

Studies on the Effect of *Rhizoctonia Solani* and
Trichoderma Viride on Cotton and Black Gram

BY

Venkateswari J.

A DISSERTATION SUBMITTED TO AVINASHILINGAM INSTITUTE FOR
HOMESCIENCE AND HIGHER EDUCATION FOR WOMEN
(DEEMED UNIVERSITY) COIMBATORE 641 043.
(ERSTWHILE AVINASHILINGAM HOMESCIENCE COLLEGE
AFFILIATED TO BHARATHIAR UNIVERSITY)
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE

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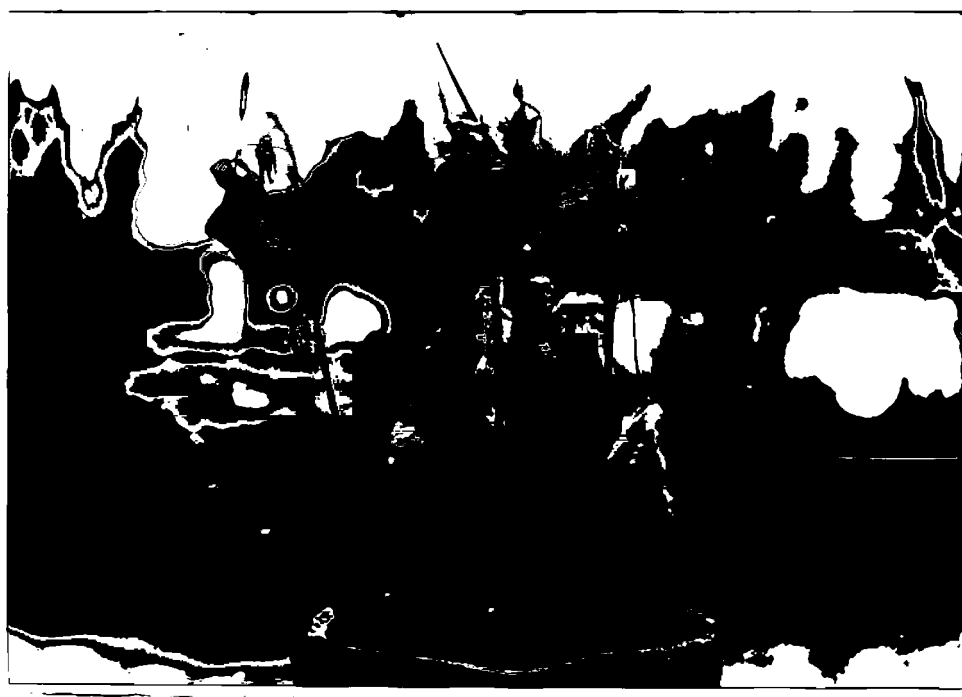
Introduction



1. Root rot of cotton caused by R. solani

1. R. solani infected. 2. Control.

1b



2. Root rot of Blackgram caused by R. solani

INTRODUCTION

Cotton (Gossypium hirsutum. L), the valuable fibre crop and blackgram (Vigna mungo, L., Hepper), the important pulse crop are cultivated extensively in many parts of India. At present in India it covers an area of about 10 million hectares, with an annual production of about 5 million tons of fibre (Rangaswami, 1984). Tamil Nadu accounts for 8.0 per cent of the total production of cotton in the country (four lakh bales of lint) which is released from 3.5 lakh hectares (Anonymous, 1988). In 1986, the area under black gram was 3.12 million ha and production was 1.24 million tonnes which accounts for 13 per cent of total pulse area and 10 per cent production in India (Saini and Sharma, 1986). The yield potential of the crop is 875 kg/ha against the actual average yield of 312 kg/ha leaving a performance gap of 64 per cent (Ashok kumar, 1986). One of the constraints in achieving the expected yield of these crops is various diseases like leaf spot, root rot, wilt and several virus diseases that affect the crops.

Cotton and blackgram grown as irrigated or rainfed crops are often severely affected by root rot caused by

Rhizoctonia solani kuhh and farmers are not in a position to take up chemical control. Moreover, there is no suitable resistant variety for this pathogen. Hence, there is a need to evolve a suitable, cheap and effective control measures.

In recent years, use of biological agents to control soil borne pathogenic fungi is attractive possibility. Biological control is the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists (Baker et al., 1974).

There have been reports on successful use of antagonist against Rhizoctonia solani (Chet and Baker, 1981; Elad et al., 1981). Biological control of Rhizoctonia solani was demonstrated with organic soil amendments containing Trichoderma spp (Sangeetha Panicker, 1988; Sesan et al., 1988).

The exact role of this antagonist against Rhizoctonia solani and various biochemical changes due to these organisms have not been studied in detail. Hence the following line of investigation were carried out.

1. Determining the mode of antagonism of Trichoderma viride against Rhizoctonia solani kuhn.
2. Study its potential use for the control of damping-off and root rot in black gram and in cotton.
3. Effect of toxin of antagonist on seed germination.
4. Effect of toxin on the growth of the pathogen.
5. To find out whether the antagonist affect the plant growth or not.
6. Biochemical changes in cotton and black gram due to Trichoderma viride and Rhizoctonia solani application.

Review of Literature

REVIEW OF LITERATURE

The review of literature pertaining to the study of "Biological control of plant disease caused by Rhizoctonia solani" Kuhn using "Trichoderma viride" is presented under the following headings:

1. Efficacy of the antagonistic organism against various diseases.
2. Mass production of antagonist
3. Mechanism of biological control
4. Effect of culture filtrate of Trichoderma spp. on plant pathogens.
5. Biochemical changes due to Trichoderma spp and Rhizoctonia solani.

1. Efficacy of the antagonistic organism against various diseases.

The literature on the efficacy of antagonistic organism in controlling various diseases caused by R. solani in different crops is briefly reviewed below.

i) Cotton:

Sesan et al (1988) reported that the application of Trichoderma viride reduced the infection caused by R.solani in cotton. Howell and stipanovic (1979) found that Pseudomonas fluorescens was antagonistic to R.solani in cotton and that treating cotton seeds with P.fluorescens increased seedling survival from 30 to 70 per cent. Seed coating of cotton with Trichoderma spp. was as effective as PCNB treatment in controlling R.solani (Elad et al., 1982). Akhtar et al. (1982) reported that addition of T.harzianum followed by application of substrate alone before sowing gave satisfactory control of Rhizoctonia root rot. Lewis and Papavizas (1985) found that mycelial preparation of the isolates of Trichoderma and Gliocladium virens reduced survival of R.solani. They also found that T.hamatum and G.virens were more effective than T.harzianum or T.viride. Alagarsamy et al. (1987) reported that seed pelleting of cotton with antagonists like T.viride and T.harzianum increased germination and reduced post emergence mortality.

ii. Pulses:

The role of T.harzianum in biocontrol of beans

damping-off has been reported by a number of scientists (Hadar et al., 1979; Elad, 1983; D'Ercote et al., 1985). Seed treatment of soybean with T.harzianum or T.pseudo-koningii and methyl cellulose increased germination per cent and reduced R.solani incidence (Wu, 1980).

Tu and Vaartaja (1981) reported that Gliocladium virens formed appressoria when it came in contact with R.solani, penetrated the hyphae and caused collapse and death of the pathogen in white beans.

Marshall (1982) reported that biocontrol of beans damping - off using T.harzianum depended on soil reaction and inoculum concentration of the pathogen and that the antagonist was most effective at pH 3.5. Kommendahl and Windels (1976) evaluated the effect of Biological seed treatment for controlling root rot of pea caused by R.solani and found 37 isolates of fungi and 22 isolates of bacteria were very effective. Seed treatment of radish and pea with T.hamatum in a methocil slurry protected seeds and seedlings from R.solani. It gave a control equivalent to fungicidal treatment. Seed treatment with a combination of T.hamatum and Chaetomium globosum was more effective than either used alone

(Harman et al., 1980). Subsequently in 1981 they reported that treating seeds with T.hamatum spore suspension containing 10^6 conidia/ml was more effective and increased population density of antagonist in soil. Addition of chitin or cell wall of R.solani to seed coat increased the ability of mycoparasite. Chu and Wu (1981) reported that pea seeds when treated with a binding substance and T.pseudokoningii or T.hamatum decreased disease severity and increased seedling weight.

iii) Potato:

Chu and Wu (1980) reported that hyphae of Trichoderma pseudokoningii, T.longibrachiatum, T.hamatum and Penicillium spp. from field soils and potato tubers twisted round R.solani and they produced antibiotics inhibitory to R.solani. Seed dip of potato in a suspension of Trichoderma spp. or Gliocladium increased yield and reduced canker incidence (Logam et al., 1983). In long Aston, potato tubers treated with spore suspension of T.viride or T.harzianum had much less black sculf incidence. Beagle et al., (1985) reported that both soil borne and tuber borne propagules of R.solani were effectively controlled by seed treatment as well as soil application of fermentor biomass of T.viride and G.virens in a mixture of pulverized prophyllite.

iv) Vegetables:

Mihuta and Rowe (1985) evaluated different delivery systems for Trichoderma in controlling R. solani in radish and found that fluid drilling of Trichoderma suspension added with seed to gel matrix natrosol was effective on all types of soil and with all isolates. Culturing Trichoderma on wheat bran for 2-3 days and then placing them in shallow furrows prior to sowing was not very effective. Drilling of dry seeds previously coated with Trichoderma spores in methocellulose and drenching the soil with Trichoderma suspension was intermediate in their effect.

2. Mass production of Antagonist:

Direct purposeful introduction of antagonistic microorganisms for biological control has been stimulated by the progress made in bacterization of leguminous seeds. But one of the obstacles to biological control of plant pathogens by direct massive soil augmentation has been the lack of suitable substrate as well as method for mass culturing and delivery of antagonists to soil. Scientists around the world are attempting to fill up this lacuna.

Wells et al., (1972) used grain bran medium for growing T.harzianum for control of Sclerotium rolfsii. The medium consisted of 1 g ground annual rye grass seeds (Lolium multiflorum), 10 g Tifton sandy loam sifted through a 2 mm mesh screen to 2 ml water autoclaved for 1 h at 120°C. for two successive days. This medium was seeded with T.harzianum and shaken for 10 days. It was comminuted with an equal amount of medium just before use. Backman et al. (1975) used diatomaceous earth granules impregnated with 10 per cent molasses solution for growth of T.harzianum. This antagonist was grown on the sterile granules for four days and applied to field 70 - 100 days after planting groundnut at the rate of 140 kg/ha. This was found to be as effective as application of PCNB granules at the rate of 112 kg/ha. They found that use of granular carrier was better than organic food base for T.harzianum 15 g molasses and 2.5 g of brewyer's yeast, mixed with 500 ml water and auto claved at 121°C for 1 h for two successive days. To this medium Trichoderma and Gliocladium were inoculated and shaken in a rotary shaker for five days.

Upadhyay and Mukhopadhyay (1986) used sorghum grain substrate for multiplication of T.harzianum. Sorghum grains were pre soaked in 2 per cent sucrose solution

overnight and sterilized at 126°C for 30 min. To this T.harzianum was inoculated and incubated at 30°C for 15 days. They were then mixed with equal amount of freshly boiled sorghum seeds 8h prior to soil application. This was applied to soil at the rate of 30g/meter row before sowing for control of Sclerotium rolfsii causing root rot of sugar beet.

Root rot of sugarcane seedlings was effectively controlled by application of T.viride multiplied in sand-sorghum medium. A mixture of 20g sorghum seeds and 100g sand were moistened and sterilized. To this T.viride was inoculated and incubated for seven days. This was then mixed with 2 kg soil and applied at 220g per bed (45 x 26 cm) before sowing (Padmanabhan and Alexander, 1987). Kousalya and Jeyarajan (1980) used Tapioca rind or thippi as substrate for T.viride and T.harzianum for control of root rot of blackgram caused by R.bataticola. This medium was prepared by mixing 50 g of powdered tapioca rind with 50 ml tap water. It was autoclaved at 126°C for 2 h for two successive days. To this T.viride or T.harzianum was inoculated and incubated for 15 days. This inoculum was applied to soil at five per cent level before sowing. They also reported the use of sand-maize medium for growth of L.arvalis for biocontrol of root rot of blackgram caused by R.bataticola.

Krishnamoorthy (1987) used sorghum grain culture for the mass production of T.viride, T.harzianum and L.arvalis for biocontrol of damping-off of tomato seedlings caused by Pythium indicum.

Mukhopadhyay et al. (1987) developed a standard preparation of T.harzianum and T.koningii in wheat bran-saw dust medium which can be very conveniently grown in autoclavable plastic bags of different sizes. Huang (1980) used autoclaved barley, rye and sunflower seeds for the multiplication of T.viride.

Some of the growth media used for the mass production of the antagonist - Trichoderma spp. were.

<u>Growth medium</u>	<u>Reference</u>
Grain bran	Wells <u>et al.</u> , 1972.
Wheat straw	Akhtar, 1977.
Wheat bran	Henis <u>et al.</u> , 1978.
Wheat bran-Sawdust	Elad <u>et al.</u> , 1980.
Sorghum grain	Mukhopadhyay and Upadhyay 1983, 1986.
Wheat bran-Sawdust modified medium	Mukhopadhyay <u>et al.</u> , 1986.

Liquid fermentation
Technology (molasses
and brewer's yeast)

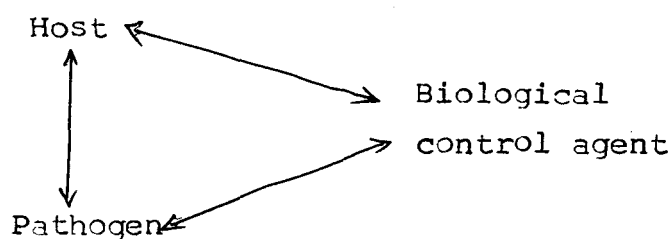
Papavizas et al., 1984.

Lignite and Stillage

Jones et al., 1984.

C. Mechanism of biological control

In plant pathology the overwhelming hypertrophy of the disease triangle involving interactions of pathogens, host environment and vectors provides more complicated system. Biological control agents are also greatly influenced by these triangle factors (Garrett, 1965) are presented in diagrammatic form.



Trichoderma spp. control mainly by being mycoparasitic and aggressive competitors with pathogens, growth of mycelia of Trichoderma spp. along and coiled around hyphae of host fungi has been observed by many workers (Chet et al., 1981; Liu and Baker, 1980; Weindling, 1932).

Much of the early literature attributed the biological control by Trichoderma viride (T.viride) by the production of the antibiotics gliotoxin and viridin. Some Trichoderma isolates do produce antibiotics, especially at low pH (Dennis and Webster, 1971a, 1971b).

Krishnamoorthy (1987) reported that T.viride, T.harzianum and L.arvalis showed greater antagonism towards Pythium indicum isolates and he found that T.viride and L.arvalis parasitized P.indicum by coiling, puncturing and invading the host hyphae T.harzianum inhibited Pythium by producing an extracellular thermostable component.

Chet and Baker (1980) showed that T.harzianum and T.hamatum acting as mycoparasite of R.solani and S.rolfsii produced B (1-3) glucanase and chitinase that caused exolysis of the host hyphae, antibiosis was not observed. T.hamatum also produces cellulase, which perhaps explain its ability to parasitize Pythium spp (Chet and Baker, 1981).

Jones and Watson (1969) found that B (1-3) glucanase produced by T.viride solubilized mycelia of Sclerotinia sclerotiorum. Ridout et al (1987) found that Chitinase,

B (1-3) glucanase produced by T.viride and T.harzianum may be involved in the degradation of Rhizoctonia solani cell wall.

Upadhyay et al (1985) showed that the type of interactions between the antagonist and pathogen were hyphal coiling, entry through haustoria like structure and direct entry in the hyphae and sclerotia of S.rolfsii the host fungus.

Mukhopadhyay (1986) studied antagonistic activities of an isolate of T.harzianum Rifai against tobacco isolate of Pythium aphanidermatum, he found that T.harzianum directly attacks the hyphae of Pythium aphanidermatum causing lysis.

Morshed (1986) reported that the growth of T.viride was vigorous in dual culture and it was an effective hyperparasite, penetrating and coiling its hyphae around the host parasite. Upadhyay and Rai (1986) reported that hyphal coiling was recorded in R.solani by T.viride.

Wu et al (1987) reported that the T.harzianum caused lysis of R.solani by coiling very tightly round the hyphae of R.solani, he also reported that hyperparasitism was also evident in natural soils.

D. Effect of culture filtrate Trichoderma spp on plant pathogens.

Dennis and Webster (1971) reported that isolates from different species, groups of Trichoderma were tested for production of non-volatile antibiotics, by an agar layer technique. Many isolates produced non-volatile antibiotics active against a range of fungi. The ability to produce such antibiotics varied between isolates of the same species group as well as between isolates of different species groups.

The toxic metabolites produced by Trichoderma spp have been the subject of intensive study ever since weindling (1934) reported that culture filtrates of T.lignorum were toxic to R.solani.

Pande (1986) found that the culture filtrates of 3 species of Aspergillus and T.viride retarded growth of Alternaria alternata, R.solani and Sclerotium rolfsii. The effect of the filtrate was proportional to their concentration in potato dextrose agar medium.

Krishnamoorthy (1987) reported that T.harzianum inhibited Pythium spp by producing an extracellular thermo-

stable component. He also reported that the culture filtrate of T.harzianum in a synthetic medium suppressed the linear growth of P.aphanidermatum by 83 per cent compared to 8 per cent inhibition by the culture filtrate of T.hamatum

Lewis and Papavizas (1986) reported that the mycelial preparations of Trichoderma spp and G.virens reduced the populations of R.solani

Lewis and Papavizas (1987) reported that the water extracts of germlings (Young, actively growing hyphae on bran) of isolates of Trichoderma spp and G.virens affected growth of R.solani in liquid culture. T.viride isolate prevented the growth as effectively as 0.2 mM Hg Cl₂.

Biochemical Analysis of Plant Materials:

1. Effect on shoot length and Rootlength:

Apart from controlling P.indicum treatment with T.viride and T.harzianum increased the shoot and root length and also dry matter production of tomato seedlings (Krishnamoorthy, 1987). Bedlan (1986) reported that tests with T.viride a natural antagonist of R.solani, gave some

yield increase and some reduction of infection compared with untreated control.

2. Effect on Carbohydrate content

Patel and Vaishnav reported that there is an increase in water soluble sugar in rust infected leaves when compared to control. Similar trend was reported by Reddy and Ramagopal (1982), Ekbote and Mayee (1983).

There is increase in reducing sugar with age is more in susceptible cultivars than in resistant one. But the reducing sugar level decreases in leaves of the groundnut plants affected by tikka disease (Brahmachari and Kotte, 1987).

3. Effect on phenol content:

Total phenols continue to decrease with age of plants in both resistant and susceptible varieties (1987). But there was a reverse in case of OD phenol.

Gangopadhyay and Wyllie (1974) reported that total and water soluble phenolic compounds were higher in internodal than in nodal tissue of cotton plants infected

by Rhizoctonia solani. Root tissue had less phenolic compounds than the above ground tissue and the more resistant varieties had greater quantities than more susceptible varieties. Murugesan and Mahadevan (1982) reported that total phenols were increased with age in cotton plants, groundnut plants and also in R.solani and Alternaria spp. infected plants. He also reported that total phenols, phenolase were found to be increased in plants inoculated with trace elements for the control of R.bataticola.

4. Effect on Protein and amino acid content

Patel and Vaishnav (1986) reported that there is an increase in protein in leaves infected with rust as compared to healthy leaves.

Increase in protein was reported by earlier workers (Reddy and Ramagopal, 1982; Ekbote and Mayce, 1983).

There is an increase in amino acid content in infected tissues when compared to control (Murugesan and Mahadevan, 1982).

Biochemical studies were done by Lewis and Papavizas (1987) using the culture filtrate or the

germling extracts of Trichoderma spp on the growth of R.solani. They reported that the leakage of compounds from mycelial mats of R.solani was induced after mats were exposed to germling extract of T.viride. The chemical composition of the leaked materials (soluble protein, carbohydrates, aminoacids and salts) were determined. The rate of leakage indicated an immediate and gradually increasing loss of materials from the pathogen hyphae, leakage was accompanied by a reduction in the mycelial weight of R.solani.

Materials and Methods

MATERIALS AND METHODS

The materials and methods pertaining to the study of studies on the effect of T.viride and R.solani on cotton and blackgram is presented under the following headings.

I. Isolation of Pathogen and Antagonist:

- a. Isolation of pathogen
- b. Isolation of Antagonist

II. Preparation of Culture Media

- a. Potato Dextrose Agar (PDA) medium
- b. Czapek's - Dox Medium (Czapek's Sucrose - nitrate medium)
- c. Preparation of media for soil application
- d. Mass production

III. Effect of toxin from Trichoderma viride (T.viride) on host and pathogen.

- a. Extraction of toxin
- b. Purification of toxin
- c. Effect of toxin on Rhizoctonia solani
- d. Effect of toxin on cotton and blackgram
 - i. Leaf spot bioassay

- ii. Seed germination inhibition assay
- iii. Radicle and Plumule inhibition assay

IV. Effect of Trichoderma viride (T.V), Rhizoctonia solani (R.S) and T.V + R.S. on cotton and blackgram seedlings on application.

- a. Seed germination inhibition assay
- b. Radicle and plumule inhibition assay
- c. Biochemical changes in cotton and blackgram due to application of T.V, R.S. and T.V. + R.S.
 - a. Extraction of plant tissues in alcohol
 - b. Evaporation of the alcohol from the extract
 - c. Estimation of carbohydrates
 - i. Estimation of total soluble sugars
 - ii. Estimation of Reducing sugar
 - iii. Estimation of starch
 - d. Estimation of Proteins
 - i. Estimation of total soluble proteins
 - ii. Estimation of Aminoacids.
 - e. Estimation of Phenol



3. Culture of R. solani

MATERIALS AND METHODS

a. Isolation of pathogen

Pathogen Rhizoctonia solani (R. solani) were isolated from the respective host plants viz., cotton and blackgram and used as test organism in the present study. Portions of infected tissues were cut into small bits, surface sterilized with 0.1% Mercuric chloride solution for 2 min, washed in three changes of sterile distilled water, plated on potato dextrose agar (PDA) medium and incubated under laboratory conditions ($28 \pm 3^{\circ}\text{C}$). On the second day, the fungal growth from the infected tissues were transferred to PDA slants. The isolates were further purified by the hyphal tip method, identified and maintained on PDA with periodic subculturing. These two isolates were tested for the pathogenicity on the respective host plants, reisolated and found to be stable in their virulence.

b. Isolation of Antagonist

Trichoderma viride (T. viride) was isolated from cotton field soil by dilution plate technique on peptone dextrose - rose bengal agar medium (Martin, 1950).

II. Preparation of Culture Media:

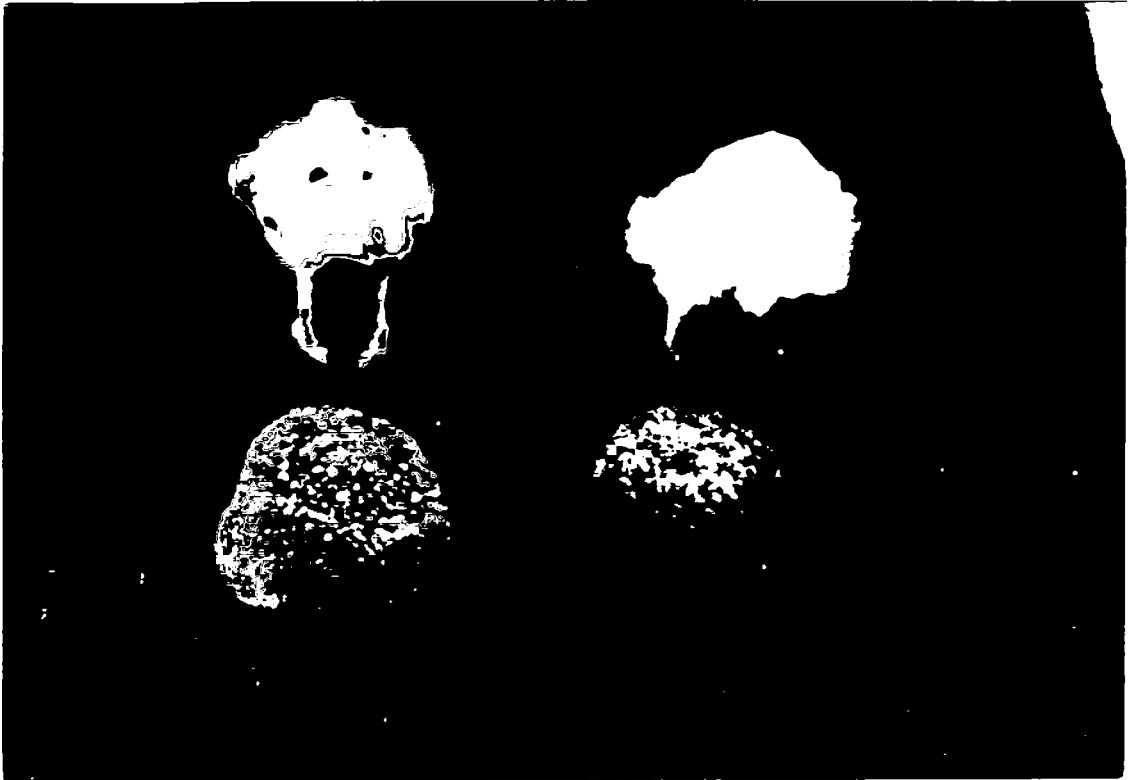
a. Potato Dextrose Agar (PDA) Medium:

Peeled and Sliced potato	:	250.00 g
Dextrose	:	20.00 g
Agar	:	20.00 g
pH	:	6.8 - 7.0

Cooked the peeled and sliced potato in 500 ml of water in a steamer or boiled for 30 min. killed the broth through cheese cloth and make upto 500 ml. At the same time, melted the agar in 500 ml of water by autoclaving or heating in a steamer. Based the potato broth into the agar and restored to 1000 ml with distilled water, added and dissolved the sugar. Adjusted the pH to 6.8 - 7.0. Sterilized the medium in an autoclave.

b. Czapek's - Dox Medium (Czapek's sucrose-nitrate medium)

KH_2PO_4	:	1.00 g
NaNO_3	:	2.00 g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$:	0.50 g
KCl	:	0.50 g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$:	0.01 g



4. Mass culture of R. solani T. viride

Sucrose	:	30.00 g
Distilled Water	:	1 litre
pH	:	6.8 - 7.0

C. Preparation of Media for soil application:

Sorghum	:	1 kg
Sucrose	:	20 g
Water	:	1 litre

Sorghum grains were soaked in 2% sucrose medium and allowed it to stand overnight and then they were transferred to conical flasks and they were autoclaved.

d. Mass production:

The sterilized flasks were inoculated with 5 mm disc of T.viride and R.solani in separate flasks. This was done in duplicates. Then the flasks were incubated at room temperature for 7 days.

III. Effect of toxin from T.viride on host and pathogen

a. Extraction of toxin:

The fungus T.viride was grown in Czapek's - Dox liquid culture media. Fifty ml of the medium was taken in each 250 ml Erlenmeyer flask and inoculated with 5 mm disc of inoculum, grown on potato dextrose agar medium. The flasks were incubated at room temperature for 10 days. Cell free culture filtrate of 10 day old culture was obtained by filtration through whatman No.1 filter paper. This culture filtrate was used as the source of toxin.

b. Purification of toxin:

The toxin was purified by extraction with Chloroform in a separatory funnel. Solvent was conveniently used in three portions each equal to the volume of the filtrate. After distilling the chloroform from the seeds soaked in sterile water served as control. The percentage of seed germination was recorded 72 hrs after incubation.

C. Effect of toxin on Rhizoctonia solani

The toxin was added at 1%, 3%, 6% and 9% concentrations separately into the sterilized Czapek's-Dox liquid media. Then 5 mm disc of the pathogen was inoculated into the liquid media, 15 days after incubation the dry mycelial weight of the pathogen was assessed.

d. Effect of toxin on cotton and Blackgram:

i. Leafspot bioassay:

Leafspot bioassay was done by detached leaf technique. Cotton and blackgram leaves were kept in moist chamber and inoculated with toxin (80%) or without pinprick injury. Sterile water served as control. Symptom development was recorded at an interval of one hour for 6 hours.

ii. Seed germination inhibition assay:

Blackgram and cotton seeds were soaked separately for 5 hours in toxin (80%). Ten seeds from each were transferred to sterile filter paper inside the petridish. The filter papers were moistened with toxin and incubated at room temperature ($28 \pm 3^{\circ}\text{C}$). Seeds soaked in sterile water served as control. The percentage of seed germination was recorded 72 hrs after incubation.

iii. Radicle and plumule inhibition assay:

Cotton and blackgram seeds were soaked separately in toxin for 5 hrs. The soaked seeds were incubated inbetween moist filter papers in a petridish. Seven days after incubation the length of the plumule and radicle were measured. Seeds soaked in sterile water served as controls.

IV. Effect of Trichoderma viride (T.V), Rhizoctonia solani (R.S) and T.V + R.S. on cotton and blackgram seedlings.

Hundred grams of sorghum grain culture of T.V, R.S. and T.V + R.S. were added separately into earthen pots containing 1 kg of sterilized soil. Ten seeds of cotton and blackgram were sown separately in each pot. This was done in replicates.

Cotton and blackgram seeds were soaked separately in toxin for 5 hrs. Ten seeds were sown separately in each pot. This was done in replicates.

a. Seed germination inhibition assay:

The percentage of seeds germinated was recorded 6 days after sowing.

b. Radicle and Plumule inhibition assay:

The shoot and root lengths of the plants were measured 10, 20 and 30 days after sowing.

c. Biochemical changes in cotton and blackgram due to application of T.V, R.S. and T.V + R.S.

Hundred gram of sorghum grain culture of T.V, R.S and T.V + R.S were added separately into earthen pots containing 1 kg of sterilized soil. Ten seeds of cotton and blackgram were sown separately in each pot. Cotton and blackgram seeds were soaked separately in toxin for 5 hrs. Ten seeds were sown separately in each pot. All these were done in replicates.

Biochemical changes that occur were studied intrends of total soluble sugars, reducing sugars, starch protein, amino acids and phenol. These were estimated in both cotton and blackgram plants on 10, 20 and 30 days after sowing.

a. Extraction of plant tissues in alcohol

Materials : Fresh cotton and Blackgram plants.

Cheese cloth.

Whatman No.41 filter paper.

Reagent : Distilled ethylalcohol (96%, 80%).

Method:

Cut the tissues into pieces of 1-2 cm immediately plunged them in boiling ethyl alcohol and allowed to boil for 5-10 mm. Used 5 - 10 ml of alcohol for every gm of tissue. The extraction was done on top of a steambath or hot plate under a hood. Cooled the extract in a pan of cold water, crushed the tissues thoroughly in a mortar with pestle or in a blender for 5 - 10 min. Passed through two layers of cheese cloth and reextracted the ground tissues for 3-10 min in boiling 80 per cent alcohol using 2-3 ml of alcohol for every gm of tissue. This second extraction ensures complete removal of alcohol soluble substances. Cooled and passed through cheese cloth. Pooled both extracts and filtered through whatman No.41 filter paper. Raised the volume with 80 per cent ethanol to reduce the volume of the extract by evaporating it to represent 5-10 ml of

the extract for every gm of tissue used. This extract ("alcohol soluble") contains reducing and non-reducing sugars, some glycosides, amide and aminonitrogen, other forms of soluble nitrogen, phenols, flavones, tannins, oils, lipids, chlorophylls, carotene and Xanthophylls.

b. Evaporation of the alcohol from the extract:

Transferred the extract to a large beaker and placed on a hot water bath under the hood. Evaporated the extract till the volume is reduced by 80 - 90 per cent. Added a few ml of water and continued to evaporate. Check the volume and if no reduction in the volume of the extract is noted, cooled the extract, filtered through whatman No.41 filter paper and adjusted the volume if necessary by adding distilled water to represent 1 g of tissue for every ml of the final extract.

This extract is quantitatively and qualitatively analysed for reducing and non-reducing sugars, amino acids, total soluble sugars, proteins and phenols.

c. Estimation of carbohydrates:

1) Estimation of total soluble sugars

The amount of total soluble sugars present in

the extracts can be estimated by anthrone and phenol-sulphuric acid (Dubois et al., 1951) reagents. There is no need to hydrolyze the sample. The total soluble sugar was estimated by anthrone method.

Anthrone method:

Anthrone, 10 - Keto - 9, 10 - dihydroanthracene, a reduction product of anthroquinone, reacts by condensing with carbohydrates furfural derivative to produce a green colour in a dilute solution and a blue colour in a concentrated solution.

Reagent:

Anthrone reagent: Dissolved 2.0 g of anthrone in 1 l of Conc. H_2SO_4 . (Prepared freshly)

Method:

1.0 ml of the extracts were pipetted out into test tubes. To each tube 4.0 ml of the anthrone reagent was added by allowing the reagent to rundown the side of the test tube. The loss of water due to evaporation was prevented by placing a glass marble on top of each tube. The tubes were kept in a boiling water bath for 10 min.

The tubes were removed and brought to room temperature by keeping them in a water bath. A Reagent blank was also treated similarly. The absorbance of the blue-green solution was measured at 625 nm. The amount of total soluble sugar present in the extract was calculated by using a standard curve prepared from glucose (1.0 ml of the working standard solution contained mg of glucose).

ii. Estimation of reducing sugars:

A convenient and sensitive method for the estimation of reducing sugars, particularly when large number of samples are to be analyzed, was developed by Miller (1972) and involved the use of dinitrosalicylic acid reagent.

Reagents:

DNS reagent: Dissolved simultaneously, 1.0 g of dinitrosalicylic acid, 200 mg of crystalline phenol and 50 mg of sodium sulfite placed in a beaker with 100 ml of 1% solution by NaOH by stirring. (Prepared freshly).
40% Solution of Rochelle Salt (Sodium potassium tartrate).

Method:

3.0 ml of the extracts were pipetted out into test tubes. To this 3.0 ml of DNS reagent was added. The mixture was heated for 5 min in a boiling water bath. After the colour was developed, to each tube 1.0 ml of 40% Rochelle salt was added, when the contents of the tubes were still warm. The tubes were cooled under running tap. The absorbance was measured at 575 nm. The amount of reducing sugar was calculated using standard prepared from glucose (1.0 ml of the standard solution contains mg of glucose).

iii. Estimation of starch:Method:

Placed 100 mg of dried sample into a 50 ml centrifuge tube. To this 25 ml of 80 per cent ethanol was added and heated the tubes in a waterbath at 80-85°C for 10 min. Centrifuged at 2,000 g for 15 min. The supernatant was decanted into a 50 ml beaker. The extraction was repeated three times. The residue contained starch.

5.0 ml of water was added to the residue left behind in the centrifuge tubes, followed by 6.5 ml of 52

per cent perchloric acid. Stirred the contents constantly with a glass rod for 5 min. and then occasionally for the next 15 min. To this 20-ml of water was added and centrifuged. The supernatant was decanted into a 100 ml volumetric flask. To the residue 5.0 ml of water was added and repeated the extraction with perchloric acid. Stirred occasionally for the next 30 min. The contents of the tubes were transferred to the volumetric flasks. The volume of the flasks were made up to 100 ml with water and filtered through whatman No.42 filter paper. An aliquot of the filtrate was diluted to a known volume with water and the sugar was analysed with anthrone reagent. The sugar content was calculated in terms of glucose equivalent and a conversion factor of 0.9 was used to convert the values of glucose to starch.

d. Estimation of Proteins:

i. Estimation of total soluble proteins:

Lowry's method for Protein determination:

Lowry et al (1951) introduced a colorimetric method to determine protein. This method is based on the principle that different proteins contain different amounts of aromatic residue which react with Folin-ciocatteu reagent, giving a blue colour, which is read in a colorimeter.

Reagents:

A. Alkaline Sodium Carbonate solution :

2% Na_2CO_3 in 0.1 N NaOH.

B. Copper sulphate sodium potassium
tairate solution :

0.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1% Rochellesalt,
prepared freshly.

C. Alkaline copper reagent : 50 ml of reagent A
and 1.0 ml of reagent
B were mixed. Prepared
freshly.

D. Folin-ciocalteu reagent : Dissolved 100 g of
sodium tungstald ($\text{Na}_2 \cdot \text{Mo}_4 \cdot \text{H}_2\text{O}$) and 25 g
sodium molybdate ($\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$) in 700 ml
water in 1l flask. To this 50 ml of 85%
O-phosphoric acid (H_3PO_4) and 100 ml of Conc.
Hcl. was added. The contents of the flask was
refluxed gently for 10 hr cooled and then 150mg
of lithium sulphate (Li_2SO_4) dissolved in 5.0 ml
water and 4-5 drops of liquid bromine was added.
Cooled and diluted to volume with water and
filtered.

The reagent was golden yellow in colour. One volume of this stock solution was diluted with 2 volumes of water just before use. E. 1 N NaOH.

Method:

The protein sample was suspended in 1 ml of 1 N NaOH at 100°C for 4.5 min. To this 5 ml of reagent C was added and the mixture was allowed to stand at room temperature for 10 min. To this 0.5 ml of Folin-Ciocalteu reagent was added rapidly and the contents were mixed immediately. The absorbance was measured after 30 min at 750 nm. The amount of protein in the samples were estimated using a standard prepared from Bovineserum albumin. (1.0 ml of the standard contained 500 µg of Bovine serum albumin).

ii. Estimation of Aminoacids:

Ninhydrin method (Moore and Stein, 1948).

Amino groups react with ninhydrin (triketo hydrindene hydrate) to give a coloured derivative, diketohydrindylidene diketohydrindamine. This derivative has an absorbance maximum at 570 nm.

Method:

1 ml of the samples were pipetted out into tubes, to this a drop of methyl red indicator was added and the sample was neutralized with 0.1 N NaOH. To all the tubes 1.0 ml of ninhydrin reagent was added and mixed thoroughly, glass marble was placed on top of each tube. The contents of the tubes were boiled for 20 min. in a waterbath. 5.0 ml of the diluting solution was added to the mixture while still in the waterbath. 1.0 ml of distilled water served as blank. The tubes were removed and cooled under the running tap and the contents were mixed thoroughly. The purple colour developed was measured at 570 nm. The amount of aminoacid present in the samples were calculated by using a standard curve prepared from glycine (1.0 ml of standard contains 100 μ g of glycine).

e. . Estimation of Phenol:

Estimation of phenols with Folin-Ciocalteu reagent was based on the reaction between phenols and an oxidizing agent phosphomolybdate which results in the formation of a blue complex. (Bray and Thorpe, 1954).

The intensity of the colour was measured in a colorimeter at 650 nm.

Reagents:

1. Folin-ciocalteu reagent
2. 20% sodium carbonate

Method:

To 1.0 ml of the extract in a graduated tube added 1.0 ml of Folin-ciocalteu reagent followed by 2.0 ml of Na_2CO_3 solution. The tubes were heated for exactly 1 min. in a boiling waterbath, cooled under a running tap. The blue solution was diluted to 25 ml with water and the absorbance was measured at 650 nm in a colorimeter. A blank containing all the reagents except the plant extract was used to adjust the absorbance to zero. (1.0 ml of the standard contained 100 μg of catechol).

Results and Discussion

EXPERIMENTAL RESULTS

The influence of Trichoderma viride, T.viride + Rhizoctonia solani, R.solani and T.viride toxin on seed germination, shoot length, root length and biochemical changes in cotton and blackgram were studied and presented here in.

The effect of T.viride toxin at 1, 3, 6 and 9 per cent was tested against R.solani and the results are presented in Table - I. The data revealed that T.viride toxin at all concentrations significantly reduced mycelial dry weight of R.solani when compared to control.

LEAF SPOT BIOASSAY

Trichoderma viride toxin at various concentrations did not produce leaf spot symptom when tested on cotton and blackgram leaves.

In vitro EFFECT OF TOXIN ON SEED GERMINATION

The results of this experiment are shown in Table - II. The seed germination of cotton and blackgram was not significantly inhibited by T.viride toxin.

TABLE - I

In vitro EFFECT OF TOXIN ON R. solani.

Treatment	Dry mycelial Weight (mg)
Control	700.15
<u>T. viride</u> toxin 1%	650.30
<u>T. viride</u> toxin 3%	545.00
<u>T. viride</u> toxin 6%	280.15
<u>T. viride</u> toxin 9%	110.07

* Mean of 3 replications

TABLE - II

In vitro EFFECT OF TOXIN ON SEED GERMINATION

Treatment	% of inhibition	
	Cotton	Blackgram
Control	9.0	0
<u>T. viride</u> toxin	12.2	10.0
C.D (5%)	18.0	12.2

In Vitro EFFECT OF TOXIN ON SHOOT AND ROOT LENGTH OF
COTTON AND BLACK GRAM PLANTS

Table - III presents the in vitro effect of toxin on shoot and root length of cotton and black gram plants.

TABLE - III

In vitro EFFECT OF TOXIN ON SHOOT AND ROOT LENGTH OF
COTTON AND BLACKGRAM PLANTS

Treatment	Cotton		Blackgram	
	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)
Control	8.5	5.5	10.0	4.8
<u>T.viride</u> toxin	5.5	4.5	7.5	2.5
C.D (5%)	0.86	0.86	1.06	0.51

The result of this experiment revealed that T.viride toxin significantly reduced the shoot and root lengths of cotton and black gram.

EFFECT OF TREATMENTS ON SEED GERMINATION:

The effect of various treatments on the germination of cotton and black gram seeds is presented in Table IV.

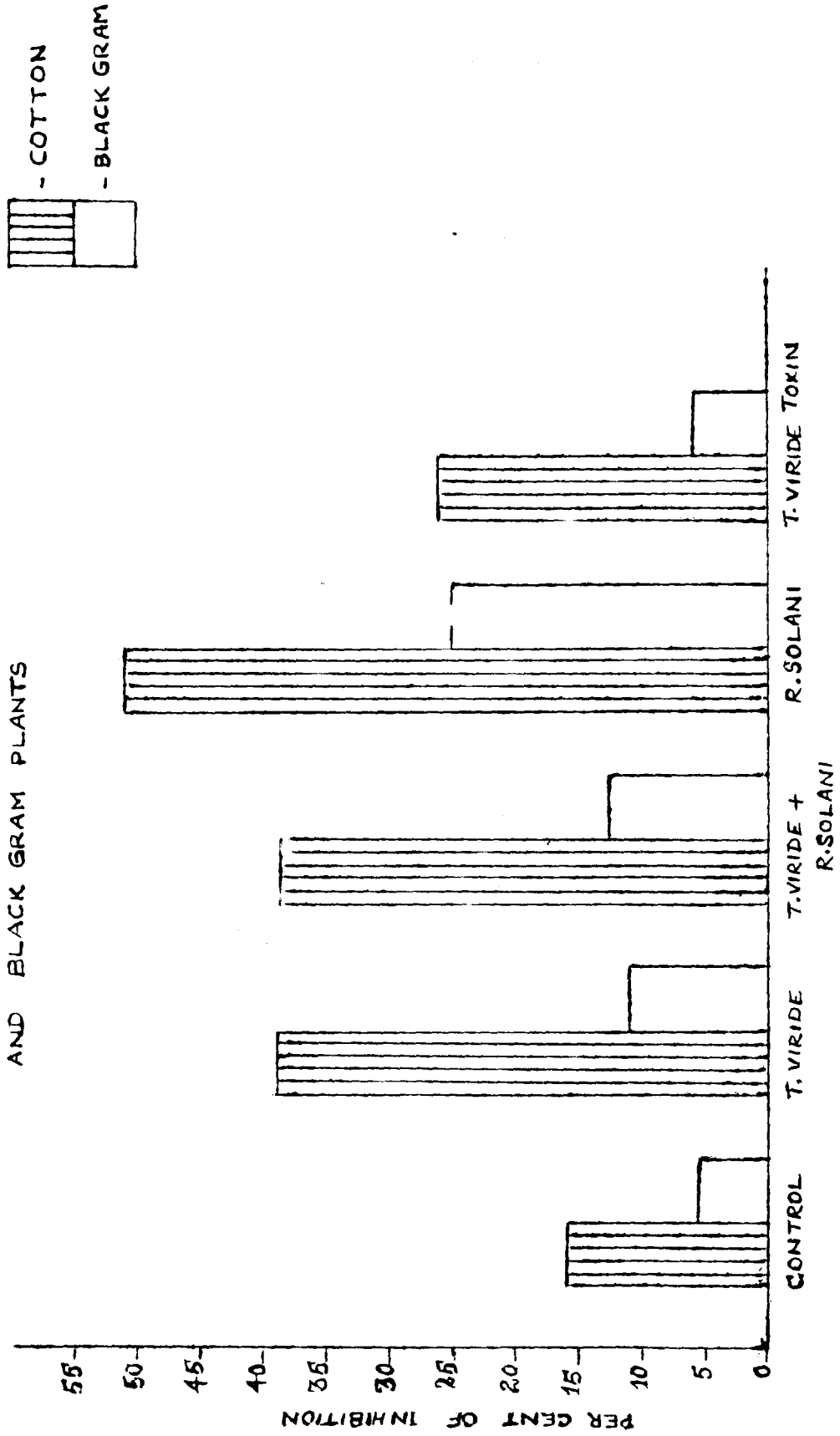
TABLE - IV

EFFECT OF TREATMENTS ON THE * GERMINATION OF COTTON
AND BLACKGRAM SEEDS

Treatment	% of inhibition	
	Cotton	Blackgram
Control	15.9	5.7
T.viride	19.2	11.3
T.viride + R.solani	28.6	12.7
R.solani	50.9	25.0
T.viride toxin	26.3	5.7
C.D (5%)	9.3	10.8

* 6 days after sowing.

Fig-1 EFFECT OF TREATMENTS ON THE GERMINATION OF COTTON AND BLACK GRAM PLANTS



The result revealed that 50.9 per cent and 25 per cent inhibition was recorded on cotton and blackgram respectively due to R. solani as against 15.9 per cent and 5.7 per cent in control. However, 28.6 per cent and 12.7 per cent seedling mortality was recorded respectively in cotton and blackgram due to combined application of T. viride and R. solani. There was no significant reduction in seed germination of cotton and blackgram due to T. viride.

EFFECT OF TREATMENTS ON SHOOT AND ROOT LENGTHS OF COTTON PLANT

The influence of various treatments on shoot and root length of cotton plant is presented in Table - V.



A - Control B - T.viride + R.solani
 C - T.viride D - T.viride Toxin.

5. Effect of Treatments on shoot and root lengths of cotton.



A - T.viride B. T.viride + R.solani
 C - Control D - R.solani

TABLE - V

EFFECT OF TREATMENTS ON SHOOT AND ROOT LENGTHS OF
COTTON PLANT

Treatment	Shoot length (cm)			Root length (cm)		
	10 DAS	20 DAS	30DAS	10 DAS	20 DAS	30 DAS
Control	10.00	22.50	26.50	12.00	14.00	16.00
T.viride	11.00	19.00	26.00	8.00	11.00	18.00
T.viride + R.solani	13.00	16.00	23.00	6.75	11.00	18.90
R.solani	5.80	14.50	17.00	4.00	7.00	10.00
T.viride toxin	14.90	19.80	25.50	6.80	11.00	15.80
C.D (5%)	1.60	1.37	1.07	0.86	0.80	0.14

DAS - Days After Sowing

Shoot length of 10 day old cotton plant was significantly increased by the treatments. T.viride + R.solani and T.viride toxin when compared to the control. There was no significant effect on shoot length due to T.viride. However, drastic reduction of shoot length

was recorded in R. solani inoculated pot culture soil. But there was a significant reduction in shoot length by all the treatments on 20 day old seedlings when compared to control. R. solani and T. viride + R. solani significantly reduced the shoot length of the 30 day old seedlings where as others were on par with the control. The root length of 10 day old plant was significantly reduced by all these treatments. Same trend was also observed on 20 day old plants. However, there was significant increase in root length was observed on 30 day old plants due to application of T. viride and T. viride + R. solani. From the data it was clear that T. viride induced root length of cotton plants and its influence has not been pronounced in shoot length.

EFFECT OF TREATMENTS ON SHOOT AND ROOT LENGTHS OF BLACK GRAM PLANTS

The effect of treatments on shoot and root lengths of blackgram is presented in Table - VI.

TABLE - VI

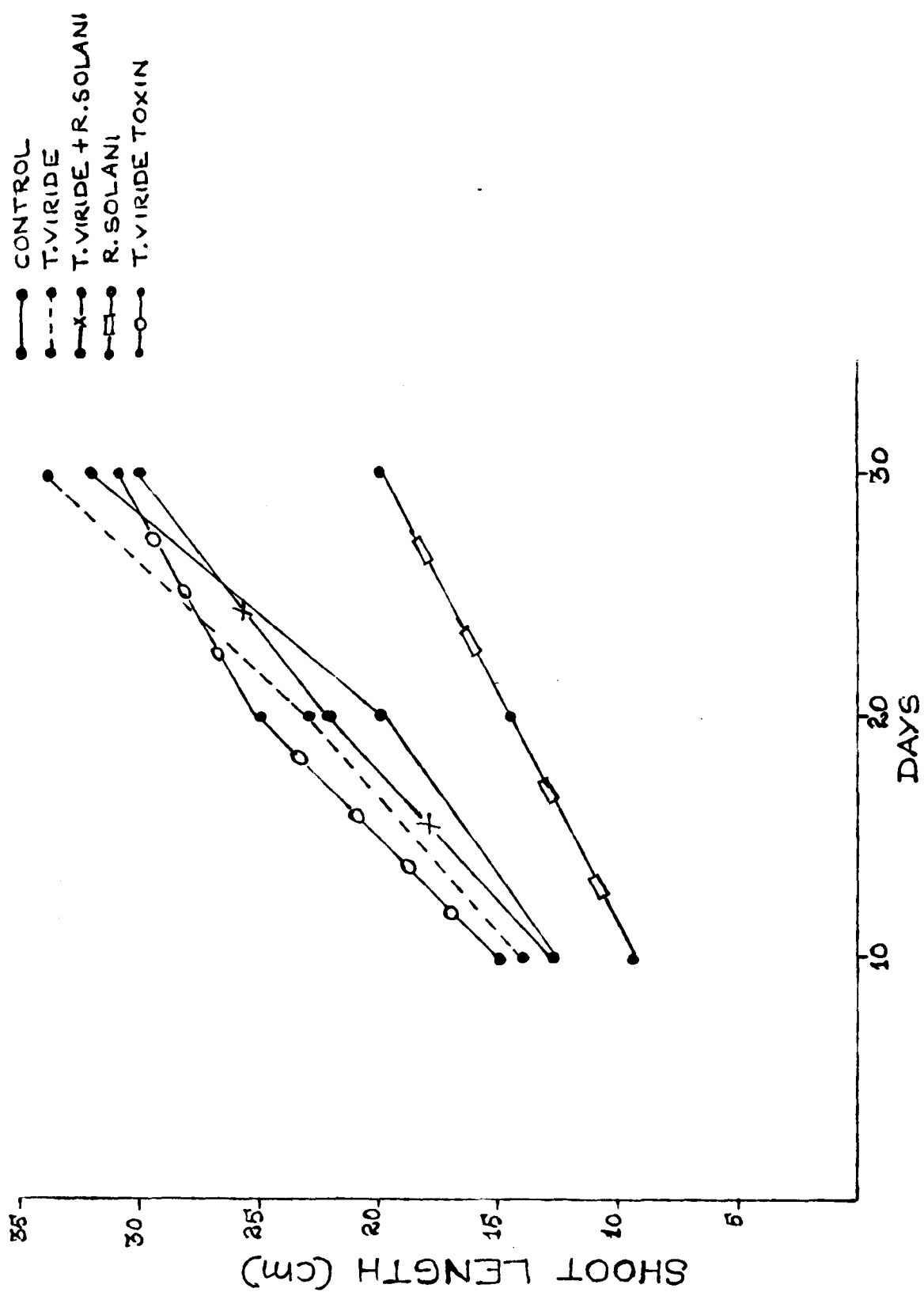
EFFECT OF TREATMENTS ON SHOOT AND ROOT LENGTHS
OF BLACK GRAM PLANT

Treatment	Shoot length (cm)			Root length (cm)		
	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS
Control	13.80	20.00	32.00	12.70	14.00	23.00
T.viride	14.00	23.00	34.00	13.00	14.00	22.00
T.viride + R.solani	13.00	22.00	30.00	7.50	12.00	20.50
R.solani	9.50	14.50	20.00	5.50	11.00	17.50
T.viride toxin	15.00	25.00	31.00	4.50	14.00	23.00
C.D (5%) :	1.90	0.85	0.25	1.50	1.04	0.87

DAS - Days After Sowing

Initially there was no significant increase in shoot length (10 day old) due to the treatments except R.solani which recorded drastic reduction in shoot length. Significant ($P < 0.05$) increase in shoot

Fig-2. EFFECT OF TREATMENTS ON SHOOT LENGTH OF BLACK GRAM



length was recorded by T.viride toxin and T.viride on 20 and 30 day old seedlings when compared to control. There was no significant increase in root length by T.viride when compared to control. It was cleared that in blackgram T.viride increased only the shoot length not root length.

BIOCHEMICAL CHANGES IN COTTON AND BLACKGRAM DUE TO THE APPLICATION OF T.viride, R.solani, T.viride + R.solani AND T.viride TOXIN

EFFECT OF TREATMENTS ON TOTAL SOLUBLE SUGARS:

The influence of various treatments on total soluble sugar content in cotton and blackgram is presented in Tables VII and VIII.

TABLE - VII

EFFECT OF TREATMENTS ON TOTAL SOLUBLE SUGAR CONTENT OF COTTON PLANTS

Treatment	Total soluble sugars mg/g								
	Leaves			Stem			Root		
	10	20	30	10	20	30	10	20	30
	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS
Control	0.70	2.95	2.50	0.40	1.80	3.20	0.42	1.80	2.40
T.viride	0.63	3.50	2.20	0.36	1.50	2.50	0.35	1.75	1.50
T.viride + R.solani	0.70	3.65	3.88	0.30	3.00	4.00	0.20	2.50	4.50
R.solani	0.99	3.70	4.43	0.60	4.00	10.00	0.90	2.20	5.00
T.viride toxin	0.99	3.30	2.90	0.40	1.40	3.50	0.30	1.00	2.00
C.D (5%) :	0.07	0.12	3.69	0.04	0.07	0.08	0.08	0.09	0.07

TABLE - VIII

EFFECT OF TREATMENTS ON TOTAL SOLUBLE SUGAR CONTENT OF BLACK GRAM PLANTS

Treatment	Total soluble sugars mg/g														
	Leaves					Stem					Root				
	10 DAS	20 DAS	30 DAS	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS
Control	0.51	1.70	8.00	0.50	0.98	5.40	0.50	0.50	1.17	2.80					
T.viride	0.50	1.20	5.00	0.50	1.10	5.50	0.51	1.50	2.20						
T.viride + R.solani	0.75	1.90	8.30	0.51	1.20	8.00	0.60	2.00	5.80						
R.solani	0.60	3.20	10.80	0.51	1.80	8.50	0.70	3.00	6.30						
T.viride toxin	0.52	1.40	5.00	0.52	1.00	5.55	0.49	1.50	2.00						
C.D (5%) :	0.01	0.08	0.12	0.01	0.11	0.25	0.01	0.23	0.23						

The data revealed that in 10 day old cotton roots and stems, the total soluble sugar content was significantly high in R.solani inoculated pot culture soil. There was drastic reduction of total soluble sugar in T.viride inoculated soil and on par with un inoculated control. Same trend was also observed from the cotton leaves. In general the total soluble sugar content was gradually increased with increase in the age of the crop. In blackgram (Table - VIII) a similar observation was also recorded in the root, stem and leaves.

EFFECT OF TREATMENTS ON REDUCING SUGAR CONTENT OF COTTON AND BLACKGRAM PLANTS

The reducing sugar content in cotton and blackgram due to various treatments is presented in Tables IX and X.

TABLE - IX

EFFECT OF TREATMENTS ON REDUCING SUGAR CONTENT OF COTTON PLANTS

Treatment	Reducing sugar (mg/g)								
	Leaves		Stem		Root				
	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS
Control	0.08	1.76	1.41	0.18	1.28	0.47	0.12	1.14	0.25
T.viride	0.89	2.00	0.50	0.18	1.25	0.49	0.12	1.10	0.61
T.viride + R.solani	0.18	2.25	0.90	0.20	1.20	0.60	0.20	1.15	1.20
R.solani	0.19	1.40	2.80	0.22	1.43	3.00	0.40	1.20	2.11
T.viride toxin	0.08	2.45	1.50	0.18	1.23	0.50	0.12	1.03	0.50
C.D (5%) :	0.110	0.009	0.007	0.008	0.009	0.010	0.010	0.010	0.010

TABLE - X

EFFECT OF TREATMENTS ON REDUCING SUGAR CONTENT OF BLACKGRAM PLANTS

Treatment	Reducing sugar mg/g								
	Leaves		Stem		Root				
	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS
Control	0.05	1.40	2.10	0.04	1.07	1.30	0.05	1.10	0.85
T.viride	0.04	1.20	2.00	0.03	1.00	1.10	0.05	1.20	0.65
T.viride + R.solani	0.30	1.60	3.70	0.10	1.12	1.50	0.12	1.34	1.60
R.solani	0.40	2.00	3.92	0.87	1.13	2.50	0.13	2.00	2.10
T.viride toxin	0.05	1.20	2.00	0.05	1.00	1.10	0.04	1.00	1.05
C.D (5%) :	0.003	0.040	0.620	0.008	0.080	0.090	0.009	0.060	0.056

In roots of cotton and blackgram the reducing sugar content was significantly high in R.solani inoculated soil and this was followed by T.viride + R.solani. There was significant reduction in reducing sugar content in T.viride inoculated pot culture soil and in T.viride toxin and were on par with un inoculated control. Same trends were observed in stems and leaves of cotton and blackgram.

EFFECT OF TREATMENTS ON STARCH CONTENT OF COTTON AND BLACKGRAM PLANTS

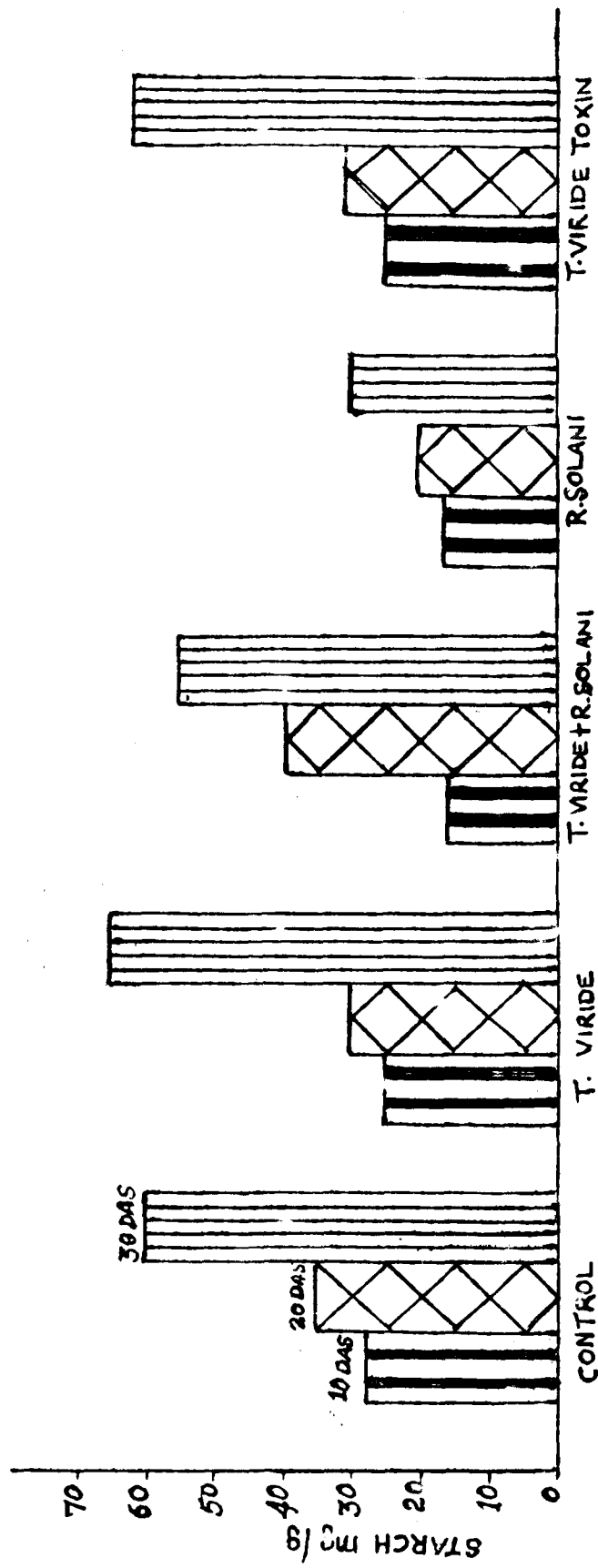
The results on starch content of cotton and black gram due to various treatments are presented in Table - XI.

TABLE - XI

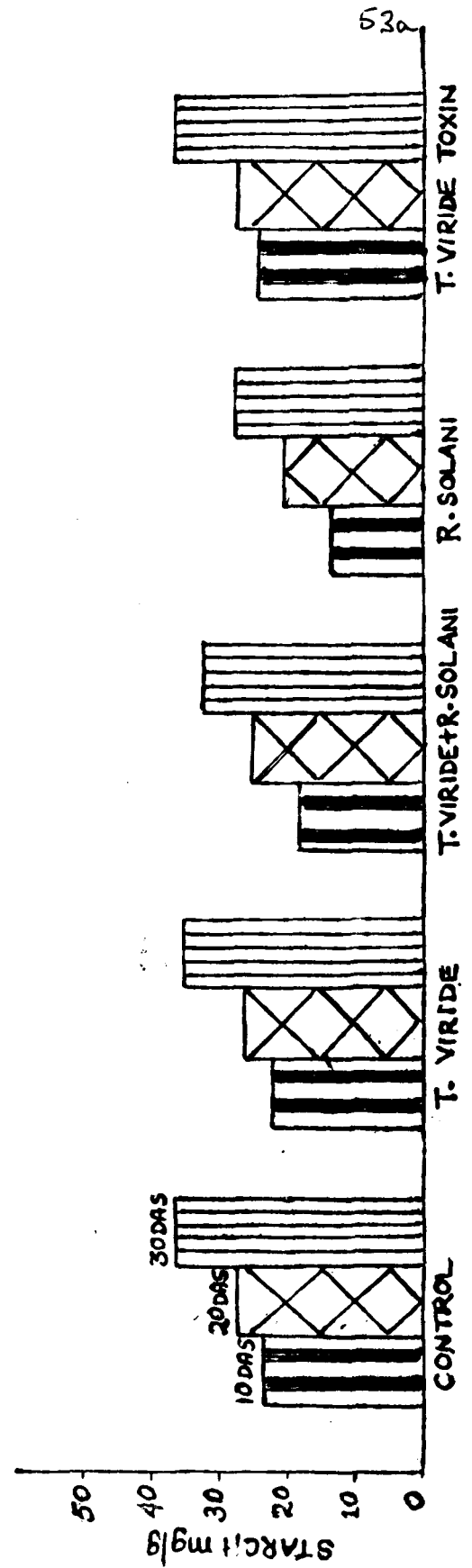
EFFECT OF TREATMENTS ON STARCH CONTENT OF COTTON AND BLACKGRAM PLANTS

Treatment	Starch mg/g					
	Cotton			Blackgram		
	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS
Control	28.00	35.20	60.00	22.50	27.10	36.00
T.viride	32.00	38.00	65.00	24.60	28.50	37.20
T.viride + R.solani	14.83	38.33	55.00	18.00	24.30	32.40
R.solani	16.00	20.00	30.00	13.50	19.80	27.00
T.viride toxin	24.80	31.33	61.50	24.30	27.00	36.00
C.D (5%) :	1.92	1.76	2.94	0.11	0.09	0.42

Fig.3. EFFECT OF TREATMENTS ON STARCH CONTENT OF COTTON PLANTS



EFFECT OF TREATMENTS ON STARCH CONTENT OF BLACKGRAM PLANTS



The starch content was greatly influenced by T.viride when compared to control. There was drastic reduction of starch content due to R.solani inoculation. In general starch content of the crops was found to be increased with age of the crop.

EFFECT OF TREATMENTS ON PROTEIN CONTENT OF COTTON AND
BLACK GRAM PLANTS

The protein contents of the root, stem and leaves of cotton and blackgram were greatly influenced by all the treatments except R.solani (Table XII and XIII). Protein content was significantly reduced in R.solani inoculated soil. There was significant increase in protein content in T.viride, T.viride + R.solani inoculated soil. They were on par with un inoculated control and T.viride toxin.

TABLE - XII

EFFECT OF TREATMENTS ON PROTEIN CONTENT OF COTTON PLANTS

Treatments	Protein mg/g								
	Leaves		Stem		Root				
	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS
Control	0.34	0.65	0.80	0.03	0.05	0.13	0.03	0.06	0.07
T.viride	0.40	0.65	0.80	0.03	0.04	0.10	0.03	0.06	0.08
T.viride + R.solani	0.20	0.45	0.60	0.02	0.05	0.10	0.04	0.05	0.08
R.solani	0.10	0.25	0.35	0.02	0.03	0.08	0.02	0.04	0.03
T.viride toxin	0.50	0.60	0.80	0.03	0.04	0.10	0.03	0.06	0.07
C.D (5%) :	0.010	0.014	0.023	0.008	0.010	0.005	0.006	0.006	0.006

TABLE - XIII
EFFECT OF TREATMENTS ON PROTEIN CONTENT OF BLACK GRAM PLANTS

Treatment	Protein mg/g								
	Leaves			Stem			Root		
	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS
Control	0.29	0.45	0.54	0.08	0.16	0.25	0.05	0.10	0.17
T.viride	0.25	0.40	0.82	0.06	0.15	0.25	0.04	0.10	0.20
T.viride + R.solani	0.23	0.33	0.75	0.05	0.13	0.28	0.03	0.11	0.19
R.solani	0.15	0.25	0.14	0.04	0.08	0.20	0.02	0.05	0.05
T.viride toxin	0.30	0.40	0.54	0.06	0.10	0.30	0.04	0.40	0.20
C.D (5%) :	0.010	0.010	0.009	0.006	0.004	0.006	0.007	0.040	0.030

TABLE -XIV

EFFECT OF TREATMENTS ON AMINOACID CONTENT OF COTTON PLANTS

Treatment	Amino acid mg/g								
	Leaves			Stem			Root		
	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS
Control	0.42	0.69	0.08	0.30	0.24	0.04	0.20	0.07	0.01
T.viride	0.40	0.68	0.09	0.30	0.23	0.04	0.20	0.06	0.02
T.viride + R.solani	0.42	0.67	0.08	0.31	0.25	0.04	0.29	0.25	0.05
R.solani	0.40	0.69	0.08	0.29	0.24	0.04	0.90	0.87	0.14
T.viride toxin	0.42	0.68	0.09	0.30	0.25	0.04	0.24	0.08	0.02
C.D. (5%)	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.02	0.02

TABLE - XV

EFFECT OF TREATMENTS ON AMINOACID CONTENT OF BLACKGRAM PLANTS

Treatment	Aminoacid mg/g								
	Leaves			Stem			Root		
	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS
Control	0.22	0.69	0.42	0.75	0.27	0.12	0.45	0.75	0.09
T.viride	0.22	0.40	0.43	0.78	0.42	0.12	0.44	0.76	0.13
T.viride + R.solani	0.29	0.73	0.80	1.05	0.35	0.26	0.80	0.90	0.40
R.solani	0.36	0.75	1.00	1.20	0.46	0.40	0.90	1.00	0.80
T.viride toxin	0.19	0.72	0.43	0.70	0.40	0.10	0.45	0.76	0.10
C.D (5%)	: 0.007	0.007	0.006	0.005	0.007	0.003	0.006	0.005	0.005

EFFECT OF TREATMENTS ON AMINOACID CONTENT OF COTTON AND BLACKGRAM PLANTS

The amino acid content was more in 10 day old cotton root when compared to 20 and 30 day old roots. Among the treatments the amino acid content was significantly high in R.solani inoculated soil (Table - XIV). There was no change in aminoacid content due to T.viride when compared to control. There was no significant difference in aminoacid content in stem and leaves. In black gram the aminoacid content was more in 20 day old roots when compared to 10 and 30 day old roots (Table - XV). Among the treatments R.solani inoculated soil recorded significantly high amount of aminoacid. This was followed by T.viride + R.solani. There was no changes in aminoacid content due to T.viride. When compared to un inoculated control. Aminoacid content was more in 10 day old stem when compared to 20 and 30 day old stem. But in the leaves maximum amount of amino acid was recorded in 30 day old plants. In both stem and leaves application of R.solani greatly influence the aminoacid content. This was followed by T.viride + R.solani.

TABLE - XVI

EFFECT OF TREATMENTS ON PHENOL CONTENT OF COTTON PLANTS

Treatment	Phenol mg/g								
	Leaves			Stem			Root		
	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS
Control	0.034	0.051	0.071	0.030	0.050	0.080	0.020	0.012	0.005
T. viride	0.049	0.077	0.081	0.040	0.080	0.090	0.034	0.016	0.012
T. viride + R. solani	0.014	0.074	0.066	0.011	0.040	0.060	0.010	0.015	0.020
R. solani	0.006	0.043	0.044	0.007	0.010	0.019	0.004	0.006	0.001
T. viride toxin	0.036	0.041	0.051	0.030	0.039	0.070	0.020	0.020	0.005
C.D (5%) :	0.001	0.002	0.015	0.025	0.001	0.004	0.001	0.002	0.002

Fig-4 EFFECT OF VARIOUS TREATMENTS ON PHENOL CONTENT OF COTTON PLANTS

- CONTROL
- - -●- - T. VIRIDE
- X-●- T. VIRIDE + R. SOLANI
- R. SOLANI
- T. VIRIDE TOXIN

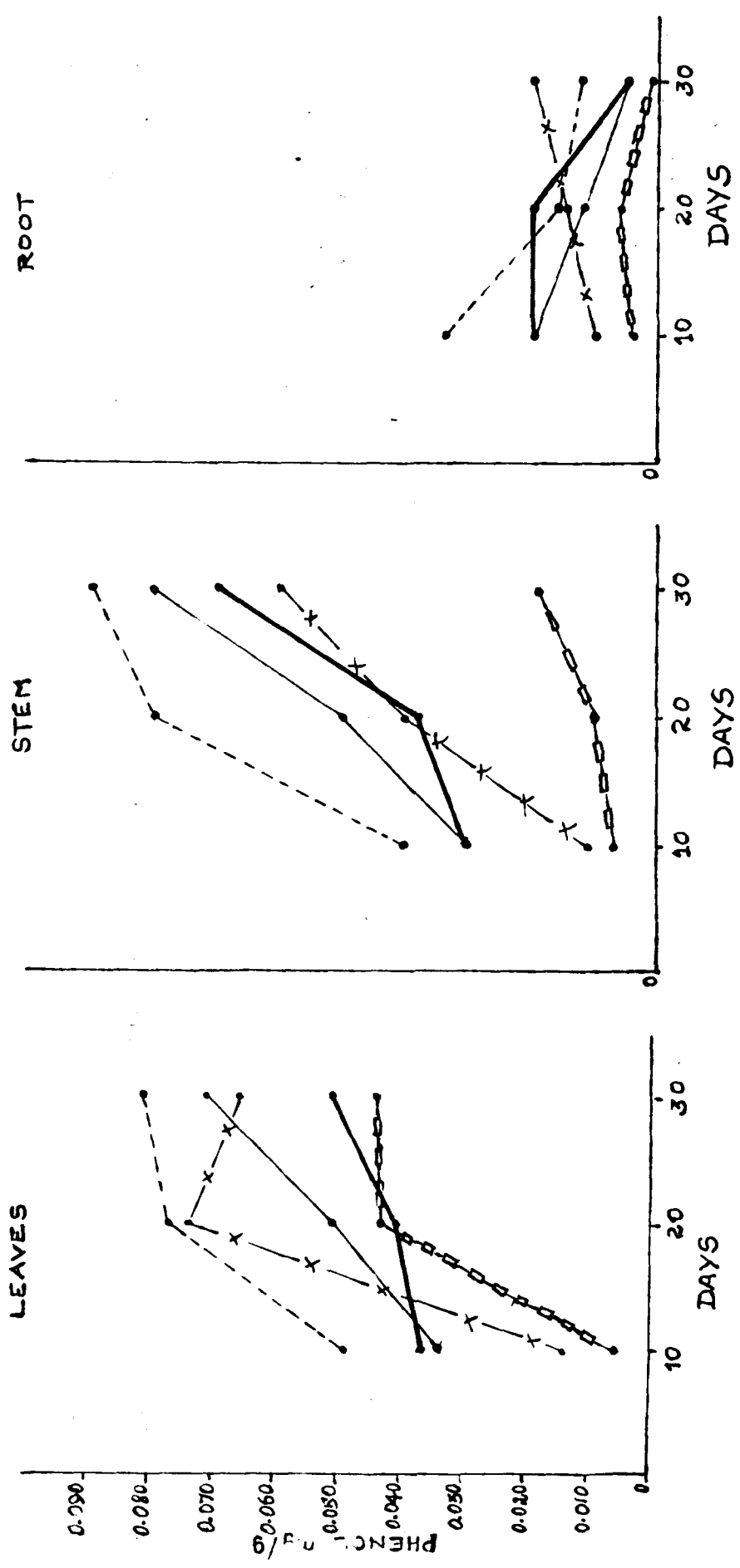


TABLE - XVII

EFFECT OF TREATMENTS ON PHENOL CONTENT OF BLACKGRAM PLANTS

Treatment	Phenol mg/g								
	Leaves			Stem			Root		
	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS	10DAS	20DAS	30DAS
Control	0.036	0.110	0.230	0.100	0.190	0.050	0.080	0.090	0.055
T.viride	0.078	0.080	0.340	0.090	0.080	0.045	0.060	0.066	0.135
T.viride + R.solani	0.024	0.160	0.620	0.180	0.100	0.086	0.123	0.086	0.150
R.solani	0.042	0.190	0.350	0.100	0.300	0.100	0.059	0.190	0.100
T.viride toxin	0.048	0.100	0.235	0.092	0.080	0.050	0.060	0.100	0.100
C.D (5%)	0.002	0.010	0.010	0.012	0.010	0.008	0.012	0.009	0.007

EFFECT OF TREATMENTS ON PHENOL CONTENT OF COTTON AND
BLACKGRAM PLANTS:

The results on phenolic content, in cotton and blackgram plants due to various treatment is presented in Tables XVI and XVII.

The data clearly showed that in roots, stem and leaves the phenolic content was significantly high due to T.viride application when compared to control and R.solani inoculated pot culture soil. Same trend was observed in black gram also.

DISCUSSION

Rhizoctonia solani Kuhn, the imperfect state of Thanatephorus cucumeris (Frank) Donk has gained the reputation of being widespread and destructive disease of cotton and blackgram in Tamil Nadu. Biological control of R. solani is rapidly becoming an acceptable technological approach in recent years (Papavizas, 1981; Cook and Baker, 1983). Biological control of plant pathogens accomplished through host resistance and continue to be a predominant disease control strategy. Though there have been several reports of successful use of Trichoderma spp. against R. solani (Chet and Baker, 1981; Elad et al., 1981), so far no work has been initiated to study the role of Trichoderma spp. on host physiology. It was against this background that the present study was undertaken and the results are discussed critically.

The role of toxin in the pathogenesis has been established by several workers (Jansen and Livingsten, 1949; Gnanamanickam and Patil, 1976). Therefore, studies on the toxigenicity of Trichoderma viride was initiated.

T.viride toxin at 1, 3, 6 and 9 per cent concentrations significantly reduced mycelial dry weight of R.solani when compared to control. Upadhyay and Mukhopadhyay (1986) reported that the production of extracellular enzymes (B (1,3) - glucanase and chitinase) capable of degrading R.solani cell walls. The inhibitory effect of Trichoderma spp. have also been reported by Hadar et al., 1979; Upadhyay and Mukhopadhyay, 1983. The inhibitory effect might be due to the presence of toxic metabolites like viridin or gliotoxin. (Parpansi, 1960; Chohan, 1971 and 1974). The toxic metabolites produced by Trichoderma spp. have been the subject of extensive study ever since Weindling (1934) reported the culture filtrate of Trichoderma were toxic to R.solani and other fungi even at high dilution. Only 2 g of 9 isolates of Trichoderma spp. tested by Dennis and Webster (1971) caused appreciable inhibition of few fungi other than Pythium aphanidermatum. Mukhopadhyay and Upadhyay (1981) reported the release of non-volatile antibiotic compounds by Trichoderma spp. were inhibitory to the growth of Sclerotium rolfsii in vitro, where as, Hadar et al. (1979), Chet, et al., (1979) and chet and Baker (1981) could not detect antibiotic activity of Trichoderma spp in vitro. This may be due to insufficient period of incubation of the antagonist.

The toxin extracted from T.viride was tested for its pathogenicity on cotton and blackgram leaves. The toxin at various concentrations didnot produce any symptom on host plants tested. However, it significantly inhibited the root and shoot length. This inhibitory effect was not pronounced on seed germination. Similar observation was also made when T.viride was applied in soil. However, when it combined with R.solani, 28.6% and 12.7% inhibition of seed germination of cotton and blackgram was recorded respectively as against 50.9% and 25% in R.solani alone-inoculated soil. More over, in cotton the root length was significantly increased and its influence has not been pronounced in shoot length. On contrary in blackgram it increased only the shoot length but not the root length. On support of the present finding Mukhopadhyay et al. (1986) reported that Trichoderma spp. significantly increased the seed germination of tobacco when applied in soil along with the pathogen. Increase in shoot and root length due to Trichoderma spp was also reported by Krishnamoorthy (1987).

Many pathogens prefer sugars for their growth (Vidhyasekaran and Parambaramani, 1971a, Vidhyasekaran and Durairaj, 1971b) and hence the quantity and quality

of sugars may play an important role in the disease resistance. The results of the present study showed that the total soluble sugars and reducing sugars were significantly less due to T.viride inoculation when compared to R.solani inoculation. Changes in sugar content of host tissue due to infection by pathogenic fungi is well known. The present finding is well supported by the work of Rosalis (1987). He reported that the total soluble sugar and reducing sugar contents were increased in tomato plants affected by Sclerotium rolfsii. Bashian (1984) recorded highest amount of soluble sugars and reducing sugars in all the nine soybean varieties susceptible to Macrophomina phaseolina on the contrary several workers reported that there was a depletion of total and reducing sugar contents due to pathogenic invasion. Bhaskaran et al. (1975). Chopra and Jhooty (1974) recorded the reduced level of total soluble sugar and reducing sugar due to pathogenic infection.

The present study revealed that there was a marked reduction in the starch content of cotton and blackgram due to R.solani infection. High level of

starch content was recorded in T.viride inoculated soil. Bhaskaran (1976) also observed such depletion with Alternaria helianthi on sunflower. Cercospora leaf spot and powdery mildew infection have also been reported to cause severe reduction in starch content in green gram (Vidhyasekaran and Kandaswamy, 1972). It is probable that the reduction in starch content may be due to inhibition of phosphorylase, the starch synthesizing enzyme in the infected tissues.

Protein contents of roots, stems and leaves of cotton and blackgram were significantly increased by T.viride when compared to R.solani. This finding agreed well with the observation of Bastian (1984). A comparative biochemical study was done by him with nine soybean varieties in relation to their susceptibility to Macrophomina phaseolina showed the presence of high level of protein in non-infected tissue of all varieties.

An extensive review on the role of amino acids in plant diseases has been made by Van Andel (1966). Amino acid contents of plants have been related to their susceptibility and resistance to the disease in several

studies (Lakshminarayan, 1955, Strech and Chappellini, 1965). In the present study, high level of amino acid was recorded in R. solani inoculated soil when compared to T. viride inoculation. Changes in aminoacids in response to infection by pathogenic fungi have been reported by several worker's (Chandramohan et al., 1967; Chopra et al., 1974 and Bhaskaran, 1976). Ragunathan et al. (1966) found that the increased level of amino acid is due to pathogenic invasion. From this study it appears that immediately after infection, free aminoacids are transported to the site of infection for the development of the pathogen. The accumulation of amino acids in the infected tissue has been reported by Patel and Walker (1963) and Krishnaswamy (1968). This may be due to the breakdown of proteins by proteolytic enzymes or the fungus may synthesize the amino acids and thus help in their accumulation.

Phenols are the known fungitoxic substances (Patil et al., 1964; Wilson and Srivastava, 1969). Mahadevan (1966) reported that pathogenic invasions invariably results in the alterations of phenolic constituents of plants and tissues. Susceptibility and resistance are very often correlated with plant phenolics. The present investigation showed that phenolic content

was significantly less in R. solani inoculated soil. T. viride significantly increased the phenols in both cotton and blackgram. Many workers attributed the seasons for the reduction in the level of phenolics in the diseased tissue. Phenols involved in metabolic changes in host plants (Tomiyama et al 1967). Quinones are highly toxic even at lower concentrations and it is responsible to postulate that quinone from phenols may be more responsible for resistance (Patel et al, 1964). Quinones produced with phenolic oxidation are of high physiological activity and can prevent the development of microorganisms by inactivating their enzymes (Rubin and Artsikhouskaya, 1964). Many workers have tried to attribute seasons for the reduction in the level of phenolics in the diseased tissue. Mahadevan (1974) pointed out that many pathogenic fungi are able to cleave the benzene ring of phenols oxidatively and the resulting cis-cis muconic acid is utilised as an energy source. The growth of several fungi with some of the phenolic compounds can be taken in support of this (McLean et al., 1961; Sivaprakasam, 1972; Natarajan, 1973; Padmanaban, 1973 and Bhaskaran, 1976). Similarly, in the present case also a portion of the phenolic compounds might have been detoxified and utilized as energy source by R. solani.

Summary and Conclusion

SUMMARY AND CONCLUSION

Various physiological changes in cotton and blackgram due to Trichoderma viride toxin, T.viride, T.viride + Rhizoctonia solani were studied in detail and the results have been summarized.

T.viride toxin at various concentrations drastically reduced mycelial dry weight of R.solani in vitro.

There was no significant reduction in seed germination due to T.viride toxin. The shoot length and root length of cotton and blackgram were significantly reduced by the T.viride toxin.

The effect of various treatments viz., T.viride, T.viride + R.solani, R.solani and T.viride toxin on seed germination revealed that R.solani caused 50.9% and 25% inhibition on cotton and blackgram respectively.

Effect of various treatments on shoot and root length of cotton revealed that T.viride induced root length and its influence has not been pronounced in

shoot length. Combined application of T.viride and R.solani influenced root length when compared to control and R.solani.

The present study revealed that the application of T.viride induced the shoot length of blackgram plants and there was no significant increase in root length. The combined application of T.viride and R.solani caused increase in shoot length compared to R.solani. T.viride toxin also increased the shoot length of blackgram compared to R.solani.

The results of the present study showed that the total soluble sugars and reducing sugars were significantly less due to T.viride inoculation than that due to R.solani inoculation.

A marked reduction in the starch content of cotton and blackgram due to R.solani infection was noticed. High level of starch content was recorded in T.viride inoculated soil. Combined application of T.viride and R.solani increased the starch content of both cotton and blackgram when compared with the application of R.solani alone, which revealed the antagonistic effect of T.viride on R.solani.

Protein content of roots, stems and leaves of cotton and blackgram was found to be significantly increased by T.viride when compared to R.solani. When T.viride was added along with R.solani protein content of both cotton and blackgram plants were much more increased than that caused by R.solani alone and control.

In the present study, high level of aminoacid was recorded in plants grown in R.solani inoculated soil when compared to those grown in T.viride inoculated soil.

The present investigation showed that phenolic content was significantly less in R.solani inoculated soil. T.viride significantly increased the phenols in both cotton and blackgram. Combined application of T.viride and R.solani also increased the phenolic content of both cotton and blackgram plants. This revealed that T.viride antagonises R.solani. It offers resistance by increasing the phenolic content.

This investigation clearly indicates T.viride as an efficient antagonist of R.solani. It revealed that T.viride brings about the control of the disease in

cotton and blackgram as indicated by the significant increase in shoot and root length of these plants by combined application of T.viride and R.solani. Also the biochemical parameters like starch, protein and phenols contents increased while total soluble and reducing sugar contents decreased.

Bibliography

BIBLIOGRAPHY

- Akhtar, C.M., Amin, C.M.
and Salim, C.M. 1982. Investigations of the
biological control of
R. solani and Fusarium
oxysporum causing root rot
and wilt diseases in cotton
and linseed respectively.
Pakistan Avub Agric. Res.
Inst., Faisalabad 63 pp.
- Alagarsamy, G., Mohan, S.
and Jeyarajan, R.
1987. Effect of seed pelleting
with antagonists in the
management of seedling,
diseases of cotton.
J. Biol. Control. 1 : 66 - 67.
- Allen, M.F., Boosalis, M.C.,
Kerr, E.D., Mulloor, A.E. and
Larsen, H.J.
1985. Population dynamics of
sugarbeet R. solani and
Laetisaria arvalis.
Appl. environ. Microbiol. 20:
1123 - 1127.
- Anonymous,
1985. Agrl. situation in India.
pp. 26.
- Ashok kumar, K.
1986. Imbalances in pulse produc-
tivity. Agrl. situation in
India, 41 : 23 - 27.
- Backman, P.A. and
Rodriguez - Kabana, R.
1975. A system for the growth and
delivery of biological
control agents to the soil.
Phytopathology, 65 :
819 - 821.
- Baker, K.F. and
R.J. Cook,
1974. Biological control of plant
pathogens, Freeman San
Francis Co, 433 pp.

- Bashan, Y. 1987. Phenols in cotton seedlings resistant and susceptible to Alternaria macrospora J. Phytopathology 1-10.
- Beagle-Ristaino, J.E. and Papavizas, G.C. 1985. Biological control of Rhizoctonia stem canker and black scurf of potato. Phytopathology 75 : 560-564.
- Bedlan, G. 1986. Test Report on control black rot in lettuce. Review of Plant Pathol. 65 : 283.
- Bhaskaran, R. Natarajan, C. Kohanraj, D. 1975. Biochemistry of resistance and susceptibility in cotton to Alternaria macrospora Acta. Phyto. 10 : 33 - 40.
- Bhaskaran, R. 1976. Studies on the leaf spot disease of sunflower caused by Alternaria Helianthi Ph.D. Thesis, Tamil Nadu Agricultural University, pp 127.
- Boogert, P.H.J.F., VanDen and Jager, G. 1984. Biological control of R. solani on potatoes by antagonists. Netherlands J. Plant Path. 90 : 117 - 126.
- Bramachari, and Kotte, 1983. Biochemical changes in plants affected by Tikka disease. Phytopathology, 5 : 192-193.
- Bray, H.G. and Thorpe, W.V. 1954. Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal. 1 : 27 - 52.

- Burgen, H.D. 1981. Microbial control of pest and plant diseases. Academic press. N.Y.
- Burpee L.L. and Goulty, L.C. 1984. Suppression of brown patch disease of creeping bent grass by isolates of non pathogenic Rhizoctonia spp. Phytopathology 74 : 692-694.
- Chandramohan, D. Mahadevan, A. Rangaswami, G. 1967. Studies on some biochemical properties of leaf tissues of Amaranthus tricolor as related to resistance to infection by Alternaria spp. Indian Phytopathology 20 : 109 - 113.
- Chopra, B.L. and Jhooty, J.S. 1974. Biochemical changes in a resistant and susceptible variety of watermelon due to infection by Alternaria cucumerina. Indian Phytopathology 27 : 502 - 507.
- Chet, I and Baker, E. 1981. Induction of suppressiveness to R.solani in soil Phytopathology 77 : (994-998)
- Chet, I. and Baker, R. 1981. Isolation and biocontrol potential of T.hamatum from soil naturally suppressive to R.solani. Phytopathology, 71 : 286 - 290.
- Chet, I., Horman, G.E. and Baker, R. 1981. T.hamatum: Its hyphal interactions with R.solani and Pythium spp. Microb. Ecol. 7 : 29 - 38.

- Chohan, J.S. 1971a. J.Res.Pb.Agric. Univ.
Botany and Plant Pathology
supplement 7 : 561 - 565.
- Chohan, J.S. 1971b Labdev. J. Sci. & Tech.
9 : 10 - 16.
- Chohan, J.S. 1974. Recent advances in diseases
of groundnut in India:
Current trends in plant
pathology, pp. 171 - 184.
- Chu, F.F. and Wu, W.S. 1980. Biological and chemical
control of potato black
scurf. Plant. Prot. Bull.,
Taiwan, 22 : 269 - 286.
- Chu, F.F. and Wu, W.S. 1981. Biological and chemical
control of R.solani by pea
seed treatment. Memoirs of
the college of Agriculture,
National Taiwan University
21 : 19 - 28.
- Cligg, K.M. 1956. The application of the
anthrone reagent to the
estimation of starch in cereals.
J.Sci. Food. Agric. 7 : 40 - 44.
- Conway, K.T. 1986. Use of fluid-drilling gels
to deliver biological
control agents to soil.
Plant Dis. 70 : 835 - 839.
- Cook, R.J. and Baker, K.F. 1983. The nature and practice of
Biological control of plant
pathogens. American
Phytopathological Soc., st. p.

- Darpoux, H. 1960. Plant pathology, (An advance Treatise) Vo. 3, Academic Press, New York, pp. 123.
- Dennis, C. and Webster, J. 1971 a. Antagonistic properties of species groups of *Trichoderma*, Production of non volatile Antibiotics. Trans, Brit. Myc. Soc. 57 : 25 - 39.
- Dennis, C. and Webster, J. 1971b. Antagonistic properties of species groups of *Trichoderma* production of volatile antibiotics. Trans. Brit. Myc. Soc. 57: 41-48.
- D'Ercole, N., Nipoli, P. and Sportelli, M. 1985. The use of *T.harzianum* in the control of *Rhizoctonia* bean rot. Diferi della piante, 8 : 141 - 146.
- Dubois, M. Giltes, K. Hamilton, J.K. Reber, P.A. and Smith, F. 1951. A colorimetric method for determination of sugars, Nature 168 : 157.
- Dumitras, L. and Sesan, T. 1980. Features of antagonism of *T.viride* to *R.solani*. Studii si cercetari de Biologie, Biologie Vegetala 32 : 87-92.
- Ekbote, A.V. and Mayee, C.D. 1983. Biochemical changes in Rust infected plants. Indian Phytopath. 36 : 194.

- Elad, Y. Chet, I. and Katan, J. 1980. T.harzianum: A biocontrol agent effective against Sclerotium rolfsii and R.solani Phytopathology 70 : 119 - 121.
- Elad, Y. Chet, I. and Henis, Y. 1979. A relective medium for improving quantitative isolation of Trichoderma spp from soil. Phytoparasitica 9 : 224 - 254.
- Elad, Y., Hadar, Y. Hadar, E. Chet, I. and Henis, Y. 1981. Biological control of R.solani by T.harzianum in carnation. Plant disease, 65 ; 675 - 680.
- Elad, Y., Chet, I. and Henis, Y. 1981 b. Biological control of R.solani in strawberry fields by T.harzianum. Plant soil 60 : 245 - 254.
- Elad, Y. Kalfon, A. and Chet, I. 1982. Control of R.solani in cotton by seed coating with Trichoderma spp. spores. Plant Soil, 66 : 279 - 281.
- Elad, Y. Hadar, Y. Chet, I. and Henis, Y. 1982. Prevention with T.harzianum of reinfestation by S.rolfsii by soil fumigation with methyl bromide and improvement of disease control in tomatoes and peanut. Crop production 1 : 199 - 211.
- Gangopadhyay, S. Wyllie, T.D. 1973. Indian Journal of Mycology and Plant Pathology, pp 131-140.
- Garrett, S.D. 1965. Toward biological control of soil borne plant pathogens Phytopathology 4 : 17.

- Gnanamanickam, S.S.
and Patil S.S. 1976. Bacterial growth, toxin production and leaves of Ornithine carbomoyltransferase in susceptible and resistant bean leaves inoculated with Pseudomonas phaseolicola. Phytopathology 66 : 290 - 294.
- Gray, D. 1981 Fluid drilling of vegetable seeds. Horticulture Rev. 3: 1-27.
- Hadar, Y. Chet, I. and Henis, Y. 1979. Biological control of R.solani damping off with wheat bran culture of T.harzianum. Phytopathology 69 : 64 - 68.
- Hadar, Y. Harman, G.E.
and Taylor, A.G.
1984. Evaluation of T.koningii and T.harzianum from New York soils for biological control of seed rot caused by Pythium spp. Phytopathology 74 : 106 - 110.
- Harman, G.E. Chet, I.
and Baker, R. 1980. T.hamatum effects on seed and seedling diseases induced in radish and pea by pythium spp. or R.solani. Phytopathology 70 : 1167 -1172.
- Hartley, C. 1921. Damping - off in forest nurseries. U.S.Dept. Agric. Bull. 934 : 1 - 99.
- Henis, Y. Ghaffar, A
and Baker, R. 1978. Integrated control of R.Solani damping off of radish: Effect of successive plantings, PCNB and T.harzianum on pathogen and diseases. Phytopathology 68 : 900 - 907..

- Hoch, H.C. and
Akawi, G.S. 1979. Biological control of pythium
root rot of table beet with
Corticium spp. *Phytopathology*
69 : 417 - 419.
- Howell, C.R. and
Stipanovic, R.D. 1979. Control of R.solani on cotton
seedlings with Pseudomonas
fluorescens and with an
antibiotic produced by the
bacterium. *Phytopathology* 69 :
480 - 482.
- Huang, 1980. Control of sclerotinia wilt
of sunflower by Hyperparasites
Can. Jour. Plant. Pathol. 2 :
26 - 32.
- Jensen, J. Hand
Lwinston, J.E. 1944. Variation in symptoms produced
by isolates of Phytomonas
medicaginis *Phytopathology* 44 :
475 - 480.
- Jones, D. and Watson, D.
1969. Parasitic and lysis of by soil
fungi of Sclerotiria Sclerotiorum
Nature, 224 : 287 - 288.
- Jones, R.W. Petlit, R.
Erud Taber, R.A. 1984. Lignite and stillage carrier
as substrate for application
of fungal biocontrol agents to
soil. *Phytopathology* 74 :
1167 - 1170.
- Kerr, A. 1982. Biological control of soil borne
microbial pathogens and nematodes.
Advances in Agricultural
Microbiology : 429 - 464.
- Krishnasamy , V. 1968. Studies on Blast Disease of
Rice - Factors contributing to
the breakdown of resistance in
the blast resistant variety Co.29.
Dessertation, Univ-Madras,
India, 62 pp.

- Krishnamoorthy, A.S.
1987.
Biological control of Damping off disease of Tomato caused by Pythium Indicum, Balakrishnan, M.Sc (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.
- Kommendahl, T. and
Windels, C.E.
1978.
Evaluation of biological seed treatment for controlling root diseases of pea. *Phytopathology* 68 : 1067-1095.
- Kousalya, G. and
Jeyarajan, R. 1988.
Techniques for mass multiplication of T.viride pers. Fr. and T.harzianum Rifai, National seminar on Management of crop disease with plant products, Biological agents on Jan 11-12, 1988, TNAU, A.C.& R.I., Madurai, p. 32 - 33.
- Kundu, P.K. and
Nandi, B.
1984.
Control of cauliflower damping - off by using antagonist coated seeds. *Pedobiologia* 27 : 43 - 48.
- Larsen, H.S., Boosalis,
M.G. and Kerr, E.D.
1985.
Temporary depression of R.solani field population by soil amendment with Laetisaria arwalis. *Plant Dis.* 69 : 347 - 350.
- Lakshminarayanan, K.
1955.
Some biochemical aspects of Vascular wilts. Symposium on soil micro organism and plant well being. *Proc.Indian Acad. Sci.* 41 (6) : 97 - 154.
- Lewis, J.A. and
Papavizas, G.C. 1985.
Effect of mycelial preparations of Trichoderma and Gliocladium on populations of Rhizoctonia solani and the incidence of damping off. *Phytopathology* 75 : 812 - 817.

- Lewis, J.A. and
Papavizas, G.C. 1987.
Permeability changes in
hyphae of Rhizoctonia solani
induced, by germling
preparations of Trichoderma
and Gliocladium Phytopathology
77 : 699 - 703.
- Logan, C., Cooke, L.R.
Copeland, R.E. and
Mills, P.R. 1983.
Potato disease. In annual
report on Research and
Technical work of the
Department of Agriculture
for Northern Ireland, 1982.
Belfort, Northern Ireland,
UK (1983): 201 - 205.
- Liu, S.D. and
Baker, R. 1980.
Mechanism of biological
control in soil suppressive
to Rhizoctonia solani
Phytopathology 70 : 404 - 412.
- Ludurg, R.A. 1957.
Toxin production of
Helminthosporium sativum and
its significance in disease
development. Canadian J.
Bot. 35 : 291 - 303.
- Luke, H.H. and
Wheeler, H.E. 1955.
Toxin production by
Helminthosporium victorial
Phytopathology, 45 : 453 - 458.
- Mahadevan, A. 1966.
Biochemistry of infection
and resistance.
Phytopathology, 268 : 73-80.
- Mahadevan, A. 1974.
Detoxification mechanisms
of plant pathogens. J.Sci.
Ind. Res. 33 : 131 - 138.

- Marshall, D.S. 1982. Effects of Trichoderma harzianum seed treatment and Rhizoctonia solani inoculum concentration of damping off of snap beans in acidic soil. Plant Dis., 66 : 788 - 789.
- Martin, J.P. 1950. Use of acid, rose bengal and streptomycin in the plate method for estimation of soil fungi. Soil Science 69 : 215 - 233.
- Mew, T.W. and Rowales, A.H. 1984. Relationship of soil micro organism to rice sheath blight, development in irrigated and dryland rice cultures. Technical Bulletin No. 79 11 pp.
- Mihuta - Grimm, L. and Rowe, R.C. 1985. Trichoderma spp. as biocontrol agents of Rhizoctonia damping off of radish in organic soil and comparison of four delivery systems. Phytopathology 75 : 306 - 312.
- Miller, G.L. 1959. Protein determination for large numbers of sample. Anal. Chem. 31 : 964.
- Miller, G.L. 1972. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31 : 426 - 28.
- Morshed, M.S. Invitro antagonism of different species of Trichoderma on some seed-borne fungi of bean. Rev. Plant. Pathology 65 : 345, 1986.

- Moore, S. and
Stein, W.H. 1948. Photometric method for
use in the chromatography of
amino acids. J.Biol.Chem. 176 : 376 - 388.
- Morton, D.J. and
Stroube, W.H. 1955. Antagonistic and stimulatory
effects of microorganisms
upon sclerotium rolfsii.
Phytopathology 45 : 417 - 420.
- Mukhopadhyay, A.N.
and Upadhyay, J.P. 1981. Mechanism of reduction of
Sclerotium root rot of
sugarbeet through nitrogenous
amendments and exploitation
of T.harzianum as potential
biocontrol agent. Phyto-
pathology 68 : 14 - 18.
- Mukhopadhyay, A.N.
Brahmbhatt, A. and
Patel, G.J. 1986. Trichoderma harzianum a
potential biocontrol agent for
Tobacco damping off.
Phytopathology 12 : 26-35.
- Odvoidy, G.N., Boosalis,
M.G. and Kerr, E.D. 1980. Biological control of Rhizoctonia
solani with soil inhabiting
basidiomycetes.
Phytopathology, 70 : 655 - 658.
- Padmanaban, P. and
Alexander, K.C. 1988. Control of root rot of sugarcane
seedlings by antagonistic
organism, Trichoderma viride.
National Seminar on Management
of Crop diseases with plant
products/Biological Agents on
Jan 11-12, 1988. T.N.A.U,
A.C. & R.I., Madurai.
- Pande, A. 1986. Biocontrol characteristics of
some moulds. Biocontrol News
and information 4 : 241.

- Papavizas, G.C. and Lumiden, R.D. 1980. Biological control of soil borne fungal propagules. Ann. Rev. Phytopathology, 18 : 389 - 413.
- Papavizas, G.C. 1981. Trichoderma & Gliocladium Biology, Ecology and potential for Biocontrol. Ann. Rev. Phytopathology 23 : 23 - 54.
- Papavizas, G.C. 1982. Biological control in crop production, Allanheld, Osmum and Co., publishers, New Jerry.
- Papavizas, G.C., Duhn, M.T., Lewis, J.A. and Beagle-Ristaino, J. 1984. Liquid fermentation technology for experimental production of biocontrol fungi. Phytopathology 74 : 1171-1175.
- Patil, V.A. and Vaishnav, M.V. 1986. Biochemical changes in Rust infected leaves of Groundnut. Indian Journal of Mycology and Plant pathology, 15 : 305 - 306.
- Patil, P.N. and Walker, J.C. 1963. Changes in free aminoacid and amide content of resistant and susceptible beans after infection with the Halo blight organism. Phytopathology 53 : 522 - 527.
- Patil, S.S. Powelson, R.L. Young, R.A. 1964. Relation of chlorogenic acid and free phenolin potato roots to infection by Verticillium, albo-atrum Phytopathology, 53 : 522-527.

- Ranganathan, R.
Mahadevan, A, and
Rangaswami, G. 1966. A comparative study of the resistant and susceptible banana varieties of fungal diseases. Biochemical changes in the banana fruit coat caused by Glocisporium musarum infection. Indian Phytopathology, 19 : 141-149.
- Ridout, C.J. Coleysmith,
J.R. Lynch, J.M. 1987. Enzyme activity and electrophoretic profile of extracellular protein induced by Trichoderma spp. by cell walls of R.solani. Rev.Plant Pathology, 66 : 144.
- Rosales, 1987. Biochemical changes in tomato plants caused by Sclerotium rolfsii. Ind. J. Myc. Plant pathology, 315 - 318.
- Rubin, B.A.
Artsikhovskaya, V. 1963. Biochemistry and physiology of plant immunity Mac Millan Co. New York, 358 pp.
- Saini, G.R. and
Sharma, C.D. 1986. All India final estimate of Kharif and rabi pulses, Agri. Situation in India, 41 : 700 - 704.
- Sindhan, G.S.
Jaglan, B.S. 1987. Role of phenolic compounds and carbohydrate in resistance of groundnut to Tikka leaf spot, Indian J. Myc and Plant pathology, 141 - 149.
- Sivan, A.A, Elad, Y.
and Chet, I. 1984. Biological control effects of a new isolate of Trichoderma harzianum on Pythium aphanidermatum

- Strastinov, Y, Elad, Y.
Swan, A. Rudich, Y.
and Chet, I. 1985. Control of R. solani fruit rot of tomatoes by T. harzianum rifai crop protection 4 : 359 - 369.
- Strech, A.W. and Cappellini, R.A. 1965. Changes in free amino acid and reducing sugars in high bush blue berry fruit. infected with Ulomerella cingulata Phytopathology 55 : 302 - 303.
- Synder, W.C. 1963. Root diseases biologically controlled. Science, 141 : 835 - 837.
- Tamini, K.M. and Wadwan, H.A. 1985. Biological effect of Neurospora sitophila and T. harzianum on growth of a range of sesamum wilt causing fungi in vitro. Indian Phytopathology, 38 : 292 - 296.
- Tomiyama, K. Eakai, R. Sakuma, T. and Izhizaka, N. 1967. The role of polyphenols in the defense reaction in plants induced by infection p. 165 - 182. Ann. Phytopathol. Soc.
- Tu, J.C. and Vaartaja, O. 1981. The effect of hyperparasite Gliocladium virens on Rhizoctonia solani and on Rhizoctonia root rot of white beans. Can. J. Bot. 59 : 122 - 127.
- Upadhyay, J.P. and Mukopadhyay, A.N. 1986. Biological control of Sclerotium rolfsii by Trichoderma harzianum in sugar beet. Trop. pest Management, 32 : 215 - 220.

- Upadhyay, R.S. and
Rai, B. 1986. Hyphal interaction and parasitism amongst some rhizosphere fungi of pigeon pea. *Biocontrol News. and information.* 7 : 241-242.
- Van Andel, O.M. 1966. Amino acids and plant diseases *Ann. Rev. Phytopathology,* 21 : 349 - 368.
- Vargas, R. and
Ramirez, C. 1983. Biological control of damping off caused by *Rhizoctonia solani* on cotton. *Agronomia costavricense* 7 : 73 - 75.
- Venkatasubbaiah, P.
Safeenlla, K.M. and
Somashekar, R.K. 1984. Efficiency of *T.harzianum* as a biocontrol agent for *R.solani* the inatant of collar rot of coffee seedlings.
- Velois, H. and
Jager, G. 1983. Biological control of *R.solani* on potatoes by antagonist. *Netherlands J. Plant Path.* 89 : 113 - 123.
- Vidhyasekaran, P.
and Kandasamy, D. 1971. Carbohydrate metabolism of *Phaseolus aureus* infected with obligate facultative parasites *Indian Phytopathology.*
- Vidhyasekaran, P.
and Parambaramani, C. 1971a. Mineral metabolism of alga infected plants. *Indian Phytopathology.*
- Vidhyasekaran, D.
Durairaj, P. 1971b. Quality of citrus fruits infected by *Xanthomonas citri* *Indian Phytopathology.*

- Weindling, R. 1934. *T. lignorum* as a parasite of other soil fungi. *Phytopathology* 22 : 837 - 845.
- Wells, H.D., Bell, D.K. and Jaworski, C.A. 1972. Efficacy of *T. harzianum* as a biocontrol for *sclerotium rolfsii*. *Phytopathology* 62 : 442 - 447.
- Wilson, K.I. and Srivastava, D.N. 1969. Chromatographic detection of chlorogenic acid and a histo chemical test for phenols in nodal tissues of sugarcane. *Indian Phytopathology*, 22 : 306 - 308.
- Windels, C.E. and Window, S.E. 1985. Biological control on the phylloplane, *American Phytopathological Society*.
- Wu, W.S. 1980. Biological and chemical seed treatments of soybean. *Memoirs of the college of Agriculture, National Taiwan, University*, 20 : 1-16.
- Wu, W.S., Liu, S.D., Chang, Y.C., Tschen, J. 1987. Hyper parasitic relationship between antagonists and *R. solani*. *Rev. Plant. Path.* 66 : 93 - 94.