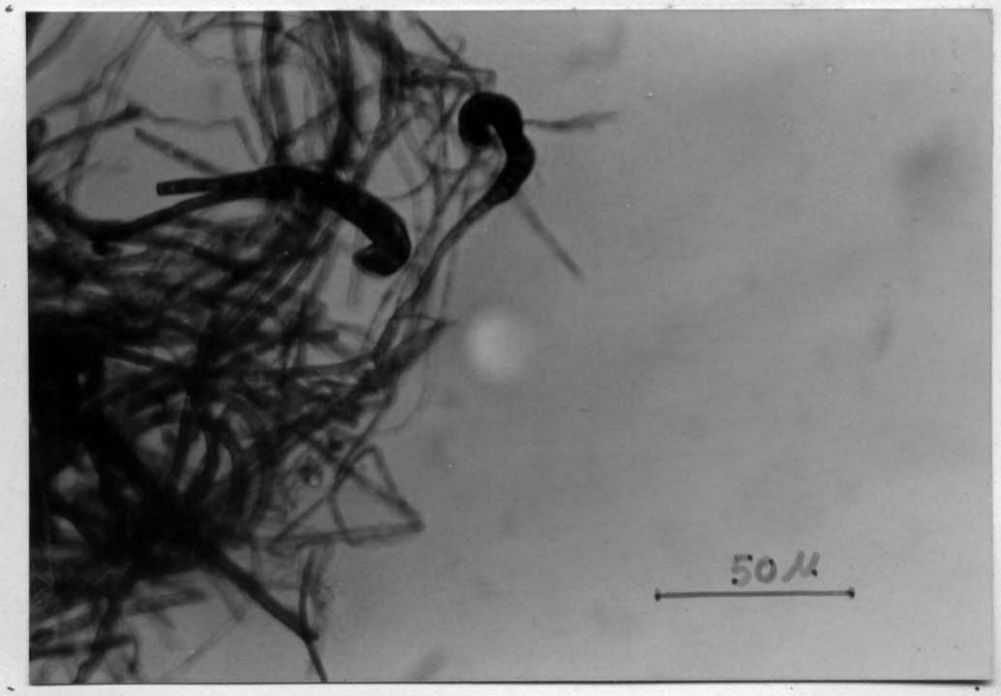


Table 18: Conidial formation and characters of different isolates of T. paradoxa.

Isolates	Occurrence	Conidial formation and characters			
		Position	Colour	Shape	Size
Nos. 1 to 5	Absent	-	-	-	-
No.6	⊛ Present	Terminal	Hyaline	Rectangular	3.6 µ x 7.2 µ to 3.6 µ x 18.0 µ

⊛ = Conidia formed on the 3rd day after inoculation on 7 media(~~xxxxxx~~ PDA, CMA, OMA, CTEA, OWA-A, DAPA and KA) and on 9th day on OWA-C medium.

Conidia were produced in terminal chains within a flask shaped conidophore (Plate 17). Conidia produced in chains are released one by one. These endoconidia are hyaline and are rectangular in shape (Plate 18). The size of conidia ranges from 3.6 µ x 7.2 µ to 3.6 µ x 18.0 µ (Table-18).

4.4.2.3. Chlamydospore formation in different isolates by T. paradoxa.

Chlamydospore formation was studied in all the six isolates of T. paradoxa in eight different culture media.

T. paradoxa isolate No.1 produced chlamydospores first on PDA and OMA on the ninth day after inoculation. In DAPA and KA media, chlamydospores were produced on 15th day after inoculation (Table-19). In CMA and OWA-C, chlamydospores are produced on twentiath day after inoculation. In OWA-A and CTEA, chlamydospores were produced on 25th and 30th day after inoculation respectively.

T. paradoxa isolate Nos. 2 and 3 produced chlamydospores on sixth and seventh day respectively after inoculation in all the eight culture media (Table-20).

In the case of isolate No.4, no chlamydospore was formed in any media even after 60 days after inoculation (Table-20).

T. paradoxa isolate-5 produced chlamydospores on 5th day after inoculation on PDA, CMA, OMA, OWA-A, DAPA and KA media. Chlamydospores were observed on the ninth day after inoculation on CTEA and OWA-C media (Table-21).

In the case of isolate no.6 of T. paradoxa, chlamydospore formation was observed on the third day itself after inoculation

Plate 17. Conidial production in T. paradoxa isolate No.6.

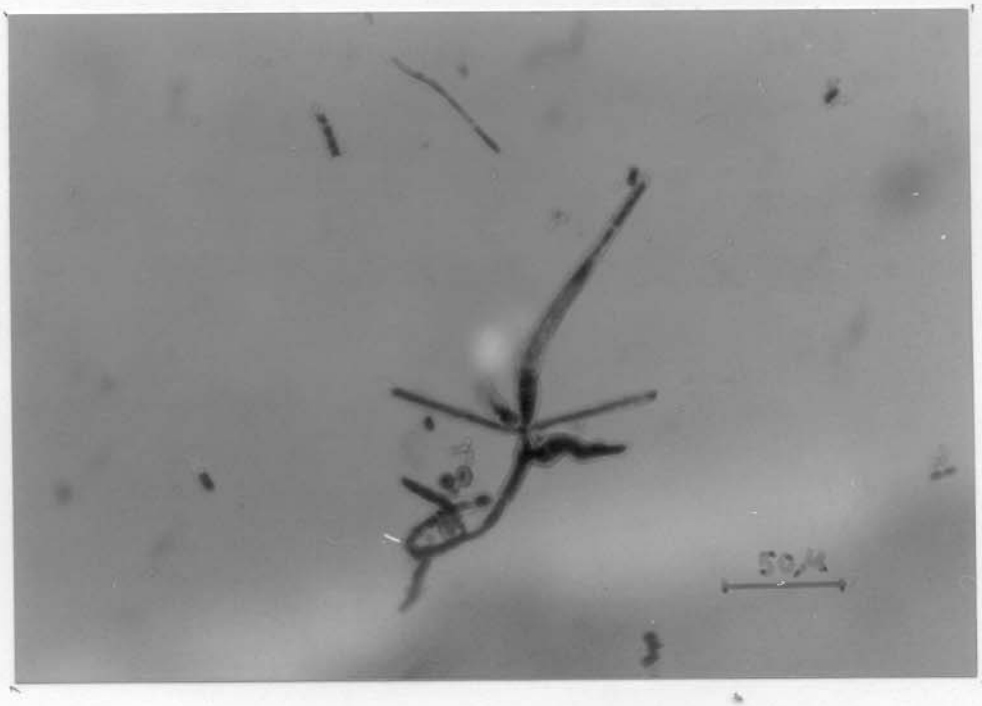


Plate 18. Conidia of T. paradoxa isolate No.6 showing the rectangular shape.

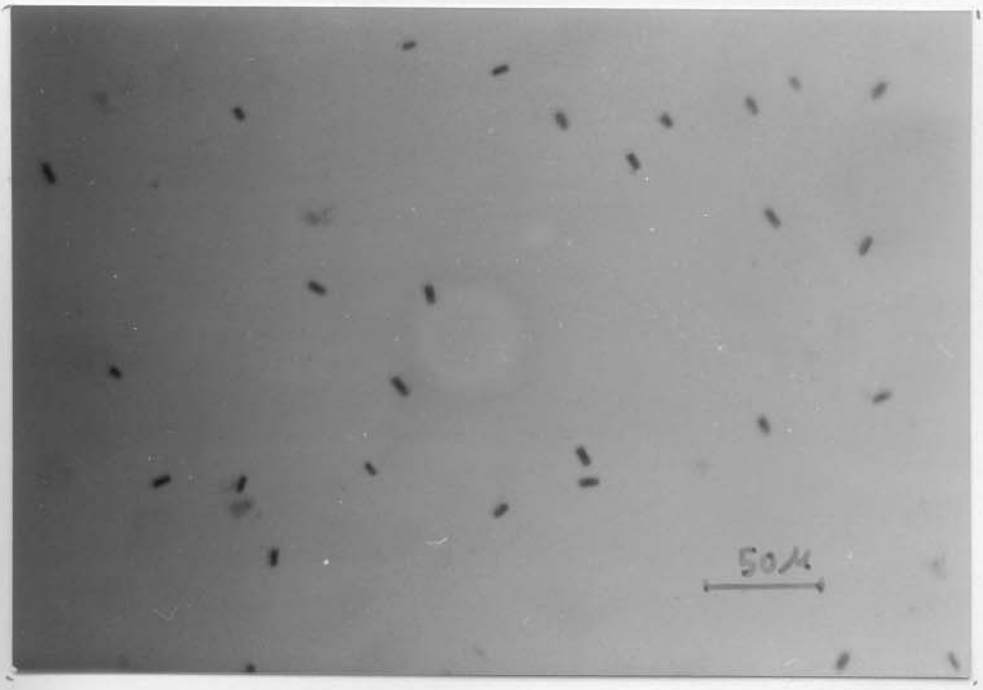


Table 19: Chlamydospore formation in T. paradoxa isolate No.1 on different culture media.

Days after inoculation	Presence or absence of chlamydospore on different culture media							
	PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
8	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
9	P	Ab	P	Ab	Ab	Ab	Ab	Ab
15	P	Ab	P	Ab	Ab	Ab	P	P
20	P	P	P	Ab	Ab	P	P	P
25	P	P	P	Ab	P	P	P	P
30	P	P	P	P	P	P	P	P

Ab = Absent
P = Present

PDA = Potato Dextrose Agar
CMA = Corn Meal Agar.
OMA = Oat Meal Agar.
CTEA = Coconut Tissue Extract Agar.
OWA-A = Oak WiltAgar-A.
OWA-C = Oak Wilt Fungus Agar-C.
DAPA = Dextrose Asparagine Phosphate Agar.
KA = Kirchoff's Agar.

Table 20: Chlamydospore formation in T. paradoxa isolate Nos.2,3 & 4 on different culture media.

Days after inoculation	Presence or absence of chlamydospore on different culture media							
	PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
				Isolate No.2				
5	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
6	P	P	P	P	P	P	P	P
				Isolate No.3				
6	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
7	P	P	P	P	P	P	P	P
				Isolate No.4				
5	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
60	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab

Ab = Absent

P = Present

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar.
OMA = Oat Meal Agar.
CTEA =Coconut Tissue Extract Agar.
OWA-A = Oak wilt Agar-A.
OWA-C = Oak Wilt Fungus Agar-C.
DAPA = Dextrose Asparagine Phosphate Agar.
KA = Kirchoff's Agar.

Table 21: Chlamydospore formation in T. paradoxa isolate Nos.5 & 6 on different culture media.

Days after inoculation	Presence or absence of chlamydospore on different culture media							
	PDA	CMA	OMA	CTEA	OWA-A	OWA-C	DAPA	KA
				Isolate No.5				
4	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
5	P	P	P	Ab	P	Ab	P	P
9	P	P	P	P	P	P	P	P
				Isolate No.6				
2	Ab	Ab	Ab	Ab	Ab	NG	Ab	Ab
3	P	P	P	P	P	NG	P	P
8	P	P	P	P	P	Ab	P	P
9	P	P	P	P	P	P	P	P

Ab = Absent
P = Present
NG= No growth of Fungus

PDA = Potato Dextrose Agar.
CMA = Corn Meal Agar
OMA = Oat Meal Agar.
CTEA= Coconut Tissue Extract Agar.
OWA-A = Oak Wilt Agar-A
OWA-C = Oak Wilt Fungus Agar-C.
DAPA = Dextrose Asparagine Phosphate Agar.
KA = Kirchoff's Agar.

in PDA, CMA, OMA, CTEA, OWA-A, DAPA and KA media. In OWA-C medium no observable growth was noticed upto seventh day after inoculation(Table-21). However, on the eighth day growth of the fungus was observed and on the ninth day, chlamydospore formation was observed.

Morphological characters of chlamydospores:

The position, shape and size of chlamydospores varied in different isolates of T. paradoxa. In T. paradoxa isolate-1 both intercalary and terminal chlamydospores were produced (Plate 19)(Table-22). Here the chlamydospores were brown, oval and the size ranged from 3.6 µ x 7.2 µ to 3.6 µ x 10.8 µ. In T. paradoxa isolate Nos.2, 3 and 5, only intercalary chlamydospores were produced which were brown in colour (Plates 20, 21, 22) The chlamydospores were oval in isolate-2, spherical to oval in isolate-3 and oval to oblong in isolate-5 respectively. The size of chlamydospore in T. paradoxa isolate-2 varied from 3.6 µ x 7.2 µ to 10.8 µ x 43.2 µ In T. paradoxa isolate-3, where both spherical and chlamydospores were noticed, the size of spherical chlamydospore ranged from 7.2 µ to 21.6 µ (Diagonal measurement)and the size of oval chlamydospores ranged from 7.2 µ x 10.8 µ to 14.4 µ x 21.6 µ. The size of oval to oblong chlamydospores in T. paradoxa isolate-5 ranged from 3.6 µ x 7.2 µ to 10.8 µ x 32.4 µ. In T. paradoxa isolate-6 chlamydospores were found to produce only terminally (Plate 23) either singly or in chains.

Plate 19. Terminal and intercalary chlamydospores of T. paradoxa isolate No.1.

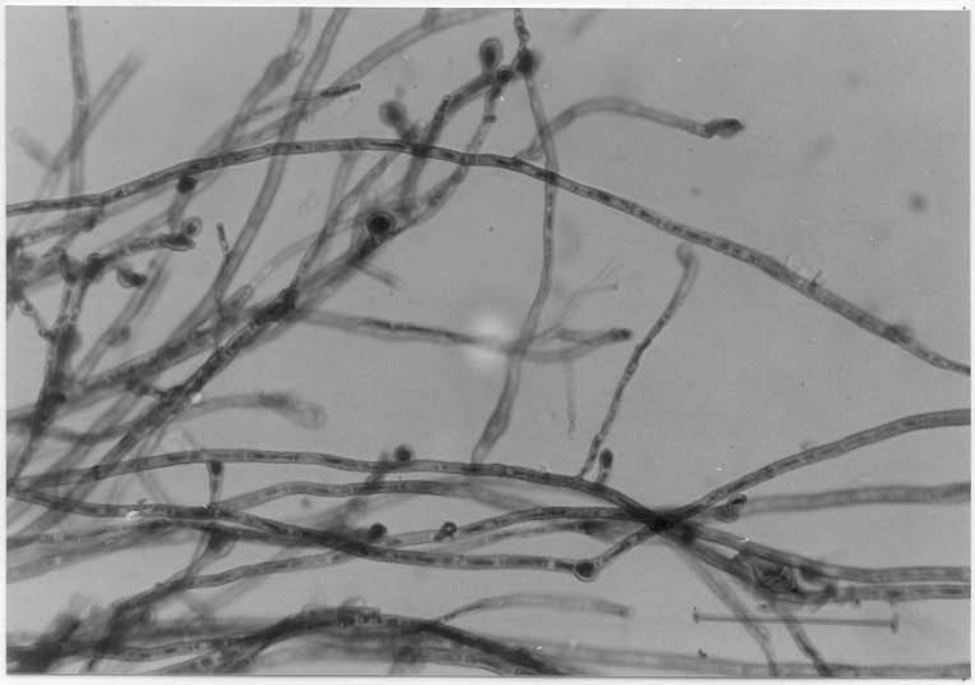


Plate 20. Intercalary chlamydospores of T. paradoxa isolate No.2.

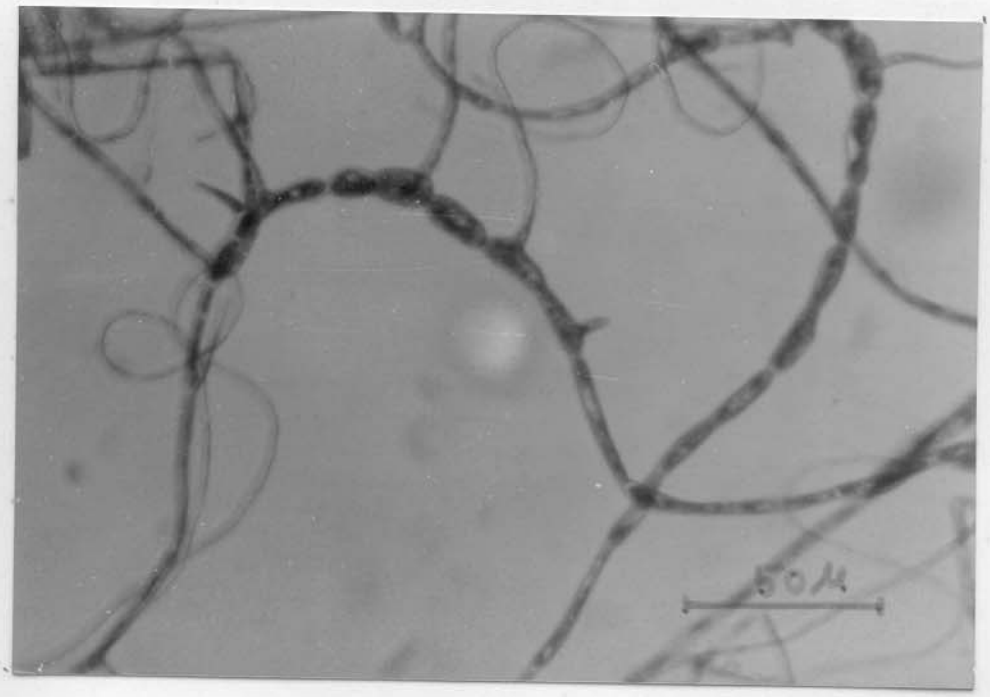


Plate 21. Intercalary chlamydospores of T. paradoxa isolate No.3.

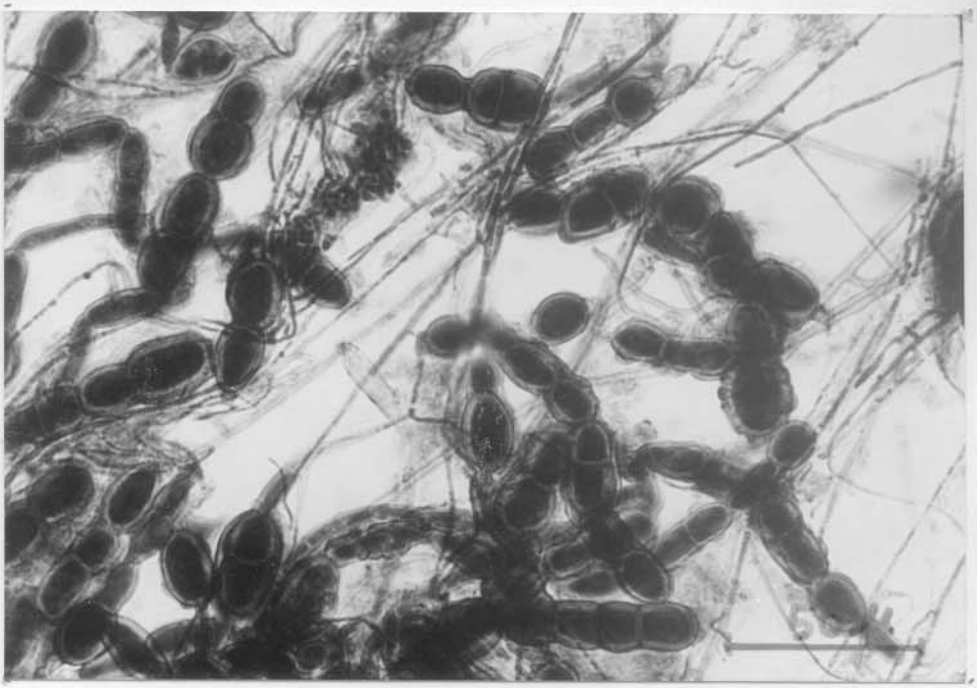


Plate 22. Intercalary chlamydospores of T. paradoxa isolate No.5.

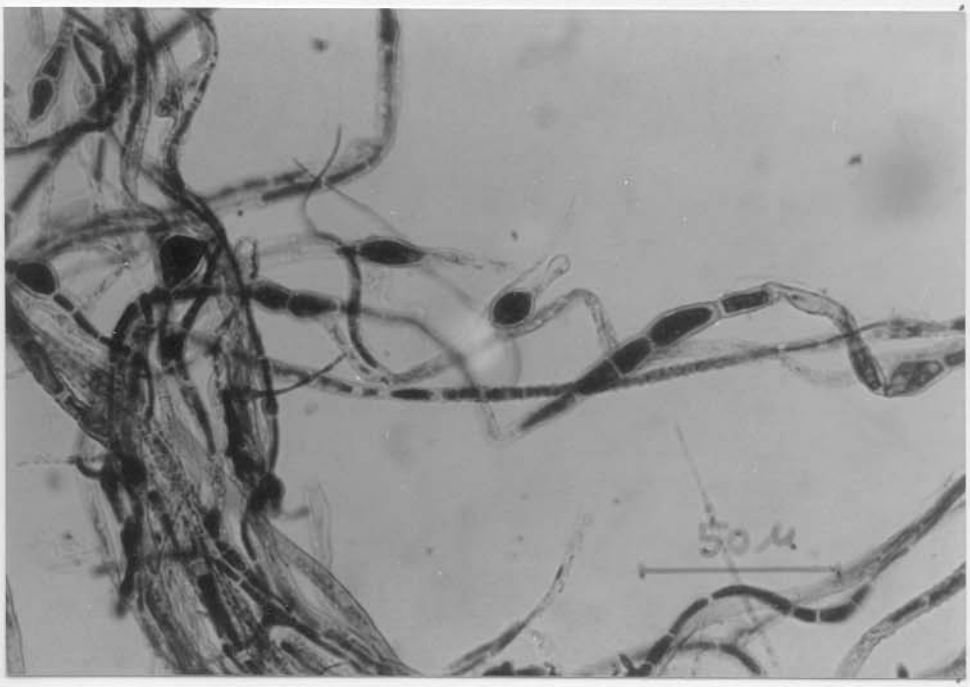


Plate 23. Terminal chlamydospores of T. paradoxa isolate No.6.

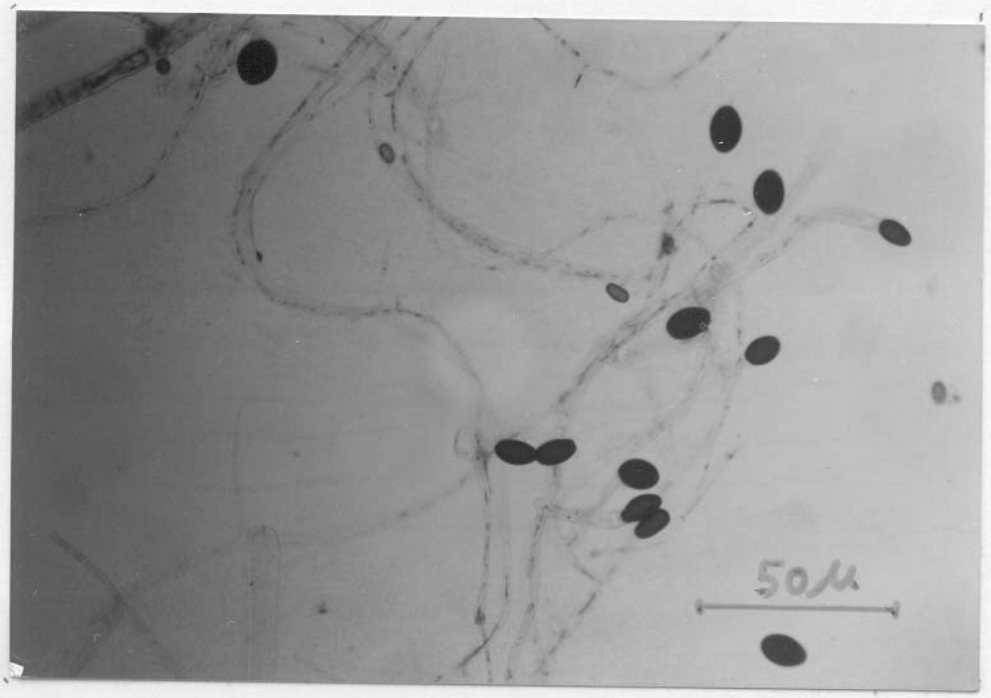


Table 22: Occurrence and morphological characters of Chlamadospores of different isolates of T. paradoxa.

Character	Isolates of T. paradoxa.					
	1	2	3	4	5	6
Occurrence	Present (9-30)*	Present (6)*	Present (7)*	Absent (even after 60 days)	Present (5-9)*	Present (3-9)*
Position	Both terminal and inter-callery	Only inter-calary	Only inter-calary	-	Only inter-calary	Only terminal either singly or in chains.
Colour of chlamadospore	Brown	Brown	Brown	-	Brown	Brown
Shape of Chlamadospore	Oval	Oval	Sperical and oval	-	Oval to oblong	Oval
Size of Chlamadospore	3.6µ x 7.2µ to 3.6µ x 10.8µ	3.6µ x 7.2µ to 10.8µ x 43.2µ	7.2µ to 21.6µ & 7.2µ x 10.8µ to 14.4µ x 21.6µ	-	3.6µ x 7.2µ to 10.8µ x 32.4µ	3.6µ x 7.2µ to 10.8µ x 25.2µ

* = Figures in parenthesis denote the range of days after inoculation when chlamadospores were present in different media.

The chlamydospores were brown in colour and oval in shape with a size range of 3.6 µ x 7.2 µ to 10.8 µ x 25.2 µ. (Table-22).

4.4.2.4 Perfect stage of T. paradoxa.

The growth studies lasted for four weeks in the case of T. paradoxa isolate Nos.1, 2, 3, 5 and 6 and for eight weeks in the case of isolate no.4. During the case of study, no perfect stage or initials of perfect stage were observed in any of the isolates on any of the growth media studied.

4.5 Studies on the interaction of the associated mycoflora with isolates of T. paradoxa in vitro:

The fungi isolated from the bark of the stem bleeding affected palms and/or from the soil from palm basin were used for the in vitro studies of their interaction with the isolates of T. paradoxa. Among the various fungi isolated, Acremonium sp (Two isolates), Aspergillus niger, A. terreus, Chalaropsis sp, Paecilomyces varioti, Penicillium citrinum, P. diverseum, P. janthinellum (two isolates), Pestalotiopsis palmarum, Trichoderma harzianum (two isolates), T. viride (three isolates) and two unidentified members of mycelia sterila were used for the interaction studies with T. paradoxa. Among the six isolates of T. paradoxa, isolate nos.1 and 4 were not taken for interaction studies due to their poor growth on various culture media. For the in vitro studies on the interaction, isolation No.2 was grown on KA medium, isolate Nos.3 and 5 on DAPA medium and isolate

No.6 on PDA medium because of their best growth in the respective media.

4.5.1 Preliminary studies on the interaction of eighteen fungi associated with stem bleeding affected palm, with the isolates of T. paradoxa.

4.5.1.1 T. paradoxa isolate-2.

Among the eighteen fungi used for the study of their interaction with T. paradoxa isolate-2, Aspergillus niger, Trichoderma harzianum and T. viride exerted maximum inhibition on the growth of T. paradoxa in vitro (Table-23). A niger caused 13.6% inhibition of growth of T. paradoxa (Plate-24). All the isolates of Trichoderma viz. T. harzianum (two isolates) and T. viride (three isolates) caused considerable reduction of growth of Thielaviopsis paradoxa. T. harzianum inhibited the growth of T. paradoxa by over 14% within 24 hours after inoculation of the dual cultures. The three isolates of T.viride reduced the growth rate of T. paradoxa isolate-2 by 15.38%, 12.5% and 25.0% respectively in 24 hrs. after inoculation. Aspergillus terreus, Paecilomyces varioti, Penicillium diverseum, P. janthinellum isolate-1, and Pestolotiopsis palmarum did not show growth inhibition of T. paradoxa by not more than 5% inhibition after a lapse of 24 hours. T. paradoxa did not show growth

inhibition of any test fungi except A. niger where mutual inhibition of growth was found for one week after inoculation (Table-23). A yellow pigmented zone was formed in between the two opposing colonies at their point of contact, after 48 hrs. of incubation. Acremonium sp., Chalaropsis sp, Penicillium citrinum, P. janthinellum (isolate-2) and two unidentified sterile fungi did not show any response on the growth of T. paradoxa and vice versa.

A.niger and all the isolates of Trichoderma harzianum and T. viride overgrew on Thielaviopsis paradoxa colony, when the dual cultures were kept for 15 days after inoculation (Table-24) (Plates 25 to 29). Intermingling of the colonies with no evidence of macroscopic or microscopic signs of interaction was found in the case of other fungi studied.

4.5.1.2 T. paradoxa isolate-3.

Twenty percent each of mutual inhibition of growth of T. paradoxa and A. niger was found after 24 hr. of incubation. (Plate 30) (Table-25). The growth inhibition was more at 48th hr. after inoculation (Table-26). A yellow pigmented zone was found at the junction of the two opposing colonies on the third day after inoculation(Table-28). A. terreus, Chalaropsis sp., P. palmarum, sterile fungus-1 and sterile fungus-2 showed 7.14%, 5.8% , 9.09%, 7.69%, 6.25% of growth inhibition of T. paradoxa colony respectively in 24 hrs. after inoculation. In 48th hr. A. terreus and P. palmarum showed 12% and 22.72% of growth inhibition of T. paradoxa.

Table 23: In vitro studies on the interaction of associated fungi with T. paradoxa Isolate No.2.
(Results 24 & 48 hrs. after inoculation)

Fungus tested	24 hrs after inoculation						48 hrs. after-inoculation
	x Colony radius of T. paradoxa (in mm)		% of inhibition of T. paradoxa	x Colony radius of test fungus (in mm)		% of inhibition of test fungus by T. paradoxa	
	Radius towards test fungus	Control		Radius towards T. paradoxa colony	Control		
1	2	3	4	5	6	7	8
Acremonium sp.1	28	28	0.0	4	4	0.0	I
Acremonium sp.2	28	28	0.0	4	4	0.0	I
Aspergillus niger.	19	22	13.6	4	5	20.0	MP
A. terreus	22	23	4.35	4	4	0.0	I
Chalaropsis sp.	20	20	0.0	4	4	0.0	I
Paecilomycs varioti	27	28	3.6	4	4	0.0	I
Penicillium citrinum	24	24	0.0	4	4	0.0	I
P.diverseum	22	23	4.35	4	4	0.0	I
P. janthinellum-1	22	23	4.35	4	4	0.0	I
P. janthinellum-2	26	26	0.0	4	4	0.0	I
Pestalotiopsis palmarum	22	23	4.35	4	4	0.0	I

contd....

Table 23 contd.

1	2	3	4	5	6	7	8
Sterile fungus-1	25	25	0.0	4	4	0.0	I
Sterile fungus-2	21	21	0.0	4	4	0.0	I
Trichoderma harzianum-1	22	26	15.38	5	5	0.0	0
T. harzianum-2	24	28	14.28	6	6	0.0	0
T. viride-1	22	26	15.38	5	5	0.0	0
T. viride-2	21	24	12.5	5	5	0.0	0
T. viride-3	18	24	25.0	4	4	0.0	0

x = Mean of four replicates.

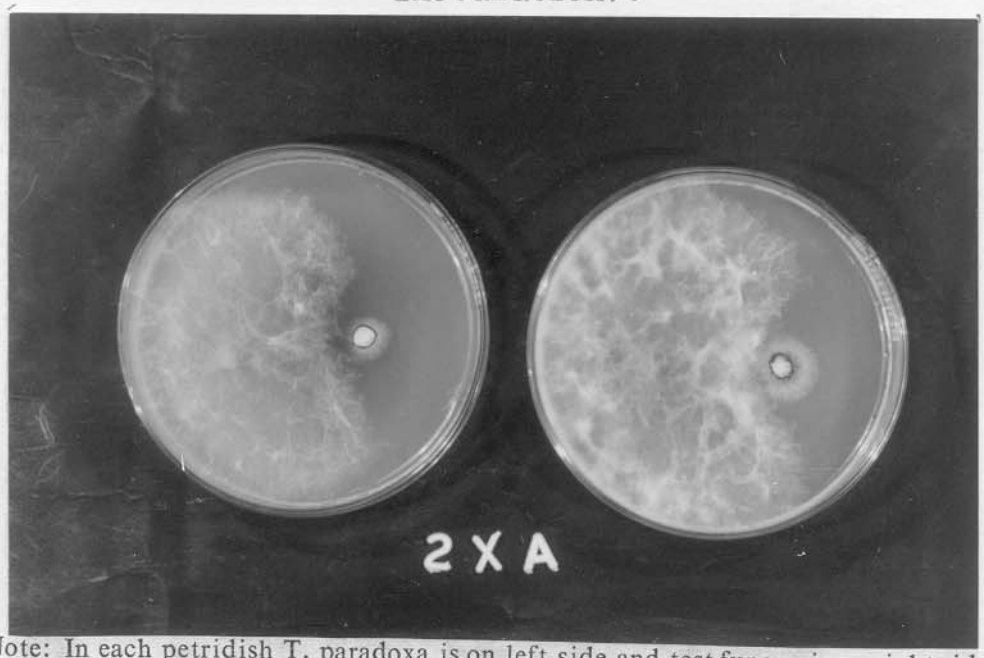
Control = radius vertically away from the opposing colony towards the side of petridish.

I.=Intermingling of T. paradoxa and test fungus colonies with no macroscopic or microscopic signs of interaction.

MP = Mutual inhibition of the growth of opposing colonies with eventual contact but no intermingling, a band of pigmented zone developing at the junction of the colonies.

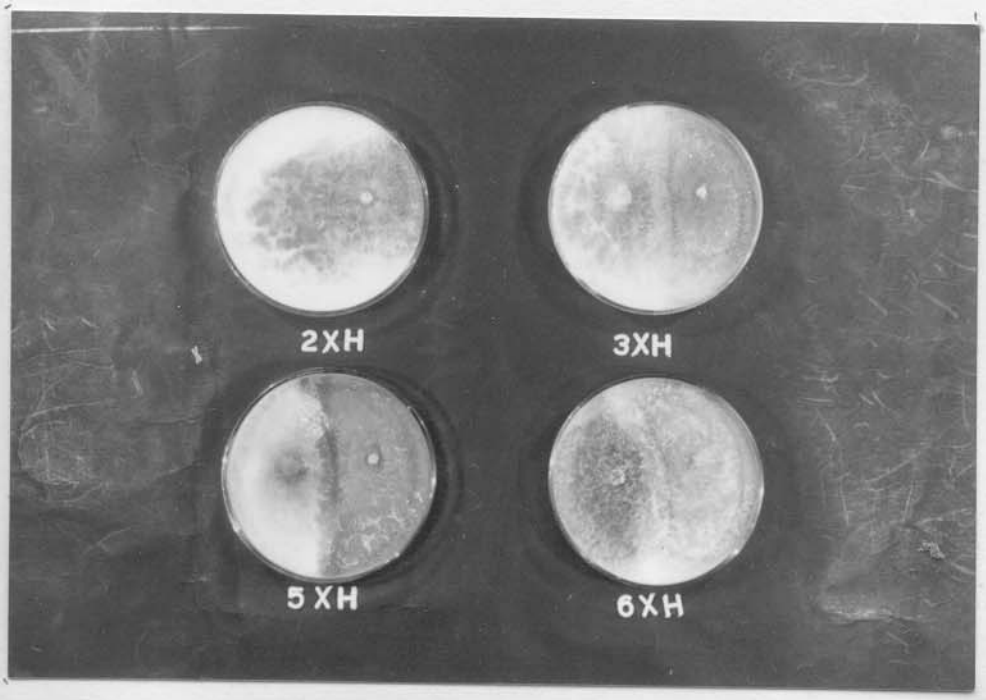
O = Over growth of T. paradoxa by the opposing fungus, usually accompanied by inhibition of T. paradoxa on or shortly after contact.

Plate 24. Mutual inhibition of T. paradoxa isolate No.2. and Aspergillus niger (Photo taken 24 hrs. after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Plate 25. Growth inhibition of T. paradoxa and overgrowth of Trichoderma viride isolate-1(15 days after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Table 24: In vitro interaction of associated fungi with T. paradoxa isolate No.2.

(15 days after inoculation)

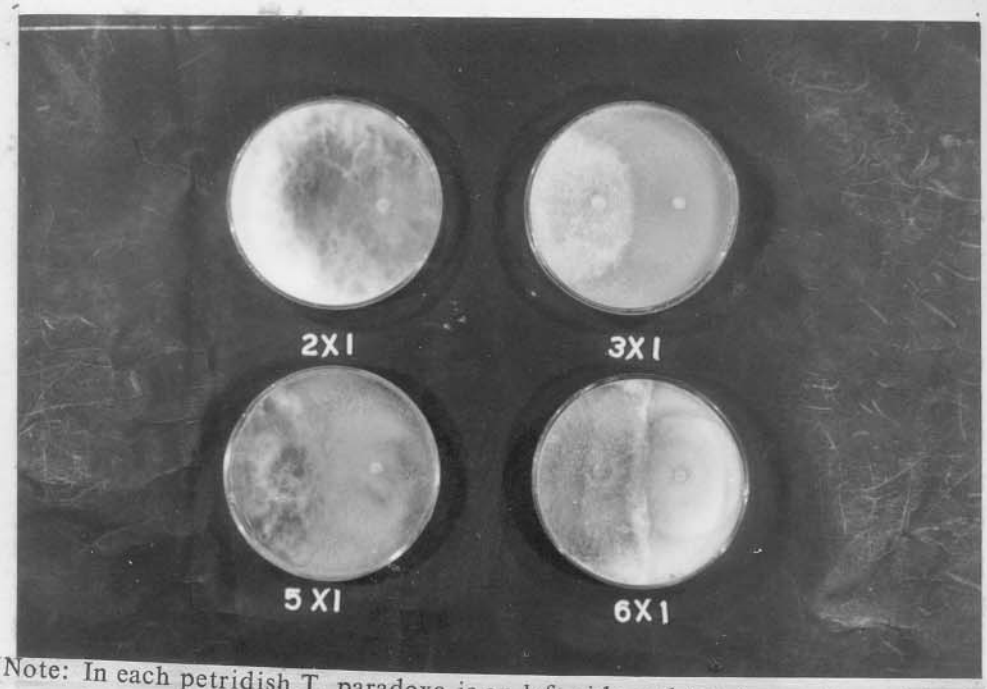
S.No.	Category	Interaction	Fungi showing the interaction with T. paradoxa.
1	2	3	4
1		Intermingling of the colonies with no macroscopic or microscopic signs of interaction.	Acremonium sp-1 Acremonium sp-2 Aspergillus terreus Chalaropsis sp. Paecilomyces varioti Penicillium citrinum P. diverseum P. Janthinellum-1 P. janthinellum-2 Pestalotiopsis palmarum Sterile fungus-1 & 2
2	O	Overgrowth of T. paradoxa colony by opposing fungi usually accompanied by inhibition of T. paradoxa on or shortly after contact.	Aspergillus niger Trichoderma harzianum-1 Trichoderma harzianum-2 T. viride-1 T. viride-2 T. viride-3
3	M.P.	Mutual inhibition of the growth of the opposing colony with eventual contact but no apparent intermingling, a band of pigmented zone often developing at the junction of the colonies.	- - - - -

contd...

Table 24 contd.

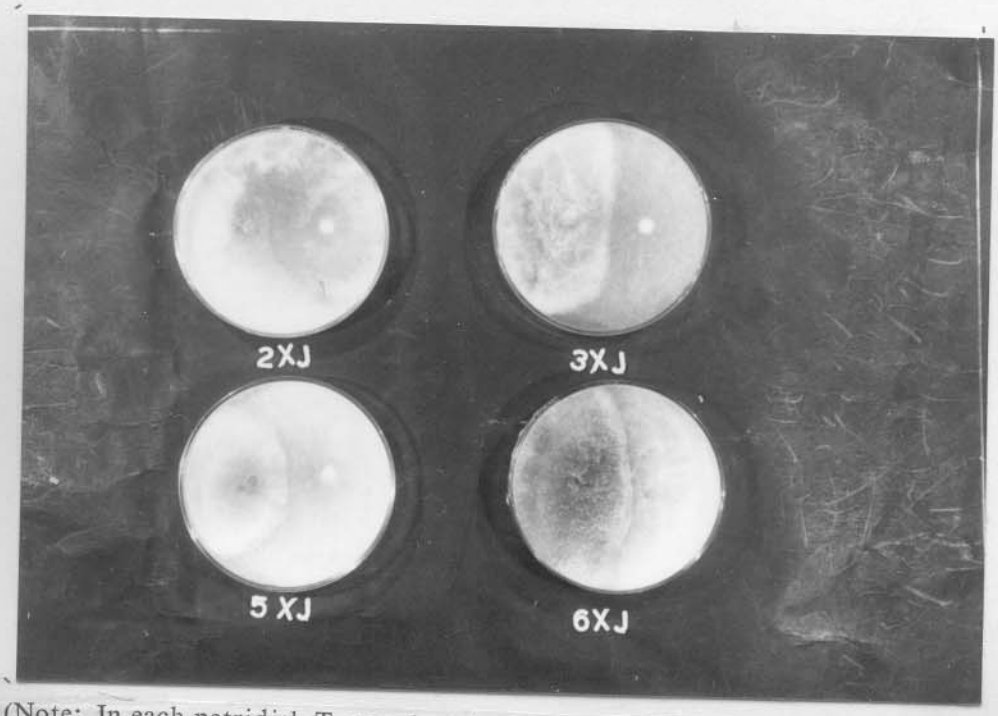
1	2	3	4
4	M.C.	Mutual inhibition of both the colony with no apparent intermingling, a clear zone remaining between the colonies.	---
5	E	Extreme inhibition of T. paradoxa colony at a distance of 5 mm or more, little or no reciprocal inhibition of the opposing colonies.	---

Plate 26. Growth inhibition of T. paradoxa and overgrowth of Trichoderma viride isolate-2(15 days after inoculation)



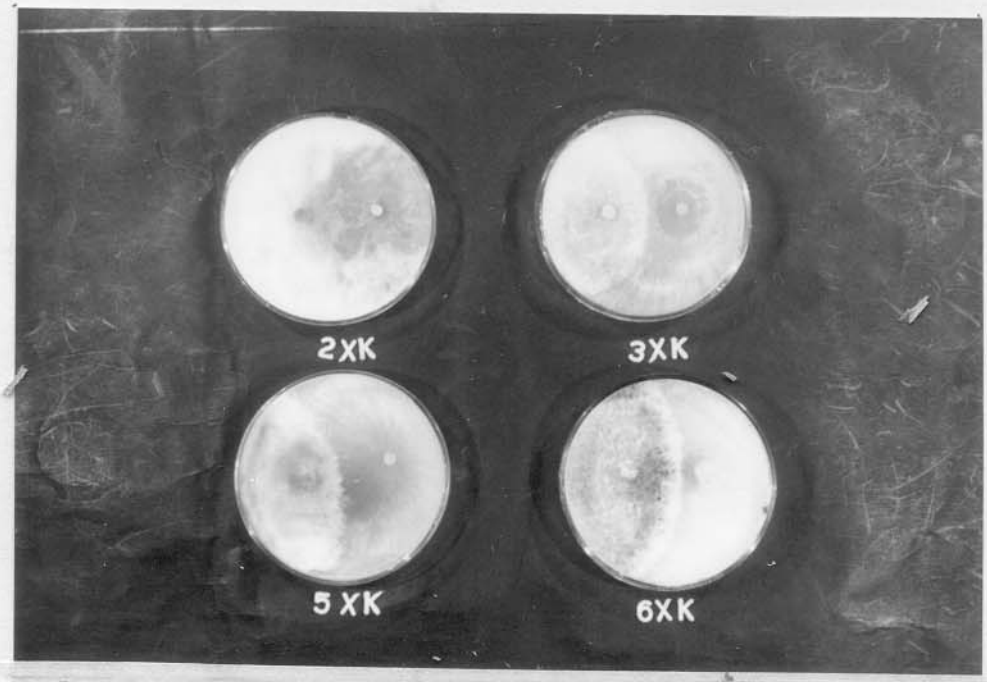
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Plate 27. Growth inhibition of T. paradoxa and overgrowth of Trichoderma viride isolate-3(15 days after inoculation)



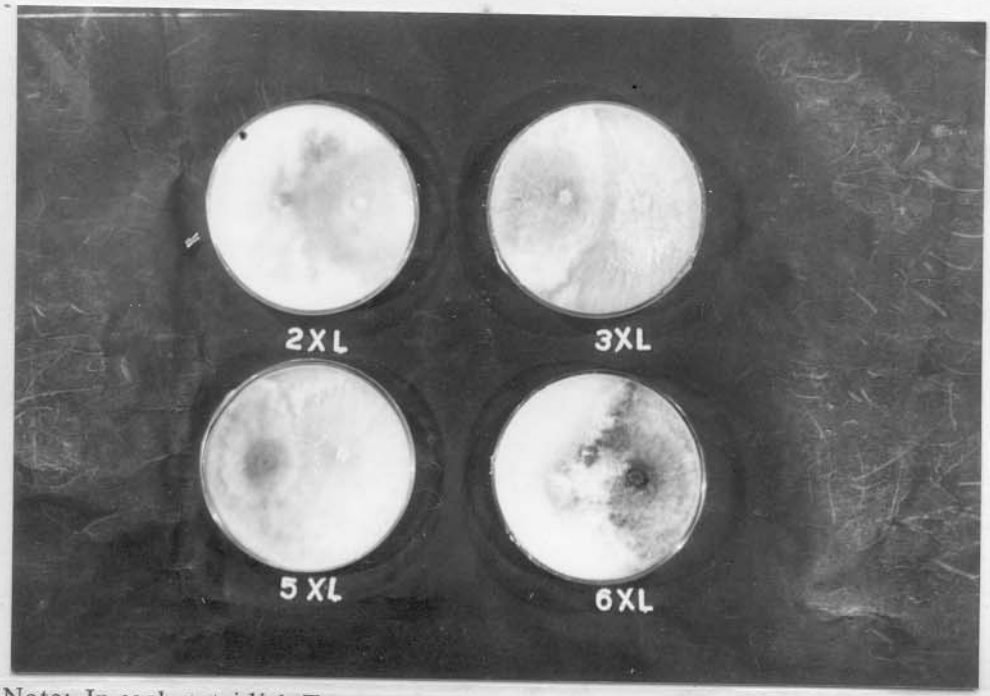
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Plate 28. Growth inhibition of T. paradoxa and overgrowth of T. harzianum isolate-1(15 days after inoculation)



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Plate 29. Growth inhibition of T. paradoxa and overgrowth of T. harzianum isolate-2(15 days after inoculation)



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Chalaropsis sp. and two species of sterile fungi did not show any sign of growth inhibition at fourty eighth hour after inoculation(Table-26). On the third day after inoculation, mutual inhibition with a clear zone in between the opposing colonies was observed in case of A. terreus. Mutual growth inhibition with a light brick red pigmented zone in between the opposing colonies was found in case of P. palmarum (Table-27)

T. harzianum isolate Nos.1 and 2 showed 16.60% and 20% of growth inhibition of Thielaviopsis paradoxa in 24 hr. and 30.76% and 43.4% of growth reduction in 48 hrs. after inoculation respectively (Table 25 & 26). T. viride isolate Nos.1,2 and 3 showed 22.22%, 14.28% and 20% respectively of growth inhibition of Thielaviopsis paradoxa in 24 hr.(Table-25). and 40%, 30.7% and 40% respectively of growth reduction of T. paradoxa in 48 hr. after inoculation (Table-26). On the third day after inoculation, mutual inhibition with a clear zone was observed in case of T. harzianum isolate-1 but overgrowth of T. harzianum isolate-2 and all the isolates of T. viride on T. paradoxa was found on the third day after inoculation. Acremonium sp. Paecilomyces varioti, Penicillium citrinum, P. diverseum, P. janthinellum (both isolates) did not show any sign of inhibition of growth of T. paradoxa (Table-25 & 26).

A.niger and all the isolates of Trichoderma harzianum and T. viride (Plates 25 to 29) overgrew on T. paradoxa colony, when the dual cultures were kept for 15 day after inoculation. Mutual inhibition of the opposing colonies with a clear zone in between them was observed in case of A. terreus and P. palmarum with

Table 25: In vitro studies on the interaction of associated fungi with T. paradoxa isolate No.3

(Results 24 hrs. after inoculation.)

Fungus tested	x Colony radius of T. paradoxa (in mm)		% of inhibition of T. paradoxa	x Colony radius of test fungus (in mm)		% of inhibition of test fungus by T. paradoxa.
	Radius towards test fungus	Control		Radius towards T. paradoxa colony	Control	
1	2	3	4	5	6	7
Acremonium sp.1	13	13	0.0	4	4	0.0
Acremonium sp.2	16	16	0.0	4	4	0.0
Aspergillus niger	12	15	20.0	4	5	20.0
A. terreus	13	14	7.14	4	4	0.0
Chalaropsis sp.	16	17	5.8	4	4	0.0
Paecilomycs varioti	14	14	0.0	4	4	0.0
Penicillium citrinum	13	13	0.0	4	4	0.0
P. diverseum	13	13	0.0	4	4	0.0
P. janthinellum-1	13	13	0.0	4	4	0.0
P. janthinellum-2	13	13	0.0	4	4	0.0
Pestalotiopsis palmarum	10	11	9.09	4	4	0.0
Sterile fungus-1	12	13	7.69	4	4	0.0
Sterile fungus-2	16	17	6.25	4	4	0.0

contd....

Table 25 contd.

1	2	3	4	5	6	7
Trichoderma harzianum-1	10	12	16.60	8	8	0.0
T. harzianum-2	8	10	20.0	7	7	0.0
T. viride-1	7	9	22.22	4	4	0.0
T. viride-2	6	7	14.28	5	5	0.0
T. viride-3	12	15	20.0	4	4	0.0

x=Mean of four replicates.

Control = radius vertically away from the opposing colony towards the side of petridish.

Table 26: In vitro studies on the interaction of associated fungi with T. paradoxa isolate No.3.

(Results 48 & 72 hrs. after inoculation)

Fungus tested	48 hrs. after inoculation						72 hrs. after-inocu-lation
	Colony radius of T.paradoxa (in mm)		% of inhi-biti-on of T. pa-radoxa	Colony radius of test fungus (in mm)		% of inhibition of test fungus by T. paradoxa	
	Radius towards test fungus	Control		Radius towards T.para-doxa colony	Control		
1	2	3	4	5	6	7	8
Acremonium sp-1	24	24	0.0	5	5	0.0	I
Acremonium sp-2	25	25	0.0	5	5	0.0	I
Aspergillus niger	20	26	23.07	5	7	28.57	M.P.
A. terreus	22	25	12.0	5	5	0.0	M.C.
Chalaropsis sp.	29	29	0.0	6	6	0.0	I
Paecilomycs varioti	25	25	0.0	4	4	0.0	I
Penicillium citrinum	23	23	0.0	5	5	0.0	I
P. diverseum	25	25	0.0	5	5	0.0	I
P. janthinellum-1	25	25	0.0	5	5	0.0	I
P. janthinellum-2	24	24	0.0	5	5	0.0	I

contd...

Table 26 contd.

1	2	2	3	4	5	6	7	8
Pestalotipsis palmarum		17	22	22.72	8	8	0.0	M.P.
Sterile fungus-1		25	25	0.0	5	5	0.0	I
Sterile fungus-2		26	26	0.0	5	5	0.0	I
Trichoderma harzianum-1		18	26	30.76	21	23	8.69	M.C.
T. harzianum-2		13	23	43.4	23	23	0.0	0
T. viride-1		9	15	40.0	11	11	0.0	0
T. viride-2		9	13	30.7	11	11	0.0	0
T. viride-3		15	25	40.0	15	15	0.0	0

x = Mean of four replicates.

Control = radius vertically away from the opposing colony towards the side of petridish.

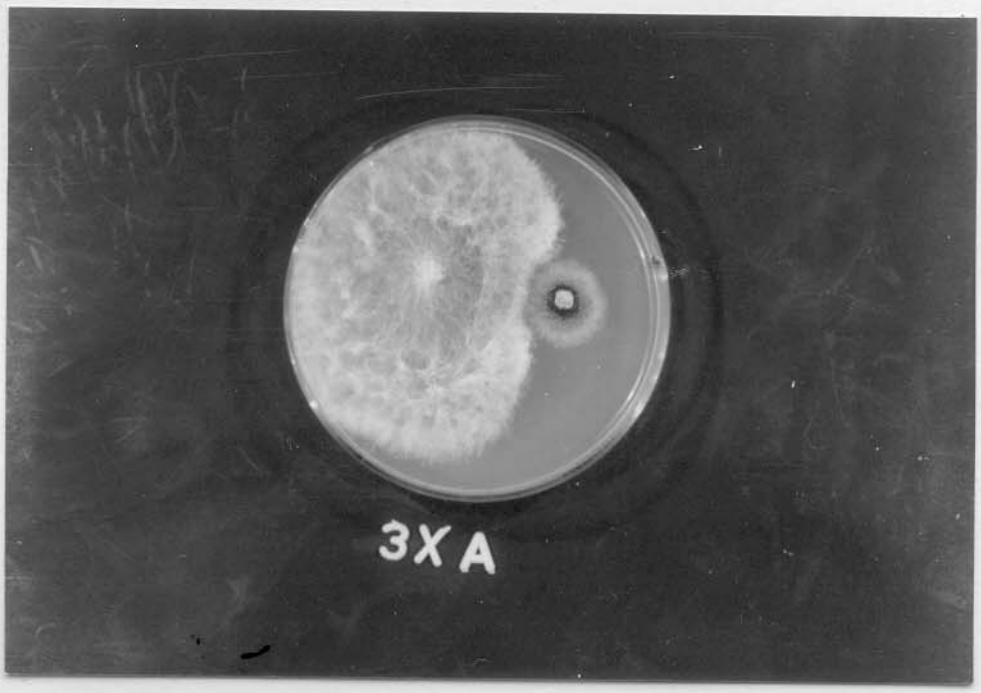
I = Intermingling of T. paradoxa and test fungus colonies with no macroscopic or microscopic sings of interaction.

M.C. = Mutual inhibition of both colonies with no intermingling, a clear zone remaining between the colonies.

M.P = Mutual inhibition of the growth of opposing colonies with eventual contact but no intermingling, a band of pigmented zone developing at the junction of the colonies.

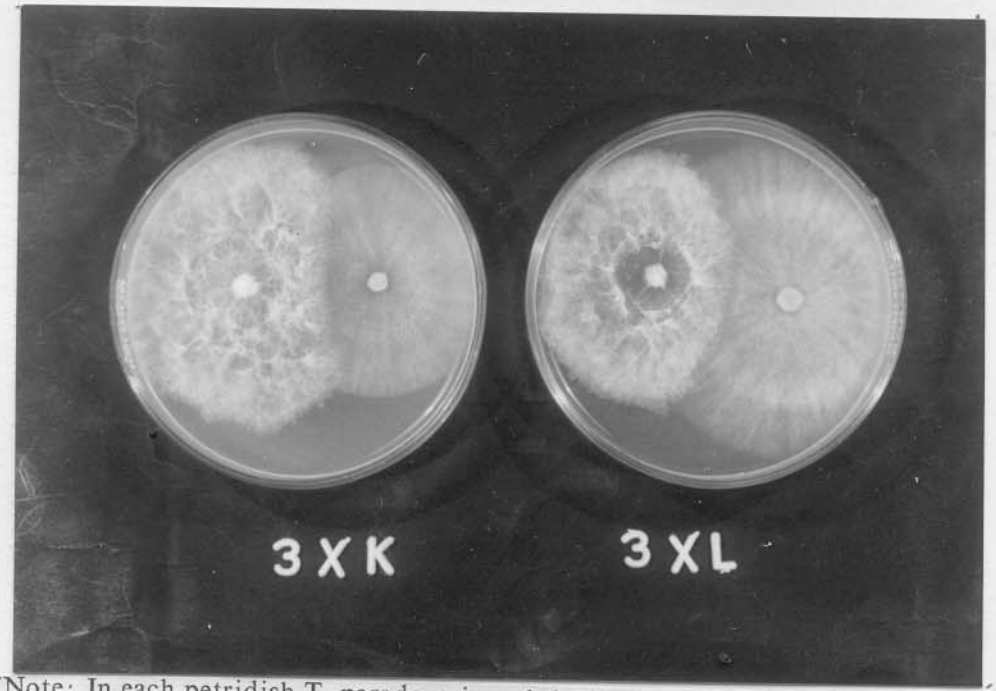
O = Over growth of T. paradoxa by the opposing fungus, usually accompanied by inhibition of T. paradoxa on or shortly after contact.

Plate 30. Mutual inhibition of *T. paradoxa* isolate-3 and *A. niger* (24 hrs. after inoculation)



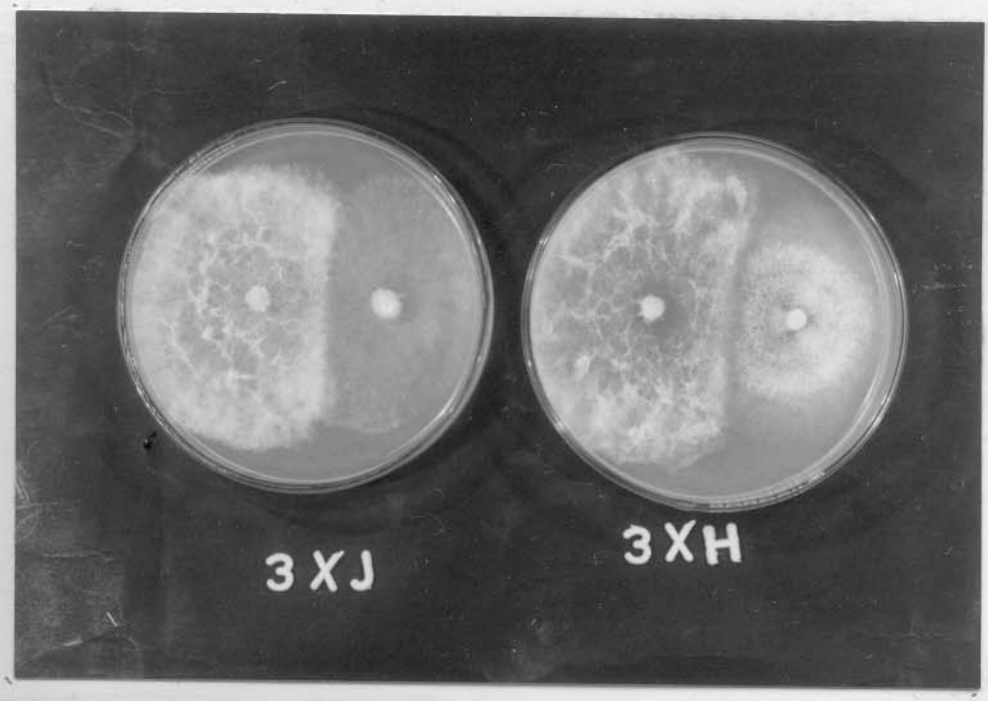
(Note: In the petridish *T. paradoxa* is on left side and test fungus is on right side.)

Plate 31. Growth inhibition of T. paradoxa isolate-3 by T. harzianum isolates 1 & 2 (48 hrs. after inoculation)



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Plate 32. Growth inhibition of T. paradoxa isolate-3 by T. viride 1 & 3(48 hrs. after inoculation)



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Table 27: In vitro interaction of associated fungi with T. paradoxa isolate No.3

(15 days after inoculation)

S.No.	Category	Interaction	Fungi showing the interaction with T. paradoxa
1	I	Intermingling of the colonies with no macroscopic or microscopic signs of interaction.	Acremonium sp-1 &2 Chalaropsis sp. Paecilomyces varioti Penicillium citrinum P. diverseum P. janthinellum-1 & 2 Sterile fungus 1 & 2
2	O	Overgrowth of T. paradoxa colony by opposing fungi usually accompanied by inhibition of T. paradoxa on or shortly after contact.	Aspergillus niger Trichoderma harzianum Isolate 1 & 2 T. viride-1 T. viride-2 T. viride-3
3	M.P.	Mutual inhibition of the growth of the opposing colony with eventual contact but no apparent intermingling, aband of pigmented zone often developing at the junction of the colonies.	----
4	M.C.	Mutual inhibition of both the colonies with no apparent intermingling, a clear zone remaining between the colonies.	Aspergillus terreus Pestalotiopsis palmarum
5	E	Extreme inhibition of T. paradoxa colony at a distance of 5 mm or more, little or no reciprocal inhibition of the opposing colonies.	----

T. paradoxa. Intermingling of opposing colonies with no apparent macroscopic or microscopic signs of interaction was found in case of other fungi studied(Table-27).

4.5.1.3 T. paradoxa isolate-5.

A. niger showed 18.18% and 20% growth reduction of T. paradoxa in 24 hrs. and 48 hrs. respectively after inoculation. Mutual inhibition of A. niger and T. paradoxa was not observed for two days after inoculation (Tables28 and 29). However, mutual inhibition was observed on the third day after inoculation (Table-30)(Plate 33).

A. terreus, Chalaropsis sp. Penicillium citrinum, P. janthinellum isolate-2 and P. palmarum showed 8.33% 10%, 9.09%, 18.18% and 10% respectively in 24 hours after inoculation (Table 28). Percentage of growth reduction of T. paradoxa by these fungi increased on the second day after inoculation except in case ofP. janthinellum isolate-2. P. janthinellum isolate-2 showed only 4.76% of growth reduction of T. paradoxa on the second day after inoculation and there was no growth reduction of T. paradoxa by this fungus on the third day or later. A. terreus showed 12% and 16.67% of growth reduction of T. paradoxa on the second and third day after inoculation (Tables29 & 30). Chalaropsis sp. P. citrinum and P. palmarum showed 10%, 13% and 13.06% growth reduction of T. paradoxa respectively on the second day after

Table 28: In vitro studies on the interaction of associated fungi with T. paradoxa isolate No.5
(Results 24 hrs. after inoculation)

Fungus tested	x Colony radius of T. paradoxa (in mm)		% of inhibition of T. paradoxa	x Colony radius of test fungus (in mm)		% of inhibition of test fungus by T. paradoxa.
	Radius towards test fungus	Control		Radius towards T. paradoxa. colony	Control	
1	2	3	4	5	6	7
Acremonium sp-1	10	10	0.0	4	4	0.0
Acremonium sp-2	10	10	0.0	4	4	0.0
Aspergillus niger	9	11	18.18	4	4	0.0
A. terreus	11	12	8.33	4	4	0.0
Chalaropsis sp.	9	10	10.0	4	4	0.0.
Paecilomyces varioti	9	9	0.0	4	4	0.0
Penicillium citrinum	10	10	9.09	4	4	0.0
P. diverseum	9	9	0.0	4	4	0.0
P. janthinellum-1	13	13	0.0	4	4	0.0
P. janthinellum-2	9	11	18.18	4	4	0.0
Pestalotiopsis palmarum	9	10	10.0	4	4	0.0
Sterile fungus-1	12	12	0.0	4	4	0.0
Sterile fungus-2	10	10	0.0	4	4	0.0

contd.....

Table 28 contd.

1	2	3	4	5	6	7
Trichoderma harzianum-1	10	12	16.66	8	9	11.11
T. harzianum-2	7	9	22.22	9	9	0.0
T. viride-1	2	10	20.0	5	5	0.0
T. viride-2	8	11	27.27	8	8	0.0
T. viride-3	8	12	33.33	5	5	0.0

x = Mean of four replicates.

Control = radius vertically away from the opposing colony towards the side of petridish.

Table 29: In vitro studies on the interaction of associated fungi with T. paradoxa isolate No.5

(Results 48 hrs. after inoculation)

Fungus tested	x Colony radius of T. paradoxa (in mm)		% of inhibition of T. paradoxa	x Colony radius of test fungus (in mm)		% of inhibition of test fungus by T. paradoxa.
	Radius towards test fungus	Control		Radius towards T. paradoxa colony	Control	
1	2	3	4	5	6	7
Acremonium sp-1	22	22	0.0	5	5	0.0
Acremonium sp-2	21	21	0.0	5	5	0.0
Aspergillus niger	20	25	20.0	5	5	0.0
A. terreus	22	25	12.0	5	5	0.0
Chalaropsis sp.	18	20	1.0	5	5	0.0
Paecilomyces varioti	13	13	0.0	5	5	0.0
Penicillium citrinum	20	23	13.0	5	5	0.0
P. diverseum	19	19	0.0	5	5	0.0
P. janthinellum-1	23	23	0.0	5	5	0.0
P. janthinellum-2	20	21	4.76	5	5	0.0
Pestaöptopüsos Pestalotiopsis palmarum	19	22	13.06	10	10	0.0

contd....

Table 29 contd.

1	2	3	4	5	6	7
Sterile fungus-1	22	22	0.0	5	5	0.0
Sterile fungus-2	18	18	0.0	5	5	0.0
Trichoderma harzianum-1	14	21	33.33	18	21	14.28
T. harzianum-2	13	20	35.0	22	22	0.0
T. viride-1	15	25	40.0	19	19	0.0
T. viride-2	14	24	41.66	23	23	0.0
T. viride-3	11	23	52.17	21	21	0.0

x = Mean of four replicates.

Control = radius vertically away from the opposing colony towards the side of petridish.

inoculation(Table-29) and 16.67%, 16.67% and 20% growth reduction of T. paradoxa respectively on the third day after inoculation(Table-30).

P. diverseum, P. janthinellum isolate-1 and sterile fungus-2 did not show growth reduction of T. paradoxa upto two days after inoculation. On the third day after inoculation, these fungi showed in significant growth reduction of T. paradoxa viz. 3.33%, 6.66% and 10.0% respectively(Table-30).

Trichoderma harzianum isolate-1 and isolate-2 showed 16.66% and 22.22% growth reduction of T. paradoxa respectively in 24 hour after inoculation and 33.33% and 35% respectively in 48 hr. after inoculation (Table 28 and 29). On the third day mutual inhibition with a clear zone in between the colonies of T. harzianum isolate-1 and T. paradoxa colony was observed. T. harzianum isolate-2 overgrew on T. paradoxa on the third day after inoculation (Table-30).

Trichoderma viride isolate Nos.1, 2 and 3 showed 20%, 27.27% and 33.33% growth reduction respectively of T. paradoxa in 24 hr. after inoculation and 40%, 41.66% and 52.17% respectively in 48 hr. after inoculation. All the three isolates of T. viride overgrew on T. paradoxa colony on the third day after inoculation(Plate-34).

Acremonium sp. (isolate Nos. 1 and 2), Paecilomyces varioti and sterile fungus-1 did not show any high of growth inhibition of T. paradoxa (Tables 28, 29 and 30).

On the fourth day after inoculation, A. niger and T. paradoxa showed mutual inhibition with a pigmented zone in between the colonies (Table-30). A. terreus, P. varioti, P. citrinum, P. palmarum and sterile fungus-2 showed mutual inhibition of growth with T. paradoxa with a clear zone in between the colonies on the fourth day after inoculation. Isolates of T. harzianum and T. viride overgrew on Thielaviopsis colony on the fourth day after inoculation. Acremonium sp. (1 and 2), Chalaropsis sp., P. diverseum, P. janthinellum (isolate 1 and 2) and sterile fungus-1 showed intermingling of their colonies with T. paradoxa colony on the fourth day after inoculation (Table-30).

A. niger and all the ioslates of T. harzianum and T. viride overgrew on T. paradoxa colony, when the dual cultures were maintained for fifteen days. A. terreus, P. varioti, P. citrinum P. palmarum and sterile fungus-2 showed mutual inhibition of growth with T. paradoxa with a clear zone in between the opposing colonies. Acremonium sp.(1 and 2), Chalaropsis sp. P. diverseum, P. janthinellum (both the isolates) and sterile fungus-1 showed intermingling of their colonies with T. paradoxa colony with no apparent macroscopic or microscopic sign of interaction (Table-31).

4.5.1.4 T. paradoxa isolate-6.

Mutual inhibition of A. niger and T. paradoxa with a pigmented zone in between the colonies was found (Plate35) A. niger showed 6.25%, 14.29% and 20% growth reduction of

Table 30: In vitro studies on the interaction of associated fungi with T. paradoxa isolate No.5

(Results 72 & 96 hrs. after inoculation)

Fungus tested	72 hrs. after inoculation						96 hrs after inoculation
	x Colony radius of T. paradoxa (in mm)		% of inhibition of T. paradoxa	x Colony radius of test fungus (in mm)		% of inhibition of test fungus by T. paradoxa.	Category of interaction.
	Radius towards test fungus	Control		Radius towards T. paradoxa colony	Control		
1	2	3	4	5	6	7	8
Acremonium sp-1	Both the colonies grew and freely intermingled.						I
Acremonium sp-2	"			"			I
Aspergillus niger	22	30	26.7	12	14	14.3	M.P.
A. terreus	25	30	16.7	7	7	0.0	M.C.
Chalaropsis sp.	25	30	16.7	10	10	0.0	I
Paecilomyces varioti	Mutual inhibition with a clear zone in between						M.C.
Penicillium citrinum	25	30	16.7	9	9	0.0	M.C.
P. diverseum	29	30	3.3	10	10	0.0	I
P. janthinellum-1	28	30	6.7	6	6	0.0	I
P. janthinellum-2	Both the colonies grew and freely intermingled.						I

contd....

Table 30 contd.

1	2	3	4	5	6	7	8
Pestalotiopsis palmarum	24	30	20	15	25	40.0	M.C.
Sterile fungus-1	Both the colonies grew and freely intermingled.						I
Sterile fungus-2	27	30	10	7	7	0.0	M.C.
Trichoderma harzianum-1	Mutual inhibition with a clear zone in between						0
T. harzianum-2	T. harzianum over grew on T. paradoxa colony						0
T. viride-1	"		"		"		0
T. viride-2	"		"		"		0
T. viride-3	"		"		"		0

x = Mean of four replicates

Control = radius vertically away from the opposing colony towards the side of petridish.

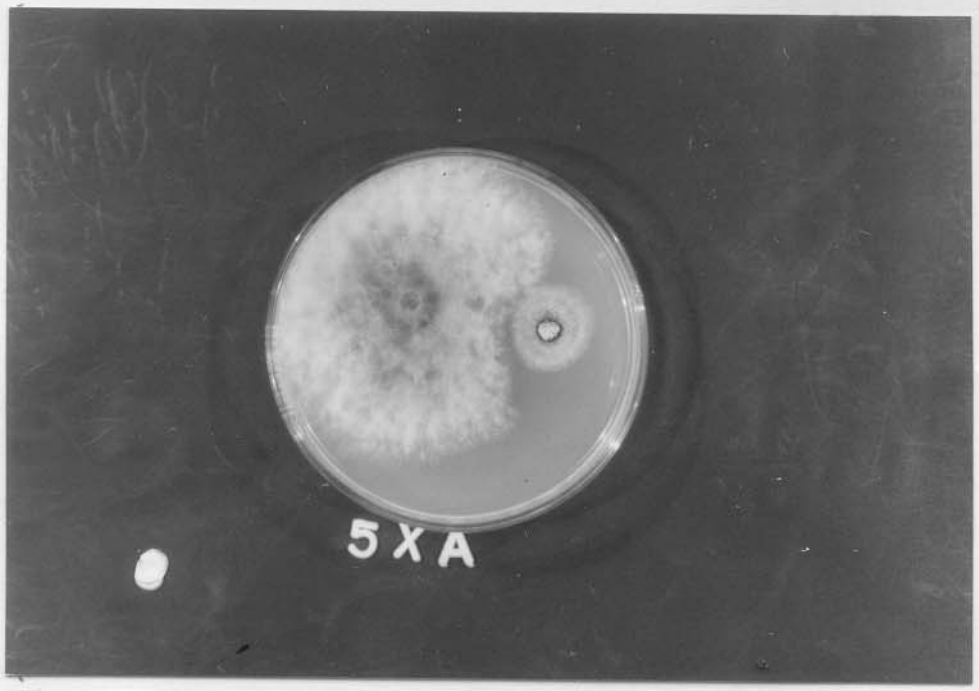
I = Intermingling of T. paradoxa and test fungus colonies with no macroscopic or microscopic sings of interaction.

M.C. = Mutual inhibition of the growth of opposing colonies with eventual contact but no intermingling, a band of pigmented zone developing at the junction of the colonies.

O = Over growth of T. paradoxa by the opposing fungus, no usually accompanised by inhibition of T. paradoxa on or shortly after contact.

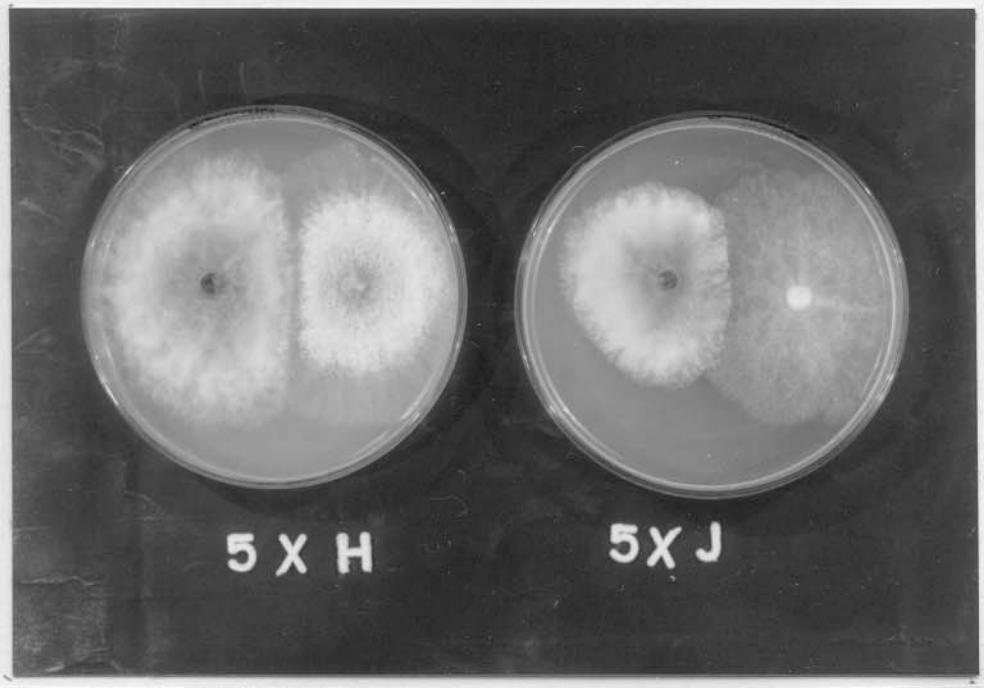
M.P. = Mutual inhibition of the growth of opposing colonies with eventual contact but no intermingling, a band of pigmented zone developing at the junction of the colonies.

Plate 33. Mutual inhibition of T. paradoxa isolate-5 and A. niger(72 hrs. after inoculation)



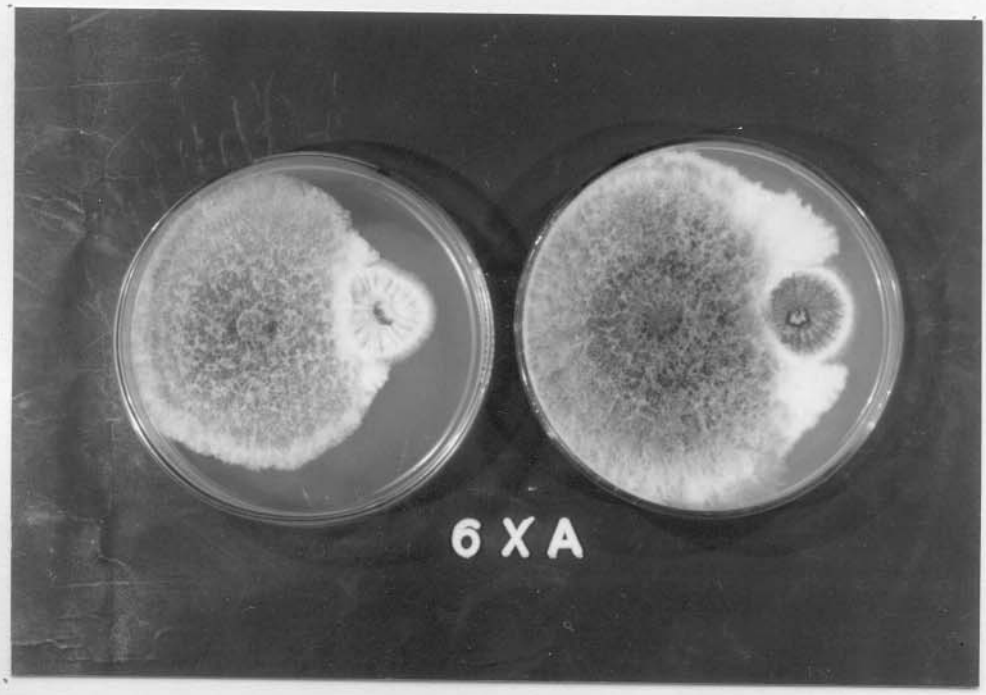
(Note: In the petridish T. paradoxa is on left side and test fungus is on right side.)

Plate 34. Growth inhibition of T. paradoxa isolate-5 by T. viride isolates 1 & 3(72 hrs. after inoculation)



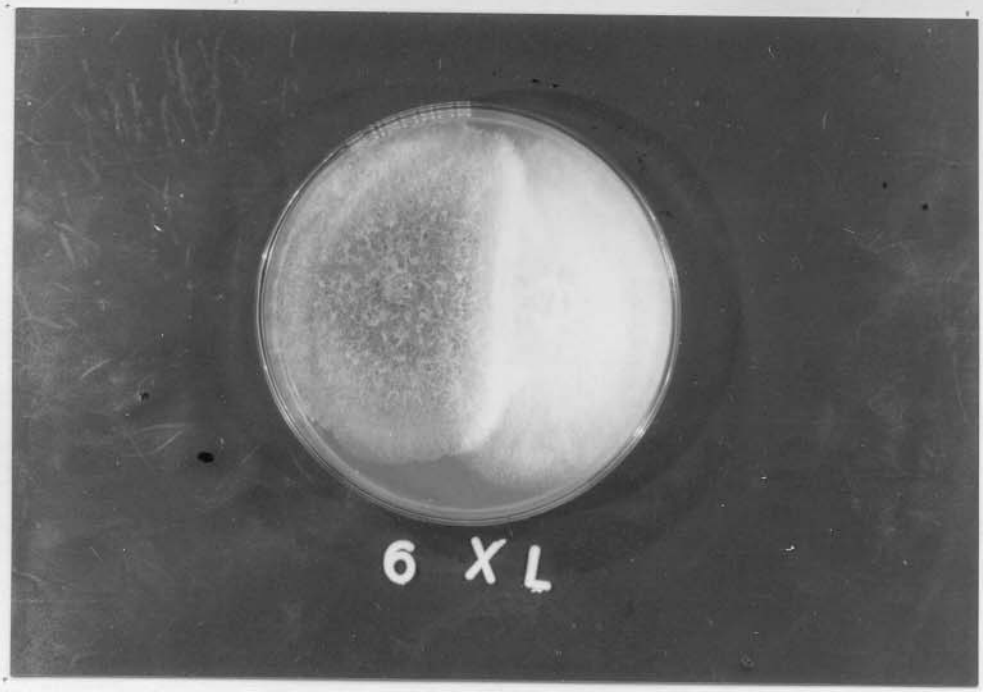
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Plate 35. Mutual inhibition of T. paradoxa isolate-6 and A. niger, 24 hrs(left) and 48hrs(right) after inoculation.



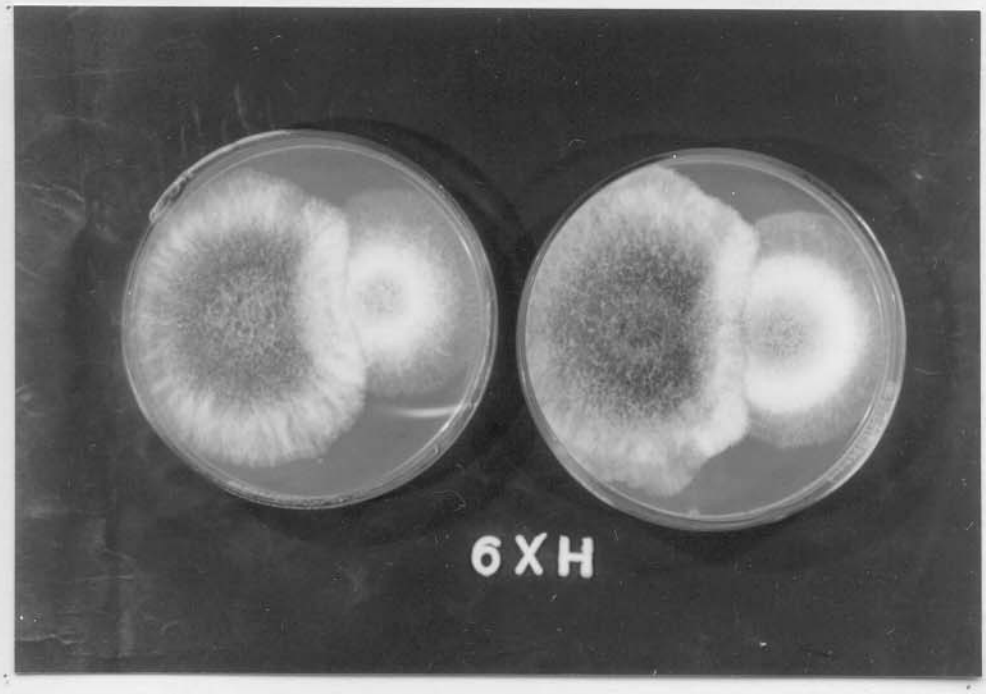
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Plate 36. Overgrowth of T. paradoxa isolate-6 by T. harzianum isolate-2(72 hrs. after inoculation).



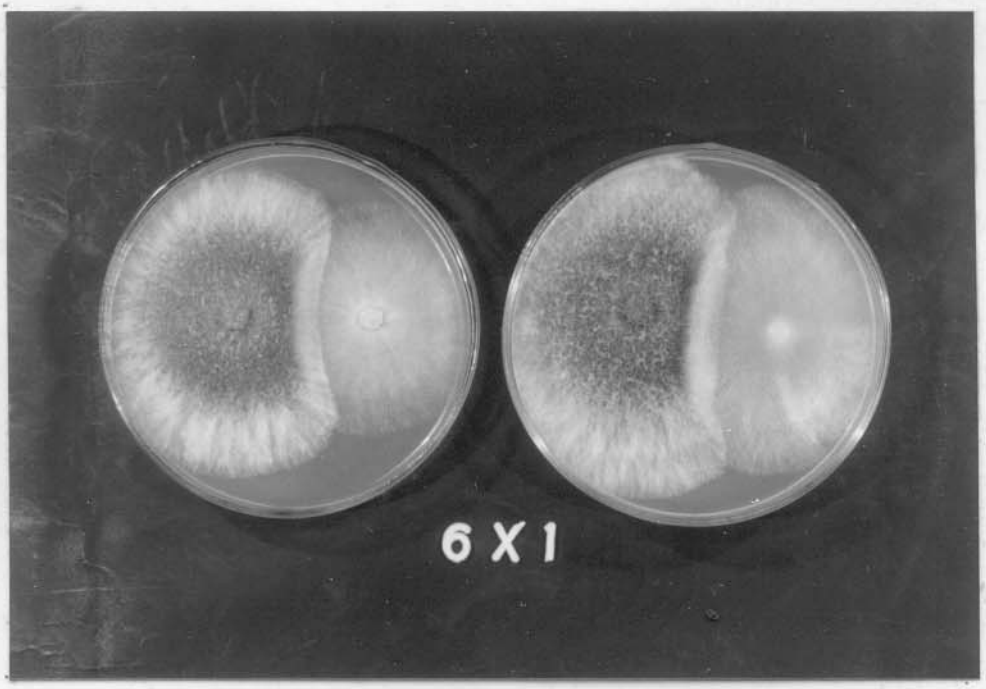
(Note: In the petridish T. paradoxa is on left side and test fungus is on right side.)

Plate 37. Overgrowth of T. paradoxa isolate-6 by T. viride isolate-1 on 4th day after inoculation.



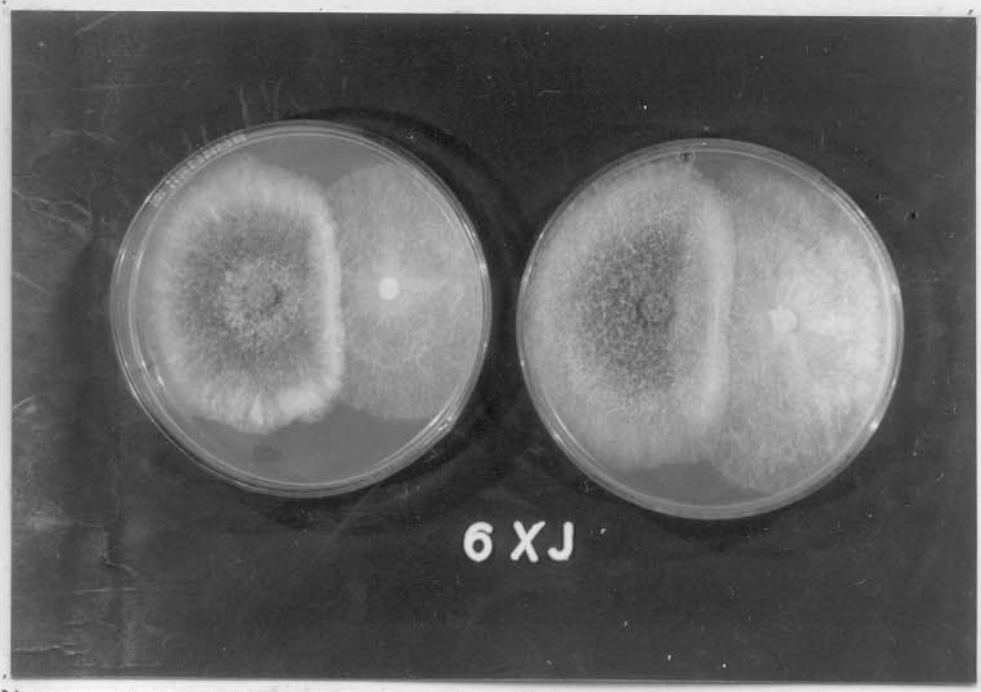
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Plate 38. Overgrowth of T. paradoxa isolate-6 by T. viride isolate-2 on fourth day after inoculation.



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Plate 39. Overgrowth of T. paradoxa isolate-6 by T. viride isolate-3 on fourth day after inoculation.



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

Table 31: In vitro interaction of associated fungi with T. paradoxa isolate No.5

(15 days after inoculation)

S.No.	Category	Interaction	Fungi showing the interaction with T. paradoxa.
1	I	Intermingling of the colonies with no macroscopic or microscopic signs of interaction.	Acremonium sp-1&2 Chalaropsis sp. Penicillium diverseum P. ienthinellum 1&2 Sterile fungus-1
2	O	Overgrowth of T. paradoxa colony by opposing fungi usually accompanied by inhibition of T. paradoxa on or shortly after contact.	Aspergillus niger Trichoderma harzianum isolates 1 & 2 T. viride-1 T. viride-2 T. viride-3
3	M.P.	Mutual inhibition of the growth of the opposing colony with eventual contact but no apparent intermingling a band of pigmented zone often developing at the junction of the colonies.	----
4	M.C.	Mutual inhibition of both the colonies with no apparent intermingling, a clear zone remaining between the colonies.	Aspergillus terreus Paecilomyces variot Penicillium citrinum Pestalotiopsis palmarum Sterile fungus-2
5	E	Extreme inhibition of T. paradoxa colony at a distance of 5 mm or more little or more reciprocal inhibition of the opposing colonies.	---

T. paradoxa colony in 24, 48 and 72 hr respectively after the inoculation. T. paradoxa reduced the growth of A. niger by 16.67% upto two days after inoculation and on the third day 33.33% growth reduction of A. niger was noticed by T. paradoxa (Tables 32, 33 & 34). There was no overgrowth of A. niger on T. paradoxa colony on the fourth day after inoculation but a pigmented zone in between the two opposing colonies was noticed.

A. terreus did not show growth inhibition of T. paradoxa upto 24 hr. after inoculation. But A. terreus showed 7.69% and 10% growth reduction of T. paradoxa at the end of the second and third day respectively after the inoculation (Tables 33 and 34). There was no mutual inhibition of the two opposing colonies upto three days after inoculation. But on the fourth day, mutual growth inhibition of A. terreus and T. paradoxa was found with a clear zone in between the two opposing colonies.

Chalaropsis sp. reduced the growth of T. paradoxa by 12.5% and 5% at the end of 24 hours and 48 hours respectively after inoculation (Tables 32 & 33). Third day onwards it did not show reduction of growth of T. paradoxa. Intermingling of colonies of Chalaropsis sp. and T. paradoxa was found from the third day onwards without showing any sign of interaction of opposing colonies on each other (Table-34).

Paecilomyces varioti did not show growth inhibition of T. paradoxa upto two days after inoculation. But on the third onwards, mutual inhibition of growth of P. varioti and T. paradoxa was found with a clear zone in between the colonies.

Table 32: In vitro studies on the interaction of associated fungi with T. paradoxa isolate No.6 (Results 24 hrs. after inoculation)

Fungus tested	x Colony radius of T. paradoxa (in mm)		% of inhibition of T. paradoxa	x Colony radius of test fungus (in mm)		% of inhibition of test fungus by T. paradoxa.
	Radius towards test fungus	Control		Radius towards T. paradoxa colony	Control	
1	2	3	4	5	6	7
Acremonium sp-1	14	14	0.0	4	4	0.0
Acremonium sp-2	15	15	0.0	4	4	0.0
Aspergillus niger	15	16	6.25	5	6	16.67
A. terreus	15	15	0.0	4	4	0.0
Chalaropsis sp.	14	16	12.5	4	4	0.0
Paecilomyces varioti	11	11	0.0	4	4	0.0
Penicillium citrinum	15	16	6.25	4	4	0.0
P. diverseum	15	15	0.0	4	4	0.0
P. janthinellum-1	15	16	6.25	4	4	0.0
P. janthinellum-2	14	15	6.6	4	4	0.0
Pestalotiopsis palmarum	16	17	5.7	4	4	0.0

contd.....

Table 32 contd.

1	2	3	4	5	6	7
Sterile fungus-1	14	15	6.6	4	4	0.0
Sterile fungus-2	15	15	0.0	4	4	0.0
Trichoderma harzianum-1	16	17	6.25	9	9	0.0
T. harzianum-2	14	15	6.67	9	9	0.0
T. viride-1	15	17	11.76	8	8	0.0
T. viride-2	16	18	11.11	9	9	0.0
T. viride-3	16	17	5.88	9	9	0.0

x = Mean of four replicates.

Control = radius vertically away from the opposing colony towards the side of petridish.

P. citrinum reduced the growth of T. paradoxa by 6.25% in 24 hrs. after inoculation, but second day onwards, it did not show any sign of growth inhibition of T. paradoxa (Tables 32, 33 & 34). P. diverseum did not show any sign of inhibition of T. paradoxa at any time after maintaining the dual culture. P. janthinellum isolates 1 & 2 showed 6.25% and 6.6% growth reduction of T. paradoxa in 24 hr. after inoculation but later there was no inhibition of growth of T. paradoxa by this fungus (Tables 32, 33 & 34). Intermingling of P. citrinum, P. diverseum and P. janthinellum (isolates 1 and 2) with T. paradoxa colony was observed on the fourth day after inoculation without any sign of inhibition of growth on each other (Table-34).

P. palmarum reduced the growth of T. paradoxa by 5.7% 8.6% and 16.67% at the end of first, second and third day respectively after inoculation (Tables 32, 33 & 34). Though mutual inhibition with a clear zone in between the colonies of P. palmarum and T. paradoxa was observed from the second day onwards, this became very obvious on the fourth day.

Sterile fungus-1 showed 6.6% growth reduction of T. paradoxa at the end of the first day after inoculation, but afterwards no sign of growth inhibition of T. paradoxa by this fungus was observed (Tables 32 to 34). Intermingling of two opposing colonies was observed from second day onwards after the inoculation

Table 33: *In vitro* studies on the interaction of associated fungi with *T. paradoxa* isolate No.6

(Results 48 hrs. after inoculation)

Fungus tested	x Colony radius of *T. paradoxa* (in mm)		% of inhibition of *T. paradoxa*.	x Colony radius of test fungus (in mm)		% of inhibition of test fungus by *T. paradoxa*.
	Radius towards test fungus	Control		Radius towards *T. paradoxa* colony.	Control	
1	2	3	4	5	6	7
Acremonium sp-1	22	22	0.0	5	5	0.0
Acremonium sp-2	23	23	0.0	5	5	0.0
Aspergillus niger	24	28	14.29	10	12	16.67
A. terreus	24	26	7.69	5	5	0.0
Chalaropsis sp.	19	20	5.0	5	5	0.0
Paecilomyces varioti	20	20	0.0	7	7	0.0
Penicillium citrinum	25	25	0.0	5	5	0.0
P. diverseum	25	25	0.0	5	5	0.0
P. janthinellum-1	22	22	0.0	5	5	0.0
P. janthinellum-2	23	23	0.0	4	4	0.0
Pestalotiopsis palmarum	21	23	8.6	9	10	10.0

contd...

Table 33 contd.

1	2	3	4	5	6	7
Sterile fungus-1	23	23	0.0	6	6	0.0
Sterile fungus-2	22	22	0.0	5	5	0.0
Trichoderma harzianum-1	19	26	26.92	23	23	0.0
T. harzianum-2	18	27	33.33	23	23	0.0
T. viride-1	17	27	37.03	21	21	0.0
T. viride-2	18	27	33.33	22	22	0.0
T. viride-3	19	26	26.92	22.	22	0.0

x = Mean of four replicates.
Control = radius vertically away from the opposing colony towards the side of petridish.

(Table 34). Sterile fungus-2 did not show any sign of growth inhibition of T. paradoxa upto two days after inoculation (Tables 32 & 33), but third day onwards, this fungus and T. paradoxa showed mutual inhibition of growth with a clear zone in between the opposing colonies(Table-34).

All the isolates of Trichoderma harzianum and T. viride inhibited the growth of T. paradoxa isolate-6, but to a lesser extent when compared to the growth inhibition of other isolates of T. paradoxa by Trichoderma sp. T. harzianum isolates1 and 2 reduced the growth of Thielaviopsis isolate-6 by 6.25% and 6.67% respectively at 24 hrs. after inoculation.and 26.92% and 33.33% respectively at the end of 48 hrs. after inoculation. Both the isolates of T. harzianum overgrew on Thielaviopsis colony on the third day onwards after the inoculation (Table-34) (Plate 36).

Trichoderma viride isolates 1, 2 and 3 showed 11.76%, 11.11% 5.88% growth reduction of Thielaviopsis paradoxa respectively at the end of first day after inoculation and 37.03%, 33.33% and 26.92% growth reduction of T. paradoxa respectively at the end of the second day after inoculation (Tables 35 & 36). All the isolates of T. viride overgrew on T. paradoxa from the third day onwards (Table 34)(Plates 37, 38 & 39).

A. niger and all the isolates of T. harzianum and T. viride overgrew on T. paradoxa when observed on the fifteenth day after inoculation (Table-35). A. terreus Paecilomyces varioti, and P. palmarum and sterile fungus-2

Table 34: In vitro studies on the interaction of associated fungi with T. paradoxa isolate No.6.

(Results 72 & 96 hrs. after inoculation)

Fungus tested	72 hrs. after inoculation						96 hrs after inoculation
	x Colony radius of T. paradoxa (in mm)		% of inhibition of T. paradoxa	x Colony radius of test fungus (in mm)		% ofinhibition of test fungus by T. paradoxa.	Category of interaction.
	Radius towards test fungus	Control		Radius towards P. Paradoxa colony	Control		
1	2	3	4	5	6	7	8
Acremonium sp-1	Both the colonies grew and freely intermingled.						I
Acremonium sp-2	"			"			I
Aspergillus niger	24	30	20	10	15	33.3	M.P.
A. terreus	27	30	10	7	7	0.0	M.C.
Chalaropsis sp.	Both the colonies grew and freely intermingled.						I
Paecilomyces varioti	Mutual inhibition with a clear zone in between						M.C.
Penicillium citrinum	30	30	0	9	9	0.0	I
P. diverseum	30	30	0	7	7	0.0	I
P. janthinellum-1	Both the colonies grew and freely intermingled.						I
P. janthinellum-2	"			"			I

contd....

Table 34 contd.

1	2	3	4	5	6	7	8
Pestalotiopsis palmarum	25	30	16.7	11	18	38.9	M.C.
Sterile fungus-1	Both the colonies grew and freely intermingled.						I
Sterile fungus-2	27	30	10	7	7	0.0	M.C.
Trichoderma harzianum-1	T. harzianum over grew on T. paradoxa colony						0
T. harzianum-2	"		"				0
T. viride-1	"		"				0
T. viride-2	"		"				0
T. viride-3	"		"				0

x = Mean of four replication

I. = Intermingling of T. paradoxa and test fungus colonies with no macroscopic or microscopic sings of interaction.

M.C. = Mutual inhibition or both colonies with no intermingling a clear zone remaining between the colonies.

M.P = Mutual inhibition of the growth of opposing colonies with eventual contact but no intermingling, a band of pigmented zone developing at the junction of the colonies.

O = Over growth of T. paradoxa by the opposing fungus, usually accompanied by inhibition of T. paradoxa on or shortly after contact.

Table-35: In vitro interaction of associated fungi with T. paradoxa isolate No.6

(15 days after inoculation)

S.No.	Category	Interaction	Fungi showing the interaction with T. paradoxa
1	I	Intermingling of the colonies with no macroscopic or microscopic signs of interaction.	Acremonium sp 1&2 Chalaropsis sp. Penicillium citrinum P. diverseum P. ienthinellum 1&2 Sterile fungus-1
2	O	Overgrowth of T. paradoxa colony by opposing fungi usually accompanied by inhibition of T. paradoxa on or shortly after contact.	Aspergillus niger Trichoderma harzianum isolates 1 & 2 T. viride-1 T. viride-2 T. viride-3
3	M.P.	Mutual inhibition of the growth of the opposing colony with eventual contact but no apparent intermingling a band of pigmented zone often developing at the junction of the colonies.	----
4	M.C.	Mutual inhibition of both the colonies with no apparent intermingling, a clear zone remaining between the colonies.	Aspergillus terreus Paecilomyces varioti Pestalotiopsis palmarum Sterile fungus-2
5	E	Extreme inhibition of T. paradoxa colony at a distance of 5 mm or more little or more reciprocal inhibition of the opposing colonies.	----

showed mutual growth inhibition with T. paradoxa with a clear zone of inhibition between the opposing colonies of the dual culture.

Intermingling of colonies of Acremonium sp.(1 and 2) Chalaropsis sp., Penicillium citrinum, P. diverseum, P. janthinellum (isolates 1 and 2) and sterile fungus-1 with T. paradoxa with no apparent macroscopic or microscopic signs of interaction was observed on the fifteenth day after inoculation (Table-35).

5.2 Interaction of selected species of fungi showing antagonism with T. paradoxa isolates:

From the preliminary studies on the interaction between fungi associated with stem bleeding disease of coconut with T. paradoxa isolates, those showing antagonism were selected for further studies. To understand the nature of their antagonism better and to simulate natural conditions, where fungal succession and combined infection are rule than exception, further experiments were formulated. In this studies dual cultures were established in the following ways:

1) T. paradoxa was inoculated first on the medium and was challenged with the antagonist 12 hrs. later.

2) Both the organisms were inoculated simultaneously.

3) Test antagonist was inoculated first and T. paradoxa followed 12 hrs. later.

The results are summarised in Tables 36 to 41.

4.5.2.1 Interaction of Aspergillus niger with T. paradoxa:

T. paradoxa isolate-2:

In the three methods of inoculation tested, inhibition of T. paradoxa was the least (7.4 in 12 hrs - 13.3%) in 72 hrs.) when A. niger was inoculated 12 hrs. after inoculation of T. paradoxa (Table-36). In simultaneous method of inoculation, the inhibition percentage ranged from 12.4 in 12 hrs. to 36.7 in 72 hrs. However, the maximum percentage of inhibition was discernible when A. niger was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate 40). The percentage of inhibition was 33.3 in 12 hrs. with 50 in 72 hrs. (Figs 8 & 9). Mutual inhibition was also noticed.

T. paradoxa isolate-3.

In the different methods of inoculation tested, T. paradoxa showed minimum inhibition (6.3-23.3% in 72 hrs) when A. niger was inoculated 12 hrs. after the inoculation of T. paradoxa(Table-36). Inhibition percentage ranged from 16.7 in 12 hrs. to 33.3 in 72 hrs. in simultaneous method of inoculation. However, maximum percentage of inhibition was obtained when A. niger was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate 41). The percentage of inhibition was 20 in 12 hrs, with 39.3 in 72 hrs.(Figs. 8 & 9). Mutual inhibition was also noticed.

Table 36: *In vitro* interaction of *Aspergillus niger* with *T. paradoxa*.

Time of observation after inoculation of both the fungi (in hrs.)	Growth inhibition of *T. paradoxa* by *A. niger* (Percentage)											
	Isolate-2			Isolate-3			Isolate-5			Isolate-6		
	A	B	C	A	B	C	A	B	C	A	B	C
12	7.4	12.4	33.3	6.3	16.7	20.0	0	0	0	0	0	0
24	10.0	28.0	36.8	16.0	27.8	33.3	19.1	23.3	25.0	8.3	12.5	20.
36	13.3	32.1	46.4	23.3	28.8	36.0	20.0	29.4	37.5	14.2	18.1	21.
48	13.3	34.5	50.0	23.3	33.3	39.3	30.0	40.0	44.0	26.7	30.0	36.
60	13.3	36.7	50.0	23.3	33.3	39.3	30.0	40.0	44.0	26.7	30.0	36.
72	13.3	36.7	50.0	23.3	33.3	39.3	30.0	40.0	50.0	26.7	30.0	36.

A = *T. paradoxa* inoculated 12 hrs. before the inoculation of *A. nig*

B = *T. paradoxa* and *A. niger* were simultaneously inoculated.

C = *T. paradoxa* was inoculated after 12 hrs. growth of *A. niger*.

Fig 8. Growth inhibition of T. paradoxa isolates by A. niger upto 72 hrs. after inoculation when T. paradoxa was inoculated after 12 hrs growth of A. niger

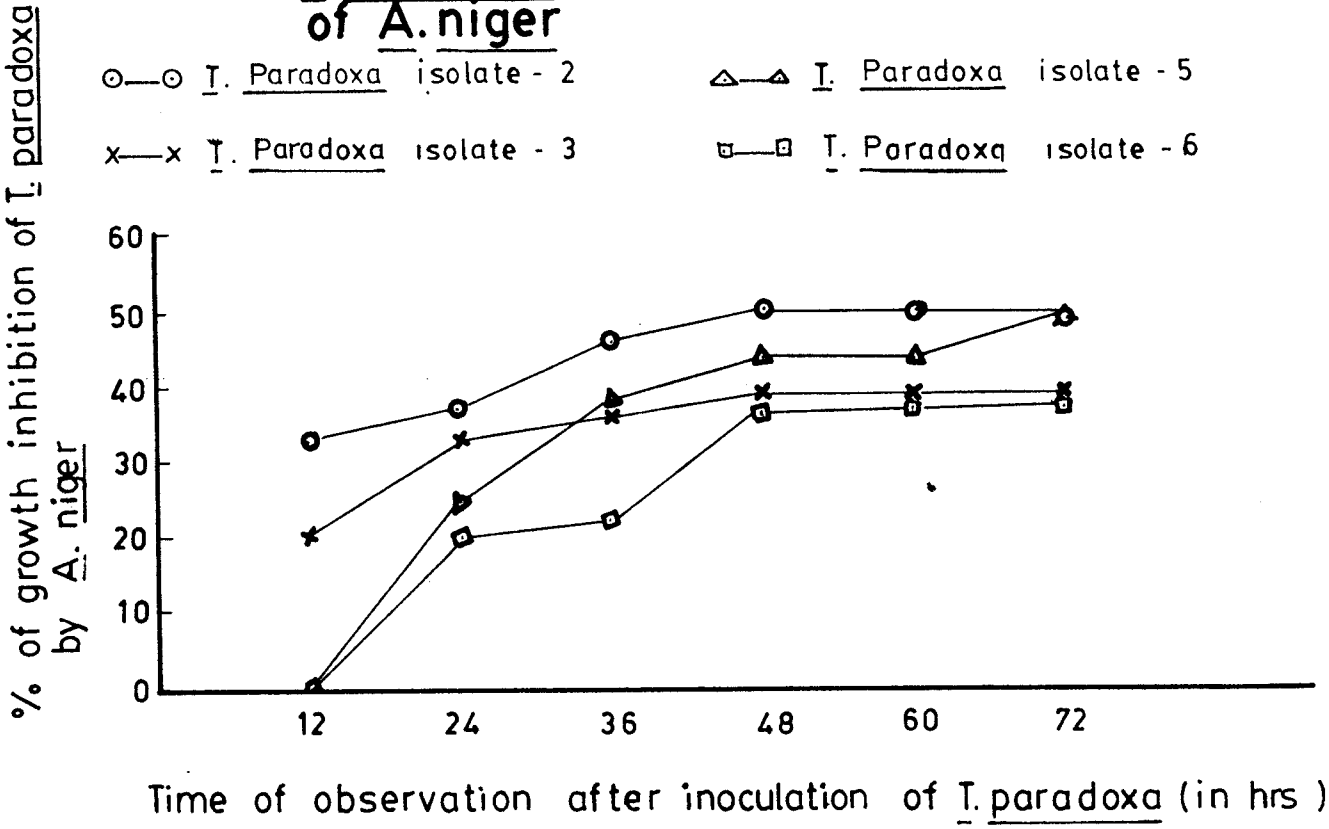


Fig.9. Growth inhibition of T. paradoxa by Aspergillus niger when T. paradoxa was inoculated 12 hrs after A. niger inoculation.

(Results 72 hrs. after inoculation)

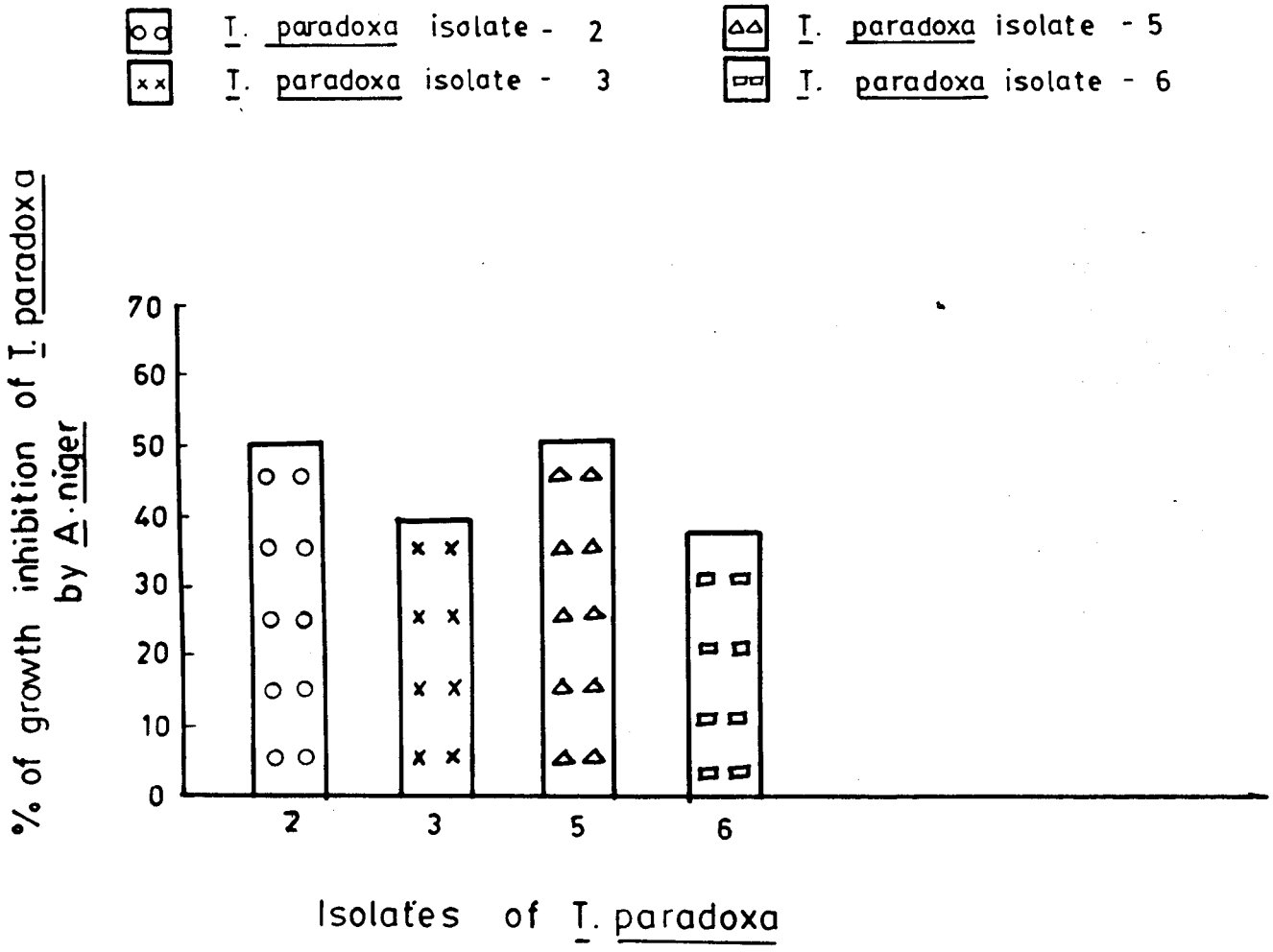
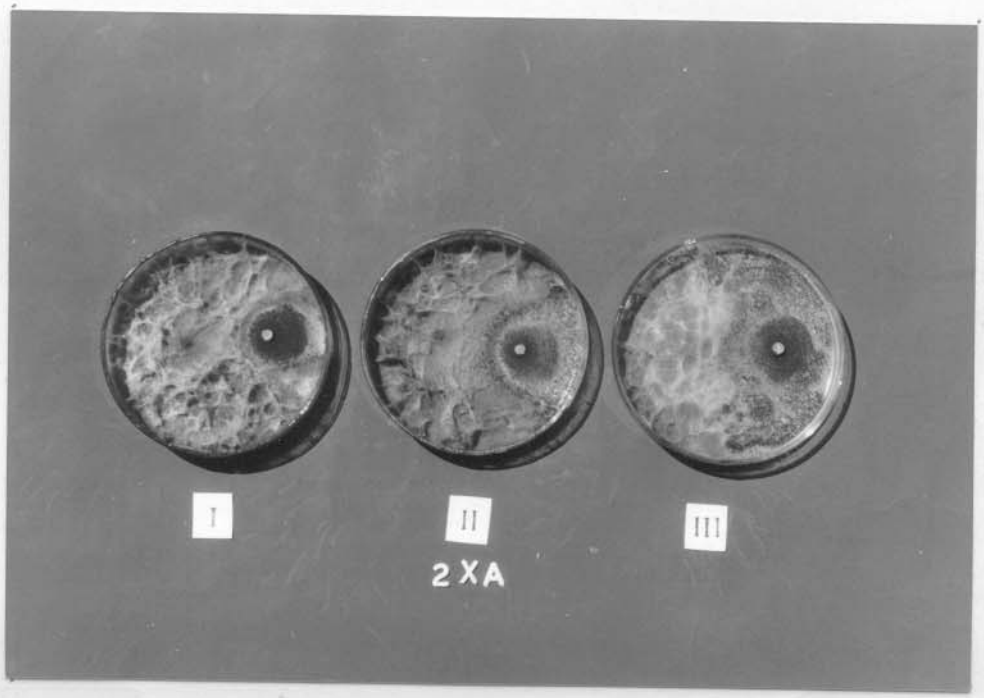


Plate 40. Three months of inoculation to test the growth inhibition of T. paradoxa isolate-2 with A. niger(7 days after inoculation).



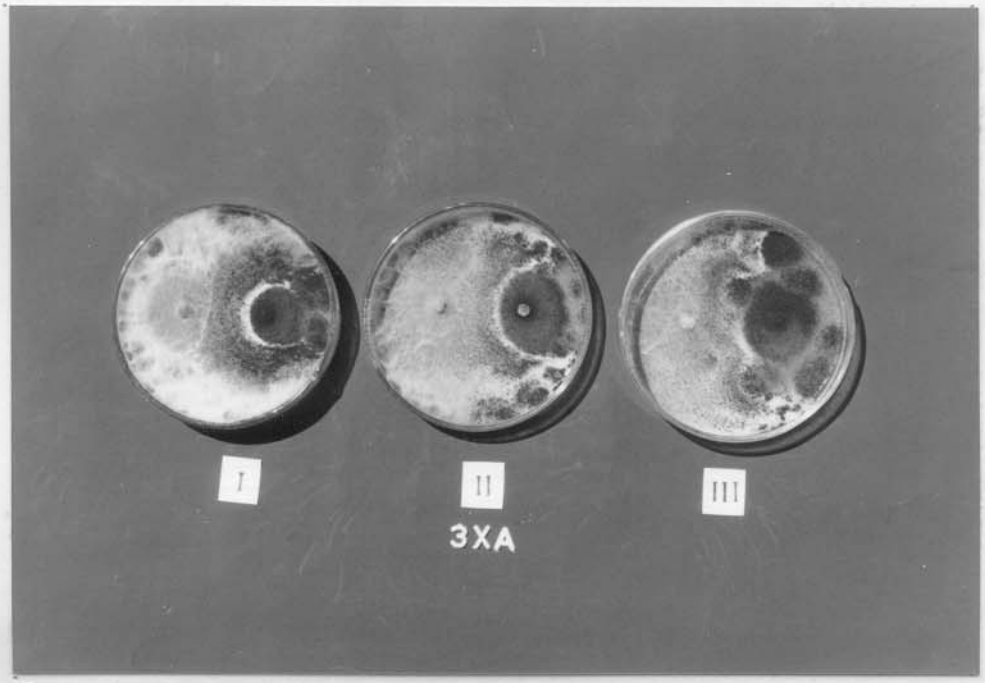
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated12 hrs. before the inoculation of A. niger.

B = Simultaneous inoculation of T. paradoxa and A. niger.

C = T. paradoxa inoculated 12 hrs. after the inoculation of A. niger.

Plate 41. Three methods of inoculation to test the growth inhibition of T. paradoxa isolate-3 with A. niger(7 days after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of A. niger.

B = Simultaneous inoculation of T. paradoxa and A. niger.

C = T. paradoxa inoculated 12 hrs. after the inoculation of A. niger.

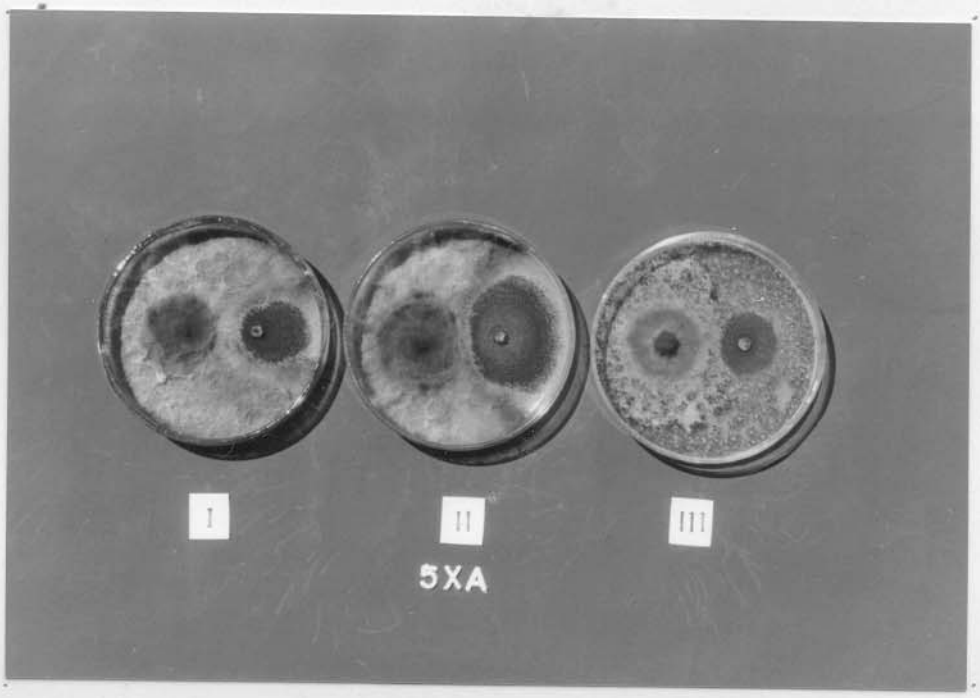
T. paradoxa isolate-5:

Among the three methods of inoculation tested, inhibition of T. paradoxa was minimum (0 to 30% in 72 hrs.) when A. niger was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-36). In simultaneous method of inoculation, the inhibition ranged from 0% in 12 hrs. to 40% in 72 hrs. But the maximum percentage of inhibition was noticed when A. niger was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate-42). There was no inhibition in 12 hrs. with 50% inhibition in 72 hrs. (Figs 8 & 9). Mutual inhibition was also discernible.

T. paradoxa isolate-6:

In the three methods of inoculation tested, inhibition of T. paradoxa was the least (0% in 12 hrs to 26.7% in 72 hrs) when A. niger was inoculated 12 hrs. after inoculation of T. paradoxa(Table-36). In simultaneous method of inoculation, the percentage inhibition ranged from 0 in 12 hrs. to 30 in 72 hrs. However, when A. niger was inoculated first and T. paradoxa was inoculated after 12 hrs maximum percentage of inhibition was discernible (Plate-43). The percentage of inhibition was o in 12 hrs with 36.7 in 72 hrs.(Figs 8 & 9). Mutual inhibition was observed in this case also.

Plate 42. Three methods of inoculation to test the growth inhibition of T. paradoxa isolate-5 with A. niger(7 days after inoculation)



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of A. niger.

B = Simultaneous inoculation of T. paradoxa and A. niger.

C = T. paradoxa inoculated 12 hrs. after the inoculation of A. niger.

Plate 43. Effect on the growth of *T. paradoxa* isolate-6 when *A. niger* was inoculated 12 hrs. before the inoculation of *T. paradoxa*.

(Note: In the petridish *T. paradoxa* is on left side and test fungus is on right side.)

4.5.2.2 Interaction of Trichoderma harzianum with T. paradoxa:

T. harzianum isolates reduced the rate of growth of T. paradoxa isolate to a considerable extent. There was no inhibition of growth of T. harzianum by T. paradoxa.

4.5.2.2.1 Interaction of T. harzianum Isolate-1.

T. paradoxa isolate-2:

Among the different methods of inoculation tested inhibition of T. paradoxa was the least (0% in 12 hrs to 23.3% in 72 hrs.) when T. harzianum was inoculated 12 hrs. after inoculation of T. paradoxa (Table-37). In simultaneous method of inoculation, the inhibition percentage ranged from 0% in 12 hrs. to 50 in 72 hrs. But, maximum percentage of inhibition was observed when T. harzianum was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate-44). The percentage of inhibition was nil in 12 hrs. with 84 in 72 hrs.(Figs.10 & 12).

T. paradoxa isolate-3:

In the three methods of inoculation tested, inhibition of T. paradoxa was minimum (0-40% in 72 hrs.) when T. harzianum was inoculated 12 hrs. after inoculation of T. paradoxa (Table 37). When inoculation was done simultaneously, the inhibition percentage ranged from 0 in 12 hrs. to 50 in 72 hrs. However, the maximum percentage ofinhibition

Table 37: In vitro interaction of Trichoderma harzianum Isolate-1 with T. paradoxa.

Time of observation after inoculation of both the fungi (in hrs.)	Growth inhibition of T. paradoxa by T. harzianum (Percentage)											
	Isolate-2			Isolate-3			Isolate-5			Isolate-6		
	A	B	C	A	B	C	A	B	C	A	B	C
12	0	0	0	0	0	0	0	25.0	25.0	5.6	12.5	14
24	13.3	21.7	42.9	10.0	18.2	40.0	26.1	33.3	54.5	8.0	17.6	25
36	13.3	44.8	73.3	13.3	25.0	57.1	37.0	42.1	58.8	23.1	44.0	60
48	16.7	46.7	73.3	38.1	47.1	70.0	40.0	52.1	72.0	30.0	46.2	64
60	23.3	46.7	76.7	40.0	50.0	80.0	40.0	56.7	75.0	30.0	46.7	66
72	23.3	50.0	84.0	40.0	50.0	80.0	40.0	56.7	85.1	30.0	46.7	66

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. harzianum.

B = T. paradoxa and T. harzianum were simultaneously inoculated.

C = T. paradoxa was inoculated after 12 hrs. growth of T. harzianum.

Fig. 10. Growth inhibition of T. paradoxa isolates by Trichoderma harzianum isolate -1 upto 72 hrs. after inoculation, when T. paradoxa was inoculated 12 hrs. after T. harzianum inoculation.

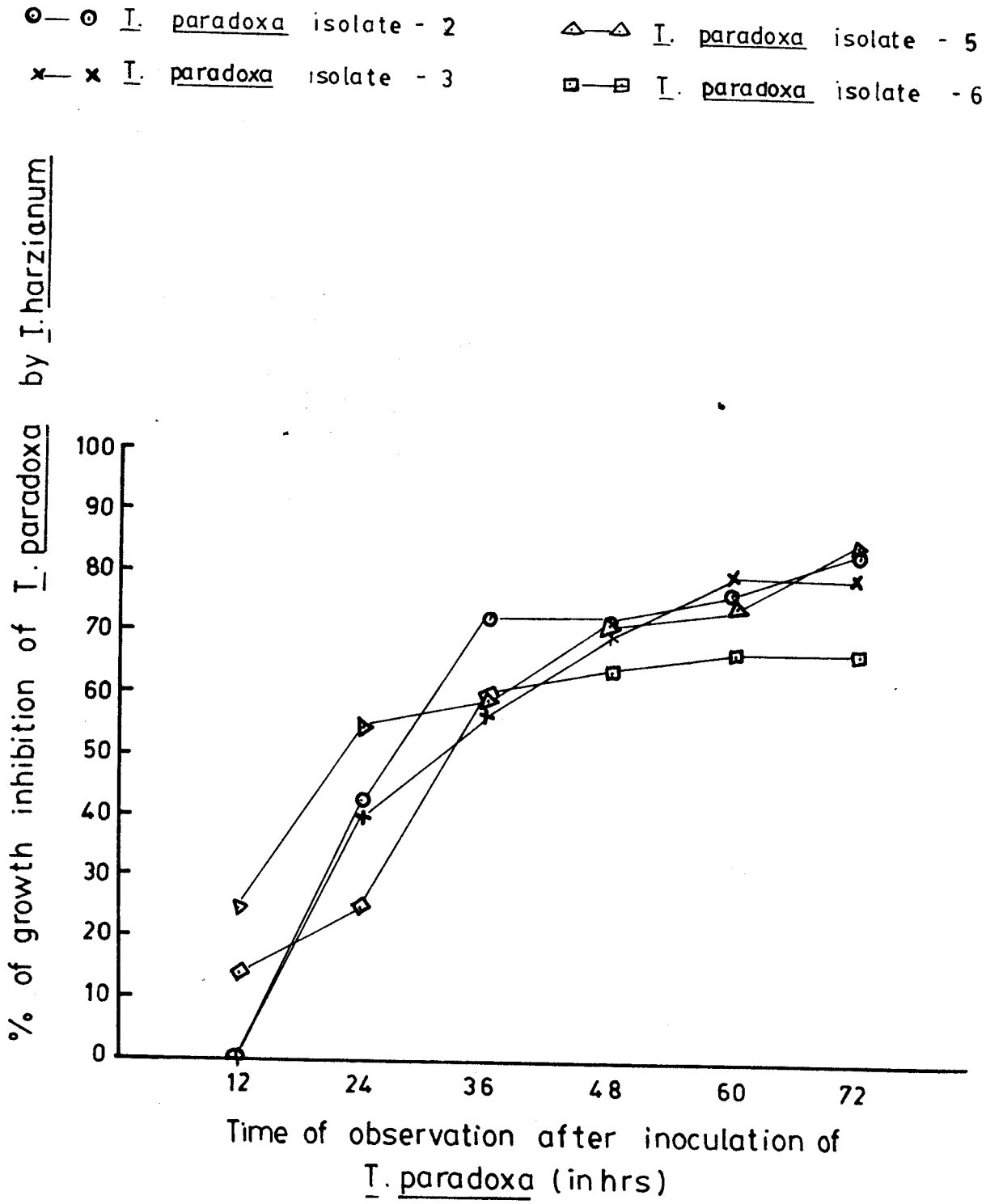
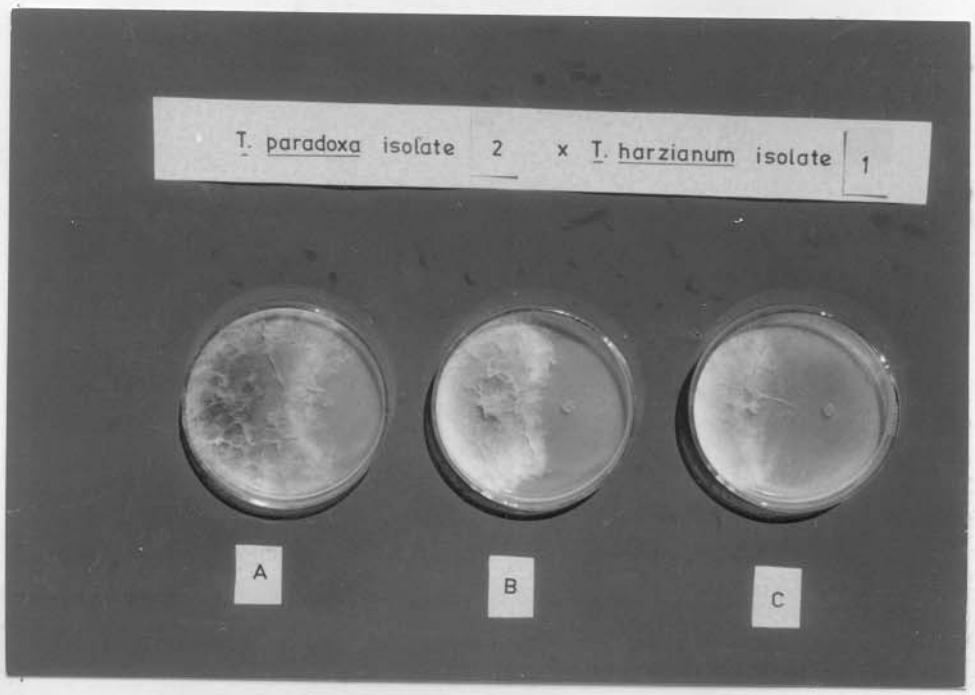


Plate 44. Effect on the growth of T. paradoxa isolate-2 by T. harzianum isolate-1 in different methods of inoculation.



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. harzianum.

B = Simultaneous inoculation of T. paradoxa and T. harzianum.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. harzianum.

Plate 45. Effect on the growth of T. paradoxa isolate-3 by T. harzianum isolate-1 in different methods of inoculation. (48 hrs. after inoculation).

(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. harzianum.

B = Simultaneous inoculation of T. paradoxa and T. harzianum.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. harzianum.

was discernible when T. harzianum was inoculated first at T. paradoxa was inoculated after 12 hrs.(Plate-45). The percentage inhibition was zero in 12 hrs. with 80 to 72 hrs. (Figs. 10 & 12).

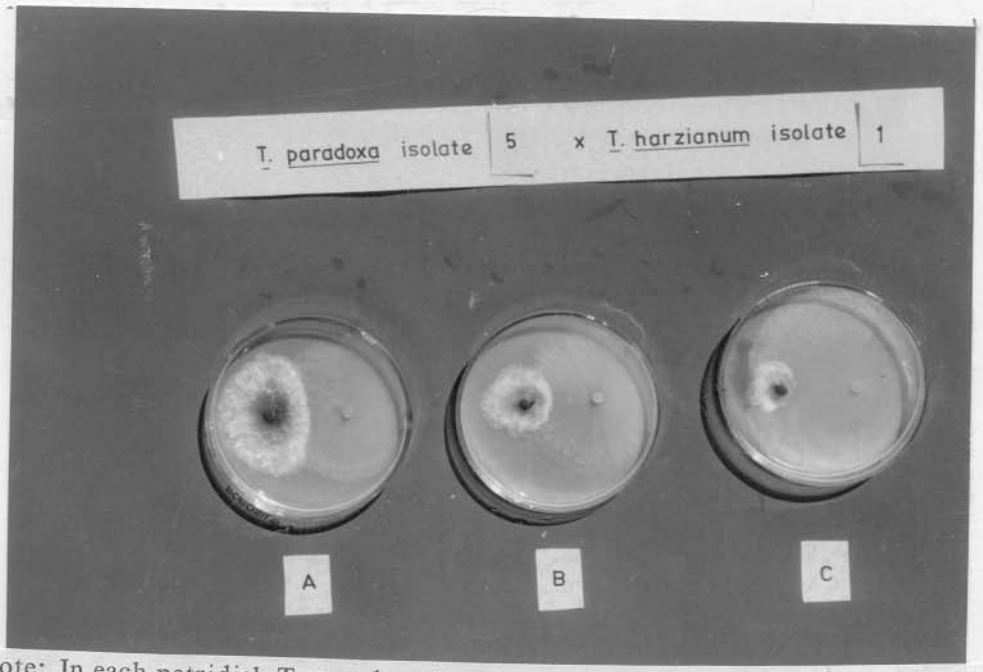
T. paradoxa isolate-5:

Among the three inoculation methods tested, inhibition of T. paradoxa was the least (0% in 12 hrs to 40% in 72 hrs.) when T. harzianum was inoculated 12 hrs. after inoculation of T. paradoxa (Table-37). In simultaneous method of inoculation inhibition percentage ranged from 25 in 12 hrs. to 56.7 in 72 hrs. However, maximum percentage of inhibition was recorded when T. harzianum was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate-46). The percentage of inhibition was 25 in 12 hrs. with 85.5 in 72 hrs.(Figs. 10 & 12).

T. paradoxa isolate-6:

In the three methods of inoculation tested, inhibition of T. paradoxa was the least (5.6%in 12 hrs.to 30% in 72 hrs.) (Table-37), when T. harzianum was inoculated 12 hrs.after inoculation of T. paradoxa. When inoculation was done simultaneously, inhibition percentage ranged from 12.5 in 12 hrs.to 46.7 in 72 hrs. But, the inhibition was maximum when T. harzianum was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate-47). The percentage of inhibition was 14.3 in 12 hrs.with 66.7 in 72 hrs.(Figs. 10 & 12).

Plate 46. Effect on the growth of T. paradoxa isolate-5 by T. harzianum isolate-1 in different methods of inoculation. (48 hrs. after inoculation).



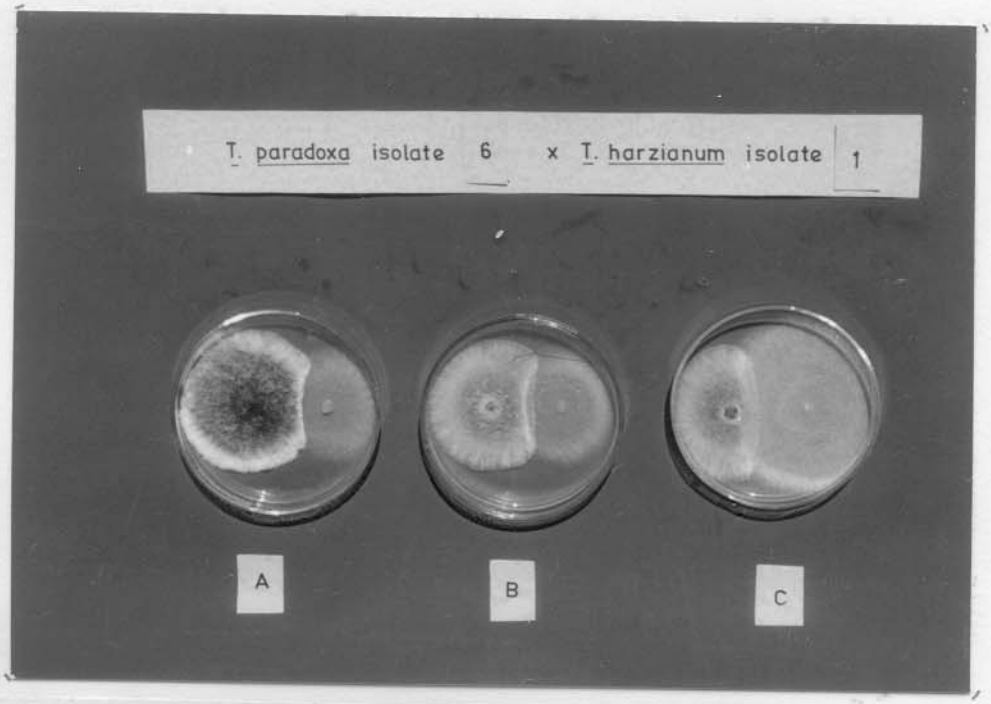
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. harzianum.

B = Simultaneous inoculation of T. paradoxa and T. harzianum.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. harzianum.

Plate 47. Effect on the growth of T. paradoxa isolate-6 by T. harzianum isolate-1 in different methods of inoculation. (48 hrs. after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. harzianum.

B = Simultaneous inoculation of T. paradoxa and T. harzianum.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. harzianum.

4.5.2.2.2 T. harzianum isolate-2:

T. paradoxa isolate-2:

Inhibition of T. paradoxa was the least (4.4% in 12 hrs. to 23.3% in 72 hrs.) when T. harzianum was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-38). In simultaneous method of inoculation, the inhibition percentage ranged from 30 in 12 hrs. to 46.7 in 72 hrs. However, the maximum percentage of inhibition was discernible when T. harzianum was inoculated first and T. paradoxa was inoculated after 12 hrs. (Plate-48). The percentage of inhibition was 40 in 12 hrs. with 86.6 in 72 hrs. (Figs. 11 & 12).

T. paradoxa isolate-3:

Inhibition of T. paradoxa was 9.1% and 28.6% at 12 hrs. and 48 hrs. after inoculation respectively when T. harzianum was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-38). But the inhibition percentage ranged from 20 in 12 hrs. to 47.4 in 48 hrs. in simultaneous method of inoculation. Percentage of inhibition was 25 and 50 in 12 hrs. and 48 hrs. respectively, when T. harzianum was inoculated first and T. paradoxa was inoculated after 12 hrs. (Plate-49). On the third day after inoculation of T. paradoxa, the percentage of inhibition was 50 in all the three methods of inoculation (Figs. 11 & 12).

Table 38: In vitro interaction of Trichoderma harzianum Isolate-2 with T. paradoxa.

Time of observation after inoculation of both the fungi (in hrs.)	Growth inhibition of T. paradoxa by T. harzianum (Percentage)											
	Isolate-2			Isolate-3			Isolate-5			Isolate-6		
	A	B	C	A	B	C	A	B	C	A	B	C
12	4.4	30.0	40.0	9.1	20.0	25.0	0	0	25.0	5.5	14.3	25.0
24	13.3	36.0	53.9	13.3	30.8	40.0	18.7	42.9	50.0	12.5	16.7	47.0
36	13.3	40.0	72.0	15.8	33.3	40.0	37.5	47.6	50.0	15.4	20.0	60.0
48	16.7	40.0	75.0	28.6	47.4	50.0	50.0	57.1	57.1	26.7	33.3	60.7
60	23.3	46.7	80.0	50.0	50.0	50.0	50.0	60.0	62.5	26.7	43.3	63.3
72	23.3	46.7	86.6	50.0	50.0	50.0	50.0	60.0	62.5	26.7	43.3	63.3

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. harzianum.

B = T. paradoxa and T. harzianum were simultaneously inoculated.

C = T. paradoxa was inoculated after 12 hrs. growth of T. harzianum.

Fig. 11. Growth inhibition of T. paradoxa isolates by Trichoderma harzianum isolate -2 upto 12 hrs. after inoculation, when T. paradoxa was inoculated 12 hrs. after T. harzianum inoculation

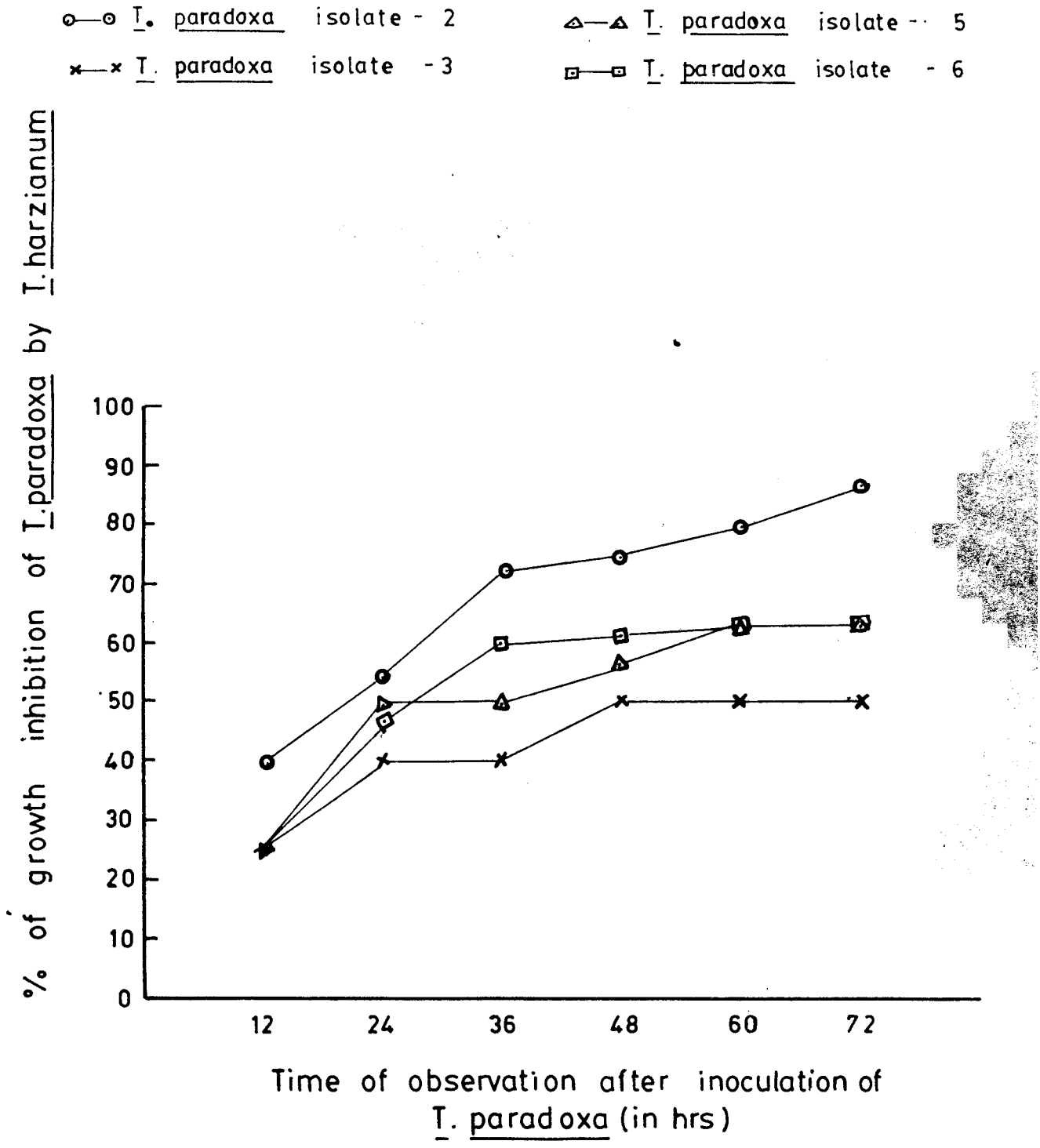


Fig.12. Growth inhibition of T. paradoxa isolates b Trichoderma harzianum isolates when T. paradoxa was inoculated 12 hrs after T. harzianum inoculation

(Result 72 hrs. after inoculation)

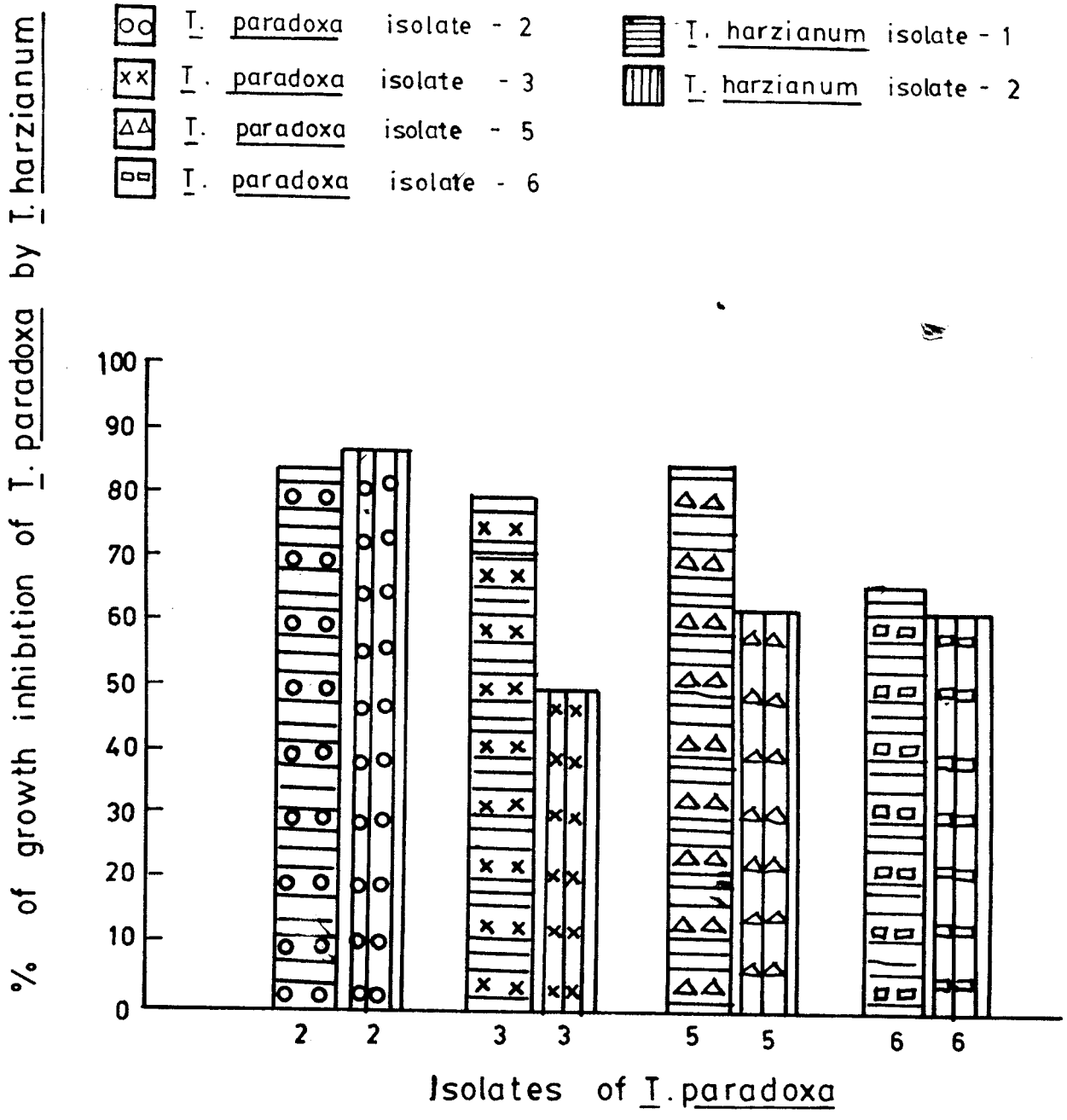
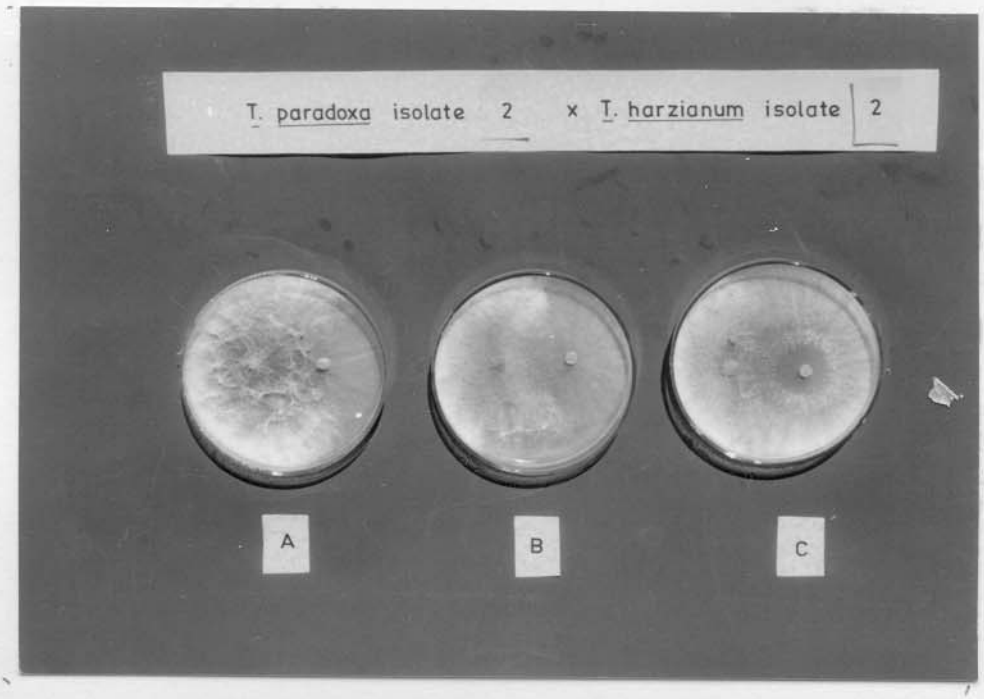


Plate 48. Effect on the growth of T. paradoxa isolate-2 by T. harzianum-2 in different methods of inoculation.
(48 hrs. after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. harzianum.

B = Simultaneous inoculation of T. paradoxa and T. harzianum.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. harzianum.

Plate 49. Effect on the growth of T. paradoxa isolate-3 by T. harzianum-2 in different methods of inoculation.
(48 hrs. after inoculation).

(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. harzianum.

B = Simultaneous inoculation of T. paradoxa and T. harzianum.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. harzianum.

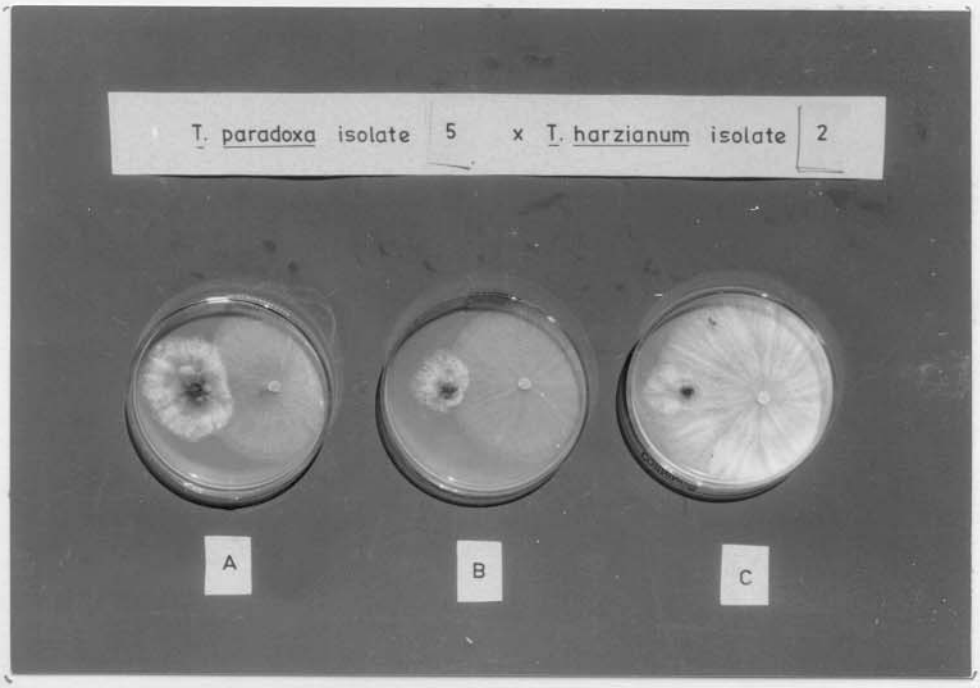
T. paradoxa isolate-5:

In the three methods of inoculation tested, inhibition of T. paradoxa was the least (0% in 12 hrs. and 50% in 72 hrs.) when T. harzianum was inoculated 12 hrs. after theinoculation of T. paradoxa (Table-38). In simultaneous method of inoculation, the inhibition percentage was nil in 12 hrs. and 60 in 72 hrs. However, the maximum percentage of inhibition was discernible when T. harzianum was inoculated first and T. paradoxa was inoculated after 12 hrs. (Plate-50). The inhibition was 25% to 12 hrs. with 62.5% in 72 hrs. (Figs. 11 & 12).

T. paradoxa isolate-6:

Among the different methods of inoculation tested, inhibition of T. paradoxa was the least (5.5% in 12 hrs. to 26.7% in 72 hrs.) when T. harzianum was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-38). When inoculation was done simultaneously, the inhibition percentage ranged from 14.3 in 12 hrs. to 43.3 in 72 hrs. However, the maximum percentage of inhibition was observed when T. harzianum was inoculated first and T. paradoxa was inoculated after 12 hrs. (Plate-51). The percentage of inhibition was 25 in 12 hrs. with 63.3 in 72 hrs. (Figs. 11 & 12).

Plate 50. Effect on the growth of T. paradoxa isolate-5 by T. harzianum-2 in different methods of inoculation.(48 hrs. after inoculation).



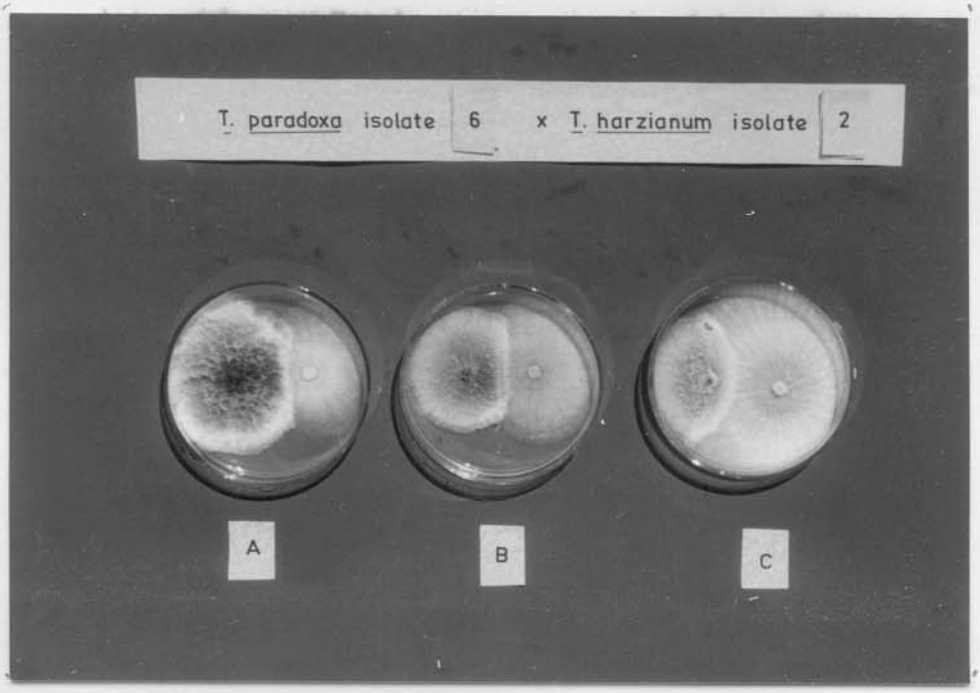
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. harzianum.

B = Simultaneous inoculation of T. paradoxa and T. harzianum.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. harzianum.

Plate 51. Effect on the growth of T. paradoxa isolate-6 by T. harzianum-2 in different methods of inoculation.(48 hrs. after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. harzianum.

B = Simultaneous inoculation of T. paradoxa and T. harzianum.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. harzianum.

A comparison of growth inhibition of T. paradoxa on the third day after the inoculation by T. harzianum isolate-1 showed that the growth inhibition of T. paradoxa isolate-6 was lesser than the growth inhibition of T. paradoxa isolate 2, 3 and 5. Similarly growth inhibition of T. paradoxa isolate-6 was lesser than of T. paradoxa isolate-2 on the third day after inoculation by T. harzianum isolate-2 (Fig.12).

4.5.2.3 Interaction of Trichoderma viride with T. paradoxa:

All the three isolates of T. viride reduced the growth of T. paradoxa isolates in vitro to a considerable extent. There was no growth inhibition of T. viride by the isolates of T. paradoxa.

4.5.2.3.1 T. viride isolate-1:

T. paradoxa isolates 2, 3, 5 and 6 were tested with T. viride isolate-1.

T. paradoxa isolate-2.

In the three methods of inoculation tested, inhibition of T. paradoxa was minimum (5% in 12 hrs. and 16.7% in 72 hrs.) when T. viride was inoculated 12 hrs. after inoculation of T. paradoxa (Table-39). In simultaneous method of inoculation the inhibition percentage ranged from 10 in 12 hrs. to 34.5 in 72 hrs. However, the maximum percentage of inhibition was discernible when T. viride was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate-52). The percentage was 20 in 12 hrs. with 56.6 in 72 hrs. (Figs. 13 & 16).

Table 39: In vitro interaction of Trichoderma viride Isolate-1 with T. paradoxa.

Time of observation after inoculation of both the fungi (in hrs.)	Growth inhibition of T. paradoxa by T. viride (Percentage)											
	Isolate-2			Isolate-3			Isolate-5			Isolate-6		
	A	B	C	A	B	C	A	B	C	A	B	C
12	5.0	10.0	20.0	6.7	16.2	20.0	7.1	20.0	25.0	0	12.5	14.
24	16.7	13.3	37.5	23.1	25.0	33.3	10.0	30.0	40.0	16	22.2	41.
36	16.7	34.5	51.9	36.6	52.0	59.3	24.0	33.3	58.9	33.3	42.3	58.
48	16.7	34.5	53.3	36.6	56.7	60.7	33.3	50.0	80.0	33.3	44.4	64.
60	16.7	34.5	56.6	36.6	56.7	64.3	33.3	60.0	82.1	33.3	46.6	76.
72	16.7	34.5	56.6	40.0	60.0	67.9	36.6	60.0	90.0	33.3	50.0	76.

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = T. paradoxa and T. viride were simultaneously inoculated.

C = T. paradoxa was inoculated after 12 hrs. growth of T. viride.

Fig.13. Growth inhibition of T.paradoxa isolates by Trichoderma viride isolate -1 upto 72 hrs after inoculation when T. paradoxa was inoculated 12 hrs.after T. viride inoculation

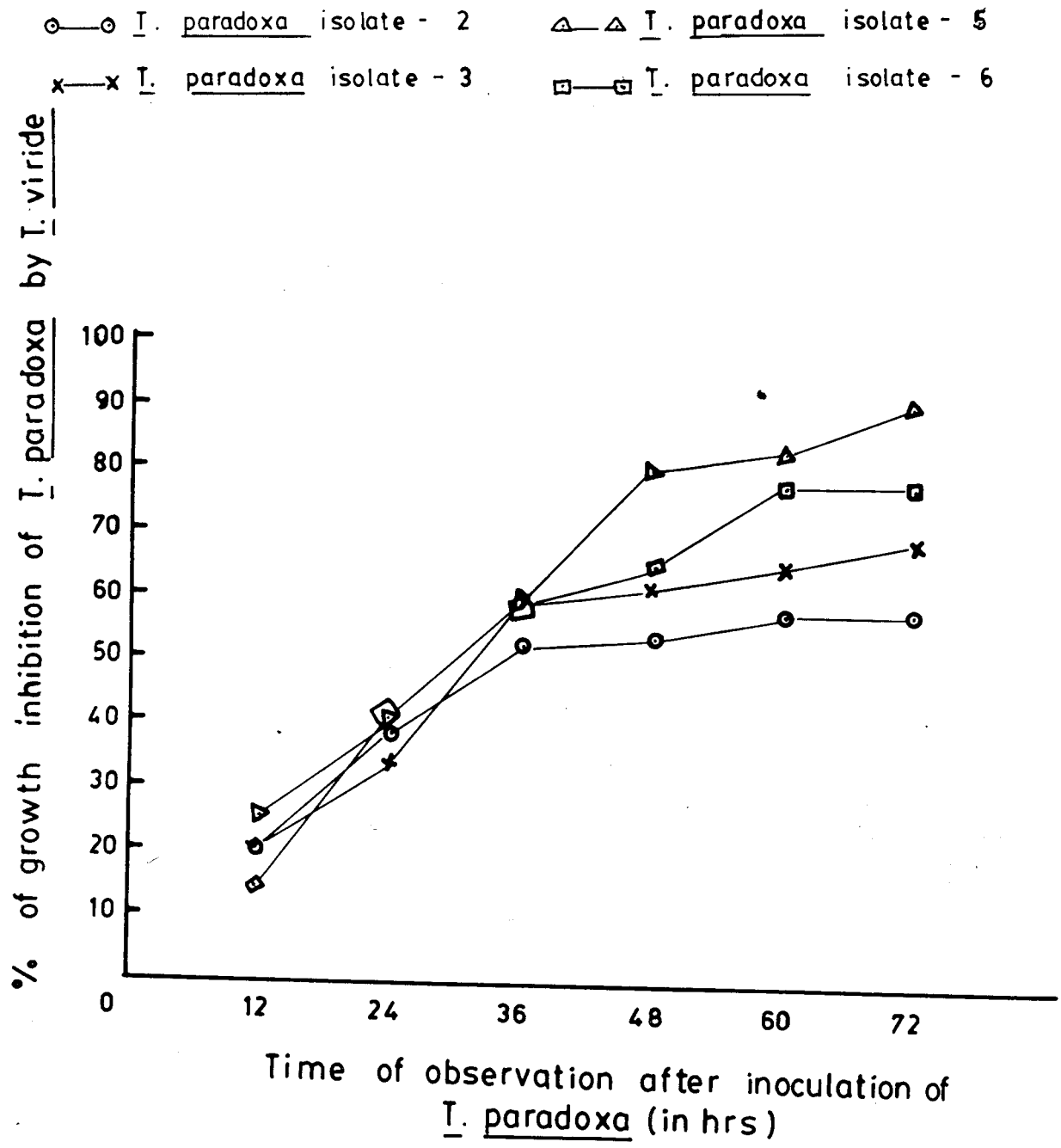


Fig. 14. Growth inhibition of T. paradoxa isolates by Trichoderma viride isolate -2 upto 72 hrs after inoculation when T. paradoxa was inoculated 12 hrs. after T. viride inoculation

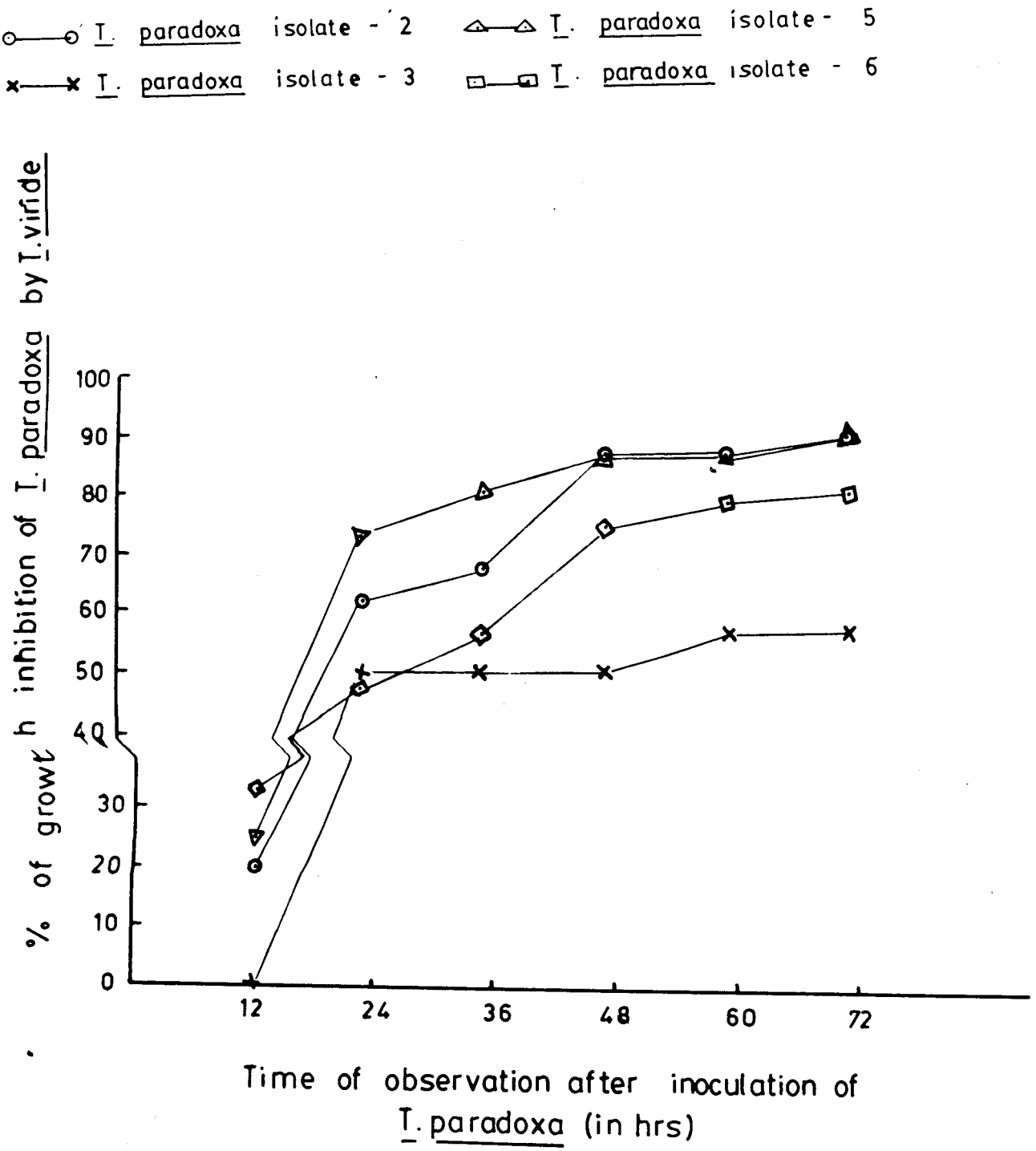


Fig. 15. Growth inhibition of T. paradoxa isolates by Trichoderma viride isolate -3 upto 72 hrs after inoculation when T. paradoxa was inoculated 12hrs. after T. viride inoculation

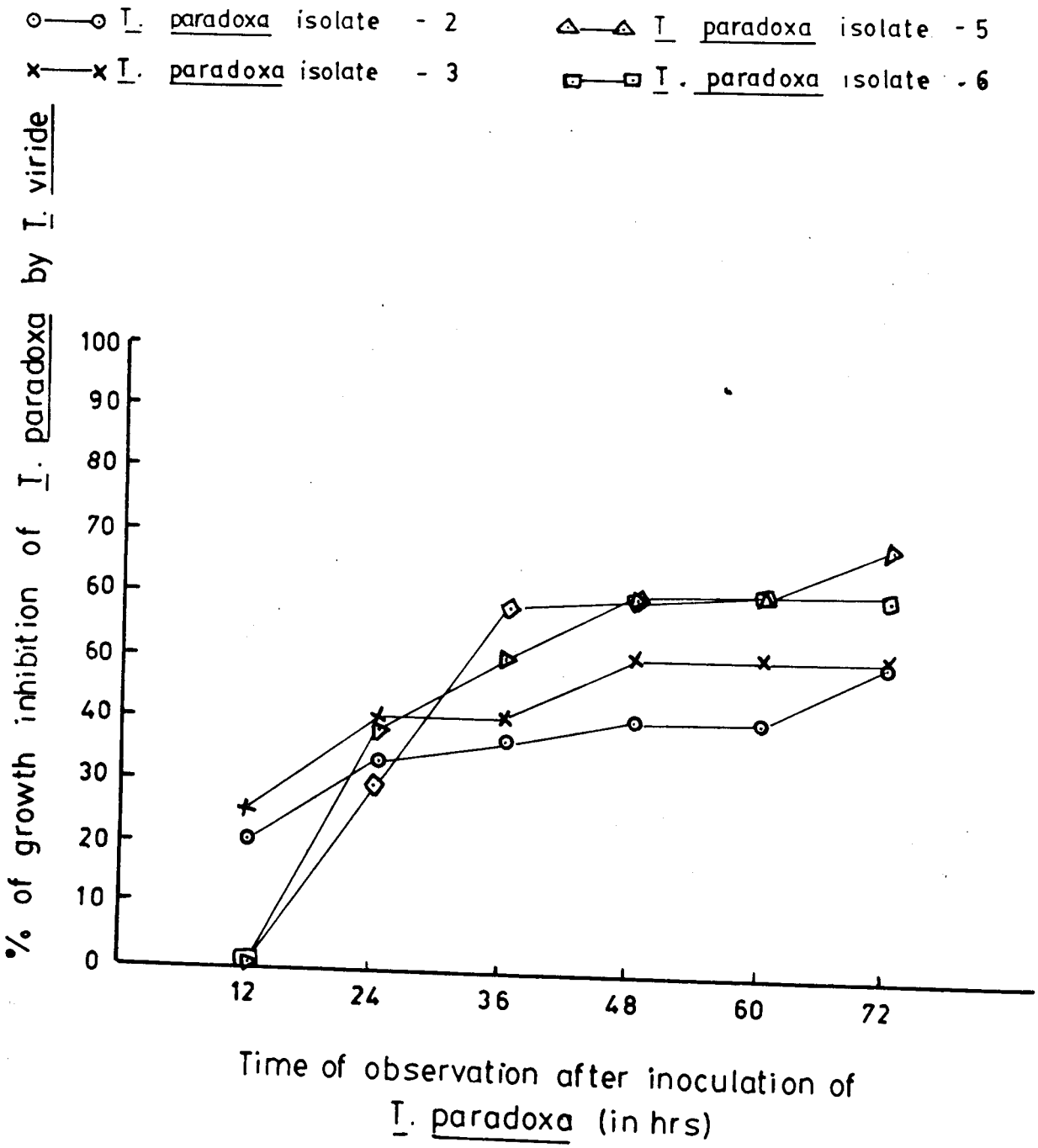


Fig.16. Growth inhibition of T. paradoxa isolates by Trichoderma viride isolates when T. paradoxa was inoculated 12 hrs. after T. viride inoculation

(Results 72hrs. after inoculation)

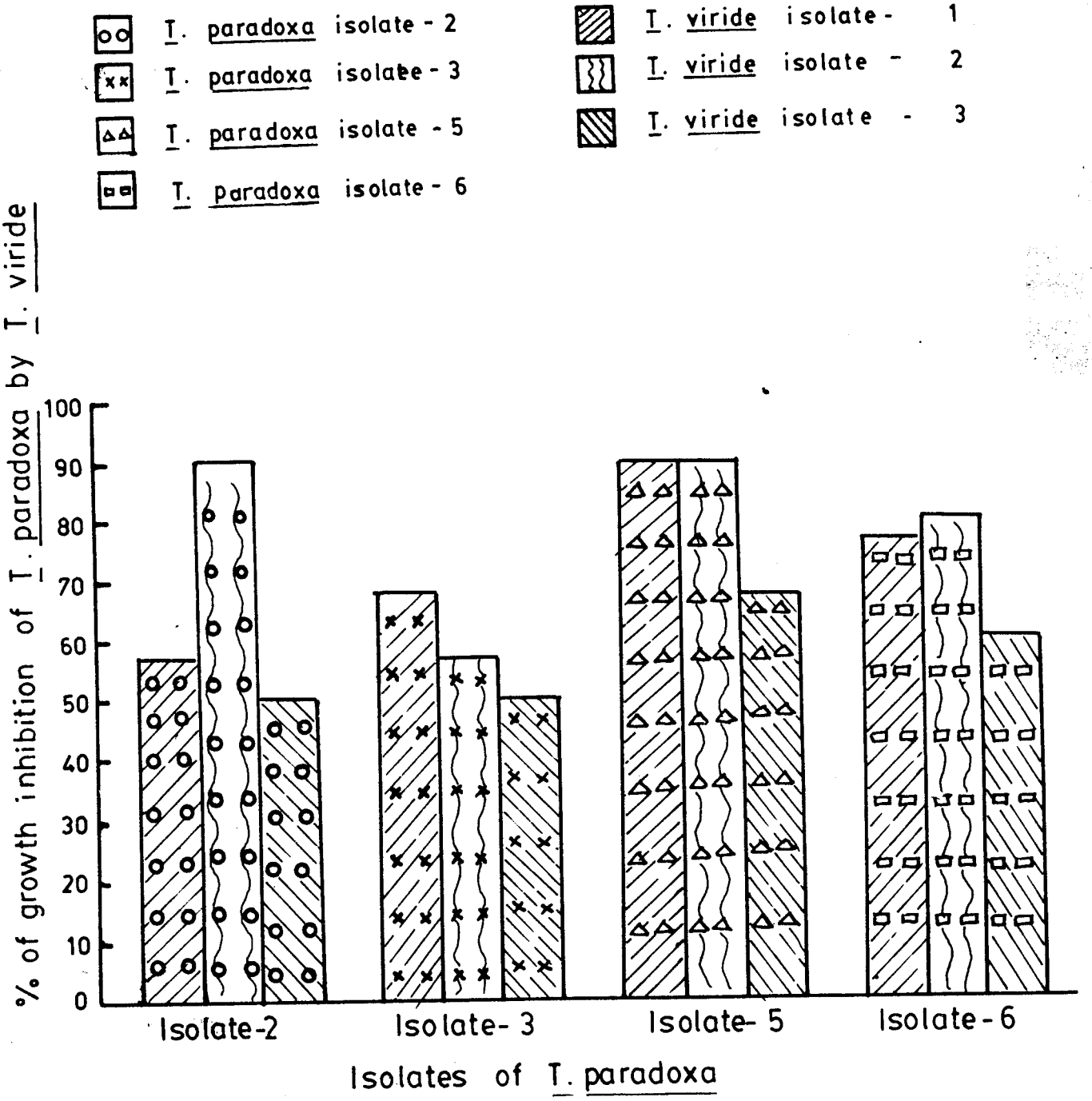
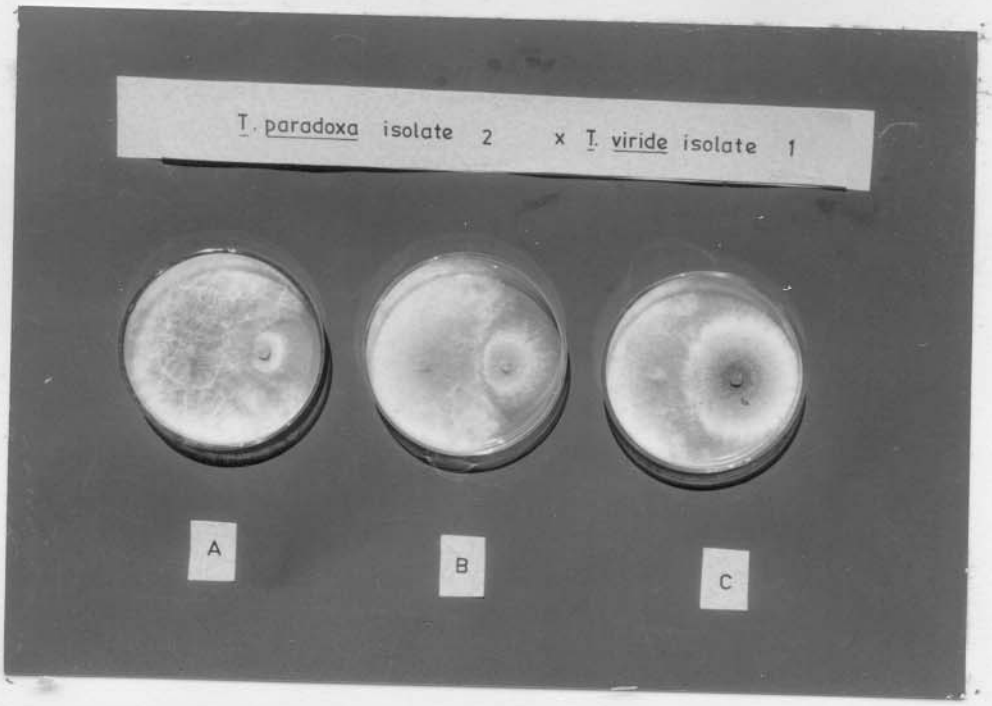


Plate-52. Effect on the growth of T. paradoxa isolate-2 by T. viride isolate-1 in different methods of inoculation. (48 hrs. after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

T. paradoxa isolate-3.

Among the three methods of inoculation tested, inhibition of T. paradoxa was the least (6.7% in 12 hrs. and 40% in 72 hrs.) when T. viride was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-39). When inoculation was done simultaneously the inhibition percentage ranged from 16.2 in 12 hrs. to 60 in 72 hrs. However, the maximum percentage of inhibition was observed when T. viride was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate-53). The percentage inhibition was 20 in 12 hrs. with 67.9% in 72 hrs.(Figs.13 & 16).

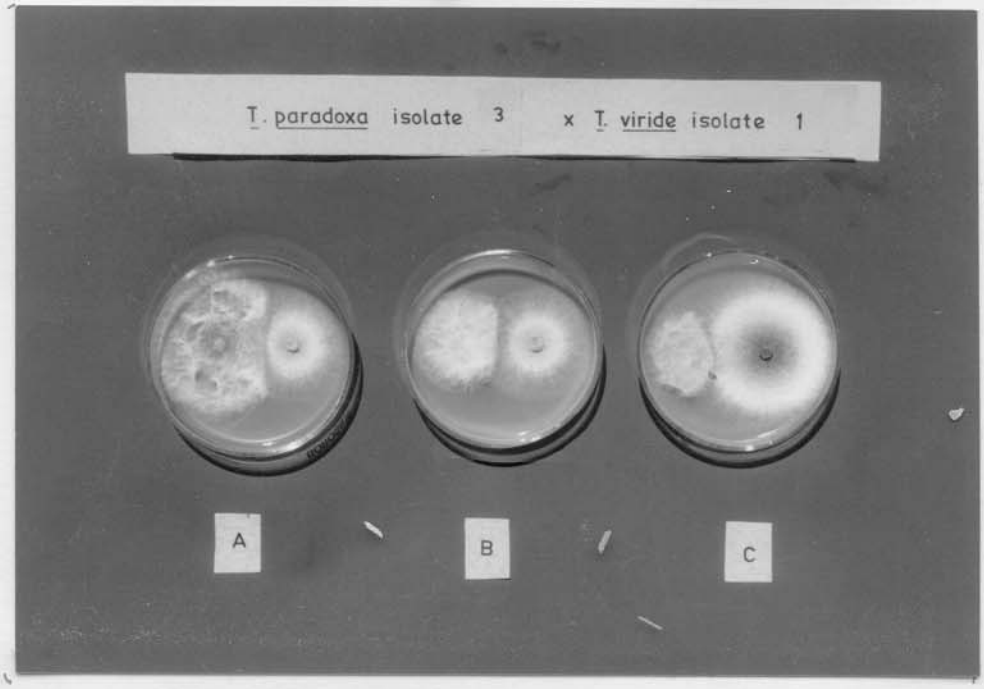
T. paradoxa isolate-5.

In the three methods of inoculation tested, minimum inhibition(7.1% in 12 hrs. and 36.6% in 72 hrs.) of T. paradoxa was observed when T. viride was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-39). In simultaneous method of inoculation, the inhibition percentage ranged from 20 in 12 hrs. to 60 in 72 hrs. However, percentage of inhibition was maximum when T. viride was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate-54). The growth inhibition was 25% in 12 hrs. with 90% in 72 hrs.(Figs. 13 & 16).

T. paradoxa isolate-6.

Of the three methods of inoculation tested, inhibition of T. paradoxa was the least (0% in 12 hrs. and 33.3% in 72 hrs.) when T. viride was inoculated 12 hrs. after the inoculation of

Plate 53. Effect on the growth of T. paradoxa isolate-3 by T. viride isolate-1 in different methods of inoculation. (48 hrs. after inoculation).



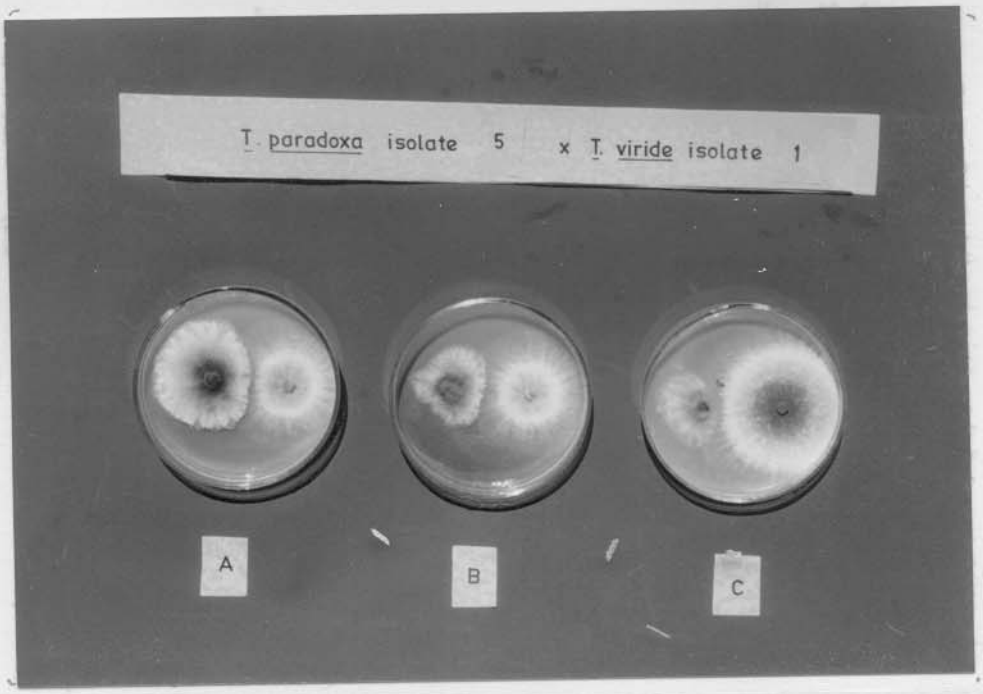
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

Plate 54. Effect on the growth of T. paradoxa isolate-5 by T. viride isolate-1 in different methods of inoculation. (48 hrs. after inoculation).



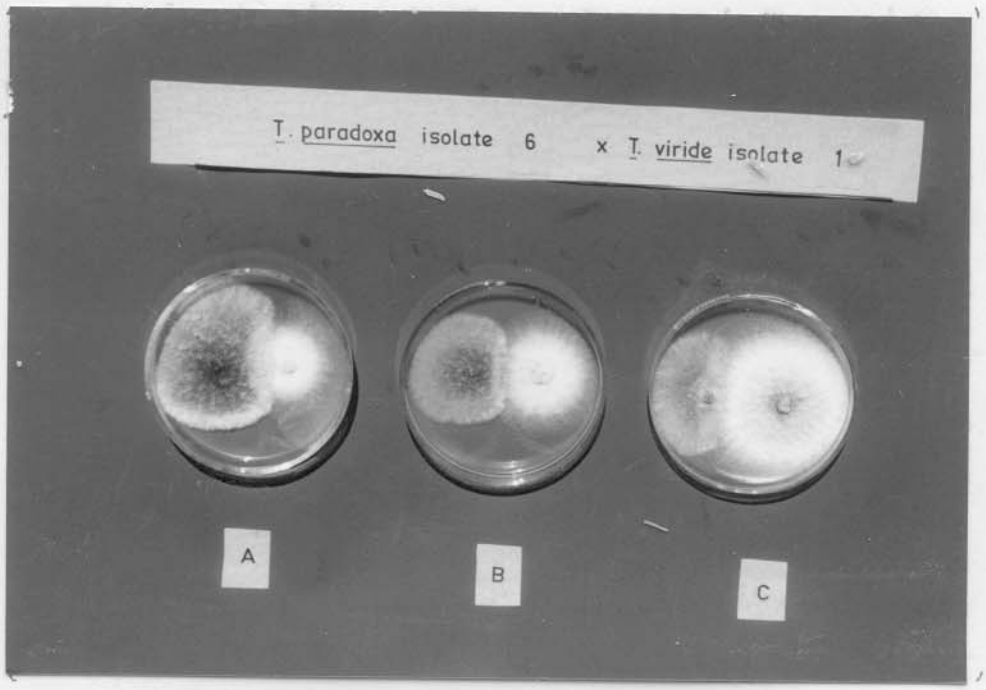
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

Plate 55. Effect on the growth of T. paradoxa isolate-6 by T. viride isolate-1 in different methods of inoculation. (48 hrs. after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

T. paradoxa (Table-39). In simultaneous method of inoculation the range of inhibition was 12.5% in 12 hrs. to 50% in 72 hrs. However, the maximum percentage of inhibition was recorded when T. viride was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate 55). The percentage inhibition was 14.3 and 12 hrs. with 76.7 in 72 hrs.(Figs. 13 & 16).

4.5.2.3.2 Trichoderma viride isolate-2.

T. paradoxa isolates 2, 3, 5 and 6 were tested with T. viride isolate-2 as in the previous case.

T. paradoxa isolate-2.

In the three methods of inoculation tested, inhibition of T. paradoxa was minimum (8% in 12 hrs. and 33.3% in 72 hrs.) when T. viride was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-40). In simultaneous method of inoculation, the inhibition percentage ranged from 18.2 in 12 hrs. to 53.3 in 72 hrs. However, there was maximum percentage of inhibition when T. viride was inoculated first and T. paradoxa was inoculated after 12 hrs. (Plate-56). The percentage of inhibition was 20 in 12 hrs. with 90 in 72 hrs. (Figs. 14 and 16).

T. paradoxa isolate-3.

Inhibition of T. paradoxa was theleast (0% in 12 hrs. and 40% in 72 hrs.) when T. viride was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-40). When inoculation was done simultaneously 50% growth reduction of T. paradoxa was found from first day onwards. There was no further reduction of growth of

Table 40: In vitro interaction of Trichoderma viride Isolate-2 with T. paradoxa

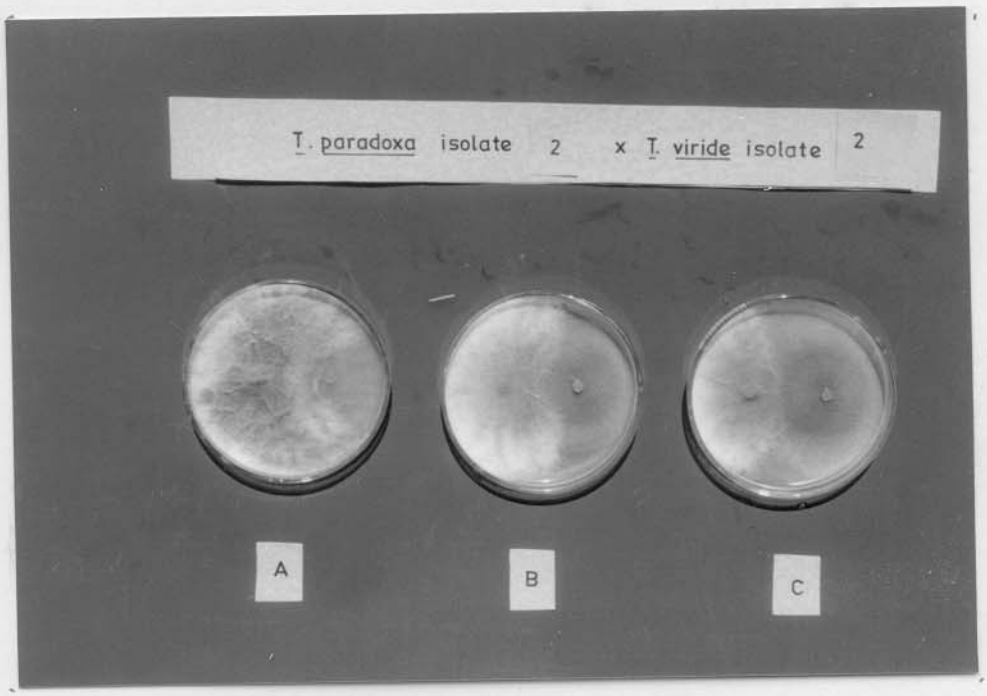
Time of observation after inoculation of both the fungi (in hrs.)	Growth inhibition of T. paradoxa by T. viride (Percentage)											
	Isolate-2			Isolate-3			Isolate-5			Isolate-6		
	A	B	C	A	B	C	A	B	C	A	B	C
12	8.0	18.2	20.0	0	0	0	0	0	25.0	0	25.0	33.
24	23.3	40.7	61.5	18.8	50.0	50.0	22.0	30.8	72.7	20.0	27.8	46.
36	26.7	50.0	66.7	30.4	50.0	50.0	32.0	40.0	80.0	25.9	33.3	55.
43	33.3	50.0	86.7	40.0	50.0	50.0	33.3	63.3	85.8	33.3	39.1	73.
60	33.3	50.0	86.7	40.0	50.0	57.1	33.3	63.0	85.8	33.3	50.0	77.
72	33.3	53.3	90.0	40.0	50.0	57.1	40.0	66.7	90.0	38.0	50.0	80.

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = T. paradoxa and T. viride were simultaneously inoculated.

C = T. paradoxa was inoculated after 12 hrs. growth of T. viride.

Plate 56. Effect on the growth of T. paradoxa isolate-2 by T. viride isolate-2 in different methods of inoculation. (48 hrs. after inoculation).



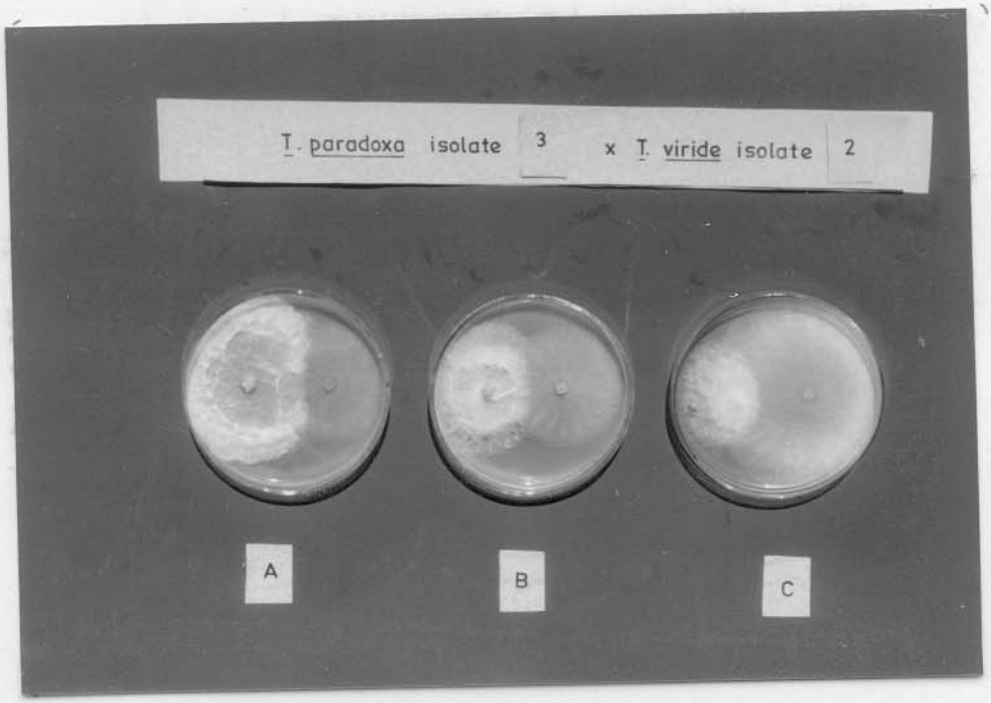
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

Plate 57. Effect on the growth of T. paradoxa isolate-3 by T. viride isolate-2 in different methods of inoculation. (48 hrs. after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

T. paradoxa. However, maximum percentage of inhibition was discernible when T. viride was inoculated first and T. paradoxa was inoculated after 12 hrs. (Plate-57). The percentage inhibition was 50 in 12 hrs. with 57.1 in 72 hrs. when the inhibition was calculated taking the colony radius towards the opposite edge of petridish as control (Figs. 14 and 16). But there was an overall reduction of the colony size of T. paradoxa on both the sides. Growth of T. paradoxa colony towards T. viride was very meager, while there was slight growth of the colony towards the opposite edge of the petridish. When T. viride was inoculated after 12 hr. growth of T. paradoxa on culture medium the colony diameter of T. paradoxa was 48 mm on the third day after inoculation of T. viride. The colony diameter of T. paradoxa was only 15 mm when T. viride and T. paradoxa were simultaneously inoculated. When T. paradoxa was inoculated after 12 hrs. growth of T. viride on the third day after inoculation of T. paradoxa, the colony diameter of T. paradoxa was only 10 mm and the growth of T. paradoxa towards T. viride was completely checked. There was no significant increase in the size of the colony of T. paradoxa.

T. paradoxa isolate-5.

In the three methods of inoculation tested, growth inhibition of T. paradoxa was the least (0% in 12 hrs. and 40% in 72 hrs.) when T. viride was inoculated 12 hrs. after the

inoculation of T. paradoxa (Table-40). In simultaneous method of inoculation, the inhibition percentage ranged from 30.8 in 24 hrs. to 66.7 in 72 hrs. However, the maximum percentage of inhibition was discernible when T. viride was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate-58). The percentage of inhibition was 72.7 in 24 hrs. with 90 in 72 hrs.(Figs. 14 & 16).

T. paradoxa isolate-6.

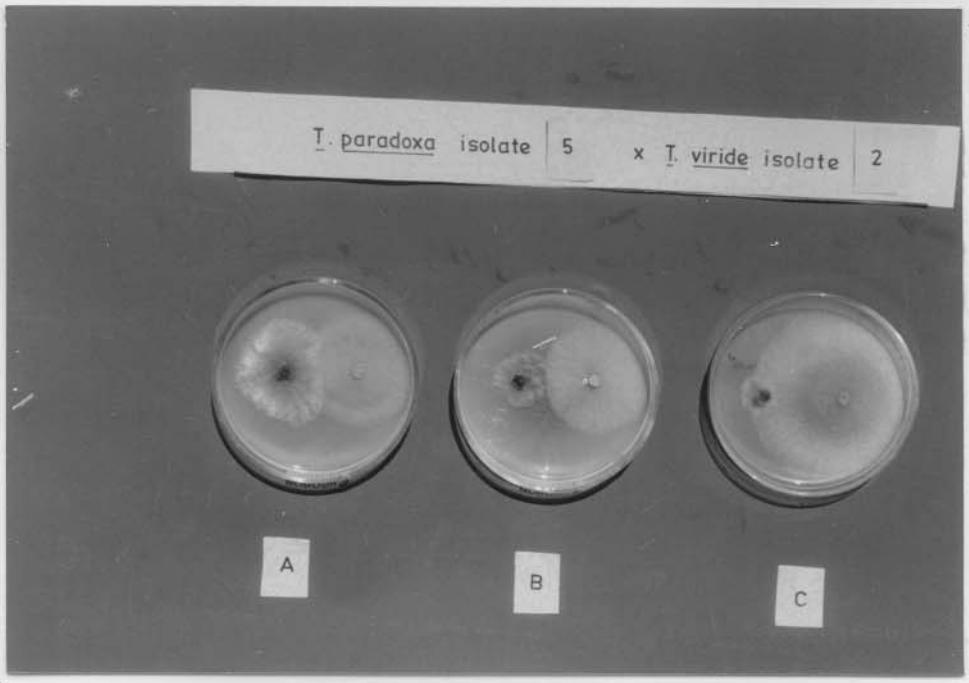
Among the different methods of inoculation tested, growth inhibition of T. paradoxa was minimum (0% in 12 hrs. and 38% in 72 hrs.) when T. viride was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-40). When inoculation was simultaneously, the inhibition percentage ranged from 25 in 12 hrs. to 50 in 72 hrs. However, the maximum percentage of inhibition was recorded when T. viride was inoculated after 12 hrs. (Plate-59). The percentage of inhibition was 33.3 in 12 hrs. with 80 in 72 hrs. (Figs. 14 & 16).

4.5.2.3.3 Trichoderma viride isolate-3.

T. paradoxa isolate-2:

In the three methods of inoculation tested, growth inhibition of T. paradoxa was the least (7.7% in 12 hrs. and 13.3% in 72 hrs.) when T. viride was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-41). In simultaneous

Plate 58. Effect on the growth of T. paradoxa isolate-5 by T. viride isolate-2 in different methods of inoculation. (48 hrs. after inoculation.)



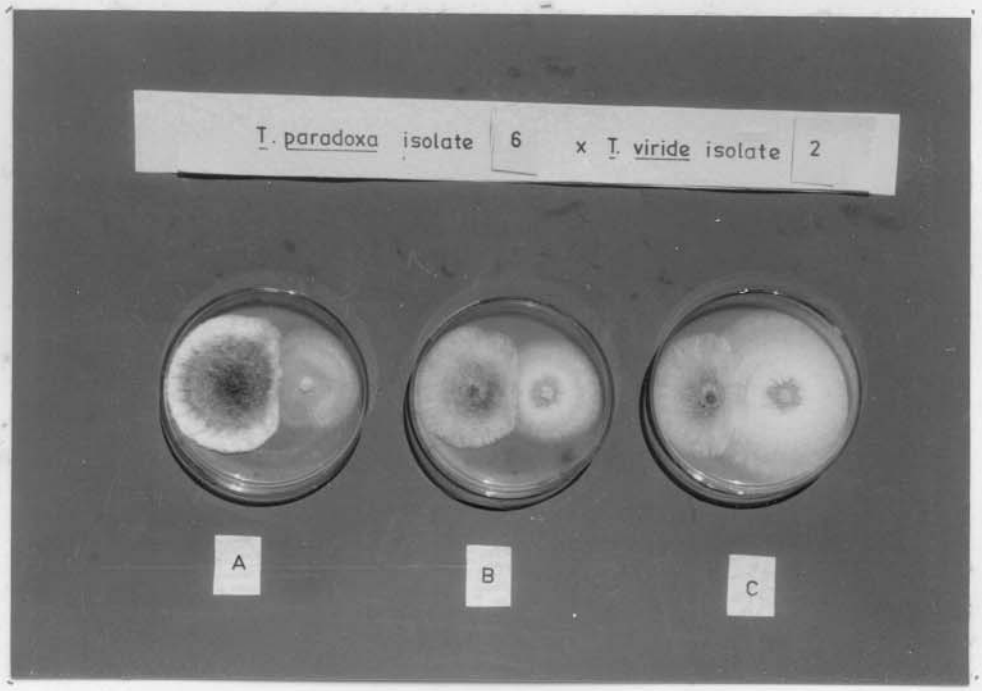
(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

Plate 59. Effect on the growth of T. paradoxa isolate-6 by T. viride isolate-2 in different methods of inoculation. (48 hrs. after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

Table 41: In vitro interaction of Trichoderma viride Isolate-3 with T. paradoxa.

Time of observation after inoculation of both the fungi (in hrs.)	Growth inhibition of T. paradoxa by T. viride (Percentage)											
	Isolate-2			Isolate-3			Isolate-5			Isolate-6		
	A	B	C	A	B	C	A	B	C	A	B	C
12	7.7	11.1	19.7	0	16.7	25.0	0	0	0	0	0	0
24	10.3	25.0	33.3	6.25	28.0	40.0	21.4	33.3	37.5	8.7	20.0	28.6
36	13.3	26.7	36.0	15.7	31.3	40.0	22.7	41.2	50.0	12.5	28.0	58.3
48	13.3	26.7	40.0	30.4	43.4	50.0	33.3	56.5	60.0	16.7	30.8	59.3
60	13.3	26.7	40.0	43.3	43.3	50.0	40.0	56.7	60.0	16.7	33.3	60.0
72	13.3	26.7	50.0	43.3	45.8	50.0	40.0	60.0	68.0	16.7	33.3	60.0

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = T. paradoxa and T. viride were simultaneously inoculated.

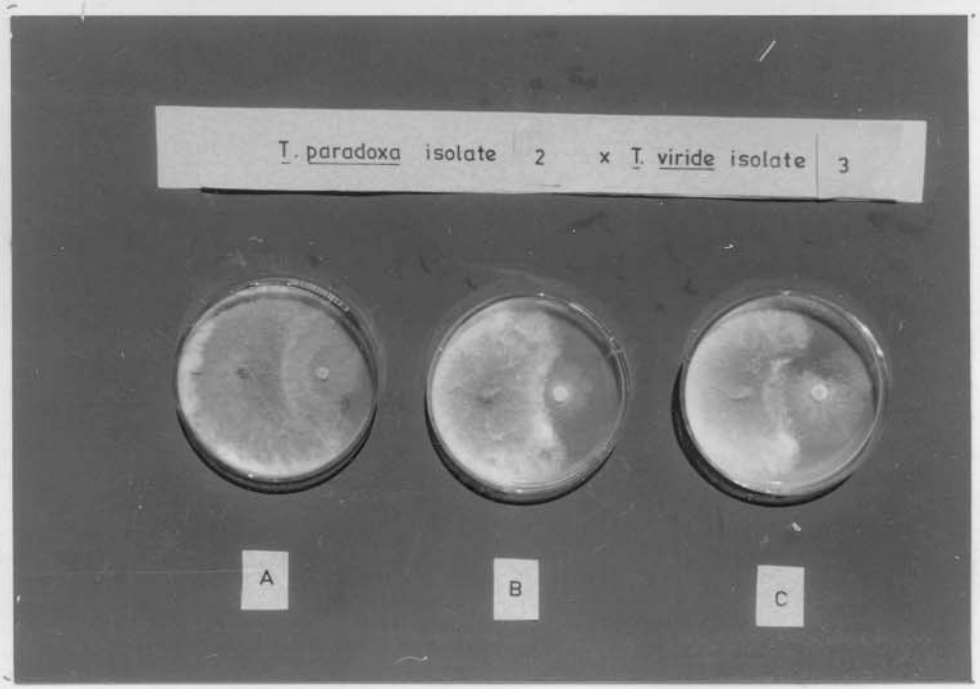
C = T. paradoxa was inoculated after 12 hrs. growth of T. viride.

method of inoculation, the inhibition percentage ranged from 11.1 in 12 hrs. to 26.7 in 72 hrs. However, there was maximum percentage of inhibition when T. viride was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate 60). The percentage of inhibition of T. paradoxa was 19.7 in 12 hrs. with 50 in 72 hrs.(Figs.15 and 16).

T. paradoxa isolate-3.

Growth inhibition of T. paradoxa was minimum (0% in 12 hrs. and 43.3% in 72 hrs.) when T. viride was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-41). When inoculation was done simultaneously, the inhibition percentage ranged from 16.7 in 12 hrs. to 45.8 in 72 hrs. However, the maximum percentage of inhibition was discernible when T. viride was inoculated first and T. paradoxa was inoculated after 12 hrs. (Plate 61). The percentage of inhibition of T. paradoxa was 25 in 12 hrs. with 50 in 72 hrs. (Figs. 15 and16). The growth of T. paradoxa was meagre towards the opposing colony of T. viride while there was slight growth of T. paradoxa on the opposite side viz. towards the petridish edge away from the opposing colony. The colony diameter of T. paradoxa was 47 mm on the third day after inoculation of T. viride in the first method wherein T. viride was inoculated after 12 hrs growth of T. paradoxa. The colony diameter of T. paradoxa was 37 mm in the third day, when both T. viride and T. paradoxa were simultaneously inoculated. When T. paradoxa was inoculated after 12 hrs. growth of T. viride the colony diameter of T. paradoxa was only 9 mm on the third day after the inoculation

Plate 60. Effect on the growth of T. paradoxa isolate-2 by T. viride isolate-3 in different methods of inoculation. (48 hrs. after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

Plate 61. Effect on the growth of T. paradoxa isolate-3 by T. viride isolate-3 in different methods of inoculation. (48 hrs. after inoculation).

(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

of T. paradoxa. There was no significant growth of T. paradoxa towards T. viride colony.

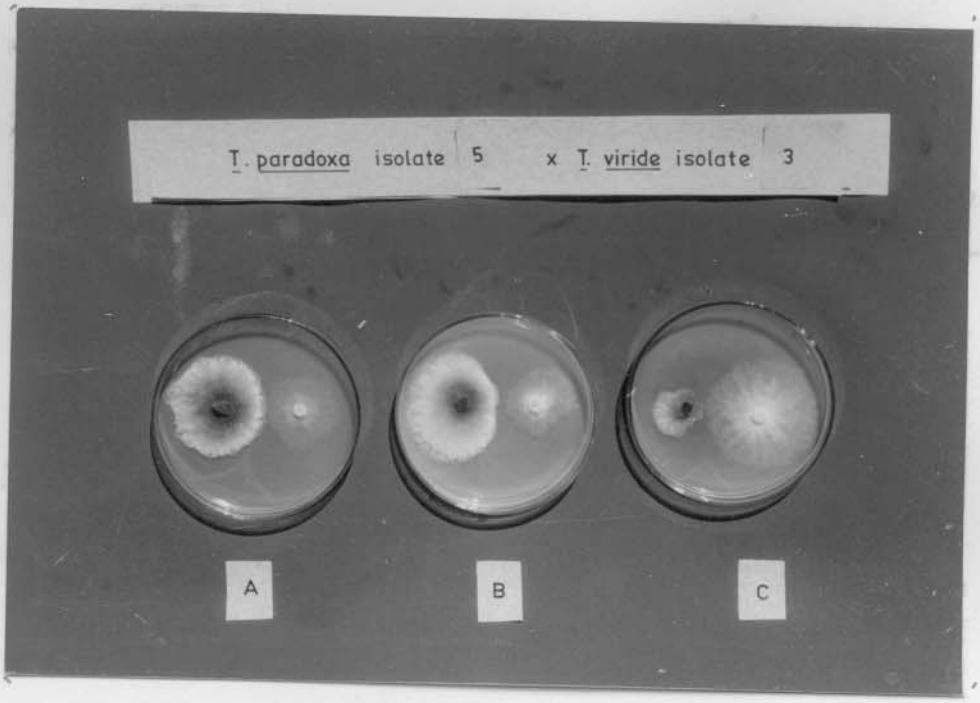
T. paradoxa isolate-5:

In the three methods of inoculation tested, growth inhibition of T. paradoxa was the least (0% in 12 hrs. and 40% in 72 hrs.) when T. viride was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-41). In simultaneous method of inoculation the inhibition percentage ranged from 33.3 in 24 hrs. to 60 in 72 hrs. However, the maximum percentage of inhibition of T. paradoxa was recorded when T. viride was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate-62). The percentage of inhibition of T. paradoxa was 37.5 in 24 hrs. with 68 in 72 hrs.(Figs. 15 & 16).

T. paradoxa isolate-6.

In the three methods of inoculation tested, minimum growth inhibition (0% in 12 hrs. and 16.7% in 72 hrs.) of T. paradoxa was observed when T. viride was inoculated 12 hrs. after the inoculation of T. paradoxa (Table-41). In simultaneous method of inoculation the inhibition percentage ranged from 20 in 24 hrs. to 33.3 in 72 hrs. However, the maximum percentage of inhibition was discernible when T. viride was inoculated first and T. paradoxa was inoculated after 12 hrs.(Plate-63). The percentage of inhibition of T. paradoxa was 28.6 in 24 hrs. with 60 in 72 hrs.(Figs. 15 and 16).

Plate 62. Effect on the growth of T. paradoxa isolate-5 by T. viride isolate-3 in different methods of inoculation. (48 hrs. after inoculation).



(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

Plate 63. Effect on the growth of T. paradoxa isolate-6 by T. viride isolate-3 in different methods of inoculation. (48 hrs. after inoculation).

(Note: In each petridish T. paradoxa is on left side and test fungus is on right side.)

A = T. paradoxa inoculated 12 hrs. before the inoculation of T. viride.

B = Simultaneous inoculation of T. paradoxa and T. viride.

C = T. paradoxa inoculated 12 hrs. after the inoculation of T. viride.

4.6. Studies on the effect of neem oil and neem oil cake on the growth of T. paradoxa in vitro.

In this study, T. paradoxa isolate-5 was taken.

4.6.1 Effect of neem oil

Growth rate of T. paradoxa was not significantly decreased by neem oil in vitro, T. paradoxa overgrew in the wells filled in the neem oil in the medium.

4.6.2 Effect of neem oil cake.

Percentage of reduction of growth of T. paradoxa colony increased with increasing concentration of neem oil cake content added to the growth medium. In 24 hrs. after inoculation, there was 18.5% growth reduction at 100 ppm 22.22% growth reduction at 100 ppm and 33.33% at 10,000 ppm of neem oil cake (Table-42). In 96 hrs. after inoculation, the growth reduction was 14.60%, 25.84%and 31.46% at 100 ppm, 1000 ppm and 10,000 ppm of neem oil cake respectively. Thus there was an appreciable difference in inhibition with the increase in concentration of neem oil cake percentage. However, at a given concentration, the inhibition percentage did not change much with increase in duration of exposure to neem oil cake from 24 hrs to 96 hrs.

Table 42: Effect of neem oil cake on the growth of *T. paradoxa* colony in vitro.

Time of observation after inoculation (in hrs.)	* Control	100 ppm		1000 ppm		10,000 ppm	
	** Diameter of colony (in mm)	** Diameter of colony (in mm)	% of inhibition of growth	** Diameter of colony (in mm)	% of inhibition of growth	** Diameter of colony (in mm)	% of inhibition of growth
24	27	22	18.5	21	22.2	18	33.3
48	58	45	22.4	43	25.8	37	36.2
72	84	69	17.8	66	21.4	58	30.9
96	89	76	14.6	66	25.8	61	31.4

* PDA medium without neem oil cake content.

** Mean of four replicates.

4.6.2.1 Effect of neem oil cake extract.

Percentage of reduction of growth of T. paradoxa colony increased with increasing concentration of neem oil cake extract added to the growth medium. In 24 hrs. after inoculation, there was 11.1% growth reduction at T. paradoxa at 5,000 ppm, 22.2% growth reduction at 25000 ppm and 29.6% growth reduction at 5000 ppm of neem oil cake (Table-43). In 96 hrs. after inoculation, the growth reduction was 5.6%, 10.1% and 13.5% at 5000 ppm, 25000 ppm and 5000 ppm of neem oil cake respectively. Thus there was appreciable difference in growth inhibition of T. paradoxa with the increase in concentration of neem oil cake percentage in the growth medium.

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Table 43. Effect of neem oil cake extract on the growth of *T. paradoxa* colony *in vitro*.

Time of observation after inoculation (in hrs.)	*Control	5,000 ppm		25,000 ppm		50,000 ppm	
	**Diameter of colony (in mm)	**Diameter of colony (in mm)	% of inhibition of growth	**Diameter of colony (in mm)	% of inhibition of growth	**Diameter of colony (in mm)	% of inhibition of growth
24	27	24	11.1	21	22.2	19	29.6
48	60	59	1.7	56	6.7	53	11.7
72	84	79	6.0	73	13.1	67	20.2
96	89	84	5.6	80	10.1	77	13.5

* PDA medium without neem oil cake content.

** Mean of four replicates.

5. DISCUSSION

One of the factors for decline in productivity of coconut is the incidence of number of diseases affecting the palm at its various stages of its growth. Among the diseases, stem bleeding disease is a very important one. Though many types of stem bleeding have been reported in coconut, in a very high percentage of cases, Thielaviopsis paradoxa was found to be was very closely associated. Only very recently it was established beyond doubt that T. paradoxa is the incitant of common stem bleeding disease of coconut (Nambiar et al., 1986).

To study a disease in detail it is important to know the variability prevalent in the pathogen. Often many organisms may colonize a diseased tissue in succession. Sometimes simultaneous infections are also noticed. All these phenomena definitely affect the symptom picture, disease development, population build up and survival of the pathogen and also damages afflicted on the host.

In the present study experiments have been designed to study the variability existing in the pathogen, to isolate the associated fungal mycoflora and further to study the nature of their interaction with T. paradoxa. Yet another objective was to study with special emphasis on those mycoflora which exhibited

innibitory influence on T. paradoxa. This would give an idea on the fungal flora which may have potential as biocontrol agents.

First set of experiment was designed at studying the variability prevailing in T. paradoxa. Therefore T. paradoxa was isolated from stem bleeding affected palms from different regions in Kerala and one locality in Dakshina Kannada (Karnataka State). Isolations were made at different stages of the disease and from a variety of treatments to avail the maximum variability. It was found that T. paradoxa could be isolated only from young untreated diseased lesions (Table-1).

Since T. paradoxa is the incitant of the disease (Nambiar et al., 1986) it is quite natural that young lesions yield the pathogen. As the disease advances, fungal succession sets in and the old lesions are invariably affected by a variety of fungi like Acremonium sp. Aspergillus niger, Chalaropsis sp. Mucur sp., Penicillium sp, Pestalotiopsis palmarum, Rhizopus sp. Trichoderma harzianum, T. viride etc.(Table-1) depending on the aerospora available in the locality. These organisms would have competed with T. paradoxa for space and nutrients or could have smothered the growth of T. paradoxa by physical contact or could have produced toxic principles arresting the growth of or even killing T. paradoxa. Such instances where antagonistic fungi competed with or affected the growth of a plant pathogen can be

found in literature. Thus Lal and Radha (1974) found it difficult to isolate Phytophthora palmivora from bud rot affected coconut in the late stages due to interference by Pseudomonas sp., Xanthomonas sp. and Erwinia sp. A variety of fungal mycoflora including Aspergillus flavus, Penicillium chrysogenum, P. frequentans, P. purpurogenum etc. were observed on peach twigs and flowers at different times of the year and found to inhibit the growth of Monilinia laxa, the pathogen of peach (Mulgarejo et al. 1985). Difficulty in isolation of the pathogen causing quick wilt of black pepper (Phytophthora palmivora) in the late stages because of fast colonization of the tissues by saprophytic mycoflora like Fusarium sp., Rhizoctonia sp., Pythium sp. etc. has been reported by Sarma and Nambiar (1982). Prior inoculation of the antagonistic fungus Peniophora gigantea to the freshly cut, nearly sterile stump surface of pine, inhibited the growth of Heterobasidion annosum, the causal agent of root rot of pine (Rishbeth, 1963; Artman, 1972; Poleshchuk and Yakimov, 1986). Since the associated fungi isolated from old lesions of coconut are prolific in their growth rate and production of conidia, attempts to isolate T. paradoxa from older lesions have been mostly failures.

Coal tar applied lesions in the coconut trunk did not yield T. paradoxa (Table-1). Even before the etiology of stem bleeding was known, it was found that removing the affected bark and applying hot coal tar could check the advancement of stem bleeding. This is mainly due to the fact that decay and damage do not penetrate

very deep into the stem tissues. Therefore when the affected portions are chiselled away, the source of inoculum is also automatically removed. Further, coal tar acts as a seal for the tissue cavities thereby preventing fungal entry to the host tissue, though temporarily. Coal tar is applied to the bark at fairly high temperatures, and this also would have killed the pathogen to an extent.

When neem cake is applied to the soil, it not only improves the soil texture but also encourages the growth of a variety of fungi which colonize and flourish in the treated soil. Many such fungi which produce airborne conidia would naturally therefore have a chance to colonize fresh lesions caused by T. paradoxa. Some of these fungi may be natural antagonists to T. paradoxa, resulting in the growth inhibition of the pathogen, either acting singly or in combination. It is also possible that some fungi would have produced chemicals/toxins which would have translocated to the site of infection through the coconut root system. It is knwon that fungi like Trichoderma sp., Gliocladium sp. etc. produce such antifungal chemicals (Papavizas, 1985). From samples of soil in which either neem cake or fertilizer cum organics have been applied, a predominance of A. niger and Trichoderma harzianum was observed (Table-2). Incidentally these organisms have been proved to be antagonistic to T. paradoxa in the later studies (Results 4.5.2), strengthening the view why isolation of T. paradoxa was poor in such soil.

Rajan and Singh (1974) reported the increased soil mycoflora by the addition of neem oil cake to the soil thereby reducing the inoculum potential of Pythium aphanidermatum in oil cake amended soil and the authors presumed that this might be due to possible toxic effect of decomposition products of neem oil cake encouraging preferably the growth of soil saprophytes which might have helped in the prevention of increase of population of the pathogen in the soil through competition. With respect to T. paradoxa further studies in this line are needed.

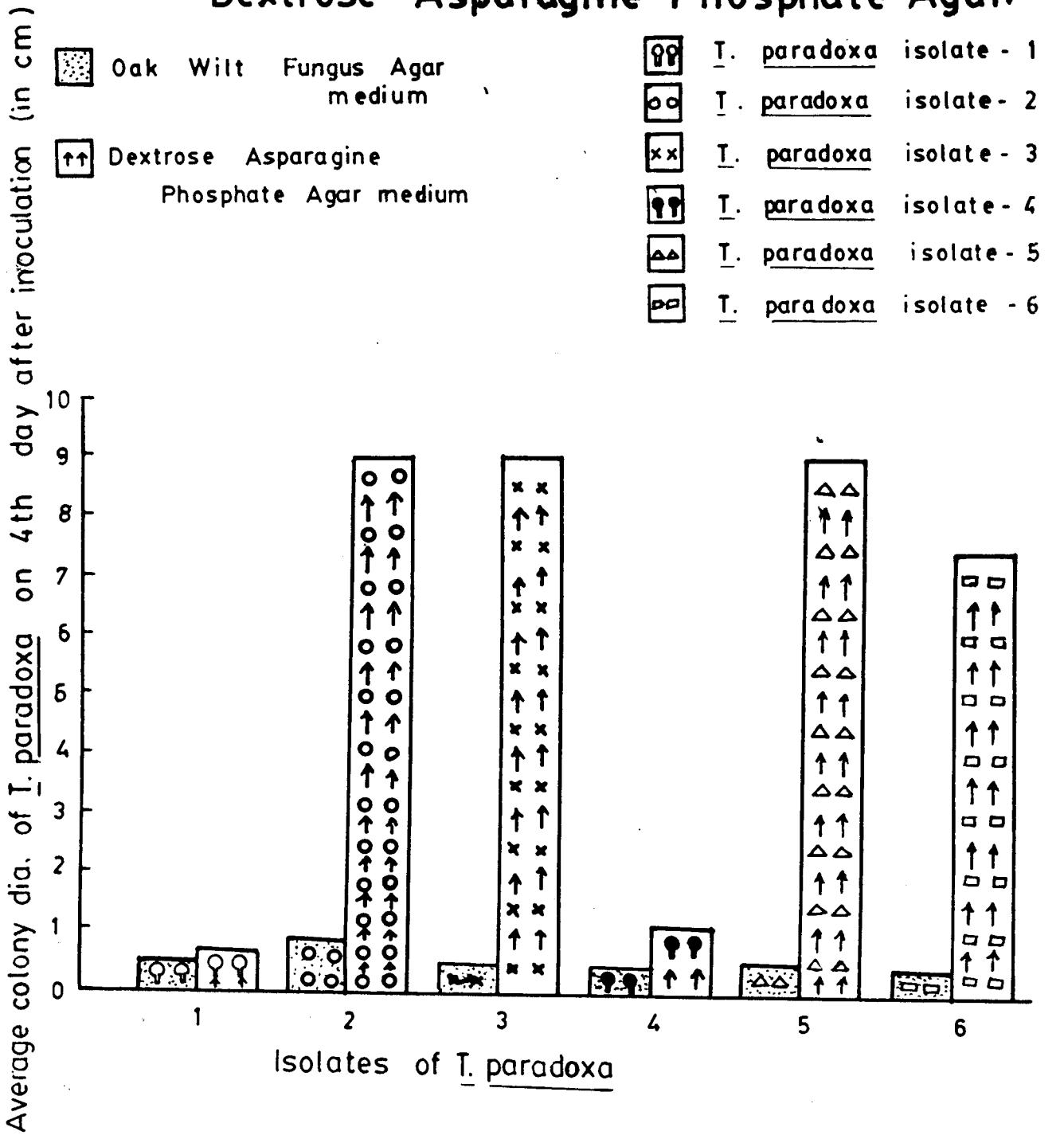
In an instance where stem bleeding was found to be very severe (Shiria in Kerala State) root samples from the affected trees, and not soil samples, yielded T. paradoxa(Tables 2 & 3). Extensive root damage was noticed in this case. This clearly indicates that T. paradoxa finds root as a very convenient substratum for its activity. Such instances have been reported in the case of soil borne pathogens. For instance, Ganoderma lucidum causing root rot in a variety of crops could not be easily isolated from soil particles because of its shy growth. However, from affected roots, the fungus could be readily isolated (Bakshi et al., 1976).

Studies on the effect of culture media on the isolates of T. paradoxa revealed that growth pattern and rate was different for different isolates on given medium (Tables 4 to 16) (Fig.7). This suggests that nutritional requirements in different isolates of T. paradoxa differed. This further suggests that eventhough gross morphology is not affected, variability for growth parameters was exhibited by T. paradoxa in different environments contributing different nutritional requirements. Similar pnenomenon has been reported in the genus, Colletotrichum, known for its variability in cultural and morphological characters (Arx, 1970). Intraspecific variations have been worked out in detail in C. falcatum on sugarcane (Abbott, 1938; Chona and Srivastava, 1960), C. lini (Zarzycka, 1976) and C. gloeosporioides on coffee (Muthappa, 1974; Hindorf, 1975) and C. gloeosporioides on cacao (Chandra Mohanan, 1983). Mordeu (1971) implicated heterokaryosis, parasexuality etc. as the causes for high degree of variability in C. gloeosporioides.

C. falcatum isolates were classified into light and dark dark types based on the cultural characters on oat meal agar (OMA) medium (Chona and Srivastava, 1960). In the present study, one isolate distinctly differed from others in that it produced a fruity smell in culture (Table-15). The isolates of Colletotrichum sp. causing coffee berry disease were divided into four groups based on cultural characteristics such as growth rate, colour and production of aerial mycelium and conidia.

In the present study of cultural and morphological characters of T. paradoxa isolates, eventhough variations of characters among the isolates were found, since the number of isolates of T. paradoxa were limited (six isolates only), more number of isolates from different places should be collected to make a classification of isolates based on morphological and cultural characters as was done in Colletotrichum. However, some generalisation can be made with regard to growth requirements of T. paradoxa isolates. None of the isolates could grow well on Oak Wilt Fungus Agar-C (Himedia). This medium was deficient in protein/aminoacids. On the contrary growth of all the isolates was favoured by Asparagine/protein containing medium like Dextrose Asparagine Phosphate Agar(DAPA) medium and Kirchoff's Agar (KA) medium (Table-16)(Fig.17). David (1987) reported Asparagine as the best aminoacid source for the profuse growth and sporulation of Ceratocystis paradoxa, the causal agent of sett rot of sugarcane. Similar instances have been observed with respect to many other fungi. Leonian and Lilly(1940) observed very good growth Phycomyces blakesleeanus on medium containining Asparagine. Lillly and Leonian(1942) investigated the effect of nitrogen source on the growth of 10 strains of Saccharomyces cerevisiae and found that different strains of the same organism responded differently to the same source of nitrogen viz. L-Asparagine. Pepton and biotin requirement of Ganoderma lucidum for profuse growth and sporophore formation in culture media was reported by Nambiar and Nair (1972). Steinberg(1942)

Fig. 17. Comparison of growth rate of isolates of T. paradoxa on Oak Wilt Fungus Agar-C and Dextrose Asparagine Phosphate Agar.



made an extensive study of growth of Aspergillus niger on 22 aminoacids. Aspartic acid and six other aminoacids were found as excellent source of nitrogen for A. niger. In literature, there are reports where species of Ceratostomella required various vitamins for their growth. More than 200 fungi were known to be partially or totally deficient in Thiamine (Fries, 1948). Among the filament fungi deficiency of pyridoxine seems to be characteristic of certain species of Ceratostomella (Robbins and Ma, 1942). Ceratostomella ulmi and C. pillfera were reported to be deficient for pyridoxine (Robbins and Ma, 1942a). Some species reported to be deficient for pyridoxine are also deficient in thiamine and biotin (C. ips, C. pini, C. montium and C. microspore), biotin only (eg. C. piceaperda) or Thiamine only (eg. C. multiannulata , C. pluriannulata) (Robbins and Ma. 1942a, 1942b). However, requirement of aminoacids and Vitamins, in case of T. paradoxa, needs further experimentation.

All the isolates of T. paradoxa were found to grow luxuriantly on sterile coconut rachillae. But a few of the isolates (isolates 2, 3 and 5) did not show good growth on Coconut Tissue Extract Agar (CTEA) medium as compared to DAPA and KA media in the initial period of growth. It is likely that the nutrient status of the bark tissues from which extraction has been made is quite different from that of the rachis. Rachis may be a rich source of nutrient as compared to bark tissues, as far as the growth of T. paradoxa is concerned. Another possible reason that the nutrients in the bark tissues would have got diluted to the extent as not to support luxurient growth of the fungus as seen on the

sterilised rachis. It is also likely that the chemical compounds in the bark tissues would have undergone changes during the process of extraction and this altered compound might be more thermolabile and would have got destroyed. In nature profuse bunch infection due to T. paradoxa has been repoted especially in regions where cooler conditions with a high relative humidity prevail(Anon, 1985).

In general, isolates of T. paradoxa (Isolates 2,3 & 5) producing fluffy colonies, grew faster and produced more mycelial growth (Tables 7, 9 & 13). As against this, T. paradoxa isolate-6 which was a shy grower with flat colonies(Table-15) produced abundant conidia and chlamydospores (Tables 18 & 21 respectively), shortly after inoculation(within 24 hrs.) thus indicating that variability existed among T. paradoxa isolates in the nature of conversion of energy. While isolate nos.2, 3 and 5 excelled in producing vegetative growth (Tables 7, 9 & 13), isolate no.6 produced innumerous conidia and chlamydospores and produced a fruity smell (Table-15) which attracted flies like Drosophila to it. As against these categories isolate nos. 1 and 4 were poor in vegetative growth as well as in producing conidia (Table-18) and chlamydospores(Tables 19 & 20). The occurrence of large number of conidia and chlamydospores in isolate-6 has a direct bearing on the virulence and also survival ability of the pathogen. Although the fast growing mycelial isolates had a distinct advantage in fast colonization

of the host tissue rapidly, the isolate-6 producing both chlamydospores and conidia had a direct advantage over the first category. Since conidia were readily produced and flies were attracted to the fungus due to fruity smell, it is quite likely that fast dissemination of the conidia would take place through these flies, which act as passive carriers. However, this needs further experimentation and confirmation. Insect dissemination of Ceratocystis ulmi was recorded in the case of Dutch elm disease. The elm bark beetle, Scolytus scolytus was reported as the most important vector of C. ulmi. (Collins, 1941). There is a likelihood of a similar possibility in stem bleeding disease also. It is known that quite often stem bleeding affected palms especially in the late stages of disease, are infested by insects like Diocalandra sp., Xyleborus etc. (Menon and Pandalai, 1960). Further studies are required to determine their role in the dissemination of the fungus.

In many fungi chlamydospores are formed during adverse climatic conditions, nutrient deficiency and due to aging. This has a direct bearing on the epidemiology. Since chlamydospores have a great survival value, they help the pathogen to tide over the unfavourable conditions till ideal conditions for disease initiation set in. Therefore an isolate which is capable of producing large number of chlamydospores has a distinct advantage of greater survival value over others who lack this capacity. Such an instance was reported in Fusarium graminearum causing the most serious crown rot of wheat in the eastern belt of Australia (Burgess et al.,1975). F. graminearum group-1 which

produced large number of chlamydospores but no perithecia was regarded as being the most serious cause oi crown rot of wheat than F. graminearum group-2 which readily produced perithecia but not chlamydospores. However, F. graminearum group-2 isolates found to be the major cause of stalk rot of maize. In Colletotrichum falcatum (Chona and Srivastava, 1960) and C. gloeosporioides (Chandra Mohanan, 1983) highly sporulating isolates showed high degree of virulence.

Often the above mentioned characters may go hand in hand with a change in the virulence in a pathogen, making it a potentially dangerous or less virulent pathogen. Presence of a highly pathogenic aggressive strain and less pathogenic nonaggressive strain, ~~xxxx~~ in the same pathogen was well reported in Ceratocystis ulmi, the causal agent of Dutch elm disease (Brasier, 1981). In Colletotrichum gloeospoioides, the isolate showing higher rate of growth were found as the least sporulating and less pathogenic than the isolates showing slower rate of growth and high degree of sporulation (Chandra Mohanan, 1983). Similar case was reported in Colletotrichum falcatum (Chona and Srivastava, 1960). In cases where less virulent isolates of the pathogen are present, there is a possibility of using them as biocontrol agents to ptotect a plant against the more virulent pathogen. There are many such instances in literature. Resistance was induced in bean (Phaseolus vulgaris) hypocotyls to Colletotrichum lindemuthianum by prior inoculation with an avirulent race of the fungus (Rahe et al., 1969). Particularly striking example of resistance

to a pathogen induced by prior inoculation with a nonpathogen is with sweet potato varieties susceptible to Ceratocystis fimbriata (Weber and Stahmann, 1966). Susceptible tissues of the sweet potato variety Jersy Orange, inoculated with a nonpathogenic isolate of C. fimbriata were immune or resistant when inoculated two days later with a pathogenic isolate of C. fimbriata. Tissues not inoculated first with the nonpathogenic isolate were fully susceptible. However, in the case of T. paradoxa though variation of cultural characters exists among different isolates, virulence among the isolate of T. paradoxa needs further investigation.

Development of a disease is affected by many factors like the host, weather, pathogen and also by other biological factors like mycoflora associated with the diseased plant/lesion. The advancement of an aggressive pathogen may be stopped by an antagonistic associate present at the site or a disease symptom/ damage may be aggravated by a fungal successor which brings about further deterioration to the plant tissue. Gliocladium roseum, Trichoderma polysporum and T. viride were reported to be capable of replacing the aggressive strain of the pathogen of Dutch elm disease, viz. Ceratocystis ulmi in the inner bark of elm trees (Webber and Hedger, 1986). On the other hand disease symptom/damage to the bud rot affected coconut palm due to Phytophthora palmivora was aggravated by the secondary colonizers viz. Pseudomonas sp., Xanthomonas sp. and Erwinia sp.; and hastened secondary rotting of the crown (Lal and Radha, 1974).

In ginger, the soft rot caused by Pythium sp. is aggravated when superimposed by Fusarium infection(Sharma and Jain, 1977). Hence it becomes an important aspect of plant pathology to study the effect of various organisms associated with an infected tissue. In the present study the inter-relationships between T. paradoxa and the associated mycoflora has revealed a wide variety of interactions. Isolates of T. paradoxa reacted differently to different fungi and vice versa (Tables 24, 27, 31 and 35). Similar studies have been reported in the case of Dutch elm disease where the in vitro interaction of different isolates of elm bark saprobes with Ceratocystis ulmi was investigated (Webber and Hedger, 1986).

In the present study, in general, four kinds of reactions were noticed as follows:

i) Commensalism where both organisms grew without affecting each other adversely but intermingling freely. Acremonium sp Chalaropsis sp. etc. belonged to this category. Cladosporium herbarum, Gliomastrix murorum, and Graphium penicillioides showed such an interaction with C. ulmi in vitro.(Webber and Hedger, 1986).

ii) Expression of mutual inhibition with the formation of a pigmented zone. In this category, it was found that mutual inhibition started shortly after inoculation. T. paradoxa (6.25% to 26.7%)inhibition) and A. niger (14.37% to 33.3% inhibition) showed such a type of mutual inhibition (Tables 23, 25, 26, 28, 29, 30, 32, 33, and 34) with a pigmented zone in the first week after inoculation. However, after

15 days, mutual inhibition was not found. This may be due to the dissolution or denaturation of the inhibitory chemical present in the pigmented zone. In the case of interaction of C. ulmi with elm bark saprobes, Apospheaeria ulmicola, Coniothyrium karstenii, Fusarium solani, Nectria coccinea, Penicillium putterillii (isolate-2), Phomopsis oblonga, Seiridium intermedium, Verticillium cinnabarinum showed this type of interaction (Webber and Hedger, 1986).

(i) Expression of mutual inhibition with a clear zone: This type of interaction was found between a) T. paradoxa isolate No.5 and Pestalotiopsis palmarum (Table-30), b) T. paradoxa isolates 3, 5 and 6, and A. terreus (Tables 26, 30 and 34). etc. Mutual inhibition may be due to the secretion of inhibitory products and their diffusion into the surrounding medium. The efficiency and concentration of these chemicals depend on the organisms involved. Sometimes this expression was delayed as in the case of interaction of T. paradoxa and P. palmarum (40%)(Table-30) in the present study. This may be due to either slow growth of the test fungus or late production of the inhibiting substance by the test fungus. Mutual inhibition with a clear zone was found in between C. ulmi and elm bark saprobes like Botryosphaeria stevensii, Epicoccum nigrum, Fusarium lateritium and Trichothecium roseum, in vitro (Webber and Hedger, 1986). However, whether this pattern of inhibition observed in ~~vitr~~ in vitro studies can be straight away translated into similar in vivo effects in the case of T. paradoxa can be judged only by real in vivo studies as has been

explained by Webber and Hedger (1986), while comparing the interactions between C. ulmi and elm bark saprobes in vitro and in vivo. All the fungi which showed mutual inhibition (with or without a pigmented zone) with C. ulmi, failed to show inhibition of growth of C. ulmi in vivo (Webber and Hedger, 1986). However, the fungi which showed mutual inhibition with T. paradoxa in vitro may be used for the study of the interaction in vivo.

iv) In the fourth category of reaction, growth of the isolates of T. paradoxa was inhibited by the challenging organims like Trichoderma harzianum (6.25% to 22.22% in 24 hrs. after inoculation) (Tables 23, 25, 28, and 32) and T. viride (5.88% to 33.3% in 24 hrs. after inoculation) (Tables 23, 25, 28 and 32). Eventhough A. niger showed mutual inhibition with T. paradoxa for 15 days after inoculation, later A. niger showed the same type of inhibition as was shown by Trichoderma sp. Fifteen days after inoculation, all the three fungi overgrew on T. paradoxa colony (Tables 24, 27, 31 and 35). This indicates the lack of defence mechanisms in T. paradoxa against the aggressors in protecting itself. This category of organisms should be thoroughly studied and searched for obtaining potential biocontrol agents.

Six organisms namely A. niger (one isolate), T. harzianum two isolates and T. viride (three isolates) expressed antagonistic reactions towards T. paradoxa. As mentioned earlier, fungal succession has a significant role in the development of diseases, survival of the pathogen, damages to the host etc. The precedence or succession of a fungus in sequencial colonization of a host decides its survival and also interaction with other organisms.

Bora (1977) tested in vitro effects of A. niger isolated from soil against Fusarium solani, Alternaria alternata and Rhizoctonia solani from egg plant. A. niger showed the greatest antagonism. Gokulapalan and Nair (1984) studied the antagonistic effect of A. niger on R. solani causing rice sheath blight and found that A. niger was contagonistic to the pathogen in vitro. Venkatasubbaiah and Safeeulla (1984) studied the in vitro interaction of A. niger with R. solani, the incitant of collar rot of coffee seedlings. A. niger hypherparasitised R. solani completely in dual culture. In the present studies using A. niger, it was observed that with all the isolates of T. paradoxa, when A. niger was inoculated first (12 hrs. earlier to T. paradoxa), maximum extent of growth inhibition ranging from 36.7% to 50% at the end of 72 hrs. after inoculation was observed (Table-36). In case where both were inoculated simultaneously, the extent of growth inhibition was lower ranging from 30% to 40% at the end of 72 hours after inoculation (Table-36). However, it was noticed that the maximum growth inhibition was obtained at 60 hrs. after inoculation and it did not increase further. In cases where T. paradoxa preceded A. niger by 12 hrs, maximum growth inhibition was reached at 48 hours. in T. paradoxa isolate Nos. 2, 3 and 6 (50%, 39.3%, and 36.7% respectively) and at 72 hrs. in Isolate No.5(50%) (Table-36). , thus indicating that for efficient expression of antagonism, the inoculation of test fungus must precede T. paradoxa by atleast 12 hrs. In certain cases, such results

have been confirmed in vivo also. For instance, A. niger showed antagonism in vivo against Fusarium solani, Alternaria alternata and Rhizoctonia solani in egg plant (Bora, 1977). Seed tratment with A. niger significantly reduced collar rot of coffee seedlings due to R. solani. (Venkatasubbaiah and Safeeulla, 1984). However, in this case, the results could not be confirmed by in vivo studies. Such studies are required to ascertain the suitability of this fungus as a biocontrol agent.

Lee and Wu (1979) reported plasmolysis of hyphae of Sclerotinia sclerotiorum by T. harzianum in vitro. T. harzianum grew more rapidly and covered colonies of S. sclerotiorum in culture. In the present study, two isolates of Trichoderma harzianum were tested as in the previous case of A. niger, against four isolates of T. paradoxa. Here also trend of the results were similar ie. extent of inhibition was maximum when the antagonist inoculation preceeded the pathogen by at least 12 hrs. In general, both the isolates of T. harzianum exerted greater growth inhibition on all the isolates of T. paradoxa than A. niger, the range being 23.3% to 86.6% (Tables 37 & 38) in comparison with 13.3% to 50% exerted by A. niger. Once again, testing these results in vivo is necessary for ascertaining the efficacy of these fungi as candidates for biocontrol of T. paradoxa. In literature plenty of instances are available where T. harzianum has been successfully used as a biocontrol agent for plant disease. Pal and Chaudhary (1975) observed a necrotrophic type of mycoparasitism between T. harzianum and

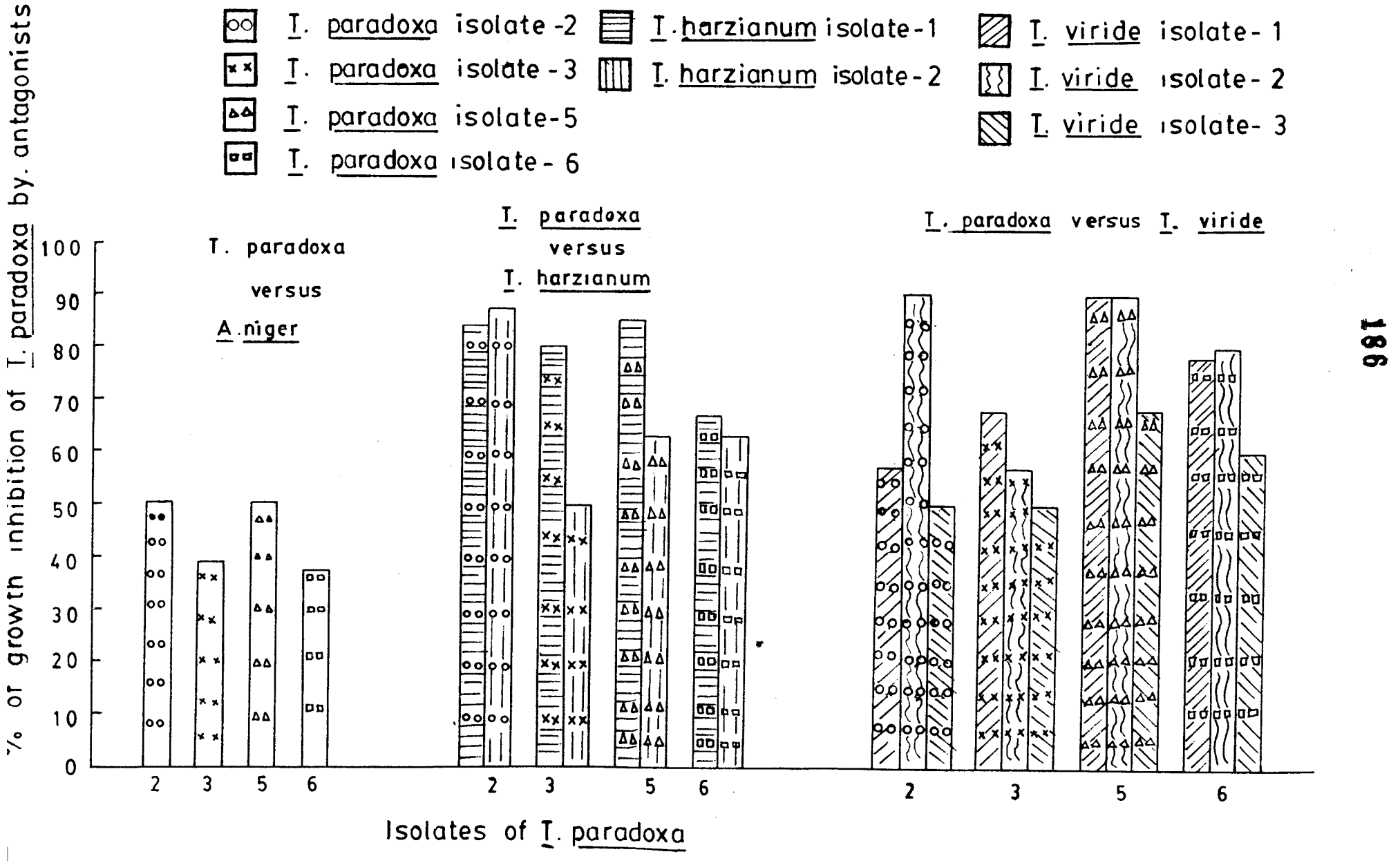
and Rhizoctonia solani in Crotalaria juncea plants. Pottle et al. (1977) reported the biological control of wound hymenomycetes in Acer rubrum by T. harzianum. Abd-Ed-Moity and Shatla (1981) reported biological control of white rot disease of onion due to Sclerotium cepivorum by T. harzianum. Application of mycelium and spores of T. harzianum decreased infection of onions in pots, glass house pots and in the field. Elad et al. (1981) reported the biological control of Rhizoctonia solani in strawberry fields by T. harzianum. The disease severity in daughter plants was reduced by 18-46% in nursery plots treated with preparations of T. harzianum. Plants treated with T. harzianum and transferred to the commercial field gave a 21-37% increase in early yield. Decreased incidence of crown rot of tomato caused by Fusarium sp. under field conditions by applying conidial suspension of T. harzianum to the roots and crowns of tomato plants at the time of planting was reported by Marois et al. (1981). Schulz (1981) investigated the biological control of Cytospora persoonii and C. cincta causing cankers on peach, plum and cherry trees. Good inhibition of the pathogen was obtained by the prophylactic treatment of trees with T. harzianum. Treatment of established cankers was unsuccessful. Smith et al.(1981) postulated the mechanism of biological control of Fomes connatus in red maple wounds treated with T. harzianum. It was reported that T. harzianum increased phenol content of the plant that inhibited decay fungus like F. connatus. With a view to determing whether similar mechanisms

may be operating in this case also, in vivo studies are required using T. harzianum against coconut pathogen. This needs in vivo study of the interaction of T. harzianum in the stem bleeding portions of the coconut palm. Venkatasubbaiah et al. (1984) studied the interaction of T. harzianum with Rhizoctonia solani, the incitant of collar rot of coffee seedlings. Seed treatment with T. harzianum significantly reduced disease incidence. Similar studies by Strashnov et al. (1985) using R. solani causing fruit rot of tomato resulted in reduction of fruit rot by 43% and 85% respectively by the application of T. harzianum to soil and by coating tomato fruits under laboratory conditions. When mixed with naturally infested soil, T. harzianum reduced the fruit rot by 27-51% in tomato. Bisiach et al.(1985) reported the reduction of incidence of Botrytis cinerea on grape in the field by 4-6 applications of conidial suspension of T. harzianum from flowering time. However, the efficacy of T. harzianum as a biocontrol agent of T. paradoxa under natural conditions needs in vivo experiments.

Trichoderma viride is one of the potential antagonistic fungi, inhibiting the growth of many plant pathogenic fungi. Bora (1977) tested in vitro effects of T. viride isolated from soil against Fusarium solani, Alternaria alternata and Rhizoctonia solani from egg plant and reported that the antagostic effect of T. viride on these pathogens. Sychev and Shaposhnik (1982) reported the growth inhibition of Rhizoctonia solani, the pathogen of Cucumis sativum, on Czapek-Dox medium,

by T. viride. The culture filtrate less than 10% was found enough for the biocontrol of the pathogen. Gokulapalan and Nair (1984) studied the antagonistic effect of T. viride on Rhizoctonia solani causing rice sheath blight and found that T. viride was antagonistic to the pathogen in vitro. During the course of the present investigations, three isolates of T. viride were obtained and subjected to in vitro studies in ascertaining their inhibition properties against the isolates of T. paradoxa. As in the two previous cases, maximum growth inhibition (50% to 90%) (Tables 39, 40 & 41) of T. paradoxa isolates was afforded when the T. viride isolates were inoculated first in.12 hours before the pathogen. With regard to the extent of inhibition afforded, T. viride faired better than A. niger in all instances. In comparison with T. harzianum also, T. viride gave better results in certain instances. Here the range forthe value of inhibition (when before, simultaneous and after inoculation of T. viride were considered) was 13.3% to 90% (Tables 39, 40 & 41) where as corresponding values for T. harzianum were 23.3% to 86.6% and for A. niger 13.3% to 50% (Fig. 18). T. viride has exhibited a wide range of inhibition from among all the three species of fungi tested. However, confirmation of these results in vitro is necessary for evaluating the efficacy of these isolates in plant protection in vivo. in literature plenty of instances are available where T. viride has been successfully used as a biocontrol agent for plant diseases. T. viride was reported to act as

Fig. 18. Interaction of T. paradoxa isolates with antagonists: antagonist challenged by T. paradoxa 12hrs. after inoculation of the former



a biocontrol agent against Armillaria root rot (Armillaria mellea) of shade trees, fruit trees etc. (Heald, 1933; Munnecke et al. 1976, Rishbeth, 1979). Musatova and Kirik (1977) reported that the introduction of T. viride into the soil limited infection of winter wheat by Fusarium sp. increasing yield. Nikitina et al. (1977) investigated the effect of culture liquids of T. viride on the growth, development and infection of maize by Ustilago maydis. Antibiotics from the fungus caused degeneration and disintegration of the pathogen. Dubos et al. (1978) studied the effect of T. viride on Botrytis cinerea and Phomopsis viticola. In vineyard at Bordeaux, a suspension of T. viride culture on oat agar, diluted to 10^8 spores/ml had an efficacy of 70% against B. cinerea and 80% against P. viticola. Bisiach et al. (1985) reported the reduction of incidence of Botrytis cinerea on grape in the field by 4-6 applications of conidial suspensions of T. viride from flowering time. Antagonistic nature of T. viride against Helminthosporium sativum infecting wheat seedlings was reported by Prasad et al.(1978). There was only 14.3% infection of seedlings when wheat seeds were treated with suspension of T. viride as compared with 96.7% in water controls. Dumitras and Fratilescu-Sesan (1979) reported the in vivo protection of beet and cotton seedlings against Pythium debaryanum by T. viride. Rebenko et al.(1979) reported that treatment of Odessa 46 winter barley with T. viride increased resistance and reduced incidence of the disease in trials using inoculation of plants with Fusarium oxysporum, Helminthosporium sativum and Ustilago hordei. Dumitras and Sesan (1980) studied the antagonistic effect of 39 isolates

of T. viride against Rhizoctonia solani on sugarbeet, bean (Phaseolus vulgaris) and cotton seedlings. In biological treatments of seeds and soils in the glass house, the results were similar to those obtained by chemical method. (Ubaidullaev (1980) reported the use of T. viride with fertilizers for the biological control of Verticillium dahliae infecting cotton. In field trials, the yield was highest when spore preparation of T. viride at 450 mg/m^2 was introduced into the soil with superphosphate after ploughing in Autumn or before sowing in spring. Schulz (1981) investigated the biological control of cytospora persoonii and C. cincta causing cankers on peach plum and cherry trees. Prophylactic treatment of trees with T. viride showed goodinhibition of the pathogen. T. viride showed the best effect four days after application. Treatments of established cankers has been successful. Ricard (1983) treated 15 elms with T. viride pellets by inserting the pellets into the trunks of elm trees affected by Dutch elm disease caused by C. ulmi, every 10 cm around the circumference. There was no progress of the disease during three growing seasons. David (1987) reported that Pythium root rot of sugarcane seedlings causing loss of valuable seedlings in nurseries after germination from the true seed (fluff) could be effectively controlled with the incorporation of Trichoderma viride to seed beds. Mass multiplication of T. viride was standardised in sand sorghum medium in polythene bags. However, the efficacy of T. viride as a biocontrol agent of

T. paradoxa under natural conditions needs *in vivo* experiments.

This preliminary studies on *T. paradoxa* indicate the possibility of using some of the associated fungal organisms as potential antagonists or biocontrol agents to the pathogen. Extensive studies in this direction are required to screen more number of candidate fungi for their possible use as biocontrol agents. On the other hand all fungi, antagonistic to the pathogen *in vitro* need not be antagonistic *in vivo* also, as has been well documented in case of Dutch elm disease (Webber and Hedger, 1986). Hence the interactions of antagonistic fungi *in vitro* should be studied *in vivo* also to use the antagonists as biocontrol agents under natural conditions.

The success of any biocontrol programme comes from the feasibility of its wide adoption. Any technique becomes popular when it is economical and is less combersome. It was observed that application of 5 kg neem oil cake/tree/year continously for 2-3 years could reduce the severity of stem bleeding disease in a few diseased palms in preliminary field tests. (Nambiar personal communication). This needs large scale field testing for confirmation. When isolations were made from young lesions from neem cake applied plants, an abundance of antagonistic fungi namely *A. niger* and *T. harzianum* were observed in those tissues.(Table-1). Thus this practice could profitably be exploited to effect biological control of *T. paradoxa*, the causal agent of stem bleeding disease of coconut, under natural conditions. To study the inhibitory properties of neem oil cake,

experiments were conducted to test the efficacy of neem oil, whole cake powder, aquous extract of neem oil cake on T. paradoxa in vitro. Results indicated that whole powder was more effective than aquous extract or oil. This gives a clue that inhibitory properties of neem oil cake may be direct as well as indirect. In direct cases, the whole powder which contains materials other than water soluble ingredients and which contains lesser level of oil may be responsible for direct inhibitory properties. To study the nature of this component various extractants should be tried for extracting the inhibitory principle. The indirect effect of neem oil cake in alleviating the symptoms may be due to the fact that it supports excellent growth of many antagonistic fungi like A. niger, T. harzianum etc. which in turn bring about inhibition of growth of T. paradoxa. However, these ideas need further experimentation and confirmation.

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6. SUMMARY AND CONCLUSIONS

1. The recent establishment of pathogenicity of *Thielaviopsis paradoxa* in causing stem bleeding disease of coconut has opened up new avenues in understanding the disease. The existence of variability in the pathogen (*T. paradoxa*) is one field, in which no work has been done. Eventhough a large number of organisms like *Aspergillus* sp, *Diplodia* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizoctonia* sp., *Trichoderma* sp., etc. have been isolated from stem bleeding lesions, their influence on the pathogen and on the disease has so far not been studied. Hence, to fill in this lacuna, *in vitro* studies on the interaction of the fungi isolated from tissues and/or soils with *T. paradoxa* was taken up with a view to mainly determining mycoflora antagonistic to the pathogen so that they might be useful in later studies in evolving biological control measures against the stem bleeding pathogen.

2. The present study consists of three parts namely:
   i) Isolation of *T. paradoxa* from different sources, and cultural and morphological characters of different isolates of *T. paradoxa*.
   ii) Isolation of different mycoflora associated with stem bleeding disease of coconut.
   iii) *In vitro* studies on the interaction of associated mycoflora with the isolates of *T. paradoxa*.

3. T. paradoxa could be isolated only from young lesions from the diseased palms and decaying roots. From old lesions or from stem tissues treated with coal tar or from soil/tissues of palms whose basins received neem oil cake application, T. paradoxa could not be isolated.

4. A total of six isolates of T. paradoxa were made from different localities in Kerala, and the cultural and morphological characters were studied in vitro.

5. Studies on the effect of culture media on the isolates of T. paradoxa revealed that growth pattern and rate were different for different isolates on a given medium. Similarly growth of each isolate in different media was also different, suggesting that nutritional requirements differed in different isolates of T. paradoxa.

6. None of the isolates of T. paradoxa could grow well on Oak Wilt Fungus Agar (OWA-C) medium which was deficient in protein/aminoacids. On the contrary, growth of all the isolates was favoured by Asparagine/protein containing media like Dextrose Asparagine Phosphate Agar (DAPA) and Kirchoff's Agar (KA) media. This showed that the isolates of T. paradoxa required organic nitrogen-supplements in their nutrition. It is likely that T. paradoxa is not able to synthesis at least a few of the essential proteins/aminoacids required by it.

7. Isolates of T. paradoxa producing fluffy colonies grew faster and produced more mycelial growth. The growth rate of T. paradoxa isolate-6 was rather slow and it produced flat colonies with a lot of conidia and chlamydospores within 24 hours after inoculation. This isolate produced a characteristic strong fruity smell, which attracted flies to the culture. Perhaps it may be one of the natural phenomena by which dissemination of conidia would take place through insects attracted to the fungus. However, this needs further experiments and confirmation. Studies are also required to determine whether the chemical which produced the fruity smell is phytotoxic in nature and is related to the virulence of the pathogen.

8. T. paradoxa isolate-6 which is capable of producing large number of chlamydospores has a distinct advantage of greater chances of survival over others. However, this aspect also needs further field studies.

9. Various saprobes namely Acremonium sp., Aspergillus niger, A. terreus, Chalaropsis sp., Mucur sp., Paecelomyces varioti, Penicillium citrinum, P. diverseum, P. janthinellum, Pestalotiopsis palmarum, Trichoderma harzianum, T. viride, etc. were isolated from old lesions/neem cake amended soils etc. Bark samples and/or soil from the neemcake ammended palms showed a high percentage of A. niger, Penicillium sp. and T. harzianum.

10. The dual culture of T. paradoxa and the test fungus was maintained on the respective medium in which the particular isolate of T. paradoxa showed very good growth in comparison to other media tested. The study revealed that different isolates of T. paradoxa responded differtially to different fungi tested in vitro. Also different isolates of the associated fungi interacted with a given isolate of T. paradoxa differently.

11. Four kinds of interactions were noticed in general, namely:

i) Commensalism where both organisms grew without affecting each other adversely and intermingled freely. eg. Acremonium sp. Chalaropsis sp. etc.

ii) Expression of mutual inhibition with a pigmented zone in between the opposing colonies. eg. A. niger in its initial stages of growth.

iii) Expression of mutual inhibition with a clear zone in between the colonies eg. A. terreus, P. varioti, P. palmarum etc.

iv) Overgrowth of test fungus on T. paradoxa colony. eg. T. harzianum and T. viride.

12. Since Acremonium sp., Chalaropsis sp. etc showed the first category of interaction where both the colonies intermingled freely without showing any antagonism, these fungi cannot be used for biocontrol of T. paradoxa.

Likewise fungi like A. terreus, P. varioti, P. palmarum etc. which showed mutual inhibition with a clear zone in between and did not overgrew on T. paradoxa, cannot be either used as potential antagonists to T. paradoxa.

13. Trichoderma harzianum (two isolates) and T. viride (three isolates) overgrew on all the isolates of T. paradoxa. This indicates a lack of defence mechanism in T. paradoxa against the aggressors in protecting itself. As such these fungi can be used as potential biocontrol agents.

14. Likewise A. niger (one isolate) which showed mutual inhibition in the initial stages only and showed overgrowth over T. paradoxa in the later stages can be used as potential antagonist.

15. Fungal succession has a significant role in the development of the disease, survival of the pathogen, damages to the host etc. It was observed that in the case of all the isolates of T. paradoxa, maximum inhibition of the fungus (36.7%to 50%). was obtained when the test fungus (A. niger) preceded the T. paradoxa inoculation by 12 hours.(Table-36).

16. Two isolates of T. harzianum were similarly tested against four isolates of T. paradoxa. Here also trend of the results was similar that maximum inhibition (50% to 86.6%) occurred when the inoculation of antagonist preceded the pathogen by at least 12 hours (Tables 37 & 38.).

17. Three isolates of T. viride were ~~treated~~ tested in vitro in ascertaining their inhibition property against T. paradoxa isolates. Maximum growth inhibition (50% to 90%) of T. paradoxa

isolates was observed when the T. viride isolates were inoculated 12 hours before the pathogen (Tables 39, 40 and 41).

18. When the extent of inhibition of the above three fungi viz. A. niger, T. harzianum and T. viride was compared, maximum growth inhibition of T. paradoxa was caused by T. viride (90%) followed by T. harzianum (86.6) and A. niger (50%) in that order when they were inoculated 12 hrs. before the inoculation of the pathogenic fungus, T. paradoxa in vitro.

19. The present studies provide the preliminary information with regard to the potential of the antagonistic fungi as possible biocontrol agents. Extensive studies in this direction are required to enlist more candidate fungi under this head.

20. The results of the present in vitro studies are to be confirmed by evaluation in in vivo studies under laboratory and field conditions.

21. The results of isolation of different mycoflora from tissues/soils from neem oil cake-ammended plots indicate the presence of potential antagonist, in such tissues/soils. This may be the reason why T. paradoxa could not be isolated from such tissues/soils. This opens up a new vista in the control of

stem bleeding disease in that this factors can be profitably be used as a tool in the biological control programme of the disease.

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