STUDIES ON THE RESISTANCE OF SOYBEAN TO *Fusarium graminearum* SCHWABE

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN SCIENCE (BOTANY)
OF THE
UNIVERSITY OF NORTH BENGAL

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Dedicated to my Parents

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February 24, 1995

This is to certify that Sri Baneswar Chandra Shil, M.Sc. has carried out his research work under my supervision. His thesis entitled "Studies on the resistance of soybean to Fusarium graminearum Schwabe" is based on his original work and is being submitted for the award of Doctor of Philosophy (Science) degree in Botany in accordance with the rules and regulations of the University of North Bengal.

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ACKNOWLEDGEMENT

It is a great pleasure to express my deep sense of gratitude to my Supervisor, Dr.B.N.Chakraborty, Department of Botany, University of North Bengal for his valuable guidance, unfailing help and constant encouragement throughout the execution of this work. I would like to express my heartfelt gratitude to Dr.Usha Chakraborty, Department of Botany, University of North Bengal for her ungrudging help and advice. I am also grateful to the Director, Centre for Life Sciences and the Head of the Department of Botany, University of North Bengal for providing necessary facilities. I am indebted to all the teachers of this Department for their help at various times.

I extend my sincere thanks to Professor N.T.Keen, Department of Plant Pathology, University of California, U.S.A. for kindly sending the authentic sample of glyceollin and Professor E.W.B.Ward, Research Centre, Agriculture, Canada, Ontario and Professor J.E.DeVay, Department of Plant Pathology, University of California, U.S.A. for providing valuable literatures. I also record my sincere thanks to the Director, Pulses and Oil seeds Research Station, Berhampore for sending soybean seeds and to the Head, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi for providing the fungal cultures.

I wish to record my thanks to all the Research Fellows of Plant Pathology Laboratory, Department of Botany for their help and co-operation. Thanks are due to Asis Nag for typing this thesis with care. I am also grateful to my parents and my wife for their constant encouragement.

Baneswar Chandra Shil.

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EXPERIMENTAL

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INTRODUCTION

Soybean (Glycine max (L.) Merrill) is an ancient crop with hundreds of food, feed and industrial uses. Today, soybeans are grown to some extent in most parts of the world and are a primary source of protein and vegetable oil. Forty percent of the world's edible vegetable oil comes from soybean, which is used in margarine, salad oil and cooking oils. Each year, soybean products become more important in formulating new, low-cost nutritionally balanced high protein foods and beverages for human consumption. As soybean acreage has expanded throughout the world, diseases have increased in number and severity. More than hundreds of pathogens are known to affect soybeans; about 35 are economically important. One or more diseases can generally be found in fields wherever soybeans are grown. Fusarium root rot occurs in most soybean growing areas of the world and is considered potentially destructive in the tropics and sub-tropics.

Fusarium graminearum Schwabe has been reported to be pathogenic and causes root rot of soybean in India (Agarwal and Sarbhoy, 1978). The disease usually develops on seedlings and young plants. Older plants generally are less susceptible than younger ones. When the disease is severe, seedling emergence is slow and poor, and affected seedlings are stunted and weak. Infection is generally confined to the roots and lower stem. Cotyledons of diseased seedlings are chlorotic and later becomes necrotic. The lower part of the tap root system may be destroyed (Plate I, Figs. A & B). The pathogen is usually confined to the cortex, but vascular elements are invaded in advance stages of disease. When soil moisture is low, infected seedlings or plants may wilt and in some instances, plants in an entire field may be wilted.

One of the problems related to soybean is that most of the available cultivars are highly susceptible, while a

very few are resistasnt to root rot disease. This differential behaviour of soybean cultivars to F.graminearum aroused interest and hence it was considered worthwhile to study the mechanisms underlying this differential disease resistance.

Disease resistance in plants depends on multiple defence mechanisms which include preformed defence barriers such as cuticle, the cell wall, or constitutive antimicrobial compounds as well as defence triggered by the invaders. Phytoalexin production is one of the most extensively studied inducible defence responses. The speed, magnitude and site of phytoalexin accumulation following penetration by microorganisms determine disease resistance in a number of plant-microbe interactions (Bhattacharyya and Ward, 1986; Ebel and Grisebach, 1988; Rouxel et al., 1989; Elgersma and Liem, 1989; Nemestothy and Guest, 1990; Chakraborty et.al, 1994). Evidence that glyceollin, the pterocarpan phytoalexin from soybeans occurs in several isomeric forms was provided by Keen et.al(1971) for preparations obtained from soybean hypocotyls inoculated with the pathogen Phytophthora megasperma f.sp. glycinea. Subsequently the structures of four isomers (Glyceollin I-IV) were established by Burden and Bailey(1975) and Lyne et.al (1976). Of these, glyceollin IV has been isolated in minor amounts only, from cotyledons treated with $CuCl_2$(Lyne and Mulheirn, 1978), and no evidence that it may play a significant role in the resistant response has been provided. Glyceollin I-III are all inhibitory to mycelial growth and zoospore germination of P. megasperma f.sp. glycinea (Bhattacharyya and Ward, 1985) and have been demonstrated to accumulate in significant amounts in soybean tissues (Kaplan et.al, 1980 ; Moesta and Grisebach, 1981 ; Purkayastha et.al, 1981; Keen et.al, 1981; Purakayastha and Ghosh, 1983 ; Hahn et.al, 1985 ; Long et.al, 1985 ; Bhattacharyya and Ward, 1986; Chakraborty et.al, 1989).

The complexities of the interactions that affect the selection of parasites and allow their establishment and survival among host cells is manifest in the frequency and variability of cell surface antigens. Some intriguing research work suggest that antigenic similarities between host and pathogen may be a prerequisite for compatible reactions, or, in other words, successful establishment of the pathogen in its host depends upon some kind of molecular similarities between the two partners (DeVay and Adler, 1976; Chakraborty and Purkayastha, 1983; Heide and Smedegaard-Peterson, 1985; Alba and DeVay, 1985; Mohan, 1988; Chakraborty, 1988; Purkayastha, 1989; Protsenko and Ladyzhenskaya, 1989; Linfield, 1993; Chakraborty and Saha, 1994). There is evidence that tolerance of parasite by the host increases with increasing antigenic similarity, whereas resistance of the host is characterised by an increasing disparity of antigenic determinants(Alba et.al, 1983; Chakraborty and Purkayastha, 1987; Purkayastha and Banerjee, 1990; Purkayastha and Pradhan, 1994). It is surprising that no work has been reported so far in the above directions involving soybean and F.graminearum.

The basic objectives of the present investigation are (a) to determine the level of glyceollin in F.graminearum infected resistant and susceptible soybean cultivars; (b) to study whether disease reactions could be altered in susceptible cultivar by extraneous supply of some selective chemicals (phytoalexin inducers); (c) to evaluate the glyceollin level in susceptible cultivar after induction of disease resistance ; (d) to estimate host parasite proteins and to analyse their protein pattern; (e) to determine serological relationship between F.graminearum and soybean cultivars; and (f) to determine the cell or tissue location of major cross-reactive antigens in soybean roots.

Before going into the details of the present work, a brief review in conformity with this study has been presented in the following pages.

Plate-I Fig:(A) Healthy (left) and F.graminearum infected (right) Soybean plants (Cv.Soymax) ; **(B)** Uprooted Soybean plants (Cv.Soymax),healthy (left) and infected with F.graminearum(right)

Plate - 1

REVIEW OF LITERATURE

It is generally accepted that the cells recognize one another through pairs of complementary structures on their surfaces : a structure on one cell carries encoded biological information that the structure on the other cell can decipher. This idea represents an extension of the lock and key hypothesis. The success or failure of infection is determined by dynamic competition and the final outcome is determined by the sum of favourable and unfavourable conditions for both the pathogen and host cells. In fact, if one considers the multitude of microorganisms to which plants are being continuously exposed in nature, the significance of specificity become more apparent. Disease resistance/susceptibility in plants exhibit a high degree of specificity at many levels. Available evidence indicates that resistance to disease in many cases is the result of activation of more than one biological defense mechanism (Ebel and Grisebach, 1988) but specific mechanism for eliciting resistance have not been established. Many plants accumulate phytoalexins as part of an inducible defense mechanism in response to pathogen invasion or treatment with biotic or abiotic elicitors. The success of some pathogenic fungi depends upon their ability to detoxify or suppress the synthesis of phytoalexins. For example, the fungus Mycosphaerella pinodes produces both an elicitor and a suppressor of the induction of pisatin, the phytoalexin in Pisum sativum. Purpose of this review is to present briefly the observations of previous workers in concord with the present line of investigation. However, this review is selective rather than comprehensive. A few aspects such as (A) evaluation of the role of phytoalexins in plant disease resistance, (B) plant disease alteration by chemical treatment and (C) serological relationship between host and parasite have been discussed in the following paragraphs.

EVALUATION OF THE ROLE OF PHYTOALEXINS IN PLANT DISEASE RESISTANCE

Phytoalexins constitute a chemically heterogenous group of substances belonging to various classes of natural products which include isoflavonoids, sesquiterpenoids, polyacetylenes and stilbenoids. Many phytoalexins are absent in healthy unchallenged plants. It was originally beleived that phytoalexins were host specific. With the evidences accumulated so far concerning the wide spread occurrence, isolation and characterization of phytoalexins during the past several years, it is now clear that more than one phytoalexin could occur in a single host species of which one may be dominant. Again, similar phytoalexins may also occur in different host species. Plant organs including roots, stem, leaves and fruits hasve been shown to respond to infection with the formation of phytoalexins. Among plant pathogens, fungi, some bacteria and viruses are capable of inducing phytoalexin production in plants, but involvement of last two groups of organisms seems to be quite negligible in comparison with the large group of fungi. During incompatible host-parasite interaction, phytoalexin is synthesized rapidly and accumulates at the infection site. In contrast, in the compatible host-parasite interaction the plant slowly synthesizes the phytoalexin.

Accumulation of phytoalexin is believed to be an important early defense response in several plant pathogen interaction. So far, it has been demonstrated in more than 100 plant species representing 23 families. A lot of work has been done and several comprehensive reviews have appeared on phytoalexins and their role in disease resistance (Cruickshank, 1963, 1977, 1980 ; Kuc, 1966, 1972, 1976; Deverall, 1972, 1976, 1977 ; Ingham, 1972, 1973; Keen and

Brugger, 1977, Purkayastha, 1973, 1976, 1985, 1986; Bailey, and Deverall, 1983; Harborne and Ingham, 1978; Van Etten and Puppke, 1976; Van Etten et.al, 1982, 1989 ; Wood, 1982; Ward, 1986 ; Paxton, 1988; Ebel and Grisebach, 1988, Lamb et.al, 1989; Keen, 1981, 1982, 1990; Chakraborty et.al, 1995). Since it is not possible to incorporate all the work done so far in the line of phytoalexin research in this brief resume, some selected observations have been incorporated in the following paragraphs.

In 1940, Muller and Borger demonstrated that potato tubers developed localized resistance to a virulent race of Phytophthora infestans when the same tuber was preinoculated with an avirulent race of the same fungus. They postulated that a non-specific principle was formed within the host after infection which checked the growth of the virulent strain. This principle was called "Phytoalexin". Later Oku and Nakanishi(1962) demonstrated the relationship between a phytoalexin like antifungal substance in the leaf diffusate and resistance of rice plants to Helminthosporium oryzae. Resistant variety used in their experiment showed relatively higher antifungal activity than the other varieties, though the activity varied with the experiments. The upper leaves of rice plant proved to be most resistant. Resistance to lesion enlargement was also enhanced by 2,4 D or IAA, but the production of anti-fungal substance was stimulated only by IAA treatment.

A reddish brown crystalline substance was isolated from soybean plants resistant to Phytophthora sojae after inoculation with the said pathogen. The same material was produced by both resistant and susceptible soybean cultivars inoculated with either P. megasperma or P. cactorum, both species usually being non-pathogenic to soybean. This phytoalexin like substance was related to disease resistance of soybean(Klarman and Gerdemann, 1963). The production of

phytoalexin by leaf and endocarp tissues of broad bean (Vicia faba L.) was demonstrated by Purkayastha and Deverall (1964, 65). They observed that the spores of B. cinerea germinated on leaf surfaces but germ tube growth was rapidly inhibited. The inhibition of growth was due to the production of a phytoalexin like compound. This work led to the isolation and identification of wyerone acid as a compound which accumulated in the infection droplet containing B. cinerea in the seed cavity of Vicia faba(Deverall, 1967, Deverall and Vessey, 1969 ; Letcher et.al., 1970).

Gray and Klarman(1967) compared phytoalexin production by susceptible and resistant soybean varieties. The resistance of Harasoy 63 was conditioned by a single gene which also apparently provides for the production of a large amount of phytoalexin unlike the susceptible cultivar. However, the action of this resistant gene depends upon a stimulus provided by Phytophthora megasperma var. sojae or to a lesser extent by other closely related fungi. Klarman(1968) reported that resistant soybean plant Harosoy 63 produced seven times as much phytoalexin as the susceptible Harasoy plant when inoculated with P.megasperma Var. sojae when a portion of phytoalexin was removed from the inoculation wounds, Harosoy 63 plants became as susceptible as Harasoy plant. Harosoy 63 produced only twice as much phytoalexin as Harosoy when both were inoculated with a non-pathogenic isolate of P.megasperma. The non-pathogen isolate of P.megasperma was four times more sensitive to soybean phytoalexin than P.megasperma var.sajae. Similar observation was also made by Gray et.al. (1968) who also noted that when the challenging fungus was a non-pathogenic strain of P. megasperma, Harasoy 63 produced only twice as much inhibition as Harosoy. A closely related fungus P.cactorum was also non-pathogenic on soybean but stimulated equal amount of inhibitor in both the varieties.

Frank and Paxton(1970) compared the production of phytoalexin following different periods of inoculation and found that phytoalexin production and fungal development were similar in both the cultivars up to 4 h. but the disease developed rapidly and the hypocetyls collapsed within 48 h. of inoculation in susceptible cultivar. Production of phytoalexin, however, continued in the resistant cultivar and after 24 h. the plant cells surrounding the fungus became discoloured and the fungal invasion was arrested. It has been suggested that the reaction responsible for resistance or susceptibility occurs between 4 and 8 h. when phytoalexin production either increases or declines. Both resistant and susceptible hypocotyls of soybean were more resistant with age and accumulated more phytoalexin (Hydroxy phaseollin) after inoculation with P.megasperma var.sojae(Keen, 1971). The accumulation of Hydroxyphaseollin was about 10-fold higher in the inoculated hypocotyls of Harosoy 63 soybeans(Monogenically resistant to P.megasperma var. sojae) than in a near isogenic susceptible cultivar, Harosoy (Keen, et.al. 1971) ; Keen and Horsch, 1972). A substance capable of inducing phytoalexin production in soybean plants was isolated from P.megasperma var. sojae which was a glycoprotein. Production of this inducer was the key reaction attributed to the Rps gene for resistance in soybean(Frank and Paxton, 1971).

Birdge and Klarman (1973) detected hydroxyphaseollin(HP) in soybean hypocotyls 12 h. after UV irradiation when plants were maintained in darkness. Maximum concentrations of HP however, occured 96 h. after irradiation. Hypocotyls placed in light immediately after irradiation contained almost no HP. When genetically susceptible plants were irradiated they became less susceptible to the soybean pathogen P.megasperma var. sojae.

Capsidiol accumulated during an incompatible interaction between pepper fruit tissue and Phytophthora

infestans (Jones et al, 1975). The concentration of capsidiol in the tissue proved to be totally inhibitory to fungal growth after 24 hrs. of inoculation. The restriction of hyphal growth in the tissue was related to rapid accumulation of capsidiol Purkayastha and Ray(1975) noted the accumulation of an antifungal compound in jute leaves after infection by Colletotrichum corchorum. Differential antifungal activities of rice cultivars resistant and susceptible to Helminthosporium orzae, were also reported by Purkayastha and Chattopadhyay (1975) and Purkayastha and Mukhopadhyay(1976).

Metlitskii(1975) demonstrated the protective role of phytoalexin in potato, tomato and many other plants. Studies on phytoalexins and resistance of tomato to early dry spot revealed that the resistance of tomato to Alternaria solani was highest in the varieties 'Novinka' 'Marinadny' and 'Sputnik'. the main difference between resistant and susceptible variety was the rate of rishitin production. Young plants had higher phytoalexin activity and were less vulnerable to attack than old and weaker plants (Darozhkin and Nanyuk, 1976).

Association of the phytoalexin kievitone with single gene resistance of cowpea to Phytophthora vignae was demonstrated by Patridge and Keen(1976). Cowpeas monogenically resistant to P.vignae accumulated more kievitone than that required to inhibit 50% mycelial growth of P.vignae in culture.

Johnson et al, (1976) explained the possible role of phytoalexin in the resistance of sugarbeet (Beta vulgaris) to Cercospora beticola. Leaves of sugarbeet infected with C. beticola yielded two compounds viz., the flavanone betagarin and the isoflavone betavulgarin. Highly resistant cultivars appeared to produce greater quantities of betavulgarin in the

lesions than the more susceptible ones. Betavulgarin plays a significant role in the resistance of sugarbeet. Three phytoalexins viz., phaseollin, phaseollidin and phaseollin isoflavone were detected in the resistant bean plants 48 h. after inoculation with Uromyces phaseoli but not in the non-inoculated plants or in inoculated susceptible variety. Phaseollin and phaseollidin were found to inhibit germination of rust uredospores. The possible role of said phytoalexins in inhibition of fungal growth in the incompatible combination was discussed by Elnaghy and Heitifuss(1976).

The induction of phytoalexin in pea plants by treatment with sodium azide was reported by Hadwiger et.al, (1976). Treatments of rice plants with 2,2-dichloro-3, 3-dimethyl cyclopropane carboxylic acid (WL 28325) exerted systemic fungicide activity against rice blast (Pyricularia oryzae) by activating the natural resistance. The activity of WL 28325 was unique in that it did not itself stimulate phytoalexin (Momilactone A and B) production but rather increased the capacity of rice to synthesize phytoalexin in response to infection. This may be the basis for the disease protectant property (Cartwright et.al., 1977).

Medicarpin was detected in leaf tissues and stem segments of Medicago sativa infected with V.albo-atrum (Khan and Milton, 1978). The role of glyceollin in restricting fungal growth of P.megasperma var. sojae, race 1 in resistant soybean hypocotyls was discussed by Yoshikawa et.al., (1978). They observed that the pathogen grew at a similar rate in both susceptible(Harosoy) and resistant (Harosoy 63) soybean hypocotyls upto 96 h of inoculations. After 96 h., growth of fungus was rapidly inhibited in resistant hypocotyls only. When resistant cultivars were treated with blasticidin 'S', the growth of fungus was not inhibited. High and localized accumulation of glyceollin was not detected in the inoculated

hypocotyls of either cv.Harosoy or cv.Harosoy 63 treated with blasticidin 'S'. These results strongly suggest that glyceollin accumulation accounts for cessation of fungal growth in the resistant soybean hypocotyls.

Yoshikawa et.al., later reported in 1978 that the resistance of Horosoy 63 soybean hypocotyls to P.megasperma var. sajae was completely dininished by actinomycin D or blasticidin-S. Production of glyceollin was also concomitantly reduced. Actinomycin D inhibited the synthesis of Poly(A) containing messenger RNA in healthy soybean hypocotyls of cv. Harosoy 63 and in hypocotyls inoculated with P.megasperma var. sojae but had little effect on protein synthesis within 6 h. Blasticidin 'S', conversely inhibited protein synthesis in the hypocotyls without exhibiting significant effects on messenger RNA synthesis. These results indicated that the normal expression of resistance to the fungus and production of glyceollin both require de novo messenger RNA and protein synthesis early after infection. Furthermore, actinomycin D and blasticidin 'S' were also effective in suppressing resistance expression and glyceollin production in soybean hypocotyls when inoculated with various Phytophthora species that were non-pathogenic to the plants. This indicates that mechanism of general resistance to these non-pathogenic fungi also involves de novo messenger RNA and protein synthesis and production of glyceollin.

In an attempt to produce phytoalexin in potato, Derevenko and Golik(1978) used Zoospores from fresh warts for inoculation. Zoospores from fresh warts were more active inducers of phytoalexin than that of winter zoospores. The production of phytoalexin was always greater in resistant cultivars than in the susceptible one.

Keen and Littlefield(1979) isolated coniferyl alcohol and coniferyl aldehyde from leaf diffusats of flax

inoculated with incompatible race of Melampsora lini. These compounds accumulated more rapidly in all incompatible reactions than compatible ones. The evidence indicates that de novo production of said compounds may be the mechanism for restriction of fungus growth in incompatible flax leaves.

Phytoalexin production as well as degradation were related to the resistance of red clover leaves to Sclerotinia and Botrytis spp. (Macfoy and Smith, 1979). S.fructicola formed no lesions at all, whereas S.fructigena and B.allii formed flecks under the infection droplets. In case of B.fabae the lesions were larger whereas those produced by B.cinerea were restricted. S.trifoliorum, on the other hand, formed spreading lesions. Both B.fabae and S.trifoliorum which produced larger or spreading lesions degraded phytoalexins (medicarpin) at a faster rate. B.cinerea, S.fructigena, S.fructicola and B.allii which produced flecks or restricted lesions were more sensitive to phytoalexin.

It appears from the report of Yoshikawa et al., (1979) that the soybean photoalexin glyceollin accumulates more rapidly in Harosoy 63 soybean hypocotyls with an incompatible race of P.megasperma var. sojae, than with a compatible race of the fungus. But there was no significant difference between the two when the rate of glyceollin biosynthesis was estimated by pulse-labelling experiments with [^{14}C] phenylalanine. In contrast, "pulse-chase" experiments with [^{14}C] phenylalanine revealed that glyceollin biodegradation activity which was normally present in the unioculated hypocotyls appeared to be inhibited more strongly in the hopocotyls infected with incompatible race than in the hypocotyls infected with compatible race. The fungus did not appear to contribute directly to the differential glyceollin degrading activity in the infected hypocotyls since neither compatible nor incompatible race degraded glyceollin in vitro.

These results suggested that differential glyceollin degrading activity of the hypocotyls following infection was of importance in determining ultimate levels of glyceollin accumulation in the infected hypocotyls. Uninoculated hypocotyls also synthesized glyceollin after wounding but glyceollin did not accumulate, apparently due to the presence of active glyceollin degrading activity.

Poplar resistant to Melampsora larici-populina produced 2-3 times more phytoalexin than the susceptible cultivars. Fungitoxicity of diffusates gradually increased during the experiment. However, it depended on light and temperature. The fungitoxicity was markedly lower at 9°C than 24°C (Denbnovetskii and Basova 1979).

The effect of fifteen phytoalexin inducers on the severity of brown spot disease of rice seedlings was studied by Giri and Sinha(1979). In all cases disease was less than in water sprayed control. The most promising chemicals were sodium malonate, sodium molybdate, cadmium chloride, ferric chloride and DL-methionine.

Corn inbreds resistant and susceptible against Helminthosporium turcicum were tested for phytoalexin production and the results revealed that all resistant lines produced antifungal or phytoalexin like compounds three days after inoculation. The quantity of antifungal compound and the time required for maximum production differed with the inbreds, but a correlation could be established between disease rating and production of antifungal substances. It has been suggested that the phytoalexin method could be used in a breeding programme for selecting plants for disease resistance (Obi et al, 1980).

Phytoalexin accumulation in hypocotyls of two cultivars of Phaseolus vulgaris in response to infection by

three isolate of B.cinerea differing in virulence was studied. The yield of phytoalexins was always higher in the less susceptible cultivar and highest in lesions produced by the least virulent isolates. Yield was lowest in case of virulent one but when challenged with $HgCl_2$, the yield was same in both the cultivars, the accumulation patterns being different from those with B.cinerea(Fraile et al, 1980).

A series of crosses were made between isolates of Nectria haematococca mating population VI that differed in sensitivity to the pea (Pisum sativum) phytoalexin, pisatin by Tegtmier and Van Etten(1982). The progeny were tested for sensitivity to pisatin, ability to demethylate pisatin, and virulence on pea. Of the three progeny analysed, all of the moderately or highly virulent progeny were tolerent to pisatin and able to demethylate it. Therefore, either pisatin tolerance and demethylating ability are required for virulence on pea or genes for pisatin tolerance and dimethylating ability are closely linked to genes for virulence. The results of this study indirectly support the hypothesis that pisatin accumulation is an active mechanism of resistance in pea.

Purkayastha and Chakraborty (1983) correlated the resistance of soybean cultivars to charcoal rot disease caused by Macrophomina phaseolina with greater production of phytoalexin. Glyceollin content of resistant soybean cvs, UPSM-19 and DS-73-16 were 400-421 /ug/g fresh wt., while of susceptible cvs. Soymax and R-184 were 265 and 297 /ug/g fresh wt. respectively. In another study Purkayastha et al (1983) demonstrated that momilactone A was associated with the resistance of rice plants to sheath rot disease caused by Acrocylindrium oryzae. Coleoptiles of tall cultivars resistant to A.oryzae contained greater amount of momilactone A (12.9 - 21.36 /ug/g fresh wt.) while the semidwarf cultivars contained relatively lower amount (5.58-8.14 /ug/g fresh wt.).

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Difference in momilactone A content of leaf sheaths of tall CV. Mahasuri (19.36 /ug/g fresh wt.) and semidwarf cv. Jaya (8.64 /ug/g fresh wt.) was also significant.

Bhattacharyya and Ward (1985) separated and purified glyceollin isomers I, II and III from soybean cotyledons (cv. Harosoy 63) after inoculation with Phytophthora megasperma f.sp. glycinea race-1. ED_{50} values for glyceollin I, II and III were 33, 12.2 and 13.9 /ug/ml respectively. Glyceollin II (ED50 7 /ug/ml) was the most active against zoospore germination and caused zoospores to burst.

The expression of resistance and susceptibility to P.megasperma f.sp. glycinea race 1 was determined in roots, hypocotyls, and cotyledons of etiolated and green seedlings and in leaves of soybean cvs, Harosoy and Harosoy 63. Gene-specific resistance was demonstrated in all organs tested, except for cotyledons of etiolated seedlings. In each case higher concentrations of the glyceollin accumulated in resistant than in susceptible reactions ; the difference being greatest in hypocotyls and smallest in roots. The relative proportions of glyceollin I, II and III varied with the organ, exposure of the seedlings to light, the interactiion type and the incubation period. Glyceollin I was relatively the most abundant isomer in roots and to a lesser extent in hypocotyls. Glyceollin III was relatively the most abundant isomer in leaves. Major differences in accumulation rates were observed in time course experiments and after reaching a maximum, all three isomer decreased in leaves and glyceollin I decreased in hypocotyls, suggesting that concentrations and hence isomeric proportions were finely controlled by rates of biosynthesis and metabolism(Bhattacharyya and Ward, 1986).

Biosynthesis and metabolism of glyceollin I in soybean hypocotyls following wounding or incubation with

P.megasperma f.sp. glycinea were studied by Bhattacharyya and Ward(1987). They pointed out that the stimulus of wounding or infection induces a metabolic pathway in which glyceollin I is not an end product. The accumulation of higher levels of glyceollin I in resistant than in susceptible responses appeared to be due to earlier initiation and subsequently higher rate of biosynthesis in the former. The interaction of soybean, cv. Harosoy 63 with P.megasperma f.sp. glycinea race 1(incompatible) and race 4(compatible) and of the near isogenic cv. Harosoy with these races (compatible) was examined by light and electron microscopy in hypocotyl tissues fixed 2,3,5 and 7 h after inoculation (Ward et al, 1989). The extent and rate of colonization was similar in both incompatible and compatible interactions. Hyphae reached the first layer of the cortex by 2 h and the third layer by 3 h. However, there were major differences in host cell responses. Ward (1989) also demonstrated that susceptibility of immature soybean leaves to P.megasperma f.sp. glycinea was possible due to deficiencies in metabolic precursors required for the expressionm of resistance and glyceollin sysnthesis was tested by supplying sugars, glyceollin precursors and growth factors. Only glucose, galactose and sucrose were effective and promoted resistance to P.megasperma f.sp. glycinea race-1 in both cv. Harosoy 63 and Harosoy. Resistance was associated with significant levels of glyceollin accumulation. More immediate precursor of glyceollin were ineffective. In this case glucose may function as a source of energy required for the development of resistant responses or for leaf maturation rather than a source of glyceollin precursors.

Elgersma and Liem(1989) isolated two phytoalexins, the terpenoid rishitin and the polycetelene cistetradeca-6-ene-1, 3 diyne-5,8-diol from susceptible and resistant tomato lines inoculated with Verticillium albo-atrum or Fusarium oxysporum f.sp. lycopersici. Inoculation concentrations of 10^5, 10^6, 10^7 and 10^8 conidia/ml had no differential effect on

phytoalexin accumulation at 3 days after inoculation and also no difference of fungal growth both in susceptible and resistant cultivars during that period. A cultivar containing gene for Fusarium wilt resistance contained more rishitin than did susceptible plants at 2 and 3 days after inoculation but at 7 and 11 days after inoculation more rishitin had accumulated in the susceptible plants.

A phytoalexin was isolated from the leaves of Brassica juncea cv. Aurea inoculated with Leptosphaeria makculans (Kollmann et al, 1989). It prevents spore germination and hyphal growth of L.maculans at 12 /ug/ml in water. It was not detected in healthy plant tissues.

Phytoalexins (pisatin and 4-hydroxy-2,3,9-trimethoxy pterocarpan) was isolated from both Fusarium solani f.sp. pisi and Rhizobium-Fusarium- infected pea epicotyls (cv. Arkel and Sweet stringlen) 4-hydroxy 2,3,9 trimethoxypterocarpan was present in a greate amount in the latter than in tne former, while pisatin concentration was similar in samples from both treatments (Chakraborty and Chakraborty, 1989).

Nemesstothy and Guest (1990) found tne NC 2326, a cultivar of tobacco resistant to Phytophthora nicotiane var. nicotianae, respond to stem inoculation by rapidly accumulating sesquiterpenoid phytoalexins and activating phenylalanine ammonia lyse activity ast the infection front. But in susceptible cultivar both responses were slower. Pretreatments of leaf discs with propylene oxide, which kill the cells, mevinolin, a specific inhibitory of sesquiterpenoid biosynthesis, or the non-specific aminotransferase inhibitor, aminoxyacetic acid inhibit post infection phytoalexin accumulation in both cultivars and induce susceptibility cv. NC-2326. They also noted that Fosetyl-Al reduces pathogen growth in the initial stage of infection, before the resistance was expressed.

Phytoalexin accumulation in the forage crop, Medicago sativa as defence against the soil borne wilt pathogen Verticillium albo-atrum was studied by Smith and Hilton (1992). One of the two isolates of the fungus was pathogenic. Effects upon phytoalexin accumulation of elicitors prepared from cultures of the two isolates and the effect on the activity of phenylalanine ammonia lyase has also been measured.

PLANT DISEASE ALTERATION BY CHEMICAL TREATMENT

Significant levels of success has been achieved by using chemicals of widely diverse nature without any direct toxic action against many plant diseases (Sinha, 1984). Apart from chemicals, physical agents such as X-ray(Purkayastha and Ghosh, 1983), UV(Bridge and Klarman, 1973) and biological agents (Sinha & Das, 1972, Chakraborty and Chakraborty, 1989) are also known to alter disease reaction. Numerous molecules have been implicated in mediating disease resistance. There is evidence that some products either of biotic or abiotic origin are capable of activating the host's defense reactions by accumulating secondary metabolites or "stress" metabolites such as phytoalexin, in treated (physically or chemically) plants (Darvill and Albersheim, 1984; Purkayastha, 1986). Several elicitors or phytoalexin synthesis also induce the expression of other host plant defense responses (e.g., proteinase innibitor synthesis and accumulation of hydroxyprotein-rich glycoproteins). In some of the recent reviews the mechanisms of induced resistances in plants have been well documented (Ouchi, 1983; Sequeira, 1983; Halverson and Stacey, 1986; Madamanchi and Kuc, 1991).

The differential effect of maleic hydrazide on the growth of leaf and stem rust of wheat was studied by Samborski

et al., (1960). It was concluded thast this metabolic inhibition could induce susceptibility in the resistant Khalpi variety of wheat. Similarly Oku(1960) presented evidence to indicate that resistance of rice plants to cochliobolus miyabeanus could be broken down by treatment with reducing agents such as ascorbate or glutathione. The resistance of rice plants against hyphal penetration by C.miyabeanus could partially be attributed to fungal oxidation product, perhaps quionones, derived from host cells or membranes.

The influence of gibberelic acid on the seedling blight of corn was noted by Wilcoxson and Sudia (1960). they observed that treatment of maize hybrid seeds with, 5,10 and 20 ppm gibberelic acid enhanced the severity of seedling blight. foliar spray with 50 ppm of potassium gibberellate augmented disease intensity in red kidney beans inoculated with California isolates Rh-5 or Rhizoctonia solani (Peterson et al., 1961).

Use of Nickel chloride as foliar spray to tea plants (Camellia sinensis) for the control of Blister blight caused by Exobasidium vexans was demonstrated by Venkataram (1961). Percentage shoot infection was lower in nickel chloride than in the cuprous oxide treatment.

Hale et al., (1962) reported that growth regulators (viz., indole-3-acetic acid, naphthalene acetic acid, 2,4-dichlorophenoxy acetic acid and maleic hydrazide) caused an increase in size and number of leaf spots/plant on the susceptible inbred corn line K-44 and the resistant line K-41 when the plants were inoculated with Helminthosporium carbonum.

Severity of lesion development on the hypocotyls of red kidney bean increased by foliar applications of gibberelic

acid when plants were grown in soil infested with Rhizoctonia solani, isolate Rh-5. However, when the plants were treated with gibberellic acid and grown in soil infested with two other pathogenic isolates of R.solani, severity of the diseases was not affected. The increased virulence of Rh-5 was probably caused by root excretions resulting from the gibberellic acid treatments (Peterson et al., 1963).

It was speculated by Daly and Deverall (1963) that hormonal concentration in a leaf could be important in controlling the development of a pathogen. The initial establishment of the disease could be due to hormonal changes brought about by the entry of the pathogen. Sequeir (1963) also reported that diseased plants contained altered concentrations of hormones, the causes underlying these changes being unknown.

Foliar application of different concentration of IAA and GA to detached bean leaves and little effect on lesion production by Botrytis fabae and Botrytis cinerea and were ineffective in the spread of lesion by B.cinerea (Purkayastha and Deverall, 1965).

The effect of maleic hydrazide (MH) on whea and ba rley rust was studied by Joshi (1965). The solutions of maleic hydrazide(0.02 percent) were administered to wheat roots and barley seedlings at the time of emergence. The doses, however, varied between 50-120 ml/pot (10 cm diam.). Barley plants (varieties Bolivia and Oderbrucker) treated with ·2% MH solution (110 ml/pot) showed reduction in growth and higher susceptibility of plants to Puccinia hordei. The response was very poor in case of Agra local variety.

Foliar or soil application of CCC [(2-chloroethyl) trimethyl ammonium chloride] reduced the infections of bean seedlings by Sclerotium rolfsii (Tahori et al., 1965). But

Crosier and Yountburg(1967) reported that CCC (2-4 pounds of CCC/acre) was ineffective against tilletia foetida on winter wheat when used alone as foliar spray.

Sinha and Wood (1967) has shown that IAA reduced wilt disease of tomato caused by Verticillium alboatrum. On the other hand, Maleic hydrazide (300 ppm), greatly retarded growth of the plant and made them susceptible. Cytocel and naphthalene acetamide gave good control of disease over a range of concentrations when applied to the soil in which the plants were growing. Of the other growth regulating substances tested, 2,4,6, trichlorophenoxyacetic acid increased disease at some concentrations and reduced it at others.

Chalutz and Stahmann (1969) induced pisatin formation in carrot tissue by ethylene. However, production of pisatin in pea tissues in response to ethylene treatment was less than that induced by fungi. It is possible that ethylene could induce some of the enzymes (Phenylalanine deaminase) involved in the biosynthesis of pisatin. Carrot roots treated with IAA, 2,4-D and 2,4,5-T also elicited coumarin accumulation. In all cases, production of isocoumarin was related to the amount of ethylene produced by the root tissue. Foliar spray with either GA_3 or CCC (both 1 and 100 ppm) increased susceptibility of jute seedlings growing in Macrophomina infested soil. Maximum susceptibility observed when treated with GA_3 but minimum in case of CCC treated plants (Purkayastha et.al., 1972) under the influence of IAA and GA_3 some aspects of host parasite relationships were studied by Valken (1972). He reported that IAA increased the Fusarium wilt of tomato while the reverse result was obtained with GA_3.

Furrer and Staulfer (1972) demonstrated that by the application of Cycocel in combination with nitrogen, yields of spring wheat was augmented lodging and eye spot caused by

Cercosporella herpotrichoides was reduced • Bojarezuk and Ruszkowski(1972) also noted that application of cycocel (3-4 kg/nac) at the end of tillering reduced eye spot infection in wheat and rye and increased yield in both cases. The effects were more pronounced in varieties susceptible to lodging with high nitrogen fertilizer. Efficacy of cycocel against grey rot of grapevine caused by Botrytis cinerea was tested by Natalina and Svetvov in 1972. About 25-50% reduction in the incidence of disease was recorded after spraying vine with cycocel. But cycocel treatment increased the infection of jute caused by Septoria nodrum.

Artificial application of natural and synthetic chemicals could also induce disease resistance in plants. Sharma(1973) reported that application of DI-tryptophan, IAA and HCN induced resistance in some sorghum varieties to Colletotrichum graminicola. Particularly, 0.12% and 0.062% of KCN(in place of HCN) proved to be effective in inducing the resistance. The most encouraging results were obtained with 25+5, 25+10, and 50+5 ppm concentrations of DL tryptophan and zinc, respectively (Zinc is known to take part in the conversion of tryptophan to IAA). By the application of IAA (50 ppm) resistance to C.graminicola was noticed more than DL-tryptophan (25 or 50 ppm).

Sad and Rashid (1973) recorded that 16 ppm CEPHA (2-chloro-ethale phosphonic acid) co ntrolled the chocolate spot disease of potato and induced the production of small size tubers and tuber crackie(4%). Similarly, 25 ppm GA also controlled the disease but induced Knobiness (12%) and sprouting (15%) of tubers in the field. On the other hand, application of IAA (80-50 ppm) did not influence disease but induced the production of large size tubers. It is interesting to note that a mixture of CEPHA and IAA when sprayed 2 weeks asfter flowering decreased disease incidence considerably and undesirable side effects produced by CEPHA alone were not observed.

The gibberellins and tri-iodobenzoic acid decreased severity of charcoal rot disease of soybean under all experimental conditions (Oswald and Wyllie, 1973). The effects of indoleacetic acid and kinetin on the development of Verticillium wilt of cotton was explained by Abrarov et.al., (1973). These compounds innibited the spread of necrosis of leaf blades and stimulated formation of leaves and generative organs.

The role of auxins in leaf spot incidence in ragi was discussed by Vidyasekaran(1976). Young leaves of ragi (Eleusine coracana) were resistant to the blight disease caused by Helminthosporium tetramera while the older leaves were highly susceptible. Young leaves contained more auxin than the older leaves. The IAA treatment innibited spore germination and growth of the pathogen only at high concentrations.

The effect of foliar application of plant hormones on the development of anthracnose disease caused by Colletotrichum corchorum in two cultivars of jute (Corchorus capsularis) were studied by Purkayastha and Ray (1977) under identical conditions. These hormones were also tested on the growth of the pathogen in vitro. Gibberellic acid (10 and 100 ppm) and indole acetic acid (10 ppm) increased disease susceptibility in both resistant and susceptible cultivars. These compounds stimulated mycelial growth of C. corchorum at a low (0.1 ppm) concentrations. Apparently there was no correlation between mycelial growth and pathogenecity of the fungus. Inflorescences of grapevine sprayed with 10 ppm gibberellic acid signsificantly reduced Botrytis infection (Rivera and Mavrich, 1978). A fungicide known as 2,2-dichloro-3,3-dimethyl cyclopropane Carboxylic acid (WL 28325) has been found to activate the natural resistance of rice plants against blast disease caused by Pyricularia oryzae. The activity of WL-28325 is unique in that it does not

itself stimulate phytoalexin production but rather increases the capacity of rice plants to synthesize more momilactones (rice phytoalexins) in response to fungal infection. The antifungal activity of rice phytoalexin may be basis for its disease production properties (Cartwright et.al., 1977; Cartwright et.al., 1980).

Mercuric acetate caused accumulation of rishitin and lubimin in potato tuber discs. Accumulation of these terpenoids was not directly correlated to the necrotic reaction (Cheema and Haard, 1978). When two cultivars of P.vulgaris showing different degrees of susceptibility were treated with $HgCl_2$, the yield of phytoalexin was similar in both the cultivars. However, the accumulation pattern differed when inoculated separately with 3 isolates of Botrytis cinerea differing in virulence (Cheema and Haard, 1978).

In the glass house, applications of 2,4-D(40% butyl ester) and atrazine(72%) increased susceptibility of soybean to blight disease caused by Sclerotium rolfsii. Incidence was higher in plants with low or high sugar content, but lowest on those with normal sugar content. It was also noted that monosodium phosphate, zinc sulphate, 2,4-D and atrazine were mildly phytotoxic (Carlos, 1979).

Some growth retardants mitigated Verticillium wilt and increased yield of cotton. Particularly the application of chloromegnat [(2-chloroethyl) trimethyl ammonium chloride], Pix (N,N-dimethyl piperidinium chloride) and chemagro 8728 [tributyl] slightly mitigated the severity of symptoms of V.dahliae on cotton and reduced internal populations of the pathogen in the petioles, cotton yield was increased (10.29%) by these treatments (Erwin et.al., 1979).

The effect of mercuric chloride on glyceollin synthesis or degradation of glyceollin was tested by Moesta

and Grisebach, (1980). They observed that $HgCl_2$ produced only a slight effect on the biosynthetic activity but strongly inhibited glyceollin degradation.

The effect of foliar spray of bacitracin, chloramphenicol and GA on the rhizosphere microflora of pea seedlings (P.sativum L.) infected with V.dahliae were studied. The antibiotics increased fungus and actinomycetes counts and reduced the bacterial populations in the rhizosphere. Ten ppm GA reduced all three groups of micro-organisms while 100 ppm increased actinomycetes slightly. Foliar sprays also affected the percentage occurrence of particular genera of fungi in the rhizosphere, for examples, Trichoderma spp. were stimulated by all treatments, the maximum being with 10 ppm GA. Foliar spray however, markedly reduced disease severity (Ramarao and Issaac, 1980).

The effect of three growth substances 6-Furfuryl aminopurine (Kinetin), 6-Benzyl aminopurine (BAP) and gibberellic acid (GA_3) on the development of charcoal rot disease of soybean caused by Macrophomina phaseolina was studied by Chakraborty and Purkayastha (1981). Two foliar sprays with 1 or 10 ppm GA_3 at an interval of 3 days before inoculation of plants reduced the disease significantly. But the application of 10 ppm kinetin or BAP markedly augmented the disease.

Eight chemicals reported to induce phytoalexin production in plants were used for wet seed treatment in an attempt to develop resistance in susceptible rice seedlings to Drechslera oryzae, the brown spot pathogen. While all produced appreciable effects, cysteine, thioglycollic acid, cyclohexamide, sodium selenite, P-chloromercuribenzoate and lithium sulphate caused marked reduction in symptoms in rice seedlings when challenge inoculation at the age of 3-4 weeks. With sodium selenite and thioglycollic acid the induced effect persisted upto 8 weeks after sowing. A second treatment in the

form of foliar spray with these chemicals caused sharp increases on protection, but this disappeared 2 weeks after treatment. Leaf diffusates from 2 week old seedlings in different treatment showed considerable fungitoxicity, which declined with seedling age and became practically non-existent by the end of fourth week. Inoculation of treated plants at this age resulted in moderate to marked toxicity in their diffusates. Seed treatment was found to be more effective than foliar spray treatment(Sinha and Hait, 1982). Accumulation of phytoalexin in excised cotyledons of P.vulgaris was detected when treated with 10^{-4}M abscisic acid or Benzylaminopurine(BAP). In case of former, cotyledons were incubated both in light and in dark but in case of latter, they were kept under light only (Stoessel and Magnalato, 1983).

Furocoumarin (phytoalexin) was induced in celery by copper sulphate (Bier and Oertelli, 1983). Capsidiol (pepper phytoalexin) production has also been induced in fruits of Capsicum annum by 0.1 M copper sulphate, sodium nitrate and chloramphenicol (Watson and Brooks, 1984). Gibberellic acid (GA_3) induced momilactone synthesis in healthy dark grown rice coleoptiles as well as leaf sheaths and markedly stimulated momilactone biosynthesis in treated inoculated (with Acrocylindrium oryzae) leaf sheaths and coleoptiles. Since GA_3 is a degraded diterpene it may act as a precursor of gibberellin mediated enzyme (associated with momilactone biosynthesis) which may count for the elicitation of momilactone synthesis in rice plants.(Ghosal and Purkayastha, 1984).

Seed treatment of wheat with dilute concentration of nickel chloride and Barium sulphate significantly induced resistance to Drechslera sorokiniana (Chakraborty and Sinha, 1984).

Twenty out of twenty four chemicals known to induce phytoalexin production in other plants when used as seed treatment provided effective protection to 3-week-old susceptible wheat seedlings against inoculation with Helminthosporium sativum. The number of lesions was very significantly reduced by most of these treatments and there was evidence for inhibition of lesion expansion in a few. Studies with twelve of the more effective chemicals showed tha the protective effect persisted at significant levels even in 5-week-old plants and that at this stage this inhibiting effect on lesion expansion was more pronounced in most of the treatments. Different treatments led to the development of a more of a moderate to high level of fungitoxicity in young wheat seedlings which markedly declined with age of the plant and disappeared in 5-week-old plants. When inoculated at the age of 3 or 5 weeks, plants receiving most of the treatments developed appreciably higher fungitoxicity than the untreated plants (Hait and Sinha, 1986).

Chakraborty and Purkayastha, (1987) studied the effect of six metabolic inhibitors(viz., sodium iodoacetate, 2,4-dinitrophenol, sodium fluoride, sodium melonate, sodium azide and sodium molybdate) on the development of charcoal rot disease of soybean (CV.soymax). The effect of sodium azide (100 /ug/ml) was found to be the most significant among the metabolic inhibitors tested, in reducing the disease symptom. The reduction in disease was evidenced by minimum loss in weight of roots and minimum root rot index. The glyceollin content of soybean roots before and after disease reactions by sodium azide treatments was estimated and compared. The production of glyceollin was maximum when plants were treated with sodium azide followed by inoculation with M.phaseolina. Sodium azide induced glyceollin synthesis even in uninoculated soybean plants. Acumulation of phytoalexin in $CuCl_2$ and $AgNO_3$ treated leaves of Brassica juncea was also reported by Rouxel et.al., (1989).

The effect of foliar application of growth substances on the development of charcoal rot disease of soybean caused by Macrophomina phaseolina was tested by Chakraborty et.al., (1989). Among the eight growth substances (3-indole-acetic acid, 2,4-dichlorophenoxyacetic acid, 2,3,5-triiodobenzoic acid, 2-napthoxyacetic acid, L-naphthalene acetic acid, gibbrellic acid, 6-furfuryl amino purine and 6-benzyl aminopurine) examined, gibberellic acid was most successful in reducing the disease severity, followed by 3-indole acetic acid and 2,3,5-triiodobenzoic acid. Low concentrations of these compounds stimulated while high concentrations inhibited the mycelial growth of M.phaseolina in vitro. Glyceollin contents of host roots before and after treatments with gibberellic acid (10mg/L.) were estimated; this compound significantly increased glyceollin production in infected roots.

Spray with $AgNO_3$ and $CuCl_2$ solution on the leaves of Brassica juncea and B. napus also caused accumulation of phytoalexin and the effect of cyclohexamide suggested that its accumulation was associated with induced plant metabolism. Challenge with $CuCl_2$ a non-specific elicitor, phytoalexin was detected in B.juncea and B.napus 6h and 18h after challenge respectively. B.juncea always accumulated 4 to 10 times more phytoalexin than did B.napus (Rouxel et.al., 1989).

Purkayastha and Banerjee (1990) used six antibiotics (Penicillin, Cloxacillin, tetracyclin, chloramphenicol, cephaloridine, and kanamycin) as foliar spray on a susceptible soybean cultivar (Soymax) to induce resistance against anthracnose. Among the six antibiotics tested cloxacillin and penicillin induced maximum resistance against a nthracnose. Spraying the lower surface of the first true leaves of cucumber plants with 50 M K_2HPO_4 induced systemic resistance to anthracnose caused by Colletotrichum lagenarium. Correlations were made between peroxidase and chitinase activities induced by several treatments on leaf 1 and the

level of protection observed in leaf 2 after challenge with C.legenarium (Irvan and Kuc, 1990). Reduction in barley stripe disease induced by Helminthosporium gramineum was observed by Mathur and Bhatnagar (1991) when seeds were immersed in ferrous sulphate solution or moistened with sulphates of manganese and cobalt before sowing.

Wet seed treatment with phytoalexin inducer chemicals and related compounds sprotected rice plants from the attack of both brown spot and blast diseases. Such compounds were effective at dilute concentrations, mostly non-hazardous and with little or no fungitoxic effect at the concentration employed. Many of the chemicals have equally strong effective against botn diseases, some are more effective against one than against the other. Sarkar and Sinha(1991) concluded that such chemicals may be mostly acting through an induction of general host resistance and also provide in the process of broad spectrum asction effective simultaneously asgainst a group of pathogens.

Effectiveness of 19 non-conventional (mostly non-toxic) chemicals in wet seed treatment (10^{-4} to 10^{-2}M) in controlling wilt of tomato (cv.Patharkuchi) caused by Fusarium oxysporum f.sp.lycopersici was demonstrated by Mandal and Sinha(1992). While most of the compounds could reduce wilt symptoms appreciably, cupric chloride, ferric chloride, zinc chloride, manganese sulphate, mercuric sulphate, L-cysteine, IAA and DL-methionine showed very strong protective effect. These reduced leaf symptoms by 52 to 71%, prevented mortality completely and also limited vascular colonization by the pathogen. Most of the test compounds showed little or no in vitro fungitoxicity at their effective concentrations and stronger protection was often achieved at lower than higher concentration. These non conventional chemicals act in plant disease control toxic action but by inducing resistance in

in susceptible tomato plants, mediated through host tissue conditioning.

SEROLOGICAL RELATIONSHIP BETWEEN HOST AND PARASITE

Each and every living plant has its own immune system functionally simila r to that of animals. Conclusive evidences are now available to confirm the existence of phytoimmunity but unlike humoral immunity or specific target of asntibodies commonly operates in animals.

A number of reviews pertaining to the cross reactive antigens shared between host and parasite as well as their cellular location hasve been published by different workers during last four decades (Damian, 1964; DeVay et.al., 1967; DeVay et.al., 1972; Purkayastha, 1973; DeVay and Adler, 1976; Damian, 1979; Clark, 1981; Chakraborty, 1988; Purkayastha , 1989 ; Purkayastha et.al., 1991, Purkayastha, 1994.

Protein extracts of resistant (Jersey Queen) and susceptible (Early Jersey Wake) cabbage seedlings (both uninoculated and inoculated with Fusarium oxysporum f.sp.conglutinans) were subjected to electrophoretic and immuno-chemical analysis. By electrophoresis in starch gel, 4 components were separated but no significant differences was observed in uninoculated or inoculasted resistant seedling extracts. In contrast, immunochemical analysis with rabbit antisera revealed upto 7 components in extracts of infected susceptible cabbage, compared with 4 components in healthy susceptible and healthy and inoculated resistant plants. The additional components detected in infected susceptible cabbage were not original fungus protein. They may have been formed

either by the fungus after infection or more likely by the infected plant cell. On the basis of different immunological experiments it was suggested that these components were not merely breakdown products but antigenic substances (probably proteins) which differed from the normally present substances in the healthy plants (Heitefuss et.al., 1960).

The relationship between antigenic compounds produced by sweet potato in response to black rot infection and the magnitude of disease resistance was pointed out by Uritani and Stahmann (1961). Tissue extracts of healthy sliced and black rotted (Ceratocystis fimbriata) sweet potato roots of several Japanese varieties showed immunochemical precipitation lines with antisera towards corresponding extracts from an American variety(Sunnyside) . Antigenic components designated as A and C were distributed in tissue extracts of all varieties. However, B and D were produced in response to the infection. The amount produced in several Japanese varieties was correlated with the degree of resistance. In healthy root tissue B and D seemed to be present in very small amounts and increased in response to simple injury or slicing but to a much lesser extent than after infection.

The immunological responses of Verticillium albo-atrum and V.nigrescens pathogenic to cotton were compared by Wyllie and Devay(1970). On the basis of antigenic pattern Verticillium species were distinctly differentiated from one another. Defoliating strain of V.albo-atrum (T9) was shown to differ antigenically from the non-defolating strain (SS4). It appeared to be more closely related serologically to the mildly virulent V.nigrescens isolates than was the defoliating T9 isolates.

Serodiagnostic methods for the differentiation between resistant and susceptible varieties of cotton infected

with Fusarium oxysporum and citrus spp. with Phytophthora citrophthora have been described by Abd-El, Rehim and Hashan (1970) and Abd-El-Rehim et.al.(1971a). Serological and immunoelectrophoretical investigation on water-melon varieties, resistant and susceptible to Fusarium semitectum also revealed that the cultivars could be differentiated by the titre or the time after which reaction occurred between antisera specific to the pathogen and seed globulins. It was noted that a_2b globulin fraction was present only in resistant varieties (Abd-El-Rehim et.al., 1971b).

Wimalajeewa and Devay(1971) detected common antigenic relationship between Zea mays and Ustilago maydis. A pair of compatible haploid lines and two diploid solopathogenic lines of U.maydis were used in serological studies. Avena sativa var. "Victory" and Hordeum vulgare var. "California Mariout" were taken as reistant hosts. Certain antigens were found common between corn and U.maydis. A strong antigenic relationship existed between the solopathogenic lines 132 and 3-day-old Oat seedlings. Barley did not have any antigen in common with any of the U.maydis lines tested. Antigenic comparison of the four lines of U.maydis did not indicate any qualitative significant serological difference among them.

Common antigens among four varieties of cotton (Gossypium hirsutum) and isolates of Fusarium and Verticillium species were compared by Charudattan and Devay(1972). One antigenic substance was common among the varieties of cotton and isolates of F.oxysporum f.sp.vasinfectum, F.solani f.sp. phaseoli, V.albo-atrum and V.nigrescens. Cotton varieties which were resistant or susceptible to Fusarium wilt shared the common antigen with both pathogenic and non-pathogenic isolates of F.oxysporum f.sp. vasinfectum. However, the common antigen was not shared between cotton and non-pathogenic isolates of F.moniliforme. In immunodiffusion tests five to eight precipitin bands were observed in homologous reactions ; of these only one or two bands were common in heterologous

reactions between the fungal and the cotton preparations. The common antigenic determinant shared by cotton and fungal isolates does not appear to be related to the severity of wilt symptoms, but it may affect host pathogen compatibility during the process of root infection.

Charudattan and Hubbell(1973) compared the soluble antigens of three Rhizobium species with those of eight legumes representing compatible and non-compatible hosts following agar-gel double diffusion tests. Cross reactive antigens found between all the legume hosts and bacteria were not related to the specificity of compatible Rhizobium-legume associations. The cross reactive antigens were absent between Rhizobium and eight non-legume plants tested, but present between five out of eleven gram negative phytopathogenic bacteria and legumes.

Abbott(1973) determined the antigenic affinity among the saline soluble proteins of Triticum aestivum and Avena sativa and soil borne fungus Ophiobolus graminis. Single precipitin band in immunodiffusion test was formed when antisera of the wheat and oat roots were allowed to diffuse with the antigens of O.graminis.

Common antigen was also shared by both avirulent and virulent isolates of F.oxysporum f.sp. vasinfectum with disease resistant and susceptible line of cotton. In all cases, the fungal isolates invaded and parasitized cortical tissues of cotton roots, but only those fungal isolates that caused disease became established in the vascular system (Kalyanasundaram et.al., 1978).

Rabbit antisera were raised against soluble extracts of Phytophthora infestans (race 4) and tubers of " Arran Banner" and "golden wonder" potato cultivars showing field

susceptibility and resistance respectively to late blight. Their antisera were then used to test for the presence of common antigens between extracts of the fungus and various host and non-host plants(Palmerley and Callow, 1978). Cross reactive antigen was detected between P.infestans (race 4) and potato tubers of both the field susceptible and field resistant cultivars and also between the fungus and leaves of tomato and tobacco. Common antigens were not detected between P.infestans (race 4) and leaves of non-hosts(mung-bean, pea, radish, cucumber and maize), nor between potatoes and the alternative pathogen, (Fusarium solani var. aeruleum) and two non-pathogens (Ustilago maydis and Phytophthora cinnamoni).

Charudattan and Devay (1981) isolated, purified to homogenity and partially characterised a major cross reactive antigenic substance(CRA) from conidial culture of Fusarium oxysporum f.sp. vasinfectum common to roots of cotton (Gossypium hirsutum). The tissue and cellular locatioin of the CRA and their possible role in host parasite compatibility has been subsequently described by Devay et.al. (1981). Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross sections of cotton roots cut near or just below the root hair zone, the CRA was concentrated mainly around xylem elements, the endodermis and epidermal cells and was present throughout the cortex tissue. Protoplast prepared from cross sections of young cotton roots also contained the CRA which was concentrated in the region of plasmalema. Treatment of conidia and mycelia of F.oxysporum f.sp. vasinfectum with asntiserum to cotton and indirect staining with FITC indicated that the CRA was mainly present in hyphal tips and in patch like areas on conidia.

Chakraborty and Purkayastha (1983) detected cross reactive antigen shared between soybean cultivars and Macrophomina phaseolina causing charcoal rot disease. Rabbit

antisera were raised against root antigens of soybean cultivars (Soymax and UPSM-19) and M.phaseolina isolate M.P1) and tested against nomologous and heterologous antigens following immunodiffusion test. When antiserum of M.phaseolina was reacted against its own antigens and antigens of susceptible soybean cultivars (Soymax, R-184) strong precipitation reactions were observed. In case of resistant cultivars, (UPSM-19 and DS-73-16) no such reactions were observed. Reciprocal cross reactions between antiserum of the resistant cultivar and antigens of three isolates of M.phaseolina also failed to develop even weak precipitation bands. Four antigenic substances were found to be common between the susceptible soybean cultivars and isolates of M.phaseolina in immunoelectrophoretic tests, but no common antigens were detected between resistant cultivars and the fungus. Purkayastha and Chakraborty(1983) further detected that in susceptible soybean plants (cvs. soymax& R-184) a close relationship exists between lower production of glyceollin and presence of common antigens. The production of glyceollin was much higher in resistant soybean cultivars (cvs. UPSM-19 and DS-73-16) where common antigens were absent.

Common antigens were also detected in extracts of urediniospores of Hemileia vastatrix and in leaf and root extracts of coffee plant. An antigenic disparity was observed between coffee plants of physiologic group D and E. Common antigens shared between coffee plants and urediniospores of H.vastatrix and their possible involvement in such interaction were discussed by Alba et.al. (1983). Serological relationship between Colletotrichum corchori and jute cultivars (JRC-212) was detected by Bhattacharyya and Purkayastha (1985).

Haide and Smedegard-peterson(1985) prepared rabbit antisera against soluble antigens extracted from Erysiphe

graminis f.sp. hordei and barley (Hordeum vulgare). Antigens extracted from four near isogenic barley lines were cross reacted with the antisera of E.graminis f.sp. hordei which shared immunologically identical antigens.

Immunodiffusion, immunoelectrophoretic and cross immunoelectrophoretic analysis of rice antigens and their serological relationship between Acrocylindrium oryzae was determined by Purkayastha and Ghosal(1985). One precipitation band was observed when the antigen of A.oryzae was cross reacted with its own antiserum or against the antisera of four susceptible rice cultivars(Jaya, Ratna, IR-8, CR-126-42-1). No precipitation band was detected between the antiserum of the resistant cv. Mahsuri and antigen preparation from three isolates of A.oryzae or between the antigens of resistant cvs. Mahsuri and Rupsail and the antiserum of A.oryzae. Cross reactive antigens were detected in crude preparations and in purified preparations from mycelia of Phytophthora infestans race-4, and race-1,2,3,4,7 with antisera for potatoes cv. King Edward and cv. Pentland Dell by using an indirect enzyme linked immunosorbent assay (Alba and Devay 1985). They suggested that the fungal mycelia do not easily release cross reactive antigens(CRA) into synthetic media where they grow and most P.infestans CRA are thermolabile and can be concentrated by precipitation in the presence of 40% saturated ammonium sulphate(SAS). An antigenic disparity was noticed when 40% SAS from P.infestans Race-4 mycelia preparation was assayed with asntisera for cvs. King Edward and Pentland Dell. The occurrence of CRA in P.infestans mycelium and their involvement in such interactions were discussed.

The common antigenic relationship between soybean cultivars and Colletotrichum dematium var. truncata was ascertained following immunodiffusion, immunoelectrophoretic

and crossed immunoelectrophoretic tests (Purkayastha and Banerjee, 1986). Atleast one antigen was found to be common between host cultivar and the pathogen. No antigenic relationship was observed either between soybean cultivars and the non-pathogen (C.corchori) or avirulent pathogen (C.dematium).

Antigens obtained from two isolates of Macrophomina phaseolina, a pathogen of groundnut, four non-pathogens of groundnut (viz., Corticium sasaki, Colletotrichum lindemuthianum, C.corchori and Botrytis alii) and five cultivars of Arachis hypogea were compared by immunodiffusion, immunoelectrophoretic and cross immunoelectrophoretic techniques for the presence of cross-reactive antigens. Common antigens were found among the susceptible cultivars of groundnut and two isolates of M.phaseolina, but not between non-pathogens and groundnut cultivars. No antigenic similarity was found between non-pathogen and M.phaseolina isolates. Cross immunoelectrophoretic tests confirmed that atleast one antigen was common between cvs. J-11 and TMV-2; Kadiri-71-1 and TMV-2, and Kadiri-71-1 and isolates of M.phaseolina (Purkayastha and Ghosal, 1987).

Changes in antigenic patterns were detected after chemical induction of resistance in susceptible soybean cultivar (Soymax) to Macrophomina phaseolina. Sodium azide (100 µg/ml) altered antigenic patterns in cv. soymax and reduced charcoal rot disease (Chakraborty and Purkayastha 1987). Common antigenic relationship between susceptible rice cultivar(Jaya) and Sarocladium orzae could be altered by the application of gibberellic acid (100 µg/ml) and sodium azide (100 µg/ml). These chemicals reduced sheath rot disease of rice(Ghosal and Purkayastha, 1987).

Evaluation of antisera raised against pooled mycelial suspensions from five isolates (Pf-1, Pf-2, Pf-3, Pf-10 and Pf-11) representing five physiologic races of Phytophthora fragariae for detecting the red core disease of strawberries by enzyme linked immunosorbant assay(ELISA) was done by Mohan (1988). Root extracts prepared from alpine strawberry Fragaria vesca and F. ananassa cv. Cambridge Favourite infected with any of the five isolates studied produced strong reactions in ELISA. In F. vesca ELISA - positive masterial was detectable 6-8 days after inoculation before macroscopic symptoms appeared. The cultivar Red Gauntlet, (resistant to Pf-1,2 and 3 but susceptible to Pf-10 and 11) reflected differential response in ELISA. The absorbance produced by extracts of plants infected with virulent isolates was significantly higher than that obtained with the corresponding extracts of plants inoculated with avirulent isolates. The root extracts of the cultivars Hapil, Ostara and Providence (susceptible to all the five isolates) were also positive in ELISA. The ELISA test proved valuable in screening certified strawberry stock (Mohan 1988).

Cross reactivity of antiserum raised against Phytophthora fragariae with other Phytophthora species and its evaluation as a genus detecting antiserum has also been discussed by Mohan (1989). Antiserum of P.fragariae isolates (Anti PfM) reacted strongly with antigens from several Phytophthora species. Some cross reactions with antigens from Phythium species are decreased by fractionating on an affinity column of sepharose 4B bound to extracts of Fragaria vesca roots infected with P.fragariae. The affinity purified anti-PfM retained its high cross reactivity with the various Phytophthora species Anti-PfM could not be made specific for P.fragariae because it was raised against components shown to be antigenically similar in all Phytopnthora species tested. However, immunoblotting with the affinity purified anti-PfM produced distinct patterns for P.fragariae, P.erythroseptica and P.cactorum.

Kitagawa et.al. (1989) has also developed competitive types of two novel enzyme linked immunosorbent assays (ELISA) for specific detection of Fusarium oxysporum f.sp. cucumerinum as well as for general detection of ten strains of common Fusarium species that show specific pathogenicities to different plants. Antiserum against a strain of F.oxysporum f.sp. cucumerinum (F 504) was elicited in rabbits, and a highly specific, sensitive, and accurate ELISA for the homologous strains was developed by using the antiserum with B-D- gelactosidase-labelled antirabbit IgG as the secondary antibody and cell fragments of the strain attached to amino-Dylark balls as the solid-phase antigens. This assay was specific for strain F 504 and showed little cross reactivity with nine other strains of Fusarium species including strain 501 of F.oxysporum f.sp. cucumerinum. Strain F 501 possess patnogenicity against cucumber similar to that of strain F 504, although slight differences have been observed between these two strains regarding their spore formation and pigment production.

A polyclonal antiserum which was prepared by immunising rabbit with a mycelial extract of Phytophthora infestans, reacted in an enzyme linked immunosorbent assay with mycelial extracts of two Phytophthora species but not with those of ten other microorganisms found on potato. P.infestans mycelium in potato leaf tissue was readily detected by ELISA using either the plate trapped antigen or F (ab')2 antibody fragment techniques (Harrison et.al., 1990). Amount of mycelium in leaf extracts was estimated by comparing the values obtained in ELISA with those for known concentrations of P.infestans mycelium.

Similarly Mazarei and Kerr (1990) reported a rapid and convenient serological method to distinguish the two very closely related plant pathogenic bacteria, Pseudomonas sysringae pv. pisi and Pseudomonas sysringae pv. syringae associated with peas. Polyclonal antisera against P.syringae

pv. syringae and pv. pisi have a high level of specificity against their homologous antigens after cross absorbtion. By using antisera to glutaraldehyde fixed bacteria cells both in Ouchterlony gel double diffusion and in indirect ELISA following cross absorbtion, the two pathovars can be easily differentiated, using whole untreated, sonicated or heat-killed bacterial cells as test asntigens. Cross reactive antigens shared by soybean cultivars and the different strains of Myrothecium rofidum (M-1, ITCC-1143 ; ITCC-1409) were analyzed by Ghosh and Purkayastha(1990). Results of immunodiffusion revealed that common antigens were present only between virulent strain of M.roridum (M-1) and susceptible nost cultivars (DS-74-24-2 and PK-327). No cross reactive asntigen was detected in case of resistant cultivars (UPSM-19 and DS-73-16). Immunoelectrophoretic analysis showed that common antigen was shared by susceptible hosts and the virulent strain which was further confirmed by both crossed and rocket immunoelectrophoresis.

Common antigenic relationship between soybean and Colletotrichum dematium var. truncata was also studied by Purkayastha and Banerjee (1990) using immunodiffusion, immunoelectrophoresis and inidrect ELISA technique. Cross reactive antigens were detected between susceptible soybean cultivars and the virulent strain of C.dematium var. truncata but no cross reactive antigen was detected between soybeans cultivars and avirulent pathogen (C.dematium) or non pa thogen (C.corchori). Results of immunodiffusion and immunoelectrophoresis showed absence of common antigen between resistant cultivars (UPSM-19) and the pathogen, while the results of indirect ELISA indicated the presence of common antigen between the two at a very low level. They compared antigenic patterns of untreated and cloxacillin treated soybean leaves which induced resistance of soybean against anthracnose disease. Disappearance of the antigen from cloxacillin treated leaves of susceptible soybean cultivar "Soymax" was correlated with alteration of disease reaction.

New monoclonal antibody (MAb) raised against haustorial complexes (UB10) isolated from pea leaves infected with the powdery mildew fungus Erysiphe pisi recognised a 45kDa N-linked glycoprotein which was specially located in the haustorial plasma membrane. This glycoprotein was clearly distinct from a previously characterised 62KDa plasma membrane (identified with MAb UB8) which was also specially located in the haustorial plasmamembrane. These antibodies were used, along with MAb UB7 which binds to a major 62 KDa glycoprotein in the cell wall and plasma membrane of both haustoria and surface hyphae, to label haustoria within epidermal strips from infected pea leaves using inidrect immunofluorescence. Results showed that all three glycoproteins recognised by MAbs sexpressed early in haustorial development (Machie et.al., 1993). Molecular differentiation in the extrahaustorial membrane of pea powdery mildew haustoria at early and late stage development was subsequently focussed by Roberts et.al., (1993).

Polyclonal antiserum, raised against a strain of Fusarium oxysporum f.sp. narcissi (GCRI 80/26) was tested by enzyme-linked immunosorbent assay. Antiserum raised to cell wall fractions gave better recognition than that to cytoplasmic fractions. Recognition was equally good in artificially and naturally infected bulbs. Little cross-reactivity in bulb tissue was shown by three other bulb-rotting fungi. Nine isolates of F.oxysporum f.sp. narcissi from a wide geographic area gave similar results in an indirect ELISA of mycelial extracts although some cross-reactivity was observed with two other Fusarium spp. From Fusarium spp. and four other fungi showed little cross reactivity. Ten days after inoculation the pathogen was readily detected in the baie plate area of three Narcissus cultivars and points remote from the inoculation site in the most susceptible cultivar. A direct correlation was observed

between positive results in the enzymes linked immunosorbent assay and recovery of the pathogen on selected medium (Linfield, 1993).

Purkayastha and Pradhan (1994) observed that three strains of Sclerotium rolfsii were serologically different and their pathogenecities also differ markedly with host cultivars. Virulent strains 266 and 23 showed common antigenic relationship with their respective susceptible host cultivars but not resistant cultivars. Antigenic change in a susceptible cv. AK-12-24 after treatment with a systemic fungicide Kitazin was also evident. This change in host may be due to inactivation of a suppressor/inhibitor gene for resistance by the fungicidal treatment. They suggested that the resistance could be induced in susceptible plants if specific antigens are eliminated by suitable treatment.

Chakraborty and Saha (1994) detected Cross reactive antigens (CRA) shared between Camellia sinensis and Bipolaris carbonum. Antigens obtained from tea varieties, isolates of B.carbonum and non-pathogens of tea (Bipolaris tetramera and Bipolaris satariae), were compared by immunodiffusion, immunoelectrophoresis and enzyme-linked immunosorbent assay. CRA were found among the susceptible varieties(TV-9, 17 and 18) and isolates of B.carbonum (BC-1,2,3 and 4). Such antigens were not detected between isolates of B.carbonum and resistant varieties (TV-16, 25 and 26), non pathogens and tea varieties, as well as non pathogens and isolates of B. carbonum. Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross sections of leaves (TV-18), the CRA were concentrated mainly around epidermal cells. Treatment of mycelia and conidia of B.carbonum with antisera to leaves (TV-18) and indirect staining with FITC indicated the presence

of CRA in the young growing hyphal tips and conidia. Serological relationship between Glomerella cingulata and Camellia sinensis following immunodiffusion, immunoelectrophoresis as well as ELISA and their cellular location has been described by Chakraborty et.al., (1994a). Major Cross reactive antigen shared between Pestalotiopsis theae and Tocklai released tea varieties has also been detected by Chakraborty et.al. (1994b). Cross reactive antigens were found among the susceptible varieties and isolates of P.theae (Pt-1, Pt-2 and Pt-3). Such antigens were not detected between isolates of P. theae and resistant varieties. Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross section of tea leaves, the CRA was concentrated in the epidermal cells and mesophyll tissues. CRA was present in the young hyphal tips of the mycelia and on the setulae and appendages of the conidia of P.theae.

MATERIALS AND METHODS

(A) PLANT MATERIALS :

(i) **Source of seeds** : The seeds of 10 cultivars (viz., JS-2, UPSM-19, Pusa-16, PK-327, R-184, KU-254, EC-2575, EC-55865, EC-95287 and Soymax) of soybean (Glycine max (L) Merrill) were obtained from the Pulses and Oil seeds Research Station, Berhampore, West Bengal. These seeds were stored at 20°C and also at room temperature (30 ± 2°C). The seeds were disinfected with 'Agrosan-GN' in order to avoid microbial decomposition during storage. For the varietal resistance test aforesaid soybean cultivars were used and finally on the basis of their maximum and minimum resistance to root rot disease of soybean, cvs. UPSM-19, EC-55865, R-184, Soymax, JS-2 and KU-254 respectively were selected for further study. It is necessasry to mention that about 80% of the seeds of JS-2 and UPSM-19 and 60% of that of Soymax and R-184 lost their viability after one year so, seeds were procured from the said research station every year.

(ii) **Growth of plants** : Healthy seeds of soybean were treated with 0.1% $HgCl_2$ for 5 minutes to remove superficial contaminants, washed several times with sterile distilled water and sown in earthen pots (10 seeds/pot of 25 cm. diam.) containing non-infested sandy soil (soil : sand 1:1). The plants were grown in the Phyto-pathological Experimental Garden, Department of Botany of the University of North Bengal under natural conditions of day light and temperature (26-34°C). The pots were watered daily with ordinary tap water. The plants were grown during March to October. Usually 15-day-old plants were used for inoculation throughout the investigation except otherwise stated.

B. Fungal culture:

i) **Source of culture:** A virulent strain of Fusarium graminearum Schwabe(Fg.1) obtained from Division of Mycology

and Plant Pathology, Indian Agricultural Research Institute, New Delhi, was used throughout this investigation (except otherwise stated) after completion of Koch's Postulate. Another isolate of F.graminearum (Fig 22) was obtained from the stock culture maintained in the Plant Pathology Laboratory, Department of Botany, University of North Bengal which was originally isolated from naturally infected roots of soybean (Cv.JS-2) grown in the field of Pulses and Oil Seeds Research Station, Berhampore. Some other fungal species which were used in this study are enlisted in Table No. 1.

(ii) Completion of Koch's postulates : Soybean seeds were surface sterilized with 0.1% $HgCl_2$ solution for 5 minutes, washed with sterile distilled water and sown in pots containing sandy soil previously infested with conidia & mycelia of F.graminearum.

The pathogen was reisolated from infected roots after 20 days of inoculation into potato-dextrose-agar(PDA) slants, examined after 15 days of incubation (at 28°C) and the identity of the organism was confirmed after comparing it with the stock culture already made available for the purpose.

(iii) Maintenance of stock cultures:

The fungi listed in Table-1 were grown on PDA slants. After 15 days, the cultures were stored under three different conditions (5°C, 20°C and 30°C) in sterile liquid paraffin. Apart from weekly transfer for experimental work, the cultures of F.graminearum isolates were also examined at a regular interval to test its pathogenicity.

(iv) Assessment of mycelial growth in liquid media :

F.graminearum was first grown in petridishes, each containing 20 ml of PDA medium and incubated for 4 days at

Table-1 : List of Fungal isolates used

Species with isolate code	Host of origin	Source of isolate
Fusarium graminearum Schwabe Fg1 (ITCC-1805)	Glycine max	Indian Agricultural Research Institute, New Delhi.
Fusarium graminearum Schwabe Fg2	G.max	Naturally infected roots of soybean, Cv.JS-2. Pulses and oil seeds Research Station, Berhampore, West Bengal.
Glomerella cingulata (Stoneman) Spauld & Schrenk GC-1(IMI-356806)	Camellia sinensis	Tocklai Experimental Station Jorhat, Assam.
Pestalotiopsis theae (Saw.) Stey. Pt1 (IMI-356807)	C.sinensis	Tocklai Experimental Station, Jorhat, Assam.
Drechslera oryzae(Breda de Haan) Subram, & Jain HO-1(ITCC-2537)	Oryza sativa	Indian Agricultural Research Institute, New Delhi.
Bipolaris carbonum Nelson. BC-1 (IMI-298762)	C. sinensis	Naturally infested leaves of Gayabari Tea Estate, Darjeeling.
Fusarium Solani (Martives) Saccaris. FS-1 (ITCC-1804)	Glycine max	Indian Agricultural Research Institute, New Delhi.
Fusarium Oxysporum Schlecht. FO-1 (ITCC -1803)	Glycine max	Indian Agricultural Research Institute, New Delhi.

IMI - International Mycological Institute, ITCC - Indian Type Culture Collection.

30°C. From the advancing zone of the mycelial mat, agar block (4mm diameter) containing the mycelia, was cut with a sterilized cork borer and transferred to each Ehrlenmeyer flask(250 ml) containing 50 ml of sterilized Richard's medium (KNO_3, 10.0 g; K_2HPO_4, 5.0 g; $MgSO_4,7H_2O$, 2.5 g; $FeCl_3$, 0.02g; Sucrose, 30.0 g; Distilled water 1 lt.) for a desired period at 30°C. Finally, the mycelia were strained through muslin, collected in aluminium foil cup of known weight, dried at 60°C for 96 h cooled in a desicator and weighed.

(C) Chemicals :

In addition to ordinary laboratory reagents, the following chemicals were used.

Acrylamide	- Merck, German
Adjuvant complete(Freund)	- Difco Laboratories, Detroit Michigan, USA.
Adjuvant incomplete(Freund)	- Difco Laboratories, Detroit Michigan, USA.
Amido black	- Hi-Media Laboratories, Bombay, India.
Ammonium peroxodisulphate	- E.Merck(India) Ltd., Bombay,
Barbitone sodium GR	- Loba Chemicals Co., Bombay, India.
Barium chloride	- BDH
Bis acrylamide	- Merck, German.
Bovine serum albumin	- Sigma Chemical Co. USA.
Cadmium chloride	- SDS
Brilliant blue R 250	- Sigma Chemical Co. USA.
Ferric Chloride	- Sarabhai M Chemicals
Folin-Ciocaltea u's reagent	- Glaxo Laboratories, Bombay, India.
Goat antirabbit-IgG(whole molecule) fluorescein isothiocyanate conjugate.	- Sigma Chemicals Co. USA.

Goat antirabbit-IgG(whole molecule) Horse radish peroxidase conjugate.	- Sigma Chemical Co. USA.
Glycine	- Merck, German
Mercury(II) chloride	- Merck
Nickel nitrate	- E.Merck(India) Ltd. Bombay
p-Nitroaniline	- Loba Chemical Co. USA.
Riboflavin	- Sigma Chemical Co. USA.
Sea-Sand	-Ricdel-De Haen Ag Seelza-Hannover
Silica gel G	- E.Merck(India) Ltd. Bombay.
Sodium acetate	- E.Merck(India) Ltd. Bombay.
Sodium azide	- E.Merck(India) Ltd. Bombay
Sodium malonate	- E.Merck(India) Ltd. Bombay
Sodium molybdate	- E.Merck(India) Ltd. Bombay
Sodium selenite	- E.Merck(India) Ltd. Bombay
Tris	- Merck, German.

(D) Preparation of inoculum and inoculation technique :

(i) Sick pot : Sick pot method as described by Nene et.al. (1981) was adopted with modification. Pots (size 9" diam.) were filled with sandy soil (1:1). Naturally infected as well as artificially inoculated (with F.graminearum) soybean plants were chopped into small pieces and these were incorporated uniformly in the surface soil of those pots and kept for one month. After the said period of incubation, 10 seeds each of the different soybean cultivars were separately sown in each pot. Control sets were maintained by sowing soybean seeds in non infested sandy soil.

On the other hand, sand maize meal medium was prepared by mixing riverbed sand and maize meal in the ratio of 9:1 respectively and taken in 100 ml Ehrlenmeyer flasks (40 g in each flask), 10 ml distilled water was added and the medium was autoclaved at 15 lbs pressure for 20 minutes. After

cooling, each flask was inoculated with F.graminearum and incubated at 28°C for 10 days. The sand maize meal culture was throughly mixed with non-infested sandy soil in each pot. Pots were watered and kept as such for 15 days. Surface sterilized soybean seeds were sown in each pot and disease intensity was assessed.

(ii) Water culture : Fifteen surface sterilized (with 0.1% $HgCl_2$ solution for 5 min.) soybean seeds of each cultivar were sown in each pot containing autoclaved sandy soil. The seedlings were nursed until their further transfer. F.graminearum was grown in potato dextrose broth (100 ml broth/250 ml) at 30°C on a shaker (8 h . each day) for 10 days. Entire contents of a flask was dilute with sterile distilled water to get the final inoculum dilution of 2.5% (approx. 6.5×10^5 spores/ml.). In each sterilized glass tube (150 x 15 mm) 20 ml. of inoculum was poured. Subsequently, 15 days old seedlings were uprooted from the experimental garden. The root system was washed throughly in running tap water, then rinsed twice in sterilized distilled water. One seedling was transferred into each tube and plugged with cotton. After every two days sterilized distilled water was added to the tubes in order to make up the loss. In control tubes one seedling in each tube was transferred.

(E) The assessment of disease intensity :

Plants were examined after 10, 20 and 30 days of inoculation. Disease intensity was assessed on the basis of percentage loss in dry weight of roots as described by Chakraborty and Shil (1989). After desired period of incubation the plants were uprooted, washed,dried at 60°C for 96 h and weighed. Root rot index was calculated in the following way : - on the basis of percentage loss in dry weight of root in relation to control, they were graded into 5 groups and a value was assigned to each group (viz., 1-10% loss

in weight = 1, 11-25% = 2, 26-50% = 3, 51-75% = 4, 76-100%=5). The root rot index in each case was quotient of the total values of the replicate roots and the number of roots (i.e., number of plants).

(F) Method of application of chemicals :

Soybean (CV. Soymax) seeds were soaked for 24 h. either in different dilutions (10^{-3}M to 10^{-8}M) of nine chemicals or water separately before sowing them in earthen pots as described by Chakraborty and Sinha (1984).

(G) Method of extraction and separation of glyceollin from infected host roots

To extract glyceollin, the method of Keen et.al., (1971) was followed with modifications. Fifteen day-old plants were inoculated with F.graminearum following water culture method as stated earlier. After 24, 48, 72 and 96 h. of inoculation, the plants were uprooted from earthen pots, washed and roots were used for extraction of glyceollin. Thirty grams of roots were homogenised with 120 ml of 95% ethanol in an electric blendor. The extracts were filtered through filter paper and the residues were re-extracted with an equal volume of 80% ethanol and filtered. The filtrates were combined and the residues discarded. The combined filtrates were reduced in volume in a rotary evaporator at 45°C. The concentrated filtrates were extracted once with 30 ml hexane and then thrice with equal volume (30 ml) of ethyl acetate. The hexane fraction was discarded and the ethyl acetate fraction was dried at 45°C. The residues was disolved in 3 ml ethyl acetate and the aliquots were used for separation on the plates and chemical detection of glyceollin. TLC plate bioassay as well as for petridish bioassay test.

Thin layer chromatograms were prepared with Silica gel G (0.2 mm thickness) and activated for 1 h . at 80°C. Aliquots (10 µl) of ethyl acetate extract of roots, as well

as authentic glyceollin were separately spotted on the activated chromatograms and developed separately in four different solvent systems in order to select the best one (showing better separation of glyceollin). The following 4 solvent systems were used.

(i) Hexane : ethyl acetate : methanol (60:40:1)
(ii) Hexane : ethyl acetate : acetic acid (80:20:4)
(iii)Chloroform : acetone : 28% NH_3(65:35:1) &
(iv) Chloroform : acetone : acetic acid (90:10:0.5)

The solvent front was first marked in the chromatogram and after drying the plate was observed under UV light or sprayed with Diazotized, P-nitroaniline (5 ml of 0.5%) P-nitroaniline solution in 2N HCl + 0.5 ml of 5% aquous sodium nitrite solution + 15 ml of 20% aquous sodium acetate solution).

Rf value of glyceollin (obtained from root extract) was compared with authentic glyceollin (obtained from Professor N.T.Keen, University of Californea, Riverside, USA) for confirmation.

(H) Ultraviolet spectrophotometry and quantification of glyceollin :

Ultraviolet spectrophotometry - Aliquots (50 µl) of ethyl acetate extract were spotted on the plates as described and developed in hexane : ethylacetate : methanol (60:40:1) solvent system and allowed to dry. Silica gel from unsprayed chromatogram corresponding to reacting zones (reacting with reagent) were scraped off, eluted in 5 ml absolute ethanol and stored for overnight at 5°C. The eluates were centrifuged to remove the silica gel particles and the volume of supernastant was made upto 10 ml and used for UV spectrophotometric studies. UV absorption spectrum of glyceollin was determined by UV spectrophotometer (Shimadzu-model 160).

Quantification : Quantity of glyceollin ($C_{20}H_{19}O_5$) was estimated from UV-spectrophotometric curve by assuming molar extinction co-efficient of 10,800 at 286 nm as described by Bhattacharyya and Ward (1985).

$$\text{Molar extinction co-efficient} = \frac{\text{O.D. of the tested solution}}{\text{Concentration}^{(a)} \text{ of the tested solution(moles/litre)} \times \text{Path length of the cell (cm)}}$$

a = moles/litre converted to g/litre by multiplying moles with molecular weight of glyceollin. Results have been expressed in /ug/g fresh weight of roots.

(I) Bioassays of glyceollin :

(i) TLC - plate Bioassay :

For bioassay of glyceollin 10 /u1 ethyl acetate extract of infected roots were spotted on TLC plates as described and developed in hexane : ethyl acetate : methanol (60:40:1) solvent system. After drying, spore suspension of Bipolaris carbonum Nelson (Spores suspended in nutrient solution - KNO_3, 1% ; KH_2PO_4, 0.5% ; $MgSO_4$, $7H_2O$, 0.25%) and incubated for 96 h. in a moist, closed glass chamber at 25°C. Finally, inhibition zone was marked on the chromatogram.

(ii) Petridish Bioassay of Phytoalexin :

For petridish bioassay of phytoalexin (glyceollin) the method of Keen(1971) was followed. Ethyl acetate extract(0.2 ml) of infected roots containing glyceollin was taken in each of the 3 sterilized petridishes and allowed to evaporate. Subsequently 0.2 ml ethanol was poured in each petridish, agitated and 20 ml sterilized PDA medium was added to it. Each petridish was inoculated with an agar block (4mm

diam.) containing 4-day-old mycelia of F. graminearum and incubated at 30 ± 1°C. Diameter of the hyphal growth was measured and compared with control.

(iii) Slide germination

Partially purified glyceollin was assayed by measuring its effects on the spore germination of F.graminearum following the method of Rouxel et.al., (1989). Glyceollin was added to spore suspension (10^6 spores/ml) in water and drops of the suspension was placed on microscopic slides and allowed to germinate in a humid chamber. The germination rate and germ tube length was measured using a light microscope after 24 h incubation at 25°C and compared with control.

(J) Extraction of total soluble protein

(i) Root protein

Soluble protein were extracted from healthy and F.graminearum infected roots of soybean cultivars as well as from the mycelia of F.graminearum following the method as described by Chakraborty and Saha (1994). Seeds of soybean cultivars were grown in earthen pots containing Fusarium infested soil as well as in sterilized soil separately. Healthy and infected plants were uprooted after two week intervals, washed with cold water and kept at -15°C for 1 h. Finally, roots (25 g fresh weight) were crushed with sea-sand in mortar and pestle in cold (4°C) and stored at -15°C for 1 h and homogenized with 25 ml of 0.05 M Tris-HCl buffer (pH 7.4) at 4°C. Homogenate was strained through cheese cloth and then was centrifuged(12,100 g) at 4°C for 1 h and known quantity of ammonium sulphate was added to it for 100% precipitation (Green & Hughes, 1965), kept at 4°C for overnight and centrifuged (10,000g) for 15 min. at 4°C. Precipitate was dissolved in the same extractive buffer (pH 7.4) and dialysed against 0.005 M Tris solution for 24 h at

4°C. During this period 10 changes were given. The dialysate (i.e., soluble protein) was used for gel electrophoretic study.

(ii) Mycelial protein

To extract soluble mycelial protein, F.graminearum was grown in sterilized liquid medium (g/L. distilled water, sucrose, 50g; KNO_3, 10g ; KH_2PO_4, 5g; $MgSO_4$, $7H_2O$, 2.5g and $FeCl_3$, 0.02g) 12 days at 30±1°C. Mycelia (50g fresh wt.) were collected, washed with 0.2% NaCl solution, rewashed with sterile distilled water and crushed in cold (4°C), stored at -15°C for 2 h. Rest of the procedure was as described for root protein preparation. The soluble proteins were used for gel electrophoretic study.

(K) Protein estimation :

The soluble proteins were estimated following the method of Lowry et.al. (1951). Initially an alkaline mixture was prepared by mixing of 0.5 ml of 1% $CuSO_4$, 0.5 ml of 2% sodium potasium tartarate, 50 ml of 2% Na_2CO_3 dissolved in 0.1N NaOH. Finally, reaction mixture was prepared by mixing 0.1 ml of the protein sample, 0.9 ml water and 5 ml of Folin-phenol solution (Folin-phenol : water= 1:1) was added and again incubated for 15 min. In case of blank, water was used instead of protein sample. At the end of the incubation period O.D. value of each sample was determined by systronics photoelectric colorimeter 101 at 710 nm. Quantity of protein was estimated following the standard curve made with bovine serum albumin (BSA).

(L) Polyacrylamide gel electrophoresis of soluble protein :

(i) Preparation of gel solution and gel column :

Protein patterns of healthy and infected roots of both resistant and susceptible varieties of soybean as well as

mycelia of F.graminearum were determined by polyacrylamide gel electrophoresis following the method of Davis(1964) with modifications, Clean gel tubes(120m. long, 4 mm. diam.) were kept vertically on the tube stand. One end of each tube was closed with rubber cap. Mixtures of working solutions were prepared as follows :

Lower gel
(solution I:II:III=1:1:1)

Solution I
Acrylamide - 30g
Bisacrylamide-0.8g
distilled water-100 ml

Solution II
Tris - 18.15g
(N)HCl - 24.0 ml
TEMED - 0.4 ml
Dist. water - 100 ml

Solution III
Ammonium peroxide -
Sulphate - 60 mg
Distilled water - 100ml.

Upper gel
(Solution IV:V:VI=2:1:1)

Solution IV
Acrylamide - 5 g
Bisacrylamide - 1.25 g
distilled water - 100 ml.

Solution V
Tris - 2.10 g
(N) HCl - 13 ml
TEMED - 0.2 ml
Dist. water - 86.8 ml.

Solution VI
Riboflavin - 2 mg
2M Sucrose -100 ml.

Lower gel solution was poured slowly into the tube up to a height of 9 cm. and drop of water was placed on the top of the gel solution with a view to have a plain surface instead of concave one. The gel tube was kept as such until it became solidified. Water drop was soaked with filter paper and the lower gel over layered with the upper gel solution. Again, a drop of water was placed on the top of the gel solution and kept in light until the gel became solidified. The rubber cap was removed and the gel tube with gel column was fitted on to the reservior.

(ii) Electrophoresis :

Soluble protein (0.01 ml = 150 µg) was added to an equal volume of 2M sucrose and trace of bromophenol blue and eventually loaded on to the gel column. Tris glycine buffer [Tris, 6g ; glycine, 28.8g. and distilled water upto 1 L. (total volume of the solution)], pH 8.4 was poured into both the upper and lower buffer reserviors. The whole set was incubated in cold room at 4°C supplying a constant current of 3 m.amp./tube approx. for 3-4 h until the dye front reached the bottom of the gel column. The gel was stained with Brilliant blue R 250 (0.25%) in methanol : acetic acid : water = 5:1:5) for 1h and then destained with the approximate solution (methanol : acetic acid : water=5:1:5) until the protein bands become clear. Rf values of individual bands were determined.

(M) Source and maintenance of rabbits for serological works :

Male rabbits used for immunological works were supplied by M/S Sujit Sarkar, Bagdogra, animal supplier. All of them were of Australian strain, and white in colour. (Plate 2 ; Fig. A). The initial weight of the rabbits varied from 1.2-1.8 kg and their age varied from 9-10 months, before experimentation. The rabbits were kept in separate cages (60cm x 45cm x 30cm) attached with metal trays at the bottom and placed in a well ventilated cleaned room. Each rabbit was supplied with 20 g carrot (Daucus carota); 50g ; soaked gram (Cicer arietinum), 50-70g. grass (Cynodon dactylon), 4-5 leaves of lettuce (Lactuca sativa) or Cabbage (Brassica oleracea L.var. Capitata) or Cauliflower (Brassica oleracea L.var. botrytis) daily. Along with the food, fresh water was supplied proportionately with the age of rabbits. Under new environmental conditions the rabbits were kept under close observation at least for a week before immunization.

(N) Preparation of antigens :

(i) Plant antigen

The seed and root antigens were extracted following the procedure as described by Chakraborty and Purkayastha (1983). Soybean seeds and roots of both resistant and susceptible varieties were selected for the preparation of antigens. Surface sterilized seeds were first soaked in sterile distilled water for 12 h and subsequently the seeds were kept for 2 h at -20°C. Roots after thorough washing with sterile distilled water were kept for 1h at -20°C. Then the seeds and roots were crushed separately in morter and pestle at 4°C with sea sand and homogenized with 0.5 M Tris-HCl buffer (pH-7.4), and centrifuged (12,100 g) for 1h at 4°C.Known quantity of $(NH_4)_2SO_4$ was added to these supernatant for 100% precipitation (Green and Hughes, 1965), kept for 12h at 4°C and centrifuged (10,000 g) for 15 min at 4°C. The precipitate was dissolved in the same extractive buffer (pH 7.4) and dialysed against 0.005 M tris-HCl solution for 24h at 4°C. During this period 10 changes were given. The dialysate (i.e., soluble protein) was used for gel electrophoretic study.

(ii) Fungal antigen :

Discs (4 mm) of mycelium were transferred to Ehrlenmeyer flasks (250 ml) each containing 50 ml of sterilized liquid medium (g/L distilled water, sucrose, 30 g; KNO_3, 10 g : KH_2PO_4, 5g; $MgSO_4$, $7H_2O$, 2.5g and $FeCl_3$, 0.02 g) and incubated for 15 days at 30 $\pm$ 1°C. For extraction of soluble antigens, mycelial mats were harvested, washed with 0.2% NaCl and rewashed with sterile distilled water. Washed mycelia (50 g fresh wt.), were homogenized with 0.05 M sodium phosphate buffer (pH 7.4) containing 0.85% NaCl in a mortar and pestle in the presence of sea sand. Cell

homogenates were kept overnight at 4°C and then centrifuged (15000 g) for 30 min at 4°C. The supernatant was equilibrated to 100% saturated ammonium sulphate under constant stirring and kept overnight at 4°C. After this period the mixture was centrifuged (22,000 g) for 30 min at 4°C, the supernatant dissolved in 10 ml 0.05 M Phosphate buffer (pH 7.4). The preparation was dialysed for 48 h. through cellulose tubing (Sigma Chemical Co. U.S.A.) against 1 L. of 0.005 M phosphate buffer (pH 7.4) with ten changes. After dialysis the preparation was centrifuged (12,000 g) for 15 min at 4°C and supernatant was stored at -20°C until required.

The protein contents of both plants and fungal antigen preparations were determined following the method as described by Lowery et.al., (1951) using bovine serum albumin as the standard.

(O) Preparation of antisera :

(i) Immunization

Before immunization, normal sera were collected from each rabbit. Antisera against antigens of host (Glycine max) and pathogen (F.graminearum) were raised in separate rabbits (Plate 2, Fig. B) of antigen (1.5 mg/ml protein of either host or pathogen) emulsified in equal volume of Freund's complete adjuvant (Difco) and repeating the doses at 7 days intervals with Freund's incomplete adjuvant (Difco) for 7 consecutive weeks. Five days after the last injection the blood samples were collected.

(ii) Bleeding :

Bleeding of rabbit was performed by ear vein puncture. In order to bleed rabbit or to handle them during injection, the animal was taken out from the cage, placed on its back on the wooden boards (measuring 60 cm x 30 cm x 1 cm,

fixed in a 60° position) with the neck in the triangular gap and the head below the board ; legs were tied to the screws and thus the body was fixed. The hairs were removed from the vein on the ear with the help of a razor and disinfected with rectified spirit. After irritation of the ear with xylene an incision was made with a sharp sterilized blade on the border vein of the ear and about 10 ml of the blood samples were collected in a sterile glass graduated tube. (Plate 2 , Fig. C). After collection of desired quantity of blood all precautions were taken to stop the flow of blood from the punctured area of the ear. The blood samples were kept as such for 1 h at 30°C for clotting. In order to avoid loss of serum included in the clot, it was loosened from the glass surface by turning a sterile wooden stick around the glass near the glass wall. Finally, normal sera as well as antisera were clarified by centrifugation(2000 g for 10 min. at 4°C) and distributed in small amounts in sterile closed ampules and stored at -20°C for further use.

(P) Determination of titre value :

Titre of antisera against the homologous antigens and titres of antigens against homologous antisera were determined following immunodiffusion technique as described by Ouchterlony (1967) and Clausen(1969). A constant amount (5 µl) of undiluted antiserum or antigen was placed in the central well, while diluted antigens or antiserum (diluted with normal saline, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128) were pipetted into the outer well. Diffusion was allowed for 48-72 h. at 25°C in a humid chamber. Titre was expressed as the reciprocal of the highest dilution of antiserum or antigen which reacted with antigen or antiserum giving precipition lines.

Plate-2 Fig : (A) Male rabbit (9 month old); (B) Antigen preparation emulsified with adjuvant being injected intramuscularly in rabbit, (C) Blood collection by ear vein puncture.

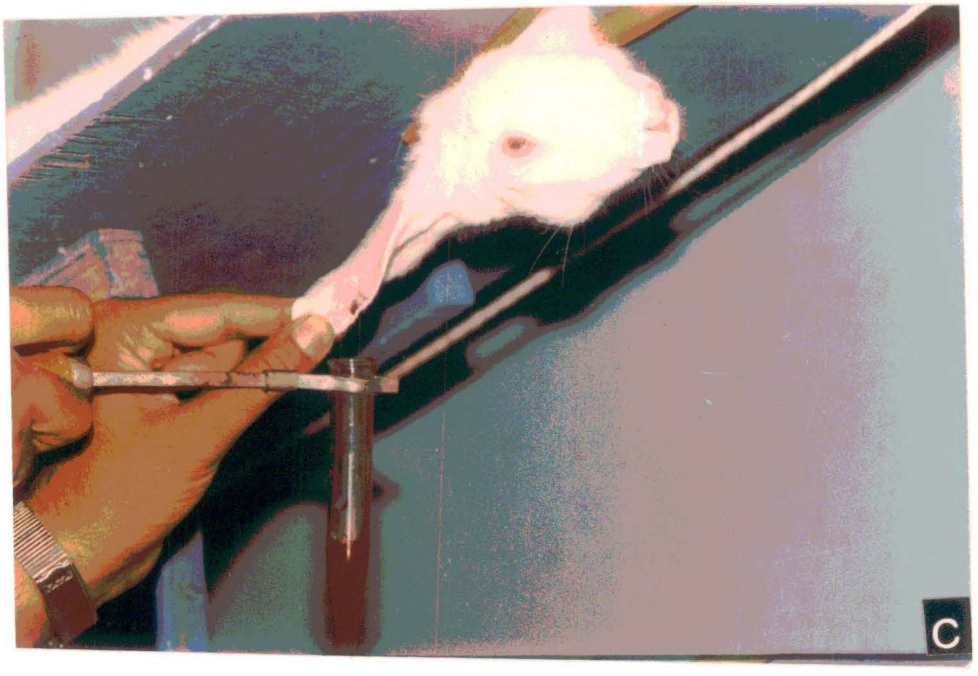


Plate- 2 .

(Q) Agar-Gel Double diffusion technique :

Agar-gel double diffusion test was performed following the method of Ouchterlony (1967). A conical flask (1 l) containing 500 ml barbitol buffer(0.05 M, pH-8.6) was placed in a boiling water bath, when the buffer was hot, it was mixed with 5 g. Difco agar during the next 40 min. The flask was repeatedly taken out and shaken thoroughly in order to prepare absolutely clear molten agar which was mixed with 0.1% (w/v) sodium azide (a bacterio static agent). The medium was dispensed in petridishes (5 ml/petridish, 5 cm. diam.) and 3-7 wells cut out with a sterilized cork borer at a distance of 5 mm. from the central well. The antigens and undiluted antisera were pipetted directly into the appropriate wells and diffusion was allowed to continue in a moist chamber for 24-28 h at 25°C. Precipitation reaction observed in the agar-gel only in cases where common antigens were present.

(R) Immunoelectrophoretic technique

(i) Preparation of agar slides :

The glass slides (7.5 x 2.5 cm) were degreased successively in 90%(v/v) ethanol; ethanol : ethyl ether (1:1 v/v) and ether. They were dried and numbered with glass marker and placed on horizontal wooden blocks. Thin and uniform layer (2 mm thickness) of fluid agar medium 0.8% Difco agar,0.1% NaN_3 dissolved in 0.05 M barbitol buffer pH-8.6) was drawn on each slide taking care that no air bubble was present in the agar medium. This was necessasry in order to avoid any irregularity which may cause asymmetrical migration and diffusion during electrophoretic separation or the immunodiffusion. The slides were placed in pairs in petridishes containing a few drops of water and stored at 4°C until use.

(ii) Electrophoretic step :

Two central wells (4 mm in diam.) were dug out from the agar plate of each slide following the conventional set up for comparison of two different antigens (Ouchterlony 1967). The slide was placed in the middle compartment of the electrophoretic box. The anode and cathode chambers were filled with barbitol buffer (0.05 M pH 8.6). Different antigens 10 /µl each were introduced into separate wells. Filter paper strips (whatman) were soaked in buffer and laid on both ends of the slides which connected the buffer solution in the anode and cathode compartments with the agar surfaces. An electric current 2.5m-amp./slide ; 10V/cm was passed through the slides for 2h in cold (4°C). After electrophoresis the current was discontinued .

(iii) Diffusion :

A longitudinal trough parallel to the long edge of the slide was cut in the agar plates in between 2 wells and the undiluted antiserum(100 /µl) was pipetted into the trough. Diffusion was allowed to continue in a moist chamber for 24-48 h at 25°C. Precipitation arcs were formed only when the common antigens were present.

(iv) Washing, drying and staining of slides :

After immunoelectrophoresis, the slides were washed with 0.9% (w/v) aqueous NaCl carefully for 48 h to remove unreacted antigen and antibody widely dispersed in the agar subsequently NaCl was washed with distilled water for 3 h dried for 30 min. at 40°C and stained either with 0.5% commasie blue or 0.5% amido black(0.5 g commasie blue or amido black, 5 g $HgCl_2$, 5 ml glacial acetic acid, distilled water 95 ml) for 30 min at room temperature (28°C), washed thrice in 2% (v/v) acetic acid for 3 h.(1 h in each item) to remove excess stain, finally washed with distilled water and dried for 30 min at 40°C.

(S) Indirect Enzyme Linked Immunosorbent Assay :-

Indirect ELISA technique described by Koening & Paul (1982) was adopted with modification. Plant and Fungal Antigens were serially diluted with loading buffer (0.05 M Carbonate Buffer. pH-9.6). Diluted antigens (100 /ul) were added to each well. The plates were incubated over night at 4°C and then washed thrice by flooding the wells with 0.15 M PBS, pH 7.2 containing 0.8% NaCl 0.02% KCl and 0.05% Tween 20 (PBS-Tween). After each washing the plates were shaken dry. Subsequently, 100 /ul PBS containing 1% BSA were added to each well in order to saturate all unbound sites (Sengupta et.al., 1989). The plates were further incubated ror 2 h at room temperature and washed thrice. Normal sera as well as plant and fungal antisera were serially diluted with PBS-tween containing 0.5% BSA and 100 /ul of diluted sera were added to each well. The plates were incubated overnight at 4°C following which the plates were washed thrice as before, shaken dry and 100 /ul of diluted (1:10,000) goat antirabbit IgG whole molecule) - Horse radish Peroxidase (HRPO, Sigma) conjugate were added to each well except the blank one & incubated for a further period of 2h at 30°C. After a further washing with PBS-tween, 100 /ul of the enzyme substrate [O-phenylene diamine (OPD), 1 /ug/ml in phosphate citrate buffer, pH-5.0, 25.7 ml of 0.2 M Dibasic Sodium phosphate (Na_2HPO_4), 24.3 ml 0.1 M Citric acid and 50 ml H_2O & 0.012% H_2O_2 was added just before use] was added to each well. Colour development was stopped by adding 50 /ul of 2M H_2SO_4 after 30 min. and absorbance was determined at 405 nm on a ELISa microplate reader, Model 700 (Cambridge Technology, USA).

(T) Fluorescent antibody staining and microscopy :

(a) Fusarium graminearum

Folowing the method of Merz et.al. (1969) with modifications mycelia of F.graminearum were grown on agar

squares (aproximately 1 cm^2) of Richards' medium (KNO_3, 10g; KH_2PO_4, 5g; $MgSO_4$; $7H_2O$, 2.5g; $FeCl_3$; 0.02g; Sucrose, 30 g; Agar, 20g; and 1 l. of distilled water). The inoculated agar blocks were placed on sterile microscopic slides, covered with sterile cover - glass, and incubated in sterile Petridishes containing filter paper for 5 days at 25°C. Finally, the agar blocks were removed, and the mycelium that had adhered to the glass slide was fixed in 95% ethanol-ethyl ether (1:1, v/v) for 10 min. at room temperature followed by 95% ethanol for 20 min. at 37°C. The fixed mycelium was flooded with appropriate antiserum, incubated in a moist chamber for 30 min. washed thrice (5 min. each) with PBS, pH-7.4. The slides were then air dried and goat anti-rabbit IgG (whole molecule) conjugated with fluorescein isothiocyanate (Sigma) diluted 1:10 with Carbonate-bicarbonate buffer, pH 9.6 was added for 30 min. The slides were then washed twice (5 min each), in PBS, pH-7.4 and once in distilled water. The slides were then air dried and mounted in a glycerol-based mounting medium, (Hardham et.al., 1986). A cover glass placed on the mycelium and sealed.

(b) Root of Glycine max. :

For fluorescence staining of root material, the technique of DeVay et.al., (1981) was followed.

Soybean seedlings grown in a sterile mixture of sand and soil (1:1) were harvested 10 days after sowing. Cross sections of the tap root at or just above the region of root hair formation were made and immediately immersed in 100 µl of normal serum, or antiserum diluted (10-fold) with phosphate buffer(0.01 M KH_2PO_4 - K_2HPO_4 containing 0.14 M NaCl, pH 7.0), and incubated for 30 min. at 27°C. The root sections were washed by shaking them in 4 ml of the buffer for 15 min and then transferring them to a 50 µl drop of goat antiserum specific to rabbit globulins and conjugated with fluorescein isothiocyanate(FITC). The sections were incubated for 30 min

in a moist chamber at 27°C. All operations with FITC labelled antibodies were made in darkness or very low light. The sections were then transferred to 4 ml of the buffer and incubated with shaking for 15 min at 27°C. This last step was repeated and then the sections were mounted on a slide in 100 /ul of the buffer diluted with 1 ml glycerol. A cover slip was placed on the tissue sections and sealed. Fluorescence of the root sections were observed using Leica Leitz Biomed microscope with fluorescence optics 020507 type equipped with ultra violet (UV) filter set I-3. Tissue sections were photographed under both phase-contrast and U.V. fluorescent conditions for comparison of treatments.

EXPERIMENTAL

PART - I

Pathogenicity test of Fusarium graminearum on different soybean cultivars :

Pathogenicity of F.graminearum was tested on ten cultivars of soybean (Viz. Soymax, KU-254, EC-2575, PK-327, JS-2, EC-95287, Pusa-16, EC-55865, UPSM-19 and R-184). Methods of inoculation and disease assessment have been described in detail under Materials and Methods and the results are given in Table-2 and Fig.1. Healthy and inoculated plants were uprooted after 10, 20 and 30 days of inoculation and percentage loss in dry weight of roots were determined and root rot index of infected roots were compute. Young plants of cv. Soymax after 10 days of inoculation with sand maize meal culture of F.graminearum, showed initially wilting symptom (Plate3, Fig.A)which was followed by necrosis (Plate 1, Fig. A). In its advance stage, infection was very much prominent in the root system. Extent of root damage by F.graminearum in relation to healthy (control) was also evident in uprooted plants of cv. Soymax (Plate1, Fig. B). The root rot index as well as percentage loss in dry weight were very low at the initial stage of infection but increased significantly with time in susceptible cultivars.

It appears from the result given in Table-2, and Fig.1 that among the ten cultivars tested against F.graminearum, cv. Soymax was found to be highly susceptible while cv. UPSM-19 was resistant. Three other cultivars viz., JS-2, KU-254 and EC-2575 were also found to be susceptible (Plate-3, Fig.B and Plate-4, Fig. A & B). Maximum loss in root weight (12-17%) was noticed in cvs. Soymax and JS-2 within 10 days of inoculation while only 2-3% loss was determined in cvs-UPSM-19 and EC-55865 under similar condition. In other cases,(cvs EC-95287, Pusa-16 and PK-327) moderate loss (4-6%)

Pathogenicity test of Fusarium graminearum on different cultivars of soybean

Cultivars	Average dry wt. of roots(mg) with S.E.[a]						%Loss in dry wt.[b]			Root rot index of[c] infected roots		
	10 days		20 days		30 days		10 days	20 days	30 days	10 days	20 days	30 days
	Healthy	Infected	Healthy	Infected	Healthy	Infected						
Soymax	135.0 ± 3.1	112.0 ± 2.8	315.0 ± 4.2	186.0 ± 2.4	460.0 ± 4.9	190.0 ±3.2	17.0	40.9	58.6	1.4	2.5	3.1
JS-2	114.0 ± 4.8	100.0 ± 3.2	266.0 ± 4.4	190.0 ± 2.3	380.0 ± 2.9	226.0 ± 2.6	12.2	28.5	40.5	1.0	1.7	2.4
KU-254	144.6 ± 3.6	128.0 ± 2.5	337.0 ± 2.4	288.0 ± 2.6	482.0 ± 3.8	300.0 ± 3.6	11.4	26.4	37.7	1.0	1.5	2.2
EC-2575	126.0 ± 4.6	113.0 ± 3.4	294.0 ± 4.8	226.0 ± 2.4	420.0 ± 3.6	280.0 ± 4.8	10.3	23.1	33.3	1.0	1.8	1.9
PK-327	112.0 ± 3.2	105.0 ± 2.6	260.0 ± 2.3	221.8 ± 3.8	374.0 ± 3.5	294.0 ± 2.4	6.2	14.6	21.3	1.0	1.1	1.7
EC-95287	97.2 ± 4.8	93.0 ± 3.2	226.0 ± 2.4	199.2 ± 3.6	324.0 ± 4.8	274.0 ± 2.4	4.3	11.8	15.9	1.0	1.0	1.3
Pusa-16	102.6 ± 3.6	98.0 ± 4.8	238.0 ± 3.6	214.0 ± 2.4	342.0 ± 4.8	290.0 ± 4.4	4.4	10.4	15.2	1.0	1.0	1.2
R-184	117.0 ± 4.4	113.0 ± 1.6	273.0 ± 2.4	246.0 ± 1.6	390.0 ± 2.4	340.0 ± 2.6	3.4	9.8	12.8	1.0	1.0	1.0
EC-55865	150.0 ± 3.8	145.0 ± 2.4	350.0 ± 5.8	316.0 ± 4.6	500.0 ± 3.8	339.0 ± 2.4	3.3	9.8	12.8	1.0	1.0	1.0
UPSM-19	112.8 ± 3.2	110.0 ± 2.8	262.0 ± 4.6	244.0 ± 4.2	376.0 ± 3.6	344.0 ± 2.4	2.4	6.2	8.5	1.0	1.0	1.0

a = Average of 50 plants/cultivar
b = In relation to control
c = Root rot index(1-10% loss in wt.= 1, 11-25%=2, 26-50%=3, 51-75%=4, 76-100%=5)

PERCENTAGE LOSS IN DRY WEIGHT OF ROOT OF DEFFERENT CULTIVARS OF SOYBEAN DUE TO INFECTION WITH *F. graminearum* .

[30 DAYS AFTER INOCULATION]

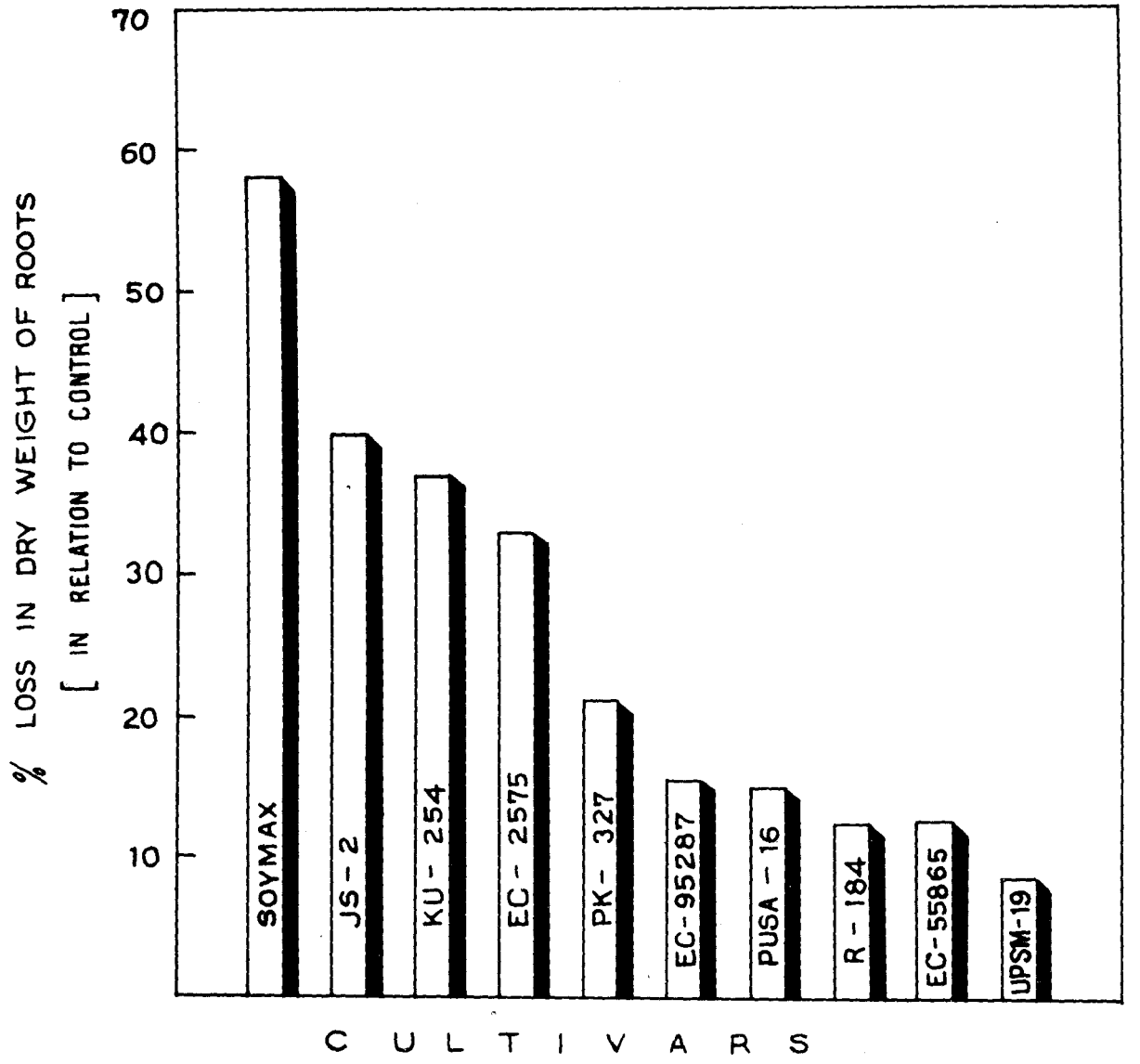


Fig. 1.

Plate-3 Fig.(A & B): soybean plants showing initial wilting symptoms after 10 days of inoculation with sand maize meal culture of F.graminearum (A) cv. Soymax ; (B) cv. JS-2.

Plate - 3

Plate-4 Fig. (A & B) : Soybean plants showing initial wilting symptoms after 15 days of inoculation with sand maize meal culture of F.graminearum **(A)** cv. KU-254 ; **(B)** cv. EC-2575.

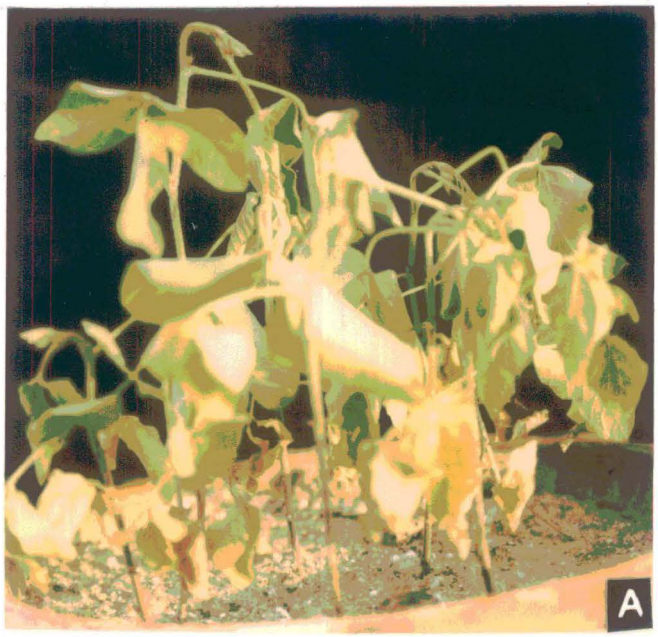


Plate - 4

in dry weight of roots was recorded in relation to control. The differences in dry weight loss between infected Cvs. Soymax, JS-2, Pusa-16 and UPSM-19 were compared after different periods (10, 20 and 30 days) of incubation. Differences between susceptible cultivars (Soymax and JS-2) and resistant cultivars (UPSM-19 and Pusa-16) in their percentage loss in dry weight of roots remained unchanged even after 30 days of incubation with regard to their inherent resistance to F.graminearum (Fig.2). On the basis of pathogenicity test of F.graminearum, on ten soybean cultivars, UPSM-19, EC-55865 and R-184(resistant) and Soymax, JS-2 and KU-254 (susceptible) were chosen for further studies.

Following sand maize meal culture or sick pot inoculation method, disease incidence could be determined only after 10 days of inoculation. However, water culture method was adopted for inoculation and detection of biochemical changes. In this case direct response of F.graminearum could be detected as early as 24-48 h of inoculation of 15 day old soybean plants (cv. Soymax) in relation to distilled water control (Plate-5, Fig. A-C). After 96 h yellowing of leaves, wilting of plants as well as discolouration of roots were very much prominent in cvs. JS-2, EC-2575 and KU-254 (Plate-6, Fig.A-F).

Plate-5 Fig.(A-C) : Soybean plants (Cv.Soymax) healthy and inoculated with F.graminearum by water culture method. **(A)** Healthy plants dipped in distilled water ; **(B)** 24h after inoculation ; **(C)** 48h after inoculation.

Plate - 5

Plate-6 Fig. **(A-F)** : Soybean plants taken out after 96h of inoculation with F.graminearum (following water culture method). **(A & D)** cv.JS-2; **(B & E)** cv. EC-2575, **(C & F)** cv. KU-254 , **(D-F)** Root portions magnified.

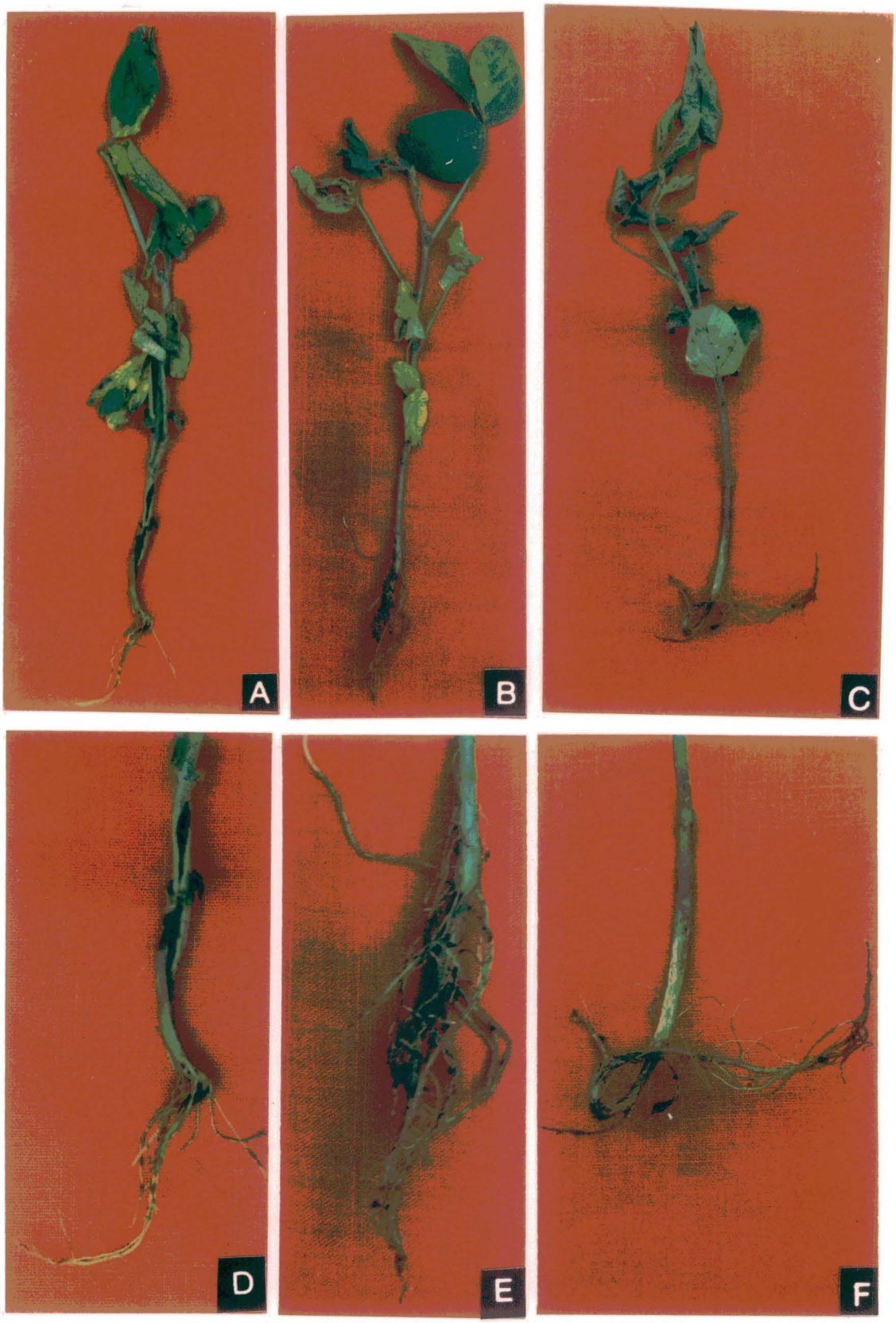


Plate- 6

EFFECT OF INCUBATION TIME ON THE DEVELOPMENT OF ROOT ROT DISEASE OF SOYBEAN

[DISEASE INTENSITY EXPRESSED AS % LOSS IN DRY WEIGHT OF ROOTS]

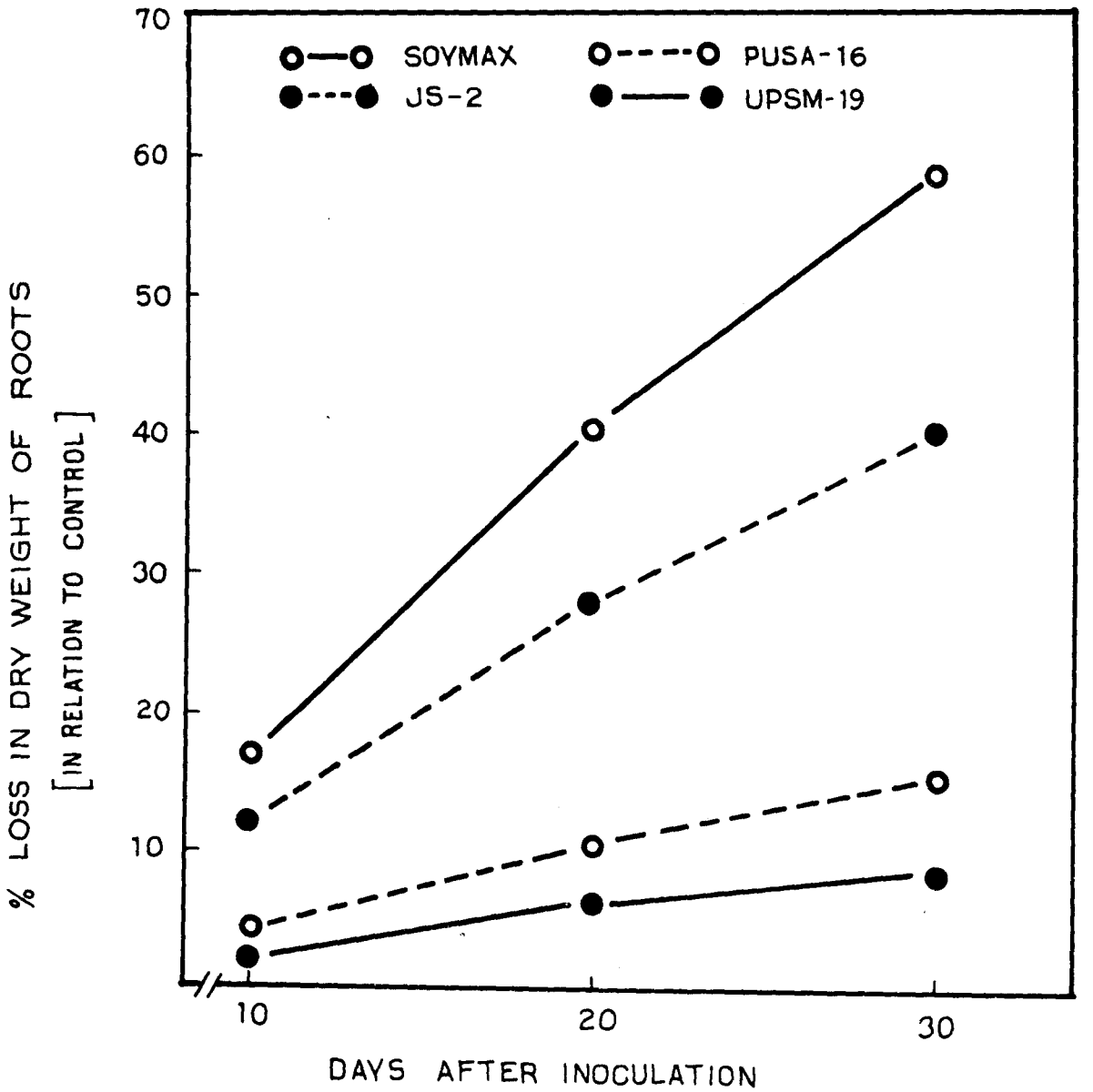


Fig. 2.

PART - II

Cultural conditions affecting growth of F.graminearum:

The colonies of F.graminearum were very fast growing on potato dextrose agar medium, reaching 9 cm diam. in 4 days at 25°C, greyish rose to livid-red to crimson (Plate-7, Fig. A & B), often becoming vinaceous with a brown tinge, aerial mycelium floccose, some-what lighter coloured and becoming brown. Sporulation often scarce, densely branched conidiophores occurring besides solitary phialides, phialides doliiform, 10-14 x 3.5-4.5 /um. Conidia slender falcate, moderately curved, with pointed and curved apical and pedicellate basal cells, mostly 5-6 septate and 41-60 x 4.0-5.5 /um (Plate-7, Fig. C-E). Chlamydospores scarce and often completely absent, mostly intercalary and in chains 10-12 /um diam. Germination of conidia on glass slides were observed after 20 h of incubation at 25°C (Plate-7, Fig.).

Effect of incubation time :

Effect of incubation time on the mycelial growth of F.graminearum was studied in vitro. F.graminearum was grown in Richard's medium for a period upto 30 days at 30±1°C. The mycelial growth of the fungus was recorded after 5,10,15,20, 25 and 30 days. The results are embodied in Table-3 and Fig.3. Maximum growth of F.graminearum was observed after 15 days of incubation and then rate of growth declined. Mycelial growth increased by 49.87% from 10 to 15 days of incubation and decreased by 5.03% from 15 to 20 days, and 18.59% from 25 to 30 days of incubation.

Effect of pH :

Initially buffer solution with pH values ranging from 4 to 8(4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 8.0) were prepared by mixing KH_2PO_4 and K_2HPO_4 each at 0.03 M

Plate-7 **Fig.(A & B)** Mycelial growth of F.graminearum on potato dextrose agar medium ; **(A)** front view and **(B)** reverse side of the petridish; (C-E) Macroconidia of F.graminearum (X 850), (F) germinated conidium (X 500).

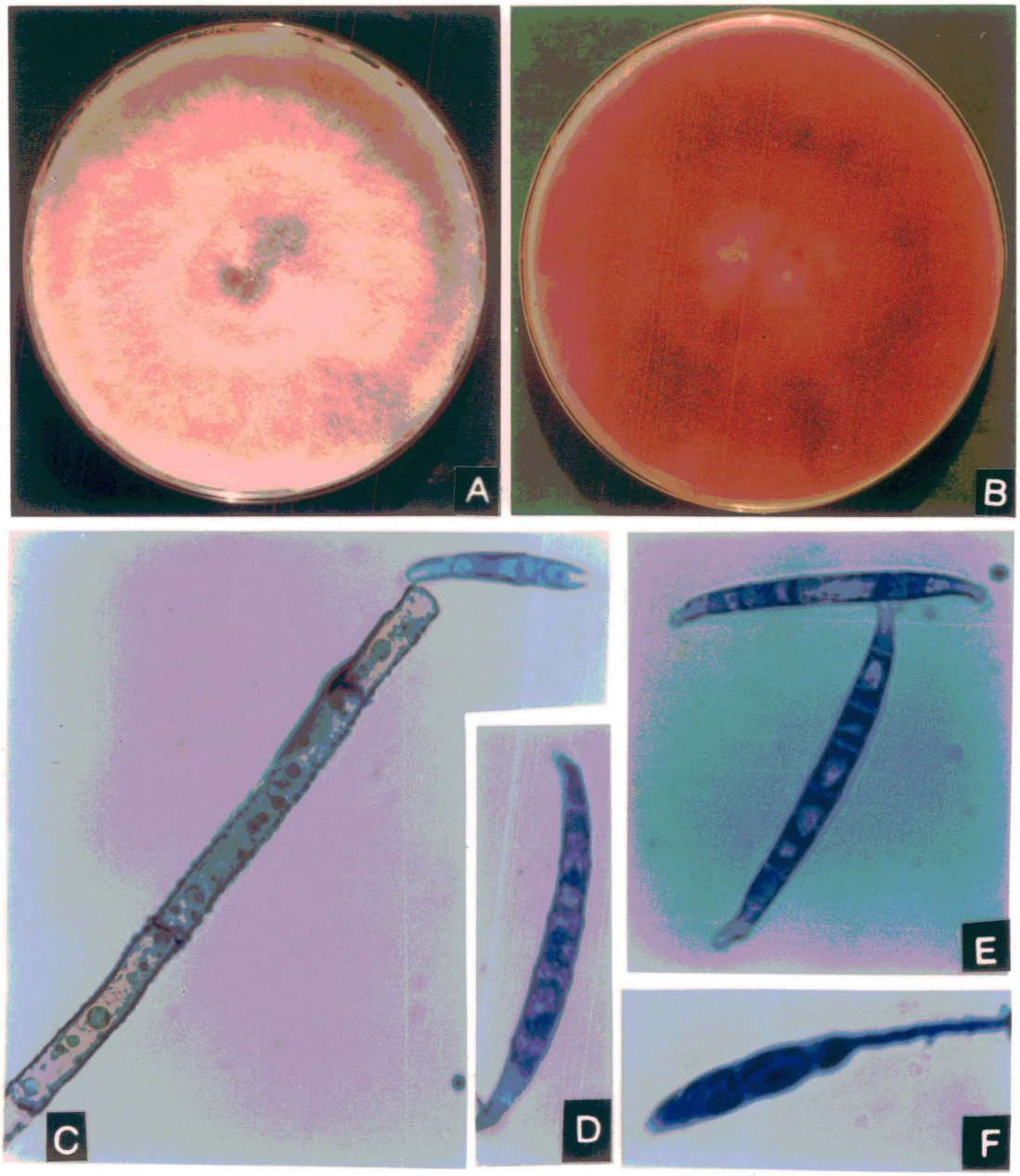


Plate - 7.

Table-3 : Effect of incubation period on the mycelial growth of F.graminearum.

Days	Average mycelial dry wt. (mg)[a]
5	210.1 ± 2.6
10	411.6 ± 1.8
15	616.2 ± 1.5
20	585.3 ± 2.4
25	554.3 ± 1.8
30	451.2 ± 2.3

Temperature - 30 ± 1°C

a - Average of 3 replicates.

concentration. The pH of the medium was adjusted using N/10 NaOH or N/10 HCl to obtain the corresponding range of pH values. Richard's medium and phosphate buffer was sterilized by autoclaving for 15 min. at 15 lbs. p.s.i. pressure and equal part of buffer and medium were mixed before use. Each flask (250 ml) containing 50 ml of the medium was inoculated with mycelial block (4 mm diam.) of F.graminearum and incubated for 15 days at 30 ± 1°C. The results are given in Table-4. It appears that F.graminearum grew over a range of pH 5.5-7.0 and showed optimum growth at pH 6.5 (Fig.4). Mycelial growth increased upto pH 6.5 and then gradually declined. The percentage increase in mycelial growth of F.graminearum from pH 6.0 to 6.5 (optimum) was 5.71% while 46.95% reduction in growth was noted from pH 7.0 to 8.0.

EFFECT OF DIFFERENT INCUBATION PERIOD ON GROWTH OF F. graminearum

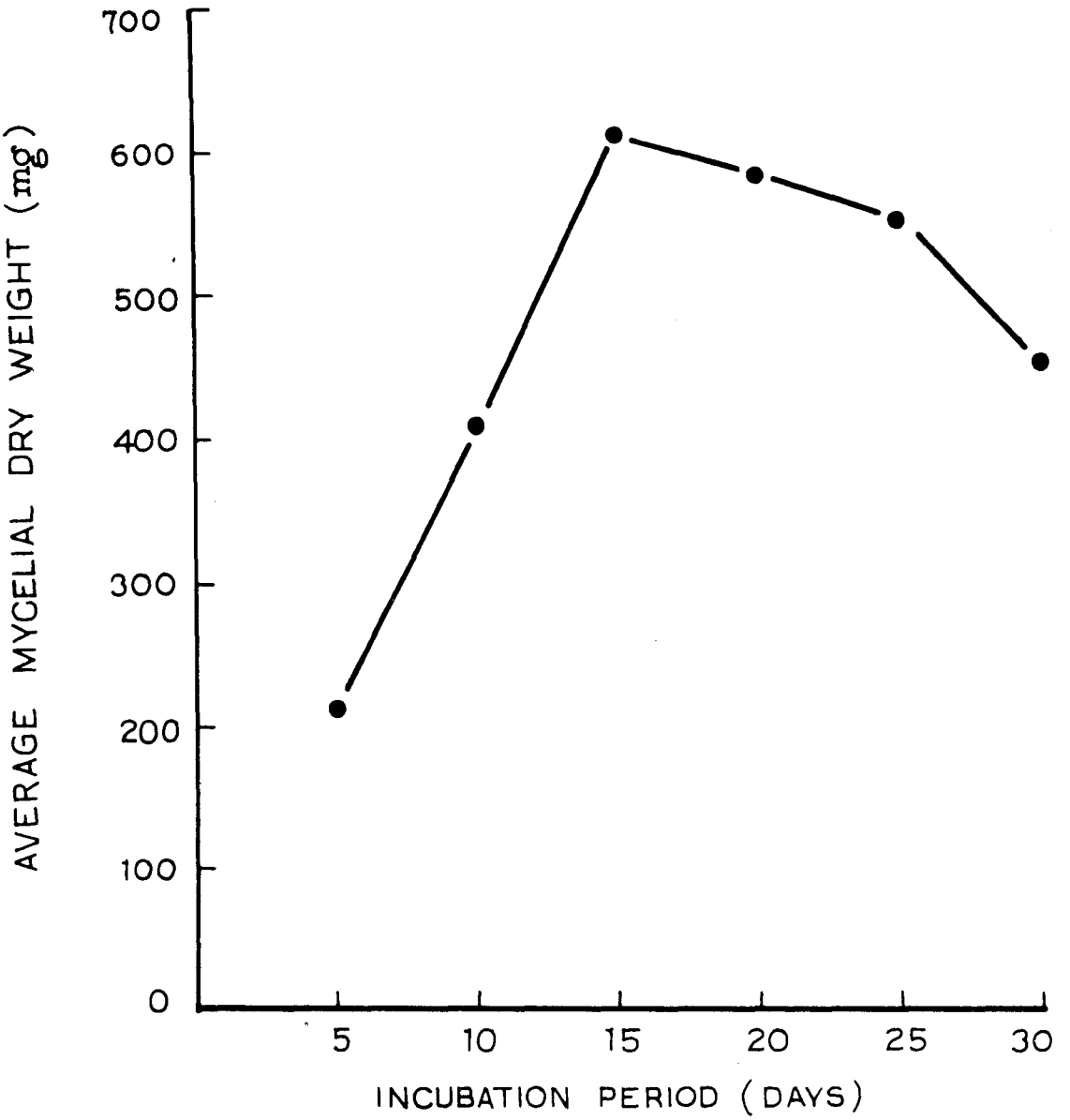


Fig. 3.

Table-4 : Effect of different pH on the mycelial growth of F.graminearum.

pH	Average mycelial dry wt.(mg)[a]
4.0	355.0 ± 2.2
4.5	425.2 ± 3.3
5.0	480.4 ± 2.5
5.5	516.6 ± 1.9
6.0	530.0 ± 2.6
6.5	560.3 ± 2.5
7.0	502.0 ± 3.8
8.0	266.3 ± 2.9

Incubation period - 15 days
Temperature - 30 ± 1°C

Effect of temperature :

Like previous experiments F.graminearum was grown in Richard's medium and incubated for 15 days at different temperatures ranging from 20°C to 40°C. Results (Table-5) revealed that F.graminearum grew over a wide range of temperature, however, maximum growth was noted at 30°C and then there was a gradual decline.

Table-5 : Effect of different temperatures on the mycelial growth of F.graminearum.

Temperature	Average mycelial dry wt (mg)[a]
20°C	387.6 ± 4.3
25°C	527.3 ± 2.8
30°C	636.8 ± 4.9
35°C	408.5 ± 3.6
40°C	141.9 ± 4.5

a - Average of 5 replicates, pH of medium 6.5, Incubation period 15 days.

EFFECT OF DIFFERENT pH ON GROWTH OF *F. graminearum* .

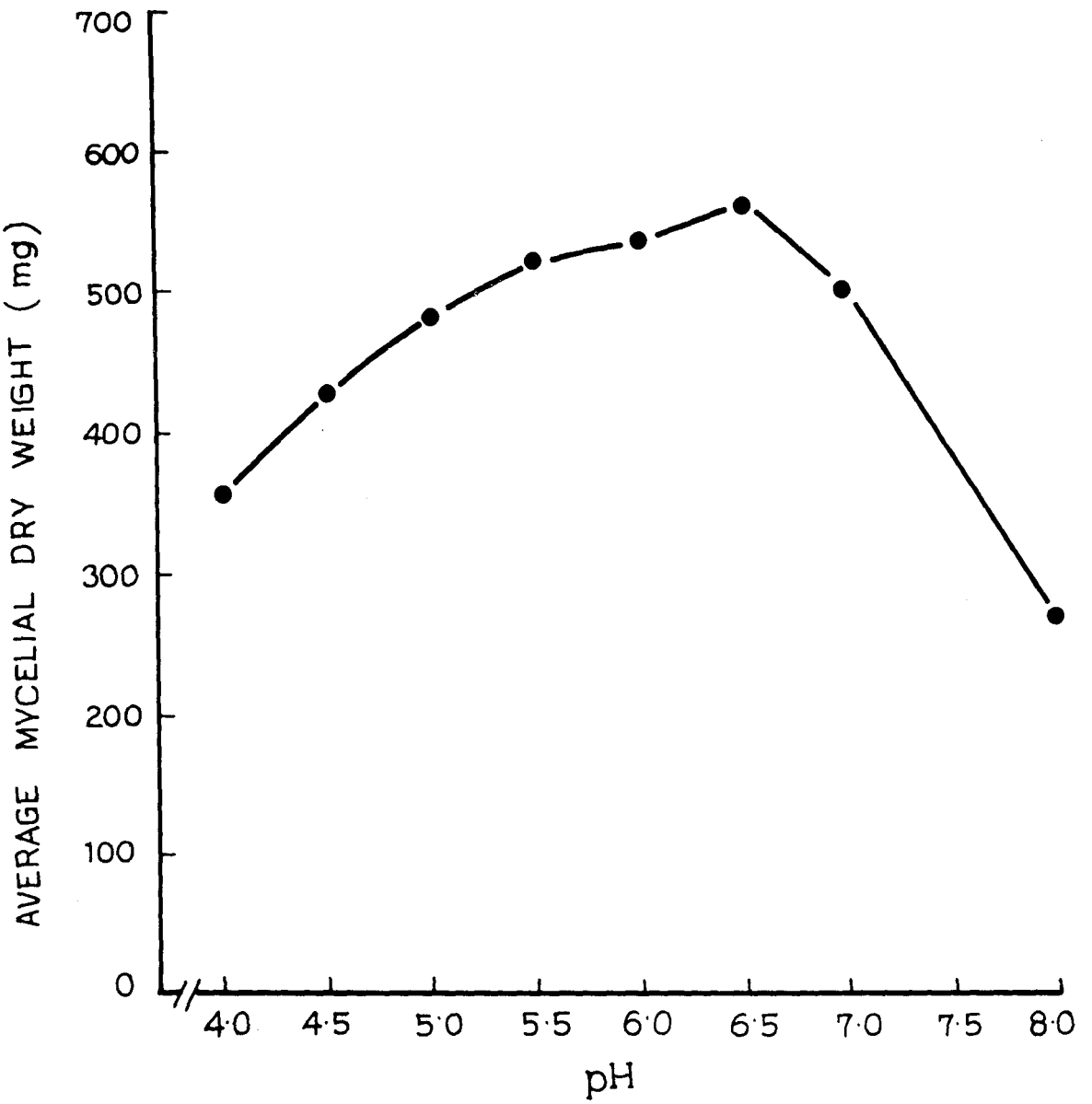


Fig. 4

PART - III

Phytotoxic effect of metabolic byproducts in the culture filtrate of F.graminearum on soybean plants :

An experiment was performed with a view to determine the phytotoxic effect, if any, of metabolic by-products of the culture filtrate of F.graminearum. The fungus was grown in Czapek-Dox medium for 20 days. Culture filtrates were collected, centrifuged and half of it was autoclaved, while the rest was cold sterilized. Fifteen-day old soybean plants of two cultivars JS-2 and Soymax were introduced into tubes containing culture filtrate, sterile distilled water and medium. Wilting index per plant was computed after 48 h of incubation. Results (Table-6) revealed that the toxic principle is partially theromolabile. Less than 50% mortality was noted (Plate-8, Fig. A & B).

Table-6 : Effect of culture filtrate of F.graminearum on Soybean plants.

Treatment	Wilting index[a]/plant[b]	
	CV-Soymax	CV- JS-2
Culture filtrate		
Heat killed	1.2	1.8
Cold sterile	3.6	3.0
Czapek Dox medium	0	0
Sterile distilled water	0	0

a - Wilting index ; 0=no wilting; 1=10% or less mortality; 2=11-20% mortality; 3=20-50% mortality; 4=51% or more mortality

b - 20 plants/treatment

Plate-8 Fig.(A) Soybean plants (cv. Soymax) in sterile distilled water ; **(B)** Soybean plants (cv. soymax) in cell-free culture filtrate of F.graminearum (48h of incubation).

Plate- 8

The culture filtrate was dialysed against 0.08% NaCl, sterile distilled water and the medium for 24h at 4°C with atleast five changes. The reason for dialysis against the medium was to maintain as far as possible the same concentration of the ingredients present except for fungal metabolites. Dialysis against salt was done because salt might act to maintain the activity of some enzymes. The dialysis against sterile distilled water was performed to see whether the fungal metabolites effective for symptom expression in plants was diffusable or not. Undialysed culture filtrate, sterile distilled water and medium as well as three dialysates were used for bioassay with 15-day old soybean plants (Table-7).

Table-7 : Effect of dialysed culture filtrates on soybean plant (cv. Soymax)

Treatment	Wilting index[a]/plant[b]
Culture filtrate	
Undialysed	3.8
Dialysed against NaCL	3.2
Dialysed against SDW	3.0
Dialysed against CDM	2.6
Control	
SDW	0
CDM	0

a - Wilting index - 0=no mortality, 1=10% or less mortality, 2=11-20% mortality, 3=20-50% mortality, 4=51%or more mortality

b - 20 plants/treatment. SDW = Sterile distilled water, CDM= Czapek Dox medium.

Plate-9 Fig.(A-E) Soybean plants (cv. Soymax) after 48h incubation in cell-free culture filtrate (20 days old) of F.graminearum ; **(A)** Left to right undialysed (first & second) ; dialysed (third & fourth) ; **(B & C)** undialysed, **(D & E)** - dialysed (root portions magnified)

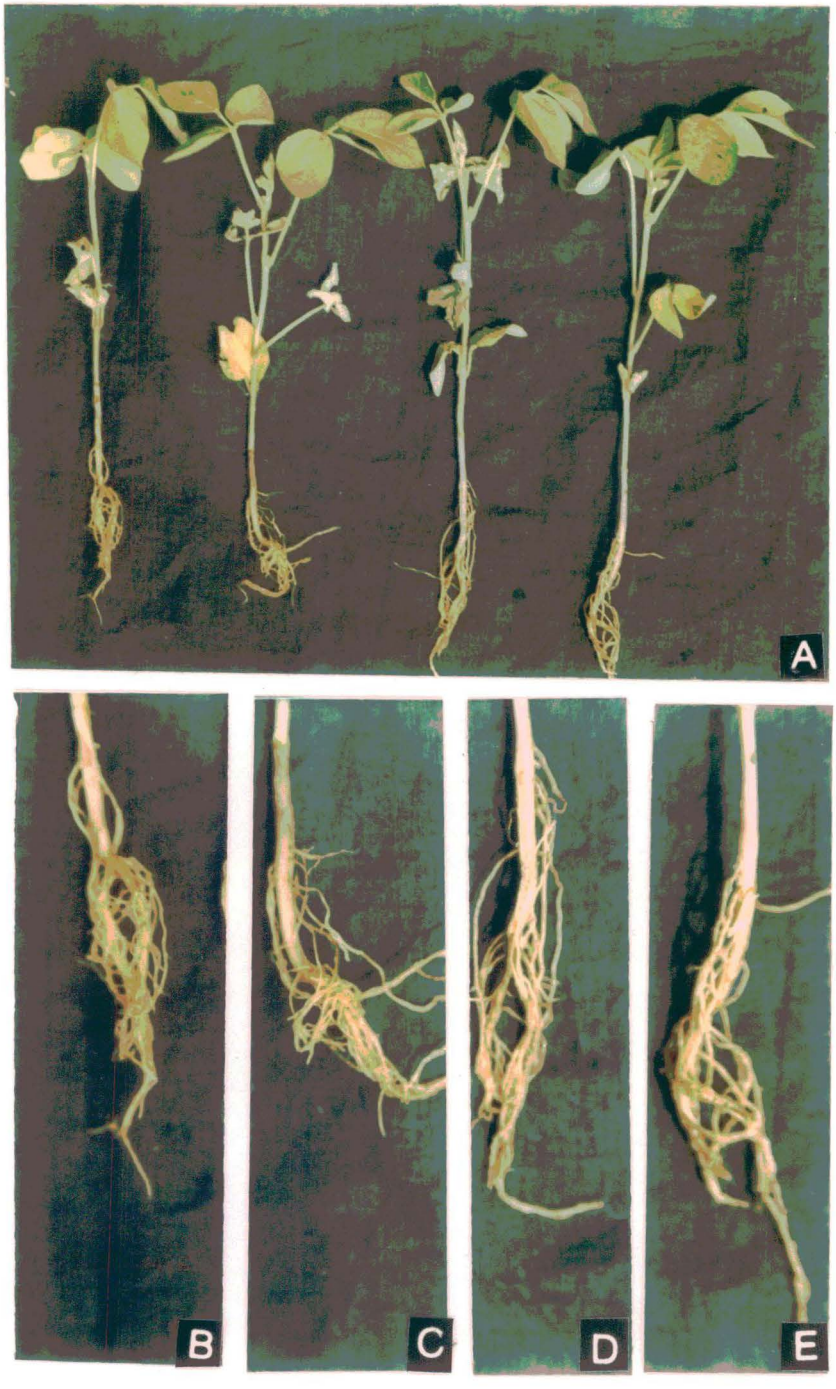


Plate - 9

Results suggest that the toxic principle is non-dialysable because wilting index both in undiaslysed as well as dialysed culture filtrate was more or less same (Plate-9, Fig.A). Roots were turned brownish in colour after 48h of incubation (Plate-9, Fig. B-E).

PART-IV

Comparison of glyceollin contents of soybean cultivars after incubation with F.graminearum.

It is evident from the results in part-I that among the ten soybean cultivars tested against F.graminearum, UPSM-19, EC-55865 and R-184 were found to be resistant while Soymax, JS-2 and KU-254 were highly susceptible. The differential resistance of soybean cultivars in response to infection with F.graminearum may be attributed to differences in their abilities to produce glyceollin (phytoalexin) in response to infection. Disease resistance of several crop plants has been correlated with the rate of production of phytoalexin by a number of previous research workers (Cruickshank, 1980; Keen, 1981; Bhattacharyya & Ward, 1986; Nemestothy and Guest, 1990; Paxton, 1995 and Avazkhodjaev et.al., 1995).

Since no work on glyceollin production by soybean plants as a result of interaction with F.graminearum has been reported so far, it was considered worthwhile to compare the glyceollin content of resistant and susceptible soybean cultivars. Glyceollin was extracted and separated from the infected roots of soybean following the scheme given below. Healthy roots were also used for extraction which was considered as control. In this case, the healthy plants grown in sterilized soil were uprooted, washed in sterile distilled water and dipped in conidial suspension of F.graminearum (Plate 10, Fig.A) following the "water culture" inoculation

Extraction and separation of glyceollin

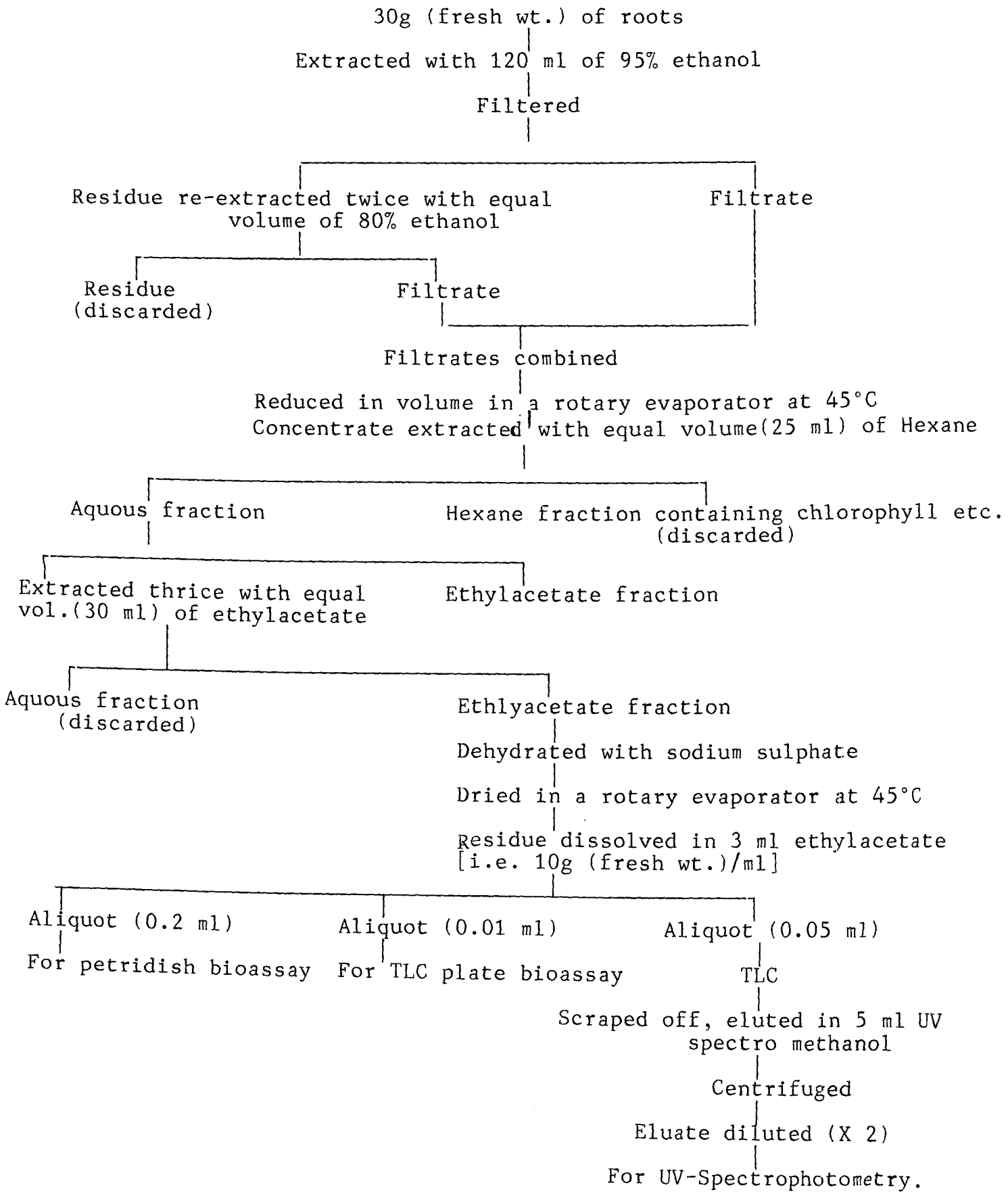


Plate-10 Fig.(A) Soybean plants (cv. Soymax) inoculated with F.graminearum following water culture method. (B) Inoculated plants 96h after inoculation ; (C) magnified portion of infected roots.

Plate - 10

technique as described under materials and methods. Yellowing of the lower leaves and browning reaction of root system was evident after 96 h of inoculation in susceptible soybean cv. Soymax (Plate 10, Fig. B & C).

In order to detect glyceollin from infected soybean roots as well as to select the best solvent system(for better separation), initially ethyl acetate fraction of root extracts loaded on TLC plates were developed in four different solvent systems as mentioned earlier (vide Materials & Methods) and sprayed with p-nitroaniline. The Rf values of glyceollin obtained from infected soybean roots (Table-8) were compared

Table-8 : Comparison of Rf values of authentic glyceollin with the glyceollin extracted from Soybean roots inoculated with F.graminearum

Solvent Systems [a]	Rf values of glyceollin	
	A	B
Hexane : ethylacetate:acetic acid (80:20:4)	0.25	0.23
Hexane:ethyl acetate:methanol (60:40:1)	0.60	0.60
Chloroform:acetone:28% NH_3 (65:35:1)	0.50	0.51
Chloroform:acetone:acetic acid (90:10:0.5)	0.48	0.45

A = Authentic glyceollin
B = Glyceollin obtained from soybean roots inoculated with F.graminearum.
a = Spray reagent - Diazotised p-nitroaniline.

after co-chromatography with authentic sample. Finally, the solvent system hexane : ethylacetate : methanol (60:40:1) was chosen.

Methanolic solution of glyceollin when examined in a UV-spectrophotometer, maximum absorption was observed at 286 nm(Fig.5). No such absorption was noted for healthy root extraction. After detection of glyceollin, its antifungal activity was tested following TLC plate assay, radial mycelial growth as well as spore germination assay methods. The procedures have already been described. In TLC plate bioassay using Bipolaris carbonum as a test organism, 12 mm diam inhibition zone at Rf. 0.60 was observed on the chromatogram after 72 h of incubation at 25°C. Silica gel corresponding to the inhibition zone (Rf 0.60) were removed from freshly prepared TLC plates and eluted by methanol. Purified eluants obtained after re-chromatography were tested for antifungal activity by spore germination assay as described under Materials & Methods. Spore Germination of F. graminearum was strongly inhibited after 24 h of incubation. The relative anti-fungal activity of glyceollin was compared against F.graminearum, F.oxysporum, F.solani, Dreschlera oryzae, Glomerella cingulata, Pestalotiopsis theae and Bipolaris carbonum following petridish bioassay method. Inhibition of radial growth of the fungal species are presented in Table-9. Thus, antifungal nature of glyceollin was confirmed.

Accumulation of glyceollin from six different cultivars (UPSM-19, JS-2, Soymax, KU-254, R-184 and EC-55865) were detected after 24, 48, 72 and 96 hours of inoculation with F.graminearum. The results are presented in Table-10. It appears from the Table-10 that resistant cultivars contained more glyceollin than the susceptible cultivars. Approximately 214.34% more glyceollin accumulation was detected in

UV- ABSORPTION SPECTRA OF GLYCEOLLIN

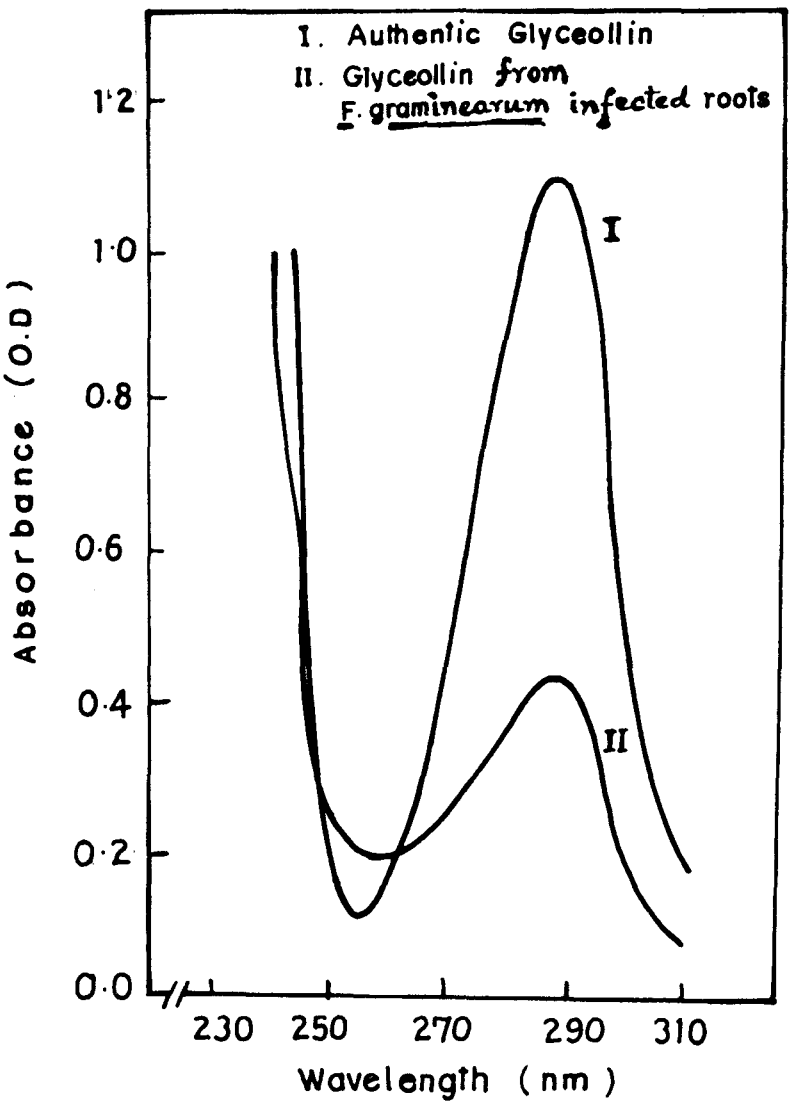


Fig. 5.

Table-9 : Inhibition of radial growth of fungi by glyceollin.

Fungi	Percent inhibition of mycelial growth (25 µg/ml glyceollin)*
F.graminearum	92
F. oxysporum	90
F.solani	84
Dreschlera oryzae	77
Glomerella cingulata	80
Pestalotiopsis theae	45
Bipolaris carbonum	64

*[Fungi were bioasayed in 2 ml of medium in petriplates (35 mm diam.). Measurements were taken when net radial growth in the controls was 30±2 mm. Growth was calculated by measuring two diameters for each of three replicate colonies and substracting the diameter of the mycelial plug(2mm) used to inoculate the plates.]

CV.UPSM-19 than the cultivar Soymax. This experiment was repeated twice and same trend in glyceollin accumulation was noticed in those six cultivars. Average glyceollin content of six cultivars after 48 h of inoculation with F.graminearum are presented in Fig.6. Highest accumulation of glyceollin at this period was noticed. Hence in further experiment this time period was considered for detection of glyceollin accumulation.

Table-10 : Comparison of glyceollin content of F.graminearum infected roots of resistant and susceptible soybean cultivars.

Cultivars	Glyceollin Content(/ug/g fresh wt. of roots			
	24 h	48 h	72 h	96h
Resistant cvs.				
UPSM-19	460.0 ± 5.9	896.5 ± 9.7	610.0 ±10.3	224.9 ± 6.2
EC-55865	327.6 ± 8.2	740.0 ± 5.5	636.5 ± 8.7	201.8 ± 9.7
R - 184	296.5 ± 4.8	705.4 ± 6.2	452.3 ± 5.8	187.2 ± 6.3
Susceptible cvs.				
Soymax	90.8 ± 4.6	285.2 ± 4.7	106.3 ± 3.9	49.9 ± 3.6
JS - 2	155.0 ± 7.2	410.5 ± 8.4	295.8 ±9.8	82.5 ± 5.7
KU - 254	236.5 ± 5.7	652.8 ± 9.7	376.2 ± 8.8	111.3 ± 6.4

PART-V

Experiments on the alteration of disease reaction by the application of various chemicals and their fungitoxic effect on F.graminearum.

It is evident from the result presented in Part IV that resistant cultivars of soybean (Cvs-UPSM-19, EC-55865 & R-184) accumulated greater amount of glyceollin than the

GLYCEOLLIN CONTENTS OF F. graminearum INFECTED ROOTS OF SOYBEAN CULTIVARS.

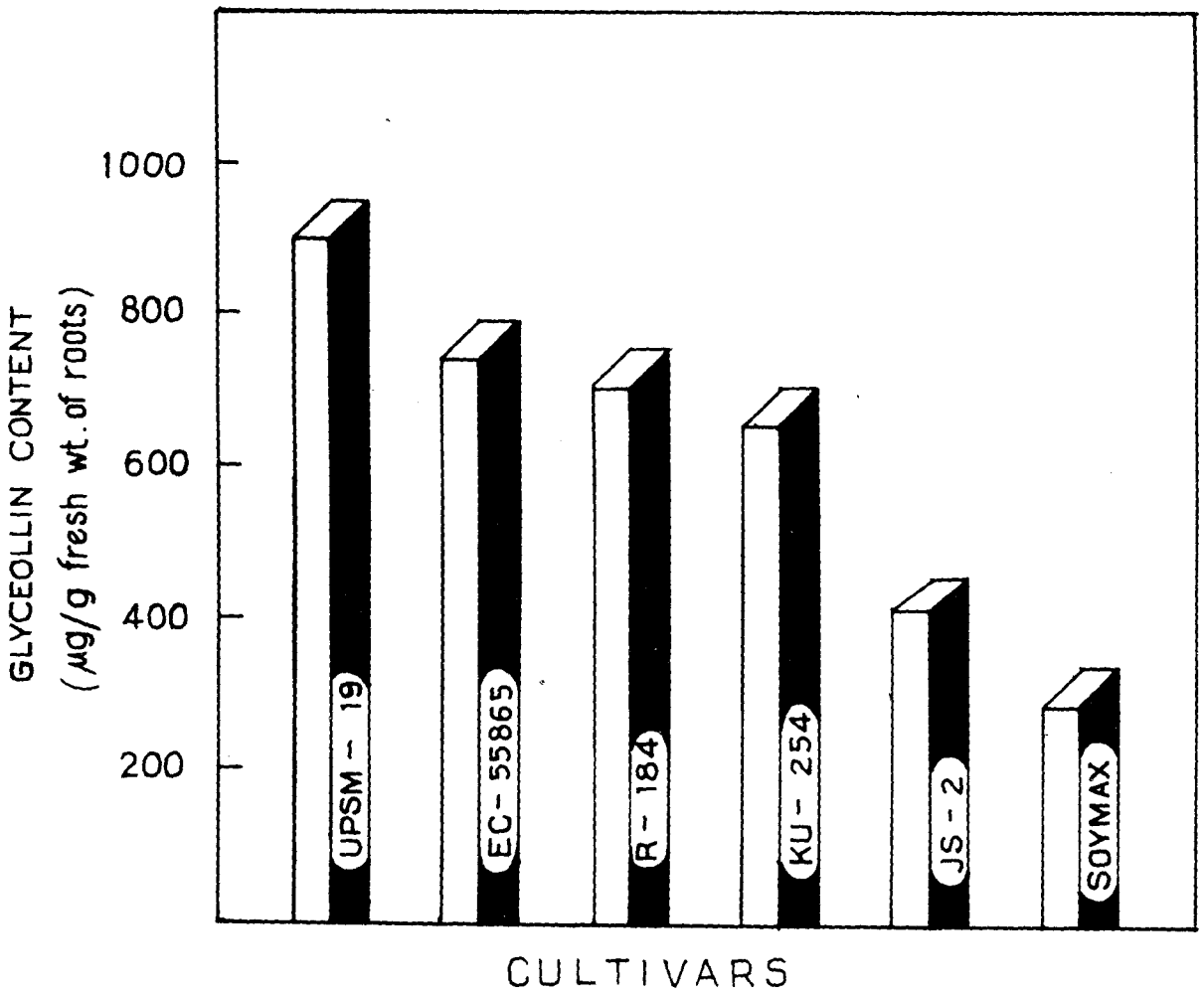


Fig. 6.

susceptible cultivars (Cvs-Soymax, JS-2 and KU-254) in response to infection with F.graminearum. The differential response of resistant and susceptible cultivars to F.graminearum clearly indicate that some host factors are involved in the development of disease. The involvement of glyceollin in the resistance of soybean plants against various other pathogens (fungi, bacteria, and nematode) have already been realized).

A variety of chemicals of widely diverse nature have been identified as phytoalexin inducers (Bell, 1967, Cheema and Haard, 1978, Purkayastha et.al. 1983; Sinha, 1984; Chakraborty and Purkayastha, 1987; Rouxel et.al., 1989). Attempts were made to alter the disease reaction by the application of some chemicals capable of inducing phytoalexin production and to examine whether alteration of disease reaction in soybean plants could be correlated with enhanced glyceollin production, which may give some insight in understanding the disease resistance mechanism. Initially twelve chemicals were tested in vitro to find out their fungitoxic effect, if any, on F.graminearum. For convenience these chemicals were screened in three separate groups e.g. (a) metal salts, (b) reducing agents and (c) metabolic inhibitors. A series of experiments were performed with nine different chemicals (4 from metal salts, 2 from reducing agents and 3 from metabolic inhibitors), to study their effects on disease development of susceptible soybean cultivar(Soymax). Seeds were treated with different chemicals and sown in F.graminearum infested soil as described under materials and methods. Disease intensity was assessed after 10,20 and 30 days of inoculation as described earlier.

Effect of metal salts:

Among the six metal salts (Barium Chloride, Cadmium Chloride, Cupric chloride, Ferric Chloride, Mercuric Chloride and Silver nitrate) tested for their effects on spore

germination of F.graminearum, Mercuric chloride completely inhibited the germination at 10^{-3}-10^{-5}M concentrations. Complete inhibition of germination was also evident at 10^{-3}M with cupric chloride and ferric chloride. Cadmium chloride and silver nitrate also markedly inhibited spore germination and germtube growth at three concentrations employed (Table-11).

Four metal salts(cupric chloride, ferric chloride, mercuric chloride and silver nitrate) were tested to determine their effects on the disease development following inoculation with F.graminearum. Three concentrations (10^{-3}, 10^{-4} and 10^{-5}M) of each chemical were used. Results are presented in Tables 12, 13, 14 and 15 and Fig. 7-10. All four metal salts reduced disease considerably at 10^{-3}M concentration after 30 days of inoculation in relation to untreated inoculated control. However, maximum reduction in disease intensity was noticed in case of plants treated with 10^{-3}M silver nitrate. In this case only 7.4% loss in dry weight was determined in treated inoculated roots, while untreated inoculated roots resulted 57.6% loss (after 30 days of inoculation) in relation to control (Fig.10). Complete inhibition of the spore germination of F.graminearum in vitro could not be correlated with in vivo experiment. Because at the same concentrations (10^{-3}-10^{-5}M) phytotoxic effects were also evident by complete inhibition of seed germination. Hence 10^{-6}-10^{-8}M concentrations of this compound was tested on the disease development which resulted into 38.5% loss in dry weight of treated roots (10^{-6}M) in relation to control after 30 days of inoculation with F.graminearum (Fig.9). Two other chemicals viz. cupric chloride and ferric chloride at higher concentration (10^{-3}M) reduced the disease to some extent. Same root rot index (1.8) was evident in both the cases (Table 12 & 13).

Table-11 : Effect of metal salts on spore germination and germtube growth of F.graminearum.

Chemical	Conc. (M)	Germination percentage[b]	Mean germtube[c] length (/u)
Water control		100	100
Barium chloride	10^{-3}	59.25(-40.75)[d]	32.20(-67.80)
	10^{-4}	68.95(-31.05)	40.25(-59.75)
	10^{-5}	71.75(-28.25)	44.27(-55.73)
Cadmium chloride	10^{-3}	0.92(-99.08)	12.05(-87.95)
	10^{-4}	1.73(-98.27)	16.00(-84.00)
	10^{-5}	2.08(-97.20)	24.15(-75.85)
Cupric chloride	10^{-3}	0	0
	10^{-4}	2.90(-97.1)	12.07(-87.93)
	10^{-5}	30.17(-69.83)	20.12(-79.88)
Ferric chloride	10^{-3}	0	0
	10^{-4}	2.41(-97.59)	8.05(-91.95)
	10^{-5}	65.99(-34.01)	18.51(-81.49)
Mercuric chloride	10^{-3}	0	0
	10^{-4}	0	0
	10^{-5}	0	0
Silver nitrate	10^{-3}	0.81(-99.19)	8.05(-91.95)
	10^{-4}	1.39(-98.61)	12.00(-88.00)
	10^{-5}	7.65(-92.35)	20.12(-79.88)

a - Results have been expressed as percentage in terms of control.

b - Average of 500 spores, c - Average of 50 germlings.

d - Values in parentheses indicate percentage reduction or increase in terms of control.

Table 12 . Effect of Cupric Chloride on the development of root rot of soybean (CV. Soymax)

Concentration (M)	Average dry wt. of roots(mg) with S.E.[a]						% loss in dry wt.[b]			Root rot index of infected[c] roots.		
	10 days		20 days		30 days		10 days	20 days	30 days	10 days	20 days	30 days
	Healthy	Infected	Healthy	Infected	Healthy	Infected						
Treated 10^{-3}	102.5 ± 4.2	96.1 ± 3.8	179.2 ± 4.9	144. ± 3.6	256.8 ± 5.6	176.0 ± 4.8	6.2	19.6	31.46	1.0	1.5	1.8
10^{-4}	148.0 + 5.8	134.4 + 3.6	259.2 + 4.2	188.0 + 3.4	371.2 + 5.2	213.6 + 4.6	9.1	27.4	42.4	1.0	1.6	2.5
10^{-5}	124.8 ± 5.4	100.0 ± 4.6	218.4 ± 5.6	131.2 ± 3.2	312.0 ± 4.4	164.0 ± 3.8	19.8	39.9	47.4	1.5	2.3	2.8
Untreated control	135.0 ± 4.8	112.0 ± 4.4	315.0 ± 5.8	186.0 ± 4.2	450.0 ± 5.6	190.5 ± 4.6	20.0	38.5	57.6	1.6	2.3	3.0

a = Average of 30 plants/treatment

b = In relation to control

c = Root rot index. [1-10% loss in weight = 1, 11-25% = 2, 26-50% = 3, 51-75% = 4, 76-100% = 5]

EFFECT OF CUPRIC CHLORIDE ON DESEASE DEVELOPMENT IN SOYBEAN (CV- SOYMAX) AFTER INOCULATION WITH *F. graminearum*.

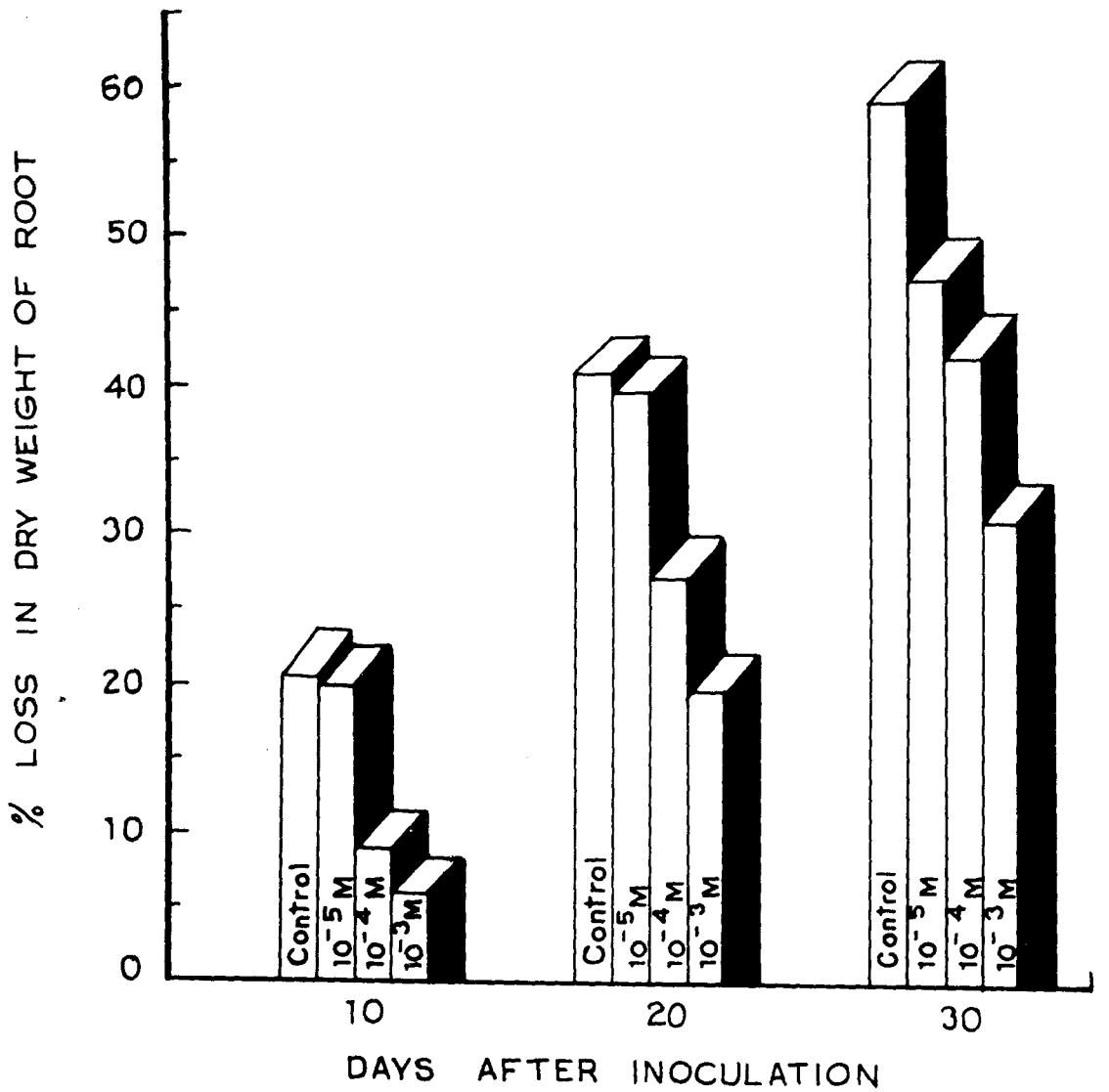


Fig. 7.

Table 13 . Effect of Ferric Chloride on the development of root rot of soybean (CV.Soymax)

Concentration (M)	Average dry wt. of roots(mg) with S.E.[a]						% loss in dry wt.[b]			Root rot index of infected roots[c]		
	10 days		20 days		30 days		10 days	20 days	30 days	10 days	20 days	30 days
	Healthy	Infected	Healthy	Infected	Healthy	Infected						
Treated 10^{-3}	142.1 ± 3.2	135.1 ± 2.6	249.0 ± 4.4	219.0 ± 3.6	357.0 ± 4.8	274.0 ± 4.4	4.9	12.0	23.2	1.0	1.0	1.8
10^{-4}	146.0 ± 4.6	128.0 ± 3.8	255.0 ± 2.2	210.0 ± 4.9	365.0 ± 3.6	255.0 ± 4.8	12.3	17.6	30.1	1.0	1.4	1.8
10^{-5}	168.0 ± 4.4	144.0 ± 3.6	311.2 ± 4.2	180.0 ± 3.8	445.7 ± 5.2	200.1 ± 4.8	18.1	42.1	55.1	1.4	2.5	2.9
Untreated control	135.0 + 4.8	112.0 + 4.4	315.0 + 4.6	186.0 + 3.2	450.0 + 5.0	190.5 + 4.6	20.0	38.5	57.6	1.6	2.3	3.0

a = Average of 30 plants/treatment

b = In Relation to Control

c = Root rot index. [1-10% loss in weight = 1, 11-25% =2, 26-50% = 3, 51-75% = 4, 76-100% = 5]

EFFECT OF FERRIC CHLORIDE ON DISEASE DEVELOPMENT IN SOYBEAN (CV.-SOYMAX) AFTER INOCULATION WITH *F. graminearum*.

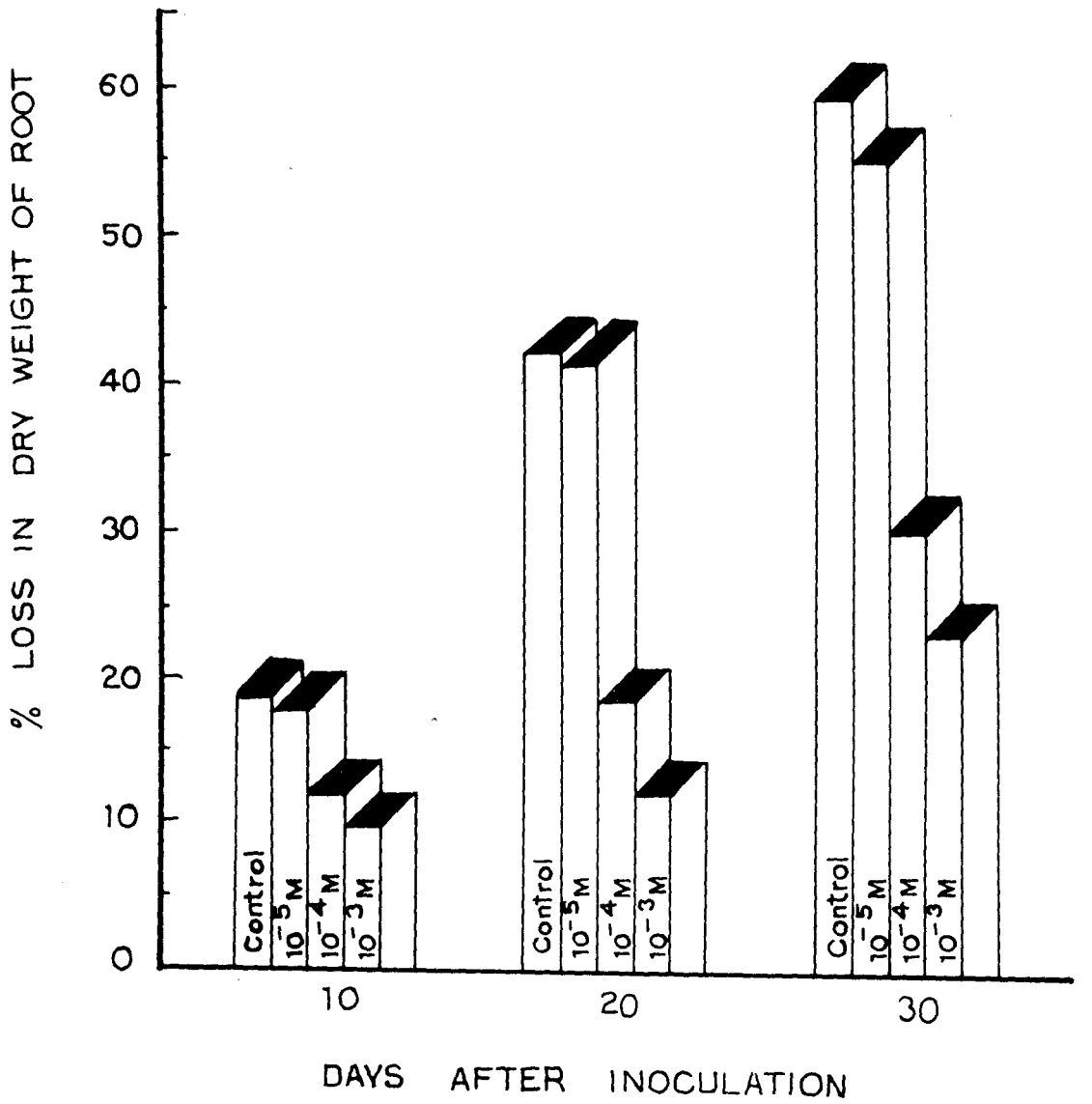


Fig. 8.

Table 14 . Effect of Mercuric Chloride on the development of root rot of soybean(CV. Soymax)

Concentration (M)	Average dry wt. of roots (mg) with S.E. [a]						% loss in dry wt. [b]			Root rot index of infected roots. [c]		
	10 days		20 days		30 days		10 days	20 days	30 days	10 days	20 days	30 days
	Healthy	Infected	Healthy	Infected	Healthy	Infected						
Treated 10^{-6}	173.0 ± 4.4	165.0 ± 3.8	325.0 ± 5.2	245.0 ± 4.6	433.0 ± 5.8	266.0 ± 4.2	4.6	24.6	38.5	1.0	1.9	2.3
10^{-7}	170.0 ± 5.8	152.0 ± 4.2	320.0 ± 4.6	215.0 ± 3.8	425.0 ± 4.4	216.5 ± 4.2	10.5	32.8	49.0	1.0	1.9	2.9
10^{-8}	185.6 ± 3.6	162.0 ± 4.2	337.0 ± 4.9	215.1 ± 3.2	438.0 ± 4.2	188.0 ± 3.8	12.7	36.0	57.0	1.0	2.1	3.0
Untreated control	135.0 ± 4.9	112.0 ± 3.4	315.0 ± 4.6	186.0 ± 4.2	450.0 ± 5.6	190.5 ± 3.6	20.0	38.5	57.6	1.6	2.3	3.0

a = Average of 30 plants/treatment

b = In relation to control

c = Root rot index. [1-10% loss in weight =1, 11-25% = 2, 26-50% =3, 51-75%=4, 76-100% = 5]

EFFECT OF MERCURIC CHLORIDE ON DISEASE DEVELOPMENT IN SOYBEAN (CV- SOYMAX) AFTER INOCULATION WITH *F. graminearum* .

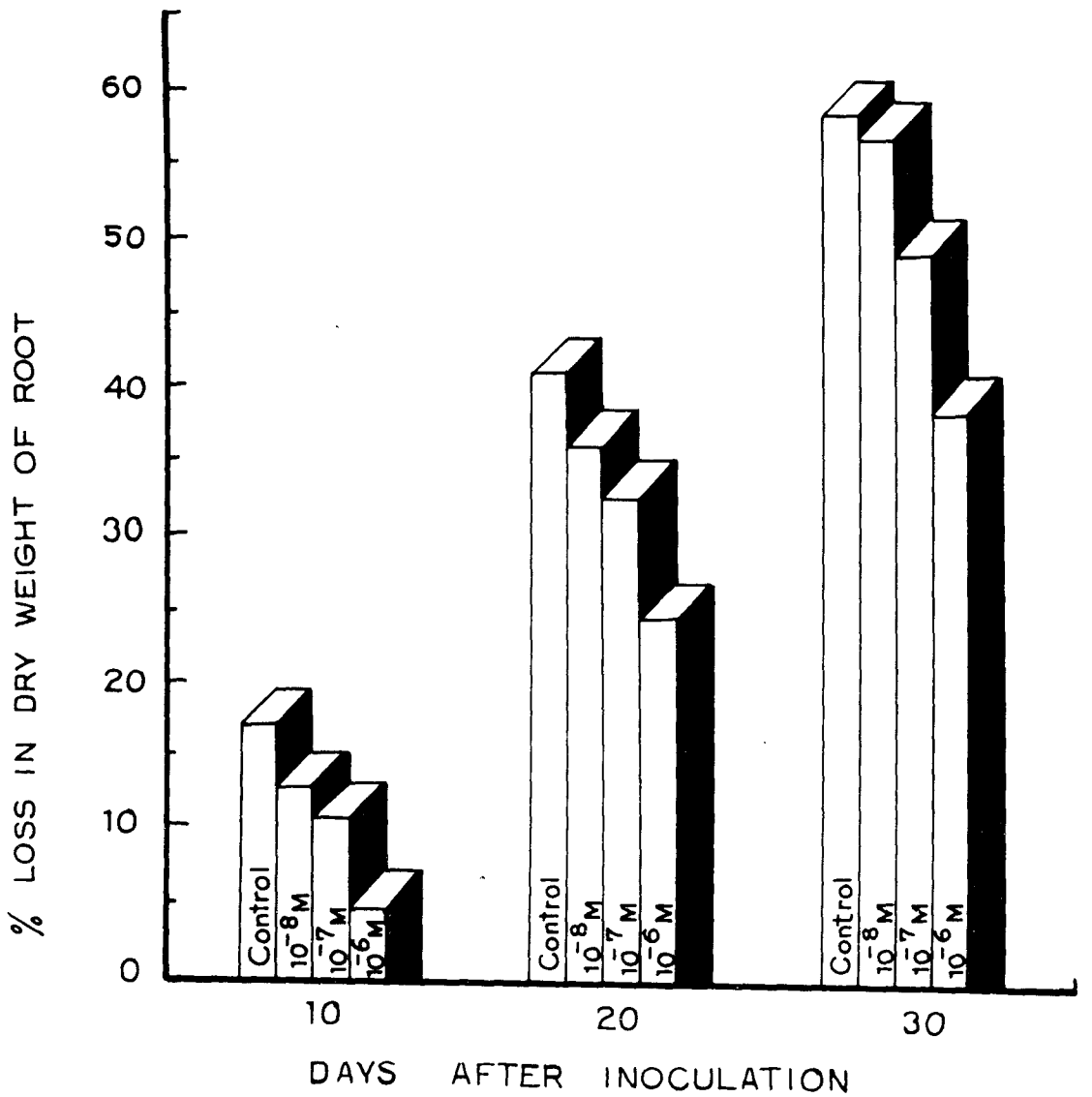


Fig. 9.

Table 15. Effect of Silver Nitrate on the development of root rot of soybean (CV. Soymax)

Concentration (M)	Average dry wt. of roots (mg) with S.E. [a]						% loss in dry wt. [b]			Root rot index of [c] infected roots		
	10 days		20 days		30 days		10 days	20 days	30 days	10 days	20 days	30 days
	Healthy	Infected	Healthy	Infected	Healthy	Infected						
Treated 10^{-4}	140.0 ± 3.4	137.1 ± 2.6	270.0 ± 4.4	255.0 ± 3.6	351.0 ± 3.2	325.0 ± 2.4	2.0	5.5	7.4	1.0	1.0	1.0
10^{-5}	144.0 ± 4.2	138.0 ± 3.6	265.0 ± 5.2	240.0 ± 4.8	360.0 ± 3.8	280.0 ± 2.6	4.1	9.4	22.2	1.0	1.0	1.7
10^{-6}	150.0 ± 5.2	126.0 ± 4.2	271.0 ± 3.8	177.1 ± 4.2	352.0 ± 5.6	182.0 ± 4.2	16.0	34.6	48.2	1.2	2.0	2.8
Untreated control	135.0 ± 4.9	112.0 ± 4.4	315.0 ± 5.6	186.0 ± 4.2	450.0 ± 3.6	190.5 ± 4.6	20.0	38.5	57.6	1.6	2.3	3.0

a = Average of 30 plants/treatment

b = In relation to control

c = Root rot index. [1-10% loss in weight =1, 11-25%=2, 26-50%=3, 51-75% =4, 76-100% =5]

EFFECT OF SILVER NITRATE ON DISEASE DEVELOPMENT IN SOYBEAN (CV- SOYMAX) AFTER INOCULATION WITH F. graminearum .

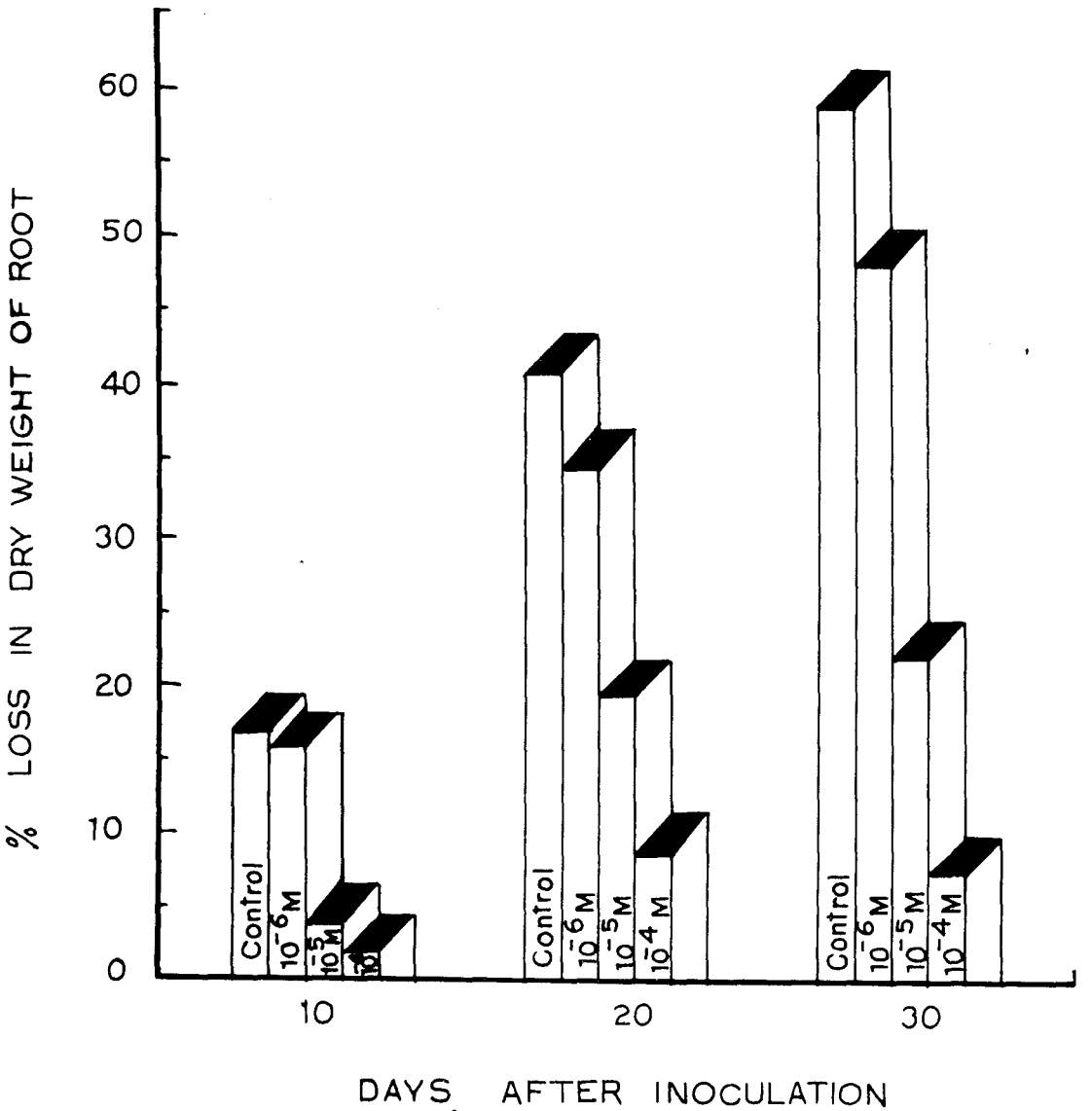


Fig. 10.

Effect of reducing agents :

Two reducing agents (Sodium selenite and sodium sulphite) were tested both *in vitro* for spore germination assay and *in vivo* for their effects on the disease development. Sodium selenite completely inhibited the spore germination at 10^{-3} and 10^{-4}M concentrations (Table 16). However, sodium sulphite also markedly inhibited spore germination and germ-tube growth at all three concentrations (10^{-3}-10^{-5}M) tested. When these two chemicals were tested on cv-Soymax to determine their effects on the disease development following inoculation with *F.graminearum*, both the chemicals reduced the disease at 10^{-3}M concentration (Table 17 & 18). However, reduction in disease development as reflected in percentage loss in dry weight of roots was more in case of sodium sulphite treated roots(9.5%) than the sodium selenite treated roots(18.9%) after 30 days of inoculation in relation to control (Fig. 11).

Effect of metabolic inhibitors :

When the four metabolic inhibitors viz. sodium azide, sodium malonate, sodium fluoride and sodium molybdate were tested to determine their effects on spore germination, none of these chemicals could completely inhibit the spore germination of *F.graminearum* at 10^{-3}-10^{-5}M concentrations. However, all three concentrations of four chemicals tested markedly reduced germination percentage (Table 19). Three chemicals viz. sodium azide, sodium malonate and sodium molybdate were selected for *in vivo* assay. Alteration of disease reaction in susceptible soybean plants(cv. Soymax) by the treatment of above chemicals were noticed (Tables 20,21 & 22). Considerable reduction in disease index was evident at all intervals in treated plants in relation to control (Fig. 12, 13 & 14). Among the three metabolic inhibitors tested,

Table 16 : Effect of reducing agents on spore germination and germtube growth of F.graminearum.

Chemical	Conc. (M)	Germination percentage	Mean germtube length (µ)
Water control	-	100	100
Sodium selenite	10^{-3}	0	0
	10^{-4}	0	0
	10^{-5}	16.58(-83.42)	12.07(-87.93)
Sodium sulphite	10^{-3}	3.24(-96.76)	8.00(-92.00)
	10^{-4}	4.05(-95.95)	9.60(-90.40)
	10^{-5}	4.86(-95.14)	9.65(-90.35)

a - Results have been expressed as percentage in relation to control.
b - Average of 500 spores.
c - Average of 50 germlings.
d - Values in parentheses indicate percentage reduction in relation to control.

pronounced protective effect was recorded with sodium azide treatment (Fig.12). Moderate effect of sodium molybdate on disease development was also noticed(Fig.14).

In the final experiment of the series, 7 among the more effective compounds, representing 3 from metal salts (cupric chloride, silver nitrate and cadmium chloride), 2 each from reducing agents (sodium sulphite and sodium selenite) and metabolic inhibitor(sodium molybdate, sodium azide) were further tested. Cold stock solution of the said seven compounds were prepared and appropriate volume was separately

Table 17. Effect of Sodium Selenite on the development of root rot of soybean (CV. Soymax)

Concentration (M)	Average dry wt. of roots(mg) with S.E. [a]						% loss in dry wt. [b]			Root rot index of infected roots [c]		
	10 days		20 days		30 days		10 days	20 days	30 days	10 days	20 days	30 days
	Healthy	Infected	Healthy	Infected	Healthy	Infected						
Treated 10^{-3}	225.0 ± 3.8	218.7 ± 2.2	525.0 ± 4.8	470.0 ± 4.6	750.0 ± 5.2	608.0 ± 4.4	2.8	10.5	18.9	1.0	1.0	1.5
10^{-4}	187.5 ± 5.4	169.9 ± 4.8	437.5 ± 4.6	321.3 ± 4.2	625.0 ± 3.4	403.8 ± 4.8	9.4	26.6	35.7	1.0	1.5	2.1
10^{-5}	157.7 ± 4.2	136.0 ± 3.8	367.5 ± 4.2	255.0 ± 3.6	525.1 ± 4.9	320.4 ± 4.4	13.8	30.6	39.0	1.1	1.8	2.3
Untreated control	135.0 ± 4.4	114.7 ± 4.2	309.0 ± 5.0	196.0 ± 4.2	551.0 ± 3.8	212.4 ± 4.6	15.0	36.5	61.5	1.2	2.1	3.2

a Average of 30 plants/treatment

b In relation to control

c Root rot index. [1-10% loss in weight =1, 11-25%=2, 26-50%=3, 51-75%=4, 76-100% =5]

Table 18. Effect of Sodium Sulphite on the development of root rot of soybean(CV. Soymax)

Concentration (M)	Average dry wt. of roots(mg) with S.E. [a]						% loss in dry wt. [b]			Root rot index of infected roots. [c]		
	10 days		20 days		30 days		10 days	20 days	30 days	10 days	20 days	30 days
	Healthy	Infected	Healthy	Infected	Healthy	Infected						
Treated 10^{-3}	104.3 ± 5.2	101.3 ± 3.8	165.0 ± 4.6	154.0 ± 4.4	214.5 ± 6.2	194.1 ± 4.2	2.8	6.6	9.5	1.0	1.0	1.0
10^{-4}	90.7 ± 4.4	83.2 ± 4.5	179.8 ± 5.8	132.0 ± 4.2	233.6 ± 5.6	158.2 ± 4.6	8.2	26.5	32.2	1.0	1.5	1.9
10^{-5}	106.0 ± 4.8	97.7 ± 4.4	170.0 ± 3.8	120.6 ± 4.2	221.0 ± 4.2	138.2 ± 3.6	15.4	29.0	37.4	1.2	1.7	2.2
Untreated control	115.0 ± 4.6	97.7 ± 4.2	159.0 ± 2.8	100.8 ± 3.2	265.0 ± 4.4	102.0 ± 3.8	15.0	36.5	61.5	1.2	2.1	3.2

a = Average of 30 plants/treatment

b = In relation to control

c = Root rot index.[1-10% loss in weight =1, 11-25%=2, 26-50% =3, 51-75% =4, 76-100%=5]

EFFECT OF REDUCING AGENTS ON THE DEVELOPMENT OF ROOT DISEASE OF SOYBEAN (CV- SOYMAX)

[DISEASE INTENSITY EXPRESSED AS % LOSS IN DRY WEIGHT OF ROOTS]

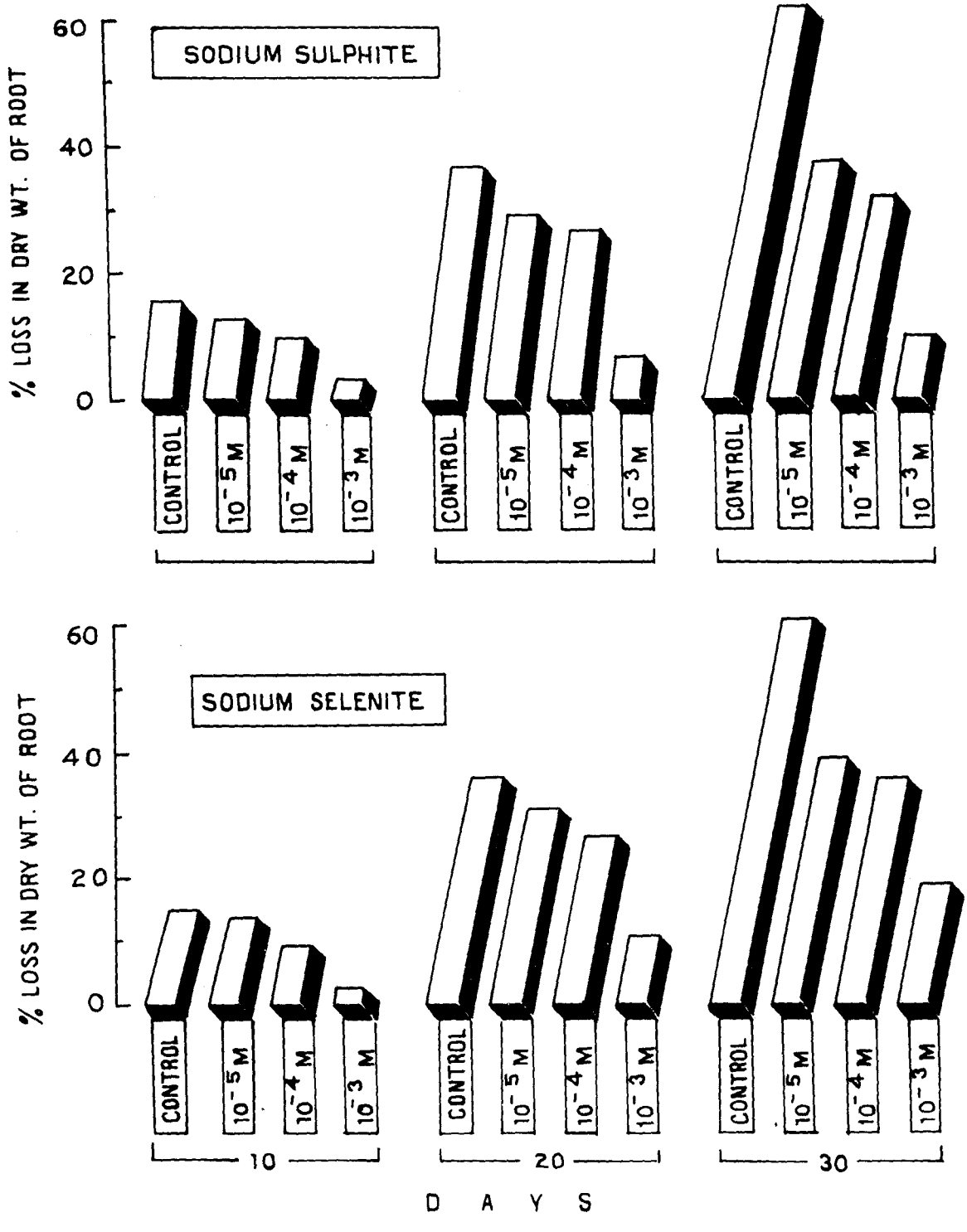


Fig. 11

Table 19: Effect of metabolic inhibitors on spore germination and germtube growth of F.graminearum .

Chemicals	Conc.(M)	Germination percentage	Mean germtube length (µ)
Water control	-	100	100
Sodium azide	10^{-3}	3.68 (-96.32)	12.07(-87.93)
	10^{-4}	4.24 (-95.76)	16.10(-83.90)
	10^{-5}	8.58 (-91.42)	36.22(-63.78)
Sodium malonate	10^{-3}	2.25 (-97.75)	16.22(-83.78)
	10^{-4}	3.47 (-96.53)	24.15(-75.85)
	10^{-5}	4.05 (-95.95)	32.20(-67.80)
Sodium fluoride	10^{-3}	0.92 (-99.08)	12.10(-87.90)
	10^{-4}	2.08 (-97.92)	20.12(-79.88)
	10^{-5}	3.47 (-96.53)	28.17(-71.83)
Sodium molybdate	10^{-3}	1.73 (-98.27)	15.00(-85.00)
	10^{-4}	2.31 (-97.69)	21.75(-78.25)
	10^{-5}	2.78 (-97.22)	40.55(-59.45)

a - Results have been expressed as percentage in relation to control

b - Average of 500 spores.

c - Average of 50 germlings

d - Values in parentheses indicate percentage reduction in relation to control.

Table 20. Effect of Sodium azide on the development of root rot of soybean(CV. Soymax)

Concentration (M)	Average dry wt. of roots(mg) with S.E. [a]						% loss in dry wt. [b]			Root rot index of infected roots [c]		
	10 days		20 days		30 days							
	Healthy	Infected	Healthy	Infected	Healthy	Infected	10 days	20 days	30 days	10 days	20 days	30 days
Treated 10^{-3}	141.6 ± 4.8	137.4 ± 3.8	330.5 ± 4.2	307.9 ± 2.6	472.2 ± 5.6	468.6 ± 4.5	2.9	6.8	7.6	1.0	1.0	1.0
10^{-4}	155.0 ± 3.2	146.8 ± 4.6	350.0 ± 5.8	320.0 ± 4.6	500.0 ± 4.8	440.0 ± 3.4	5.2	8.6	12.0	1.0	1.0	1.0
10^{-5}	118.3 ± 4.4	107.0 ± 3.9	276.2 ± 5.2	240.8 ± 4.4	394.6 ± 6.8	320.0 ± 4.2	9.6	12.8	18.9	1.0	1.0	1.5
Untreated control	135.0 ± 4.6	110.9 ± 4.2	315.5 ± 4.8	202.2 ± 4.6	473.4 ± 3.2	222.2 ± 4.6	17.8	35.9	53.1	1.4	2.1	3.1

a Average of 30 plants/treatment

b In relation to control

c Root rot index. [1-10% loss in weight =1, 11-25% =2, 26-50%=3, 51-75% = 4, 76-100% =5]

EFFECT OF SODIUM AZIDE ON DISEASE DEVELOPMENT OF SOYBEAN (CV- SOYMAX) AFTER INOCULATION WITH *F. graminearum* .

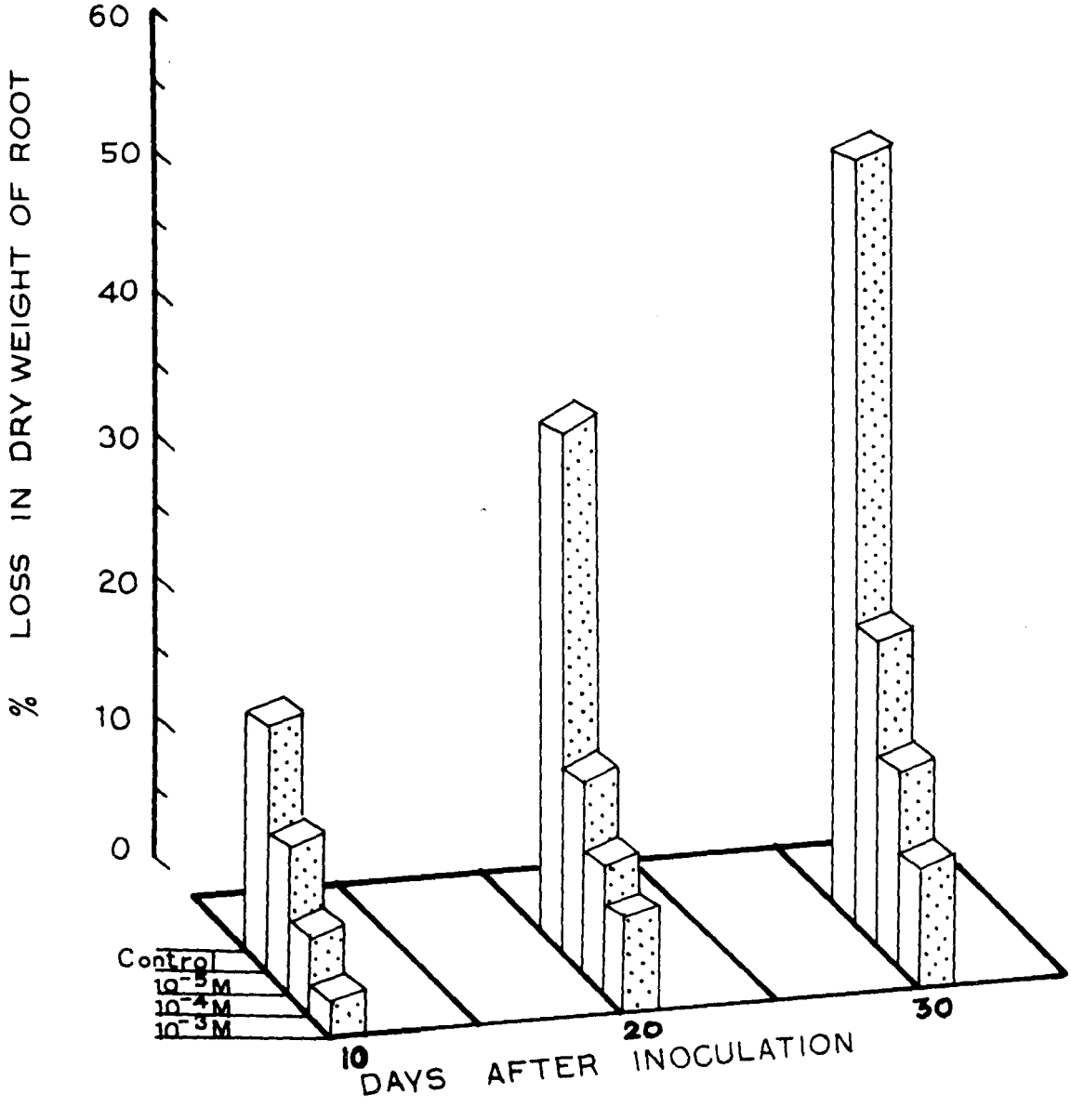


Fig. 12

Table 21. Effect of Sodium malonate on the development of root rot of soybean(CV. Soymax)

Concentration (M)	Average dry wt. of roots(mg) with S.E. [a]						% loss in dry wt. [b]			Root rot index of infected roots [c]		
	10 days		20 days		30 days							
	Healthy	Infected	Healthy	Infected	Healthy	Infected	10 days	20 days	30 days	10 days	20 days	30 days
Treated 10^{-3}	122.4 ± 5.2	116.4 ± 4.4	300.8 ± 6.6	252.8 ± 5.2	397.4 ± 4.8	324.9 ± 3.6	4.9	16.0	18.2	1.0	1.2	1.4
10^{-4}	125.7 ± 4.8	115.0 ± 4.6	312.0 ± 3.8	256.0 ± 4.6	405.6 ± 4.2	310.1 ± 2.8	8.4	17.9	23.5	1.0	1.4	1.8
10^{-5}	136.3 ± 3.8	113.1 ± 2.2	350.4 ± 4.4	272.0 ± 4.1	455.5 ± 4.6	310.4 ± 3.8	17.0	22.4	31.8	1.3	1.7	1.9
Untreated control	135.0 ± 4.6	110.9 ± 4.2	315.1 ± 4.8	198.0 ± 4.2	438.0 ± 5.1	203.3 ± 4.2	17.8	37.2	53.5	1.4	2.2	2.9

a Average of 30 plants/treatment

b In relation to control

c Root rot index. [1-10% loss in weight =1, 11-25% =2, 26-50% = 3, 51-75% =4, 76-100% =5]

EFFECT OF SODIUM MALONATE ON DISEASE DEVELOPMENT OF SOYBEAN (CV- SOYMAX) AFTER INCULATION WITH F. graminerum .

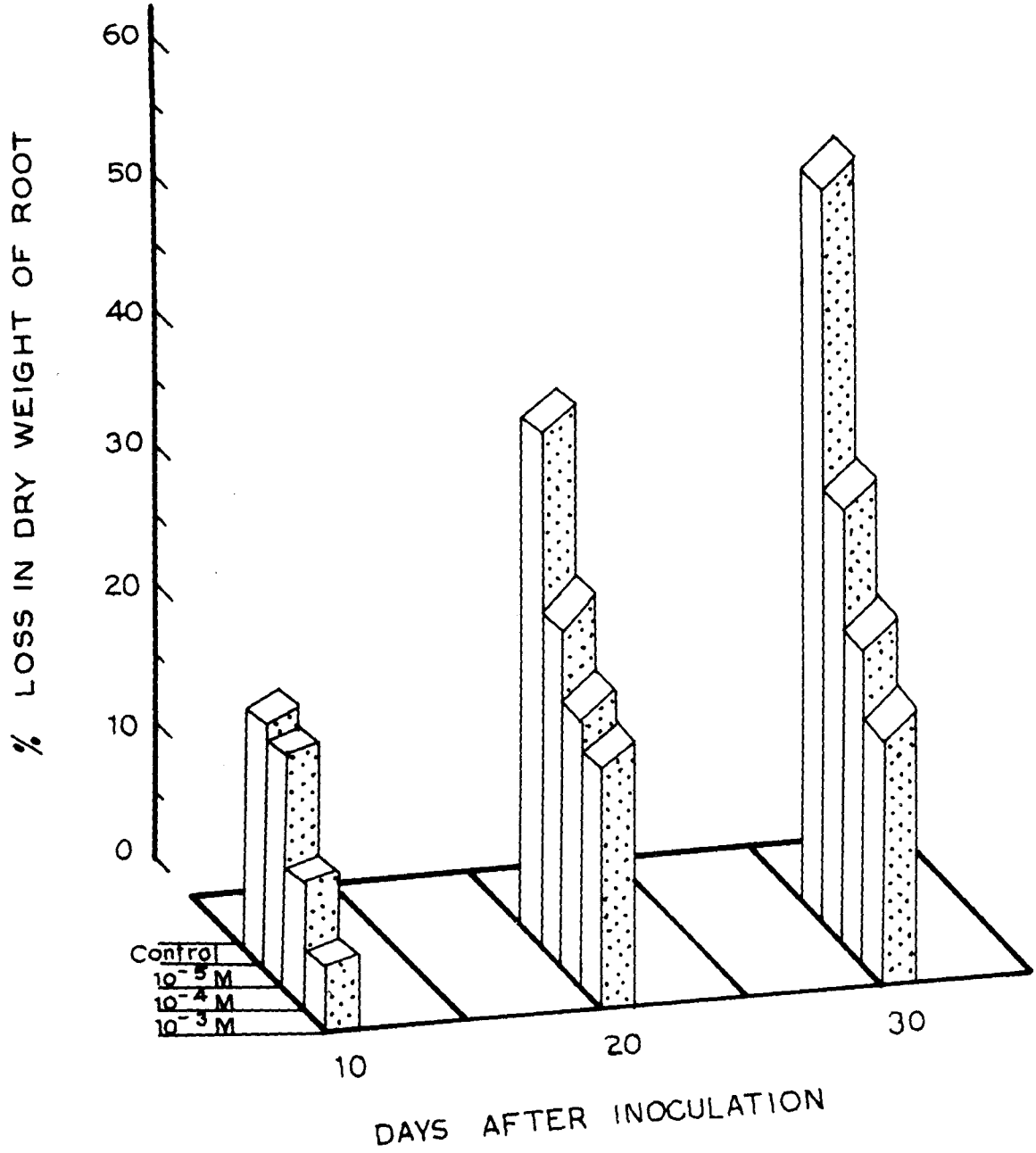


Fig . 13

Table 22 . Effect of Sodium molybdate on the development of root rot of soybean (CV.Soymax)

Concentration	Average dry wt. of roots(mg) with S.E.[a]						% loss in dry wt.[b]			Root rot index of infected root[c]		
	10 days		20 days		30 days		10 days	20 days	30 days	10 days	20 days	30 days
	Healthy	infected	Healthy	Infected	Healthy	Infected						
Treated 10^{-3}M	115.7 ± 4.6	112.5 ± 3.2	161.0 ± 5.1	152.5 ± 4.2	209.3 ± 3.9	188.5 ± 2.6	2.7	5.2	9.9	1.0	1.0	1.0
10^{-4}M	133.0 ± 4.8	125.6 ± 4.4	190.0 ± 3.8	162.5 ± 4.2	247.0 ± 3.2	205.4 ± 2.6	5.5	14.5	16.8	1.0	1.1	1.3
10^{-5}M	135.5 ± 3.8	120.5 ± 4.6	195.1 ± 4.2	150.0 ± 5.6	253.5 ± 3.9	180.0 ± 3.4	11.0	25.1	28.9	1.0	1.5	1.7
Untreated control	115.0 ± 4.6	94.5 ± 2.4	159.0 ± 4.8	99.9 ± 4.2	225.0 ± 4.8	105.0 ± 4.1	17.8	37.2	53.3	1.4	2.2	3.1

a = Average of 30 plants/treatment

b = In relation to control

c = Root rot index. [1-10% loss in weight = 1, 11-25% = 2, 26-50%=3, 51-75% = 4, 76-100%= = 5]

EFFECT OF SODIUM MOLYBDATE ON DISEASE DEVELOPMENT OF SOYBEAN (CV- SOYMAX) AFTER INOCULATION WITH *F. graminearum*.

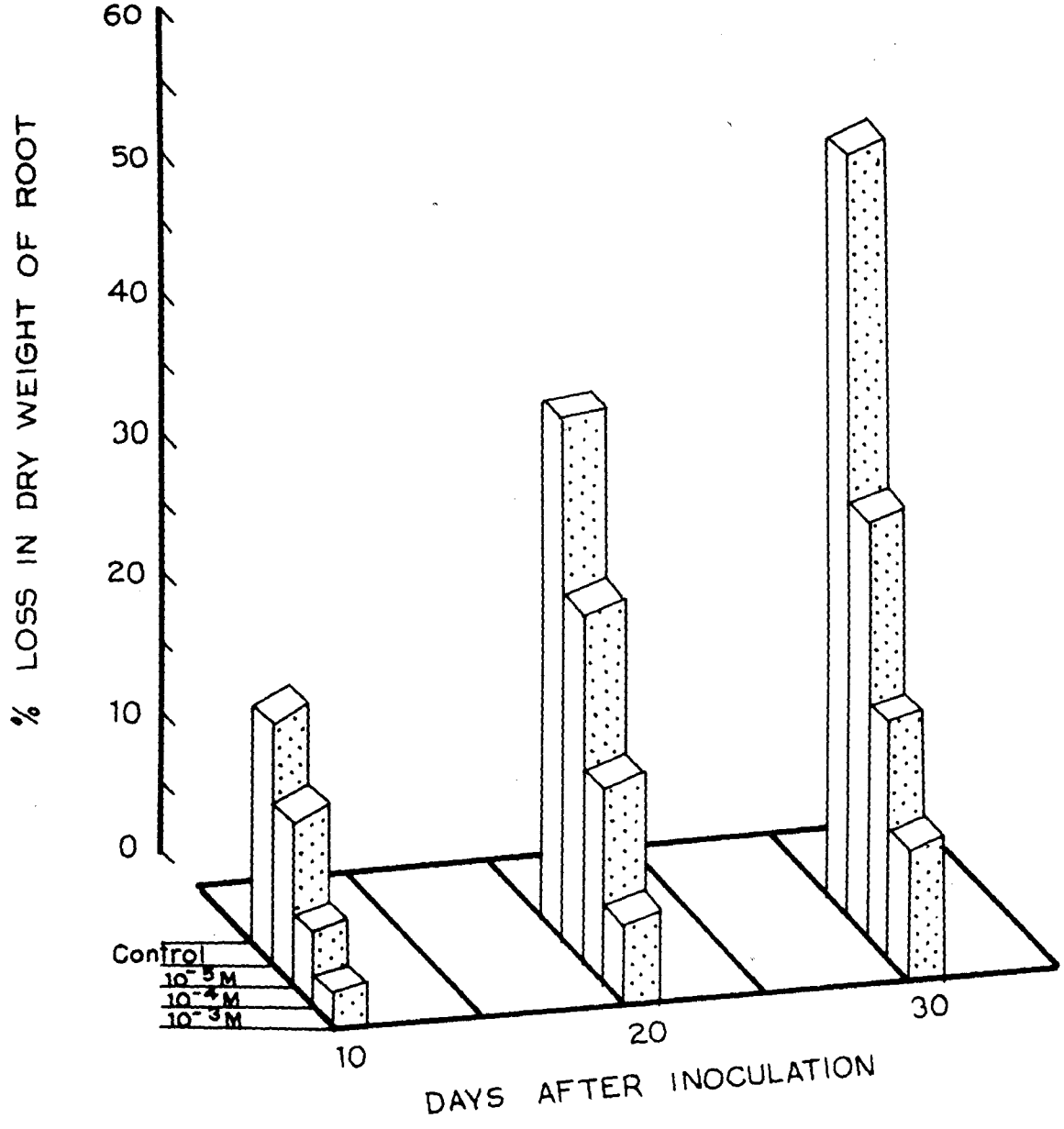


Fig. 14

Table 23 : Effect of some chemicals on mycelial growth of F.graminearum.

Chemicals	% inhibition of mycelial growth [a]		
	10^{-3}M	10^{-4}M	10^{-5}M
Metal salts :			
Cupric chloride	45.00	37.50	24.02
Silver nitrate	89.50	51.06	42.50
Cadmium chloride	95.00	90.05	82.00
Reducing agents :			
Sodium sulphite	25.68	7.70	4.40
Sodium selenite	94.03	54.12	38.89
Metabolic inhibitors:			
Sodium molybdate	98.16	44.95	12.47
Sodium azide	95.35	91.08	80.73

a = In relation to control

added to the autoclaved basal medium (Richard's medium) after cooling to get the desired concentrations (10^{-3}, 10^{-4} and 10^{-5}M) of the compound in the medium. Effect of these compounds on mycelial growth of F.graminearum was determined. Results are presented in Table-23. At higher concentration (10^{-3}M,) cadmium chloride, sodium selenite, sodium molybdate and sodium azide inhibited mycelial growth ranging from 94-98%, whereas at this concentration sodium sulphite and cupric chloride caused 25 & 45% inhibition respectively. Sodium azide and cadmium chloride at all concentrations markedly inhibited mycelial growth of F.graminearum (Fig 15 & 16).

EFFECT OF SOME SELECTED CHEMICALS ON MYCELIAL GROWTH OF *F. graminearum*.

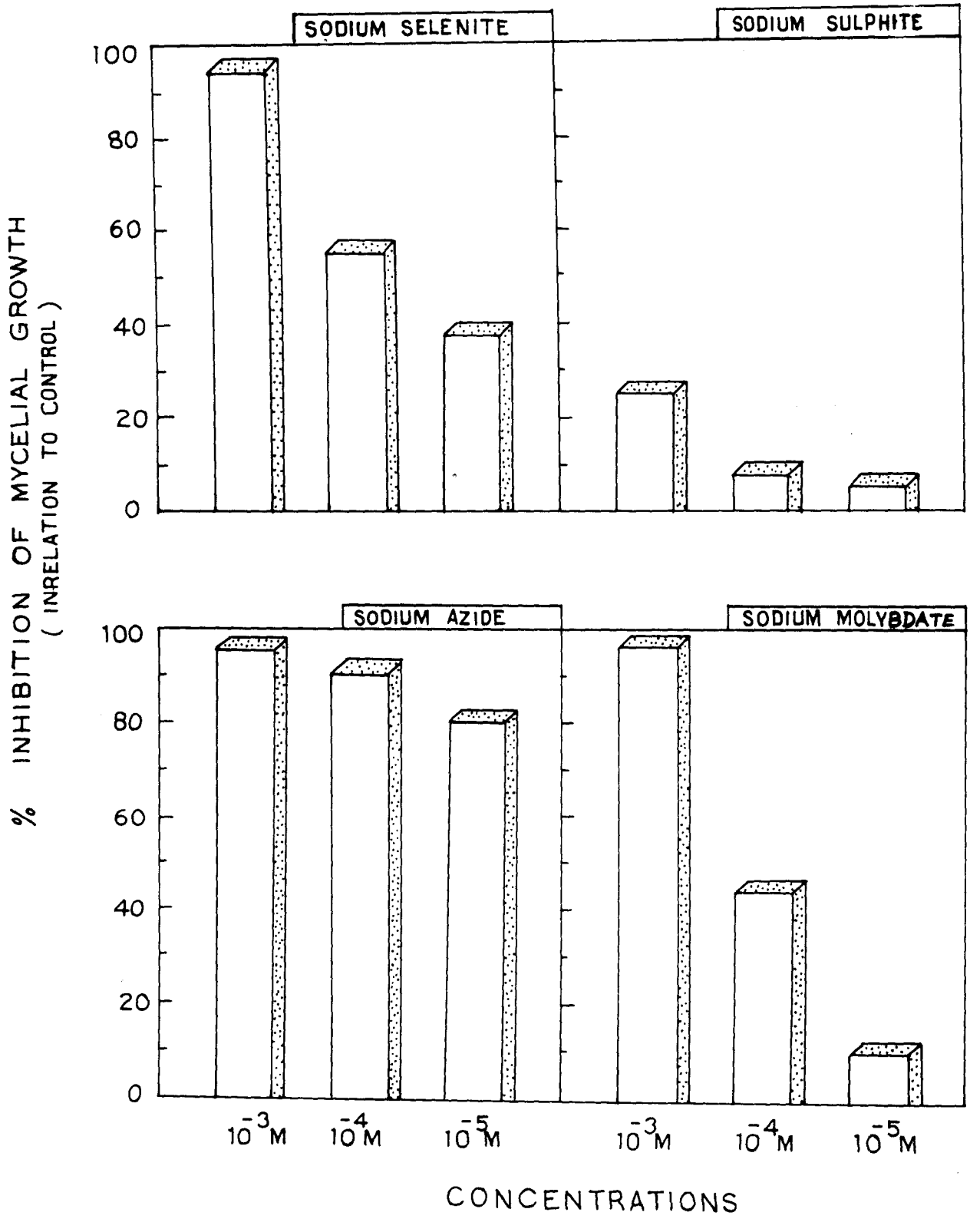


Fig. 15

GROWTH RESPONSE OF F. graminearum TO SOME METAL SALTS.

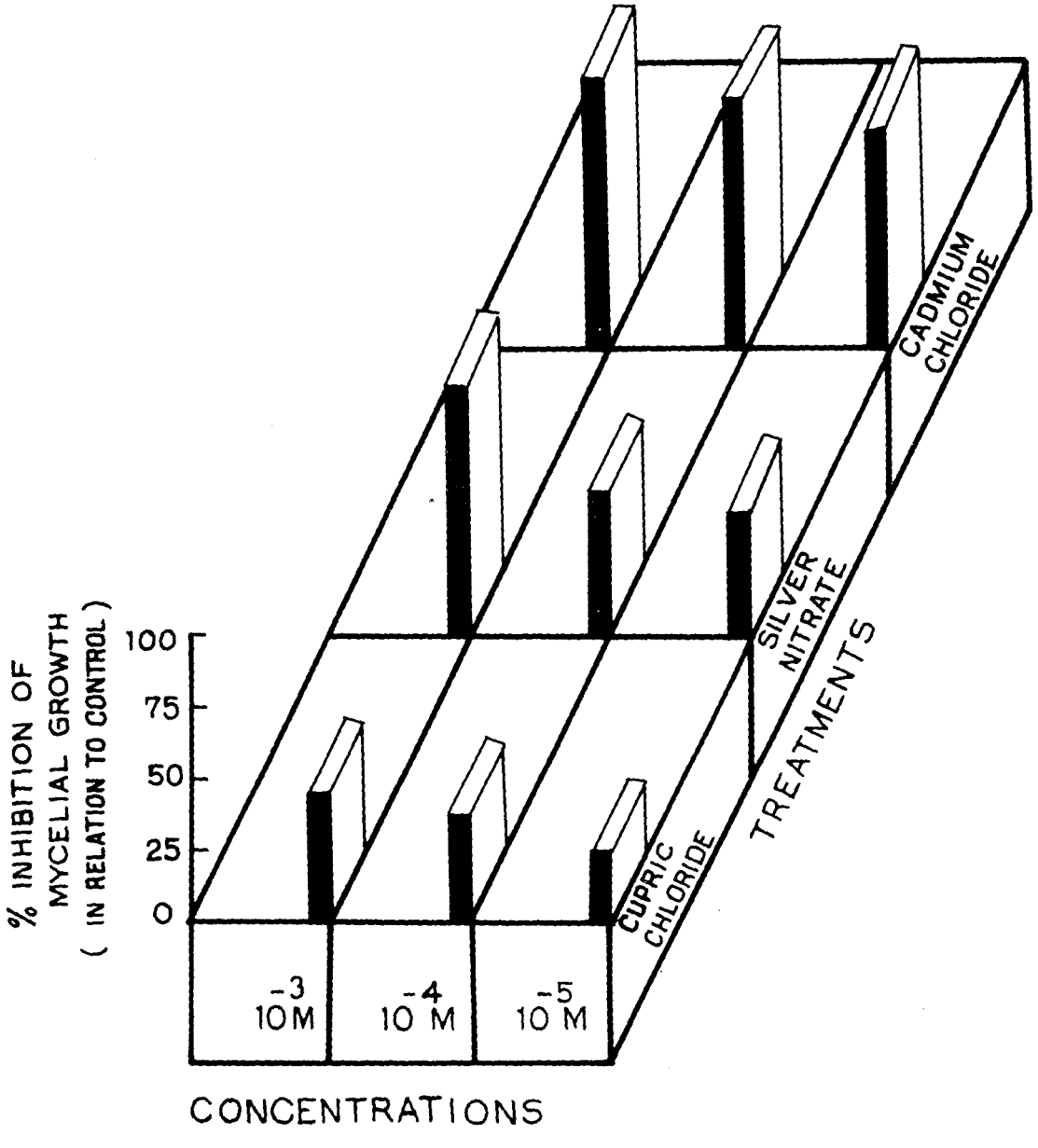
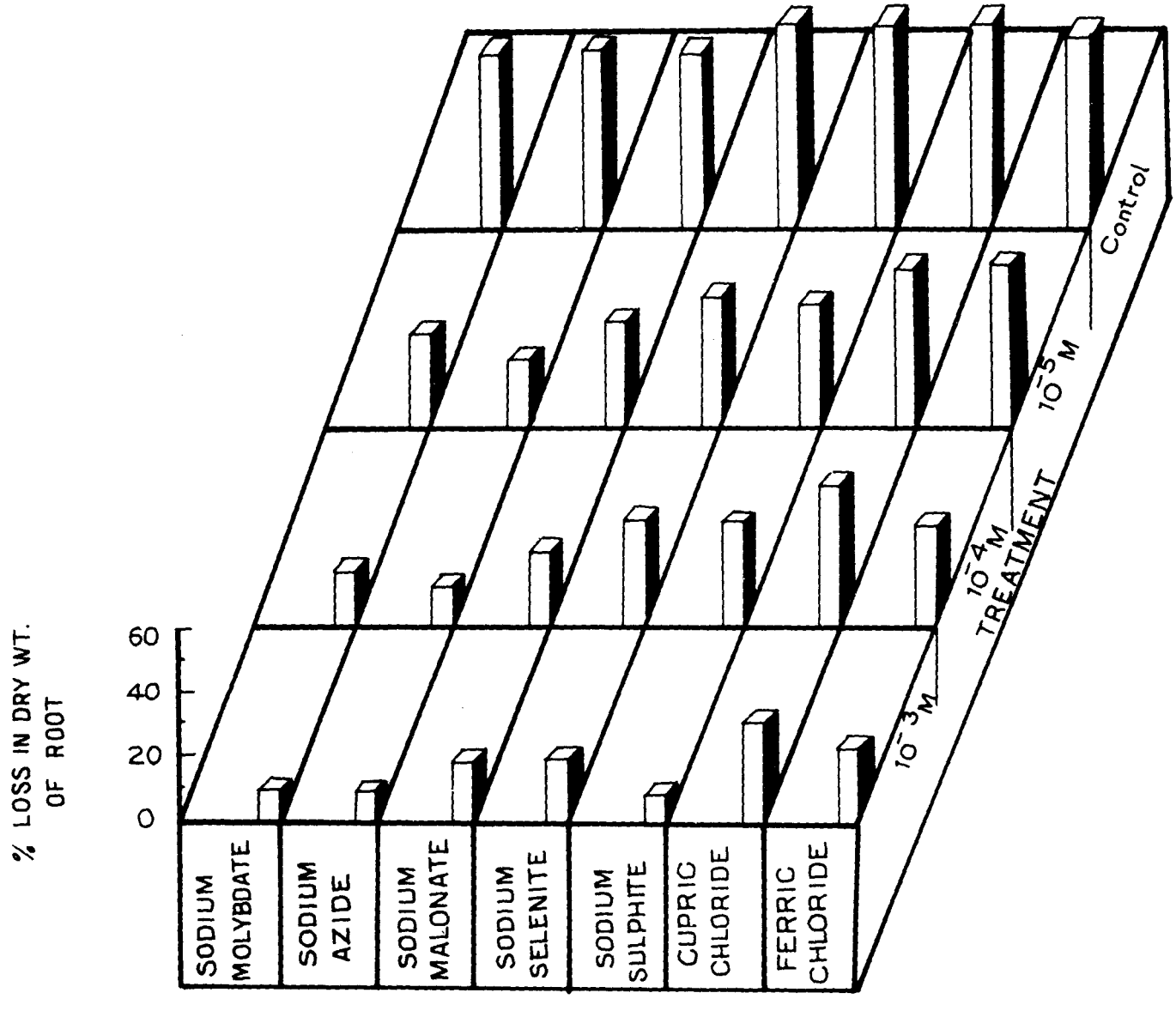


Fig. 16.

EFFECT OF SOME SELECTED CHEMICALS ON THE DEVELOPMENT OF ROOT ROT DISEASE OF SOYBEAN (CV- SOYMAX)

[DISEASE INTENSITY EXPRESSED AS % LOSS IN DRY WEIGHT OF ROOTS]



[30 DAYS AFTER INOCULATION WITH F. graminearum]

Fig. 17.

Three different concentrations of 5 selected compounds, 3 from metabolic inhibitors (sodium molybdate, sodium azide and sodium malonate), 2 from reducing agents (sodium selenite and sodium sulphite) and 2 from metal salts (cupric chloride and ferric chloride) were again tested under identical conditions to find out their effects on the development of root rot disease of soybean(cv-Soymax). Untreated and treated plants grown in sick pot infested with F.graminearum were uprooted after 30 days of inoculation and percentage loss in dry weight in relation to control was computed. results have been depicted in Fig.17. Sodium azide, sodium sulphite and sodium selenite reduced the disease markedly.

PART-VI

Comparison of glyceollin contents of soybean roots before and after alteration of disease reaction by chemical treatment.

In order to alter disease reactions in susceptible soybean cultivar - Soymax by some selected chemicals, several experiments were carried out (vide part V). The results presented in Part IV clearly indicate that resistant cultivars contained significantly more glyceollin than the susceptible cultivars when inoculated with F.graminearum. In view of this it was decided to investigate whether chemically induced resistance in Cv-Soymax was related to higher production of glyceollin. Since sodium azide and sodium selenite markedly reduced disease in susceptible soymax cultivar, it was decided to estimate glyceollin content of the same before and after treatment with the said two compounds.

To study the effect of sodium azide and sodium selenite on the production of glyceollin, seeds of Cv.Soymax were treated with either sodium azide (10^{-4}M) or sodium selenite(10^{-4}M) as described under Materials and Methods, sown in the earthen pots and subsequently the plants were uprooted and inoculated with F.graminearum as discussed in Part-III and presented in Plate 10, Fig. A-C. Both the chemicals at 10^{-4}M concentration were equally effective in reducing diseases in Cv.soymax, hance this concentration was chosen for treatment. Glyceollin was extracted from the treated and untreated inoculated roots as described (vide Scheme in Part IV). Results are given in Table-24.

Table-24 : Effect of sodium selenite and sodium azide on the production of glyceollin in soybean roots (Cv.Soymax).

Treatment	Glyceollin content[a] (µg/g fresh wt. of roots)	
	Non-inoculated	Inoculated with F. graminearum
Untreated	0	256 ± 6.9
Treated		
Sodium azide(10^{-4}M)	187.8 ± 5.2	545 ± 9.8
Sodium selenite(10^{-4}M)	98.0 ± 4.1	392 ± 5.5

a - Average of three experiment

b - Roots collected after 48 h of inoculation.

It is interesting to note that sodium azide(10^{-4}M) induced glyceollin synthesis in uninoculated soybean plants.

Similarly, sodium selenite (10^{-4}M) also induced glyceollin synthesis in uninoculated plants but in this case glyceollin production was comparatively less. The production of glyceollin was maximum in sodium azide treated plants inoculated with F.graminearum than sodium selenite treated and inoculated (with F.graminearum) plants. The results clearly indicate that glyceollin accumulation increases in soybean plants (Cv.Soymax) after induction of resistance to F.graminearum by sodium azide or sodium selenite treatment.

PART-VII

Studies on host-parasite protein :

The immunity of plant towards pathogen may also depend on the speed and extent of protein synthesis induced in the host by the pathogen (Uritani, 1976). This type of defence seems to be related to that afforded by phytoalexins. Additional proteins or enzymes may be required for the synthesis of phytoalexins although the mechanism involved in phytoalexin and protein synthesis operates separately. In case of compatible combination, however, changes in protein configuration in the hosts may induce host accessibility to the pathogen, which is related to the induced susceptibility (Joosten & Dewit, 1988). There is also evidence that alteration in the protein synthesis in the plants can lead to the development of local resistance for immune layer around infection sites. In several plants it has been demonstrated that upon infection with fungi, bacteria or viruses, development of symptoms is accompanied by the appearance of one or more, new proteins designated as pathogenesis related proteins.

In view of these findings it seems important to consider whether or not new types of pathogen induced proteins

are synthesized in soybean roots inoculated with F.graminearum In this investigation, however, host-parasite proteins have been studied for three reasons -

a) to ascertain whether protein level also change like glyceollin after treatment with selected chemicals inducing disease resistance ;

b) to determine whether soybean root proteins changes in resistant and susceptible cultivars after infection ; and

c) to evaluate protein patterns of healthy and F.graminearum infected resistant and susceptible soybean cultivars.

(a) Comparison of protein contents of roots of soybean before and after treatment with chemicals inducing disease resistance :

Among the nine different chemicals tested, sodium azide and sodium selenite reduced disease symptoms as well as induced glyceollin production in both inoculated and non-inoculated roots. In this experiment, total soluble protein content of untreated and treated (with sodium azide and sodium selenite) soybean roots of Cvs. Soymax and JS-2 were estimated as described earlier. Results (Table-25) revealed that after 7 days of treatment either with 10^{-4}M sodium azide or sodium selenite, root proteins decreased slightly in both the cultivars.

(b) Comparison of protein contents of healthy and F.graminearum inoculated soybean roots of resistant and susceptible cultivars :

Total soluble proteins were extracted from healthy and F.graminearum inoculated roots of five soybean cultivars

Table-25 : Estimation of total soluble protein content of healthy and treated roots of soybean cultivars.

Treatment	Protein content(mg/g fresh wt. of (root)	
	cv. Soymax	cv. JS-2
Untreated(healthy)	82.9	80.2
Treated		
Sodium azide(10^{-4}M)	78.3	75.6
Sodium selenite(10^{-4}M)	80.0	72.4

(Soymax, JS-2, PK-327, Pusa-16 and UPSM-19) and estimated. Experimental procedure has been described in detail under materials and methods and results are given in Table-26 and

Table-26: Estimation of total soluble protein content of healthy and F.graminearum infected roots of soybean cultivars.

Cultivars	Protein content(mg/g fresh wt. of root)	
	Healthy	Infected
Susceptible :		
Soymax	86.2	98.5
JS-2	78.5	88.7
Resistant :		
UPSM-19	85.0	89.5
PK-327	82.0	93.4
PUSA-16	75.0	80.6

Fig-18. Protein contents increased (about 13-14%) in the infected roots of cvs. JS-2, PK-327 and Soymax (susceptible cultivars) while only 5.3% and 7.5% increase in protein content were evident in infected roots of cvs. UPSM-19 and PUSA-16 respectively after 48h of inoculation with F.graminearum.

(c) Protein content of F.graminearum :

Since protein content increased in inoculated roots irrespective of resistant and susceptible cultivars, it was considered worthwhile to estimate the protein content of pathogen because protein preparations from infected roots might also contain proteins of parasite. Soluble mycelial protein of F.graminearum was extracted from 15-day old mycelia and estimated following the method described earlier. Results revealed that F.graminearum contains 1.860 mg/g fresh weight protein.

(d) Analysis of protein pattern of healthy and infected roots of Soybean :

It appears from the above results that protein content changes in resistant and susceptible soybean cultivars after inoculation with F.graminearum. Sometimes total protein content of host plant remains the same after infection, but isozyme patterns may change in response to infection. In such cases, where changes in total activities of a particular enzyme could not be detected, changes in the isozyme pattern in the diseased tissue have been detected following polyacrylamide gel electrophoresis(PAGE). In the present study, protein patterns of healthy and infected(48h after inoculation with F.graminearum) soybean roots of cvs. Soymax, JS-2, PK-327 and UPSM-19 as well as the mycelia of two isolates of F.graminearum have been evaluated by

TOTAL SOLUBLE PROTEIN CONTENT OF HEALTHY AND INOCULATED SOYBEAN ROOTS.

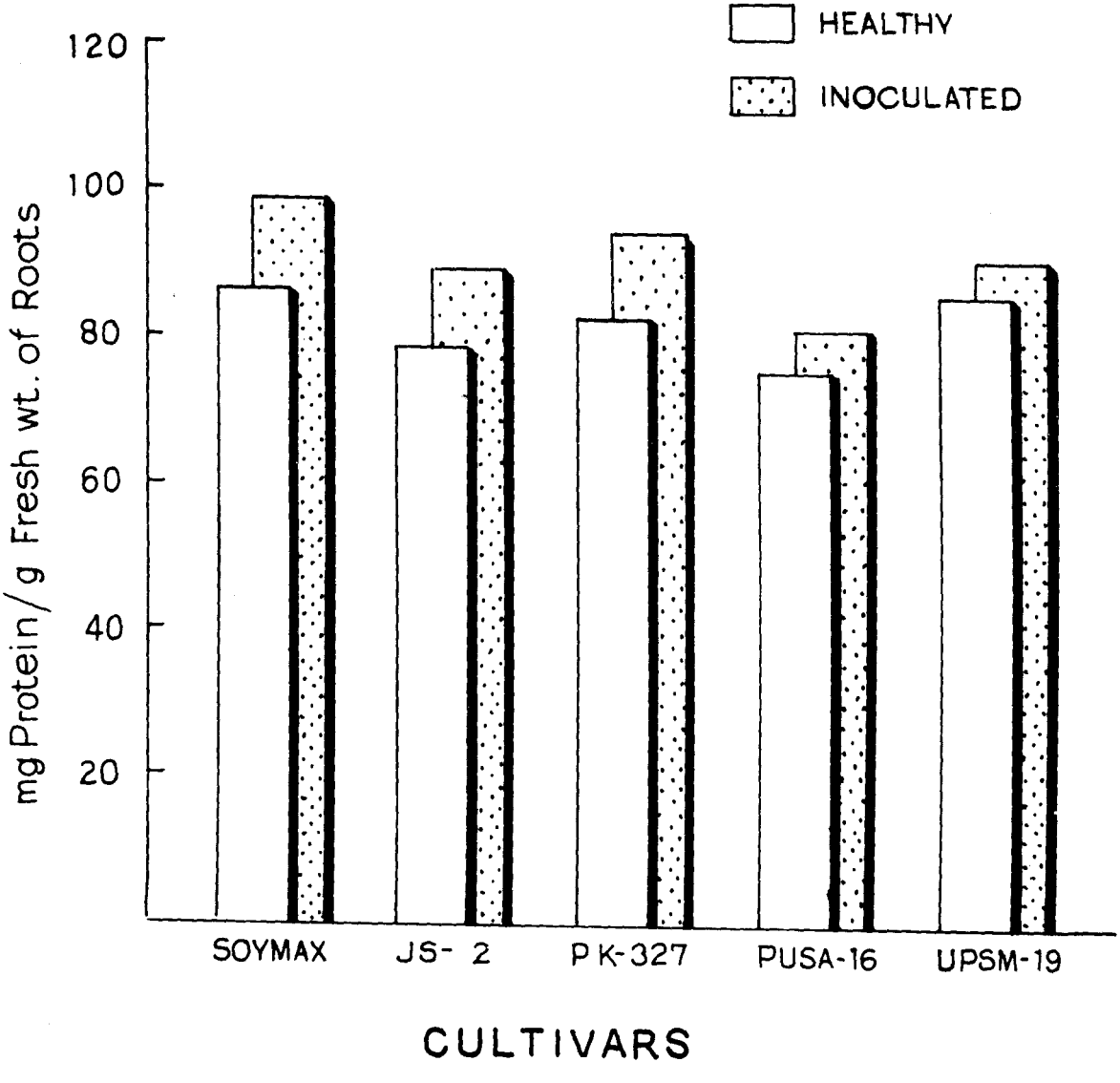


Fig. 18

polyacrylamide gel electrophoretic technique as stated earlier. The RF values of protein bands have been shown in Tables 27-29. It is interesting to note that in cvs. Soymax

Table-27: RF values of protein bands of healthy and F.graminearum infected roots of susceptible cultivars soybean evaluated by polyacrylamide gel electrophoresis.

Soybean Cultivars							
Soymax				JS - 2			
Healthy		Infected		Healthy		Infected	
Band No.	RF Value	Band No.	RF Value	Band No.	RF Value	Band No.	RF Value
1	0.05	1	0.07	1	0.07	1	0.09
2	0.07	2	0.08	2	0.12	2	0.12
3	0.10	3	0.11	3	0.21	3	0.14
4	0.15	4	0.15	4	0.23	4	0.22
5	0.31	5	0.21	5	0.29	5	0.25
6	0.38	6	0.32	6	0.37	6	0.29
7	0.49	7	0.40	7	0.39	7	0.37
8	0.51	8	0.52	8	0.47	8	0.40
9	0.64	9	0.54	9	0.50	9	0.45
10	0.73	10	0.59	10	0.57	10	0.49
11	0.75	11	0.65	11	0.60	11	0.52
12	0.77	12	0.74	12	0.65	12	0.62
13	0.86	13	0.77	13	0.76	13	0.66
14	0.93	14	0.78	14	0.88	14	0.68
		15	0.88	15	0.91	15	0.78
		16	0.95			16	0.81
						17	0.87
						18	0.89

Table-28: RF values of protein bands of healthy and F.graminearum infected roots of moderately resistant cultivars of soybean evaluated by polyacrylamide gel electrophoresis.

Soybean Cultivars							
PK - 327				UPSM - 19			
Healthy		Infected		Healthy		Infected	
Band No.	RF Value	Band No.	RF Value	Band No.	RF Value	Band No.	RF Value
1	0.09	1	0.10	1	0.13	1	0.14
2	0.12	2	0.12	2	0.21	2	0.21
3	0.18	3	0.18	3	0.26	3	0.26
4	0.21	4	0.20	4	0.31	4	0.31
5	0.29	5	0.29	5	0.39	5	0.40
6	0.32	6	0.32	6	0.42	6	0.43
7	0.40	7	0.40	7	0.46	7	0.47
8	0.43	8	0.43	8	0.51	8	0.53
9	0.47	9	0.46	9	0.56	9	0.57
10	0.52	10	0.51	10	0.60	10	0.60
11	0.56	11	0.56	11	0.69	11	0.70
12	0.60	12	0.60	12	0.75	12	0.75
13	0.67	13	0.67	13	0.81	13	0.81
14	0.70	14	0.69	14	0.89	14	0.88
15	0.72	15	0.72	15	0.93	15	0.90
16	0.76	16	0.76				
17	0.88	17	0.87				

Table-29 : RF values of mycelial protein of F.graminearum evaluated by polyacrylamide gel electrophoresis.

Mycelial protein of F.graminearum			
Isolate Fg 1		Isolate Fg 2	
Band No.	Rf Value	Band No.	Rf Value
1	0.14	1	0.12
2	0.16	2	0.17
3	0.20	3	0.20
4	0.22	4	0.27
5	0.26	5	0.34
6	0.31	6	0.36
7	0.34	7	0.38
8	0.36	8	0.43
9	0.38	9	0.52
10	0.41	10	0.57
11	0.44	11	0.64
12	0.50	12	0.70
13	0.53	13	0.73
14	0.57	14	0.82
15	0.63	15	0.89
16	0.70		
17	0.74		
18	0.84		
19	0.90		

and JS-2, 16 and 18 bands were detected in infected roots while 14 and 15 protein bands could be detected in healthy roots respectively. On the other hand, both healthy and infected roots of cvs. PK-327 and UPSM-19 exhibited 17 and 15 protein bands. The mycelial protein prepared from two isolates of F.graminearum Fg 1 and Fg 2 exhibited 18 and 15 protein bands respectively (Fig.19). However, it is not desirable to compare the protein patterns of host and parasite obtained by PAGE, because protein band showing in the same RF of host and parasite may not necessarily be similar. Their molecular weight may be different hence, it was decided to study the serological relationship between F.graminearum and soybean cultivars, existing if any.

PART-VIII :

Studies on the serological relationship between F.graminearum and soybean cultivars.

A phenomenon which has received increased interest during last twenty five years is the presence of common antigens or antigen sharing between host (plant or animal) and pathogenic micro-organisms. Existing studies on both plant or animals host and their parasites and pathogens suggests that whenever an intimate and continuing association of cells of host and parasites occurs, partners of these association have a unique serological resemblance to one another involving one or more antigenic determinants. Resistance or susceptibility of an animal to infection and disease development may be dependent on the antigenic relationship of host and pathogen

DIAGRAMMATIC SKETCHES OF THE PROTEIN PATTERNS OF HEALTHY AND INFECTED ROOTS OF SOYBEAN CULTIVARS AND Fusarium graminearum .

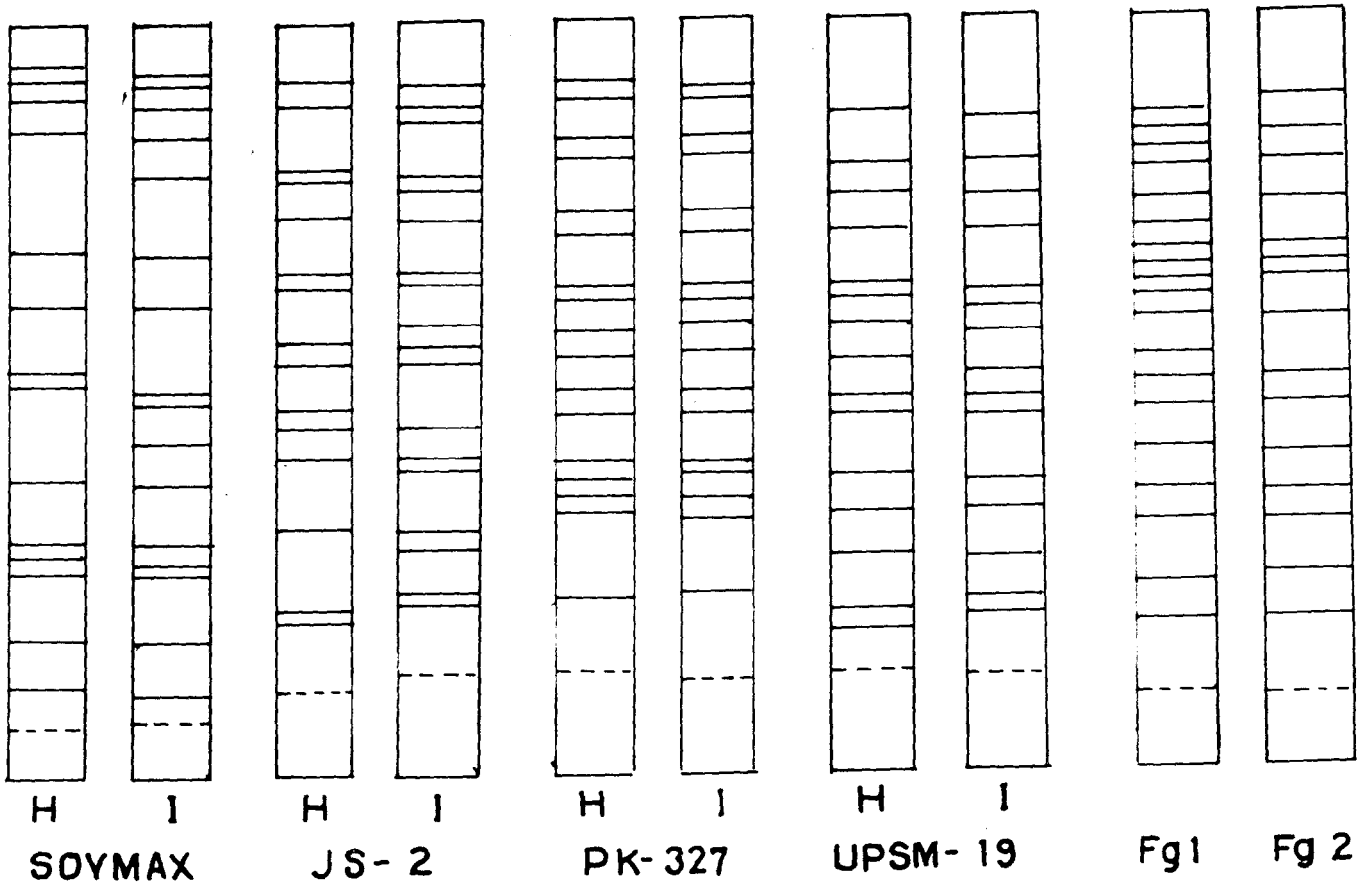


Fig- 19

(Dineun, 1963; Damian, 1964). The greater the antigenic similarity, the greater the possibility of immunological tolerance and likelyhood that the pathogen will become established in the host and cause disease. In several studies on plant pathogen systems, an analogous phenomenon have been observed. In this context common antigens between a plant and the pathogen are a notable feature of their association.

In the present investigation, attempts have been made to determine the presence of common antigens, if any, among the soybean cultivars and isolates of F.graminearum. Plant antigens were prepared from healthy roots of six soybean cultivars viz., Soymax, JS-2, KU-254, UPSM-19, EC-55865 and R-184, while fungal antigens were prepared from two isolates of F.graminearum (Fg 1 and Fg 2). Antigens were also prepared from two fungal species, viz., Glomerella cingulata and Pestalotiopsis theae, which are non pathogenic to soybean. Antisera were raised in separate male white rabbits against antigens of the representative type i.e., one susceptible (Soymax) and one resistant (UPSM-19) soybean cultivars and one isolate (Fg 1) of F.graminearum as described earlier. Normal sera were obtained from rabbits by ear - vein puncture before immunization.

(a) Agar-gel double diffusion tests :

The effectiveness of antigen preparations from roots of cvs. soymax and UPSM-19 and isolates of F.graminearum (Fg 1) in raising antibodies was checked by homologous cross-reaction. The titre value of the antisera for both the soybean cultivars were 32 and for the pathogen was 64 (Table-30). Finally, these undiluted antisera were tested against both homologous and heterologous antigens following agar-gel double diffusion technique. The results are presented in Table-31 and Plate-11 (Fig. A-F) and Plate-12(Fig. A-F). Control sets

Plate-11 Fig.(A-F) Agar gel double diffusion test using antigens and antisera of Soybean roots and F.graminearum.

(A) Central well Contained antiserum of F. graminearum (F_1A) and the surrounding wells contain antigens of isolates of F.graminearum, Fg1 (F_1a) and Fg2 (F_2a) ; **(B)** Central well contain antiserum of UPSM-19 (UA) and the surrounding wells contain root antigens of cv. UPSM-19 (Ua) ; **(C)** Central well contain antigen of UPSM-19 (Ua) and the peripheral wells contain antiserum of UPSM-19(UA) and normal serum (NS) ; **(D-F)** Central wells contain antiserum of Soymax (SA) and the surrounding wells contain root antigen of Soymax(Sa), R-184 (Ra), KU-254 (Ka), JS-? (Ja) and F. graminearum (F_1a).

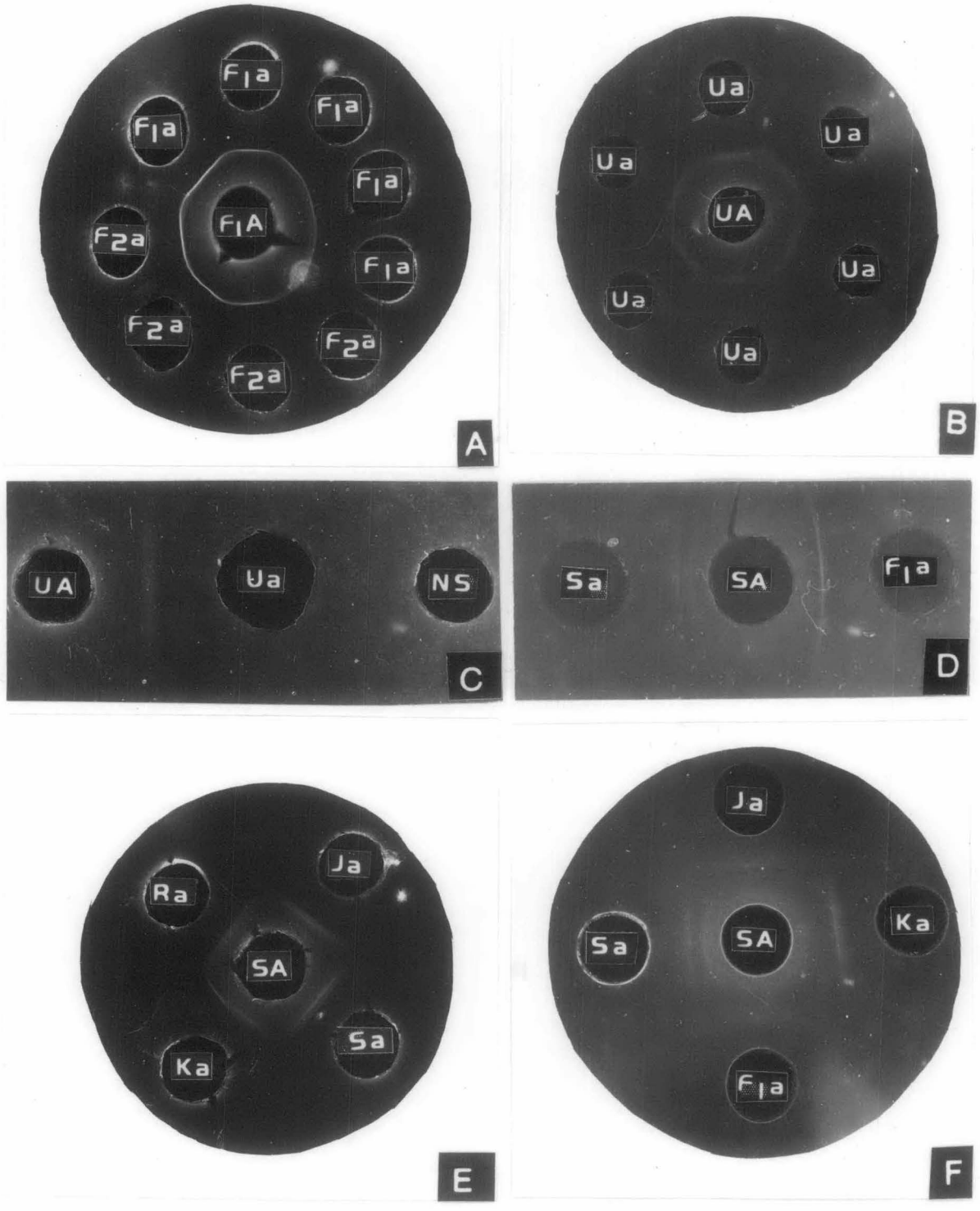

F1a
F1a
F1a
F1a
F1A
F2a
F1a
F2a
F2a
F2a
A
Ua
Ua
Ua
UA
Ua
Ua
Ua
B
UA
Ua
NS
C
Sa
SA
F1a
D
Ja
Ra
SA
Sa
Ka
E
Ja
Sa
SA
Ka
F1a
F

Plate- 11

Plate-12 Fig.(A-F) Agar gel double diffusion test using antigen and antisera of Soybean cultivars, isolates of F.graminearum and non pathogens (G. cingulata and P.theae). **(A)** Central well contain antiserum of Soymax (SA) and surrounding wells contain root antigens of Soymax (Sa), JS-2 (Ja) and KU-254(KA) ; **(B & F)** Central wells contain antiserum of F.graminearum (F_1A) and surrounding wells contain antigens of isolate Fg1 (F_1a) and Fg2 (F_2a) of F.graminearum, P.theae (Pta), G. cingulata (Gca), root antigens of Soymax (Sa), JS-2 (Ja) and KU-254 (Ka) ; **(C & D)** Central wells contain antiserum of UPSM-19 (UA) and surrounding wells contain root antigen of R-184 (Ra), EC-55865 (Ea), UPSM-19 (Ua), isolate Fg1 (F_1a), Fg2(F_2a) of F.graminearum and G. cingulata (Gca) ; **(E)** Central well contain antiserum of Soymax (SA) and surrounding wells contain seed antigens of Soymax (Sa), JS-2 (Ja) and isolate Fg1 (F_1a) of F.graminearum.

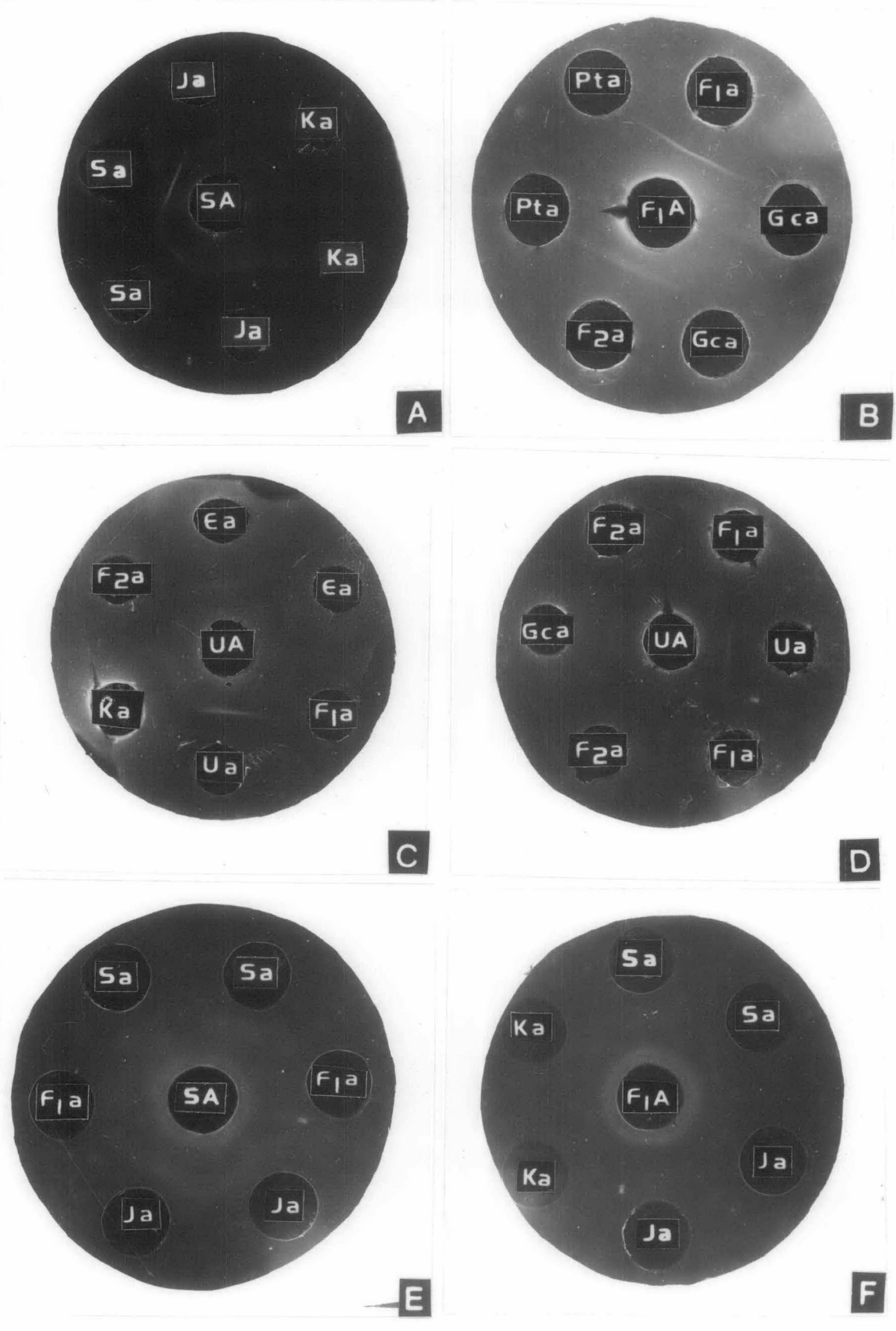

Ja
Ka
Sa
SA
Ka
Sa
Ja
A
Pta
F1a
Pta
F1A
Gca
F2a
Gca
B
Ea
F2a
Ea
UA
Ka
F1a
Ua
C
F2a
F1a
Gca
UA
Ua
F2a
F1a
D
Sa
Sa
F1a
F1a
SA
Ja
Ja
E
Sa
Sa
Ka
F1A
Ja
Ka
Ja
F

Plate - 12

Table-30 : Semi-quantitative estimation of antigens and antisera of soybean cultivars and Fusarium graminearum.

Soybean cultivars and Fungus	Titre of antigen against homologous antiserum	Titre of antiserum against homologous antigen
Soybean		
Cv. Soymax	8	32
Cv. UPSM-19	8	32
Fungus :		
F. graminearum (Fg 1)	16	64

Incubation time 72 h ; Temperature 25° ± 1°C

involving normal sera and antigens of both the host and parasite were all negative (Plate-11, Fig-C). Strong precipitin reactions occurred when antiserum of F.graminearum (F_1A) was reacted against its own antigen (F_1a) and the antigens of another isolate (F_2a) of F.graminearum (Plate-11, Fig-A and Plate-12, Fig-B). When antiserum of F.graminearum (F_1A) was cross-reacted with antigens of susceptible cultivars (Sa, Ja & Ka), precipitin bands were evident near the central wells and showed complete identity, since no spurs in band fusion were evident (Plate-12, Fig.F). Antigen prepared from seeds were weakly reactive with antiserum of cv. Soymax forming diffused precipitin bands (Plate-12, Fig.E). To confirm the presence of cross-reactive antigens between F.graminearum (isolate Fg 1) and susceptible cultivars (Soymax JS-2 and KU-254), antiserum of Soymax(SA) was cross-reacted

Table-31 : Detection of cross reactive antigens among soybean cultivars and isolates of F.graminearum

Antigens of host	Code	Antisera of host and parasite with code		
		F.graminearum (Fg 1A)	Soymax (SA)	UPSM-19 (UA)
Isolates of F. graminearum				
Fg 1	F_1a	+	+	±
Fg 2	F_2a	+	±	-
Susceptible Cultivars				
Soymax	Sa	+	+	+
JS-2	Ja	+	+	+
KU-254	Ka	+	+	+
Resistant Cultivars				
UPSM-19	Ua	-	+	+
EC-55865	Ea	-	±	+
R-184	Ra	±	+	+
Non-Pathogen				
1. G.cingulata	Gca	-	-	-
2. P.theae(Pt-1)	Pta	-	-	-

(+) Common precipitin band present
(±) Weak precipitin band
(-) Common precipitin band absent.

with antigens (F_1a, Sa, Ja, Ka & Ra). Strong precipitin reactions were discerned in all cases (Plate-11, Figs. D-F; Plate12,Fig.A) whereas weak precipitin reactions were observed with isolate Fg 2 (Table-31). Strong precipitin reactions were

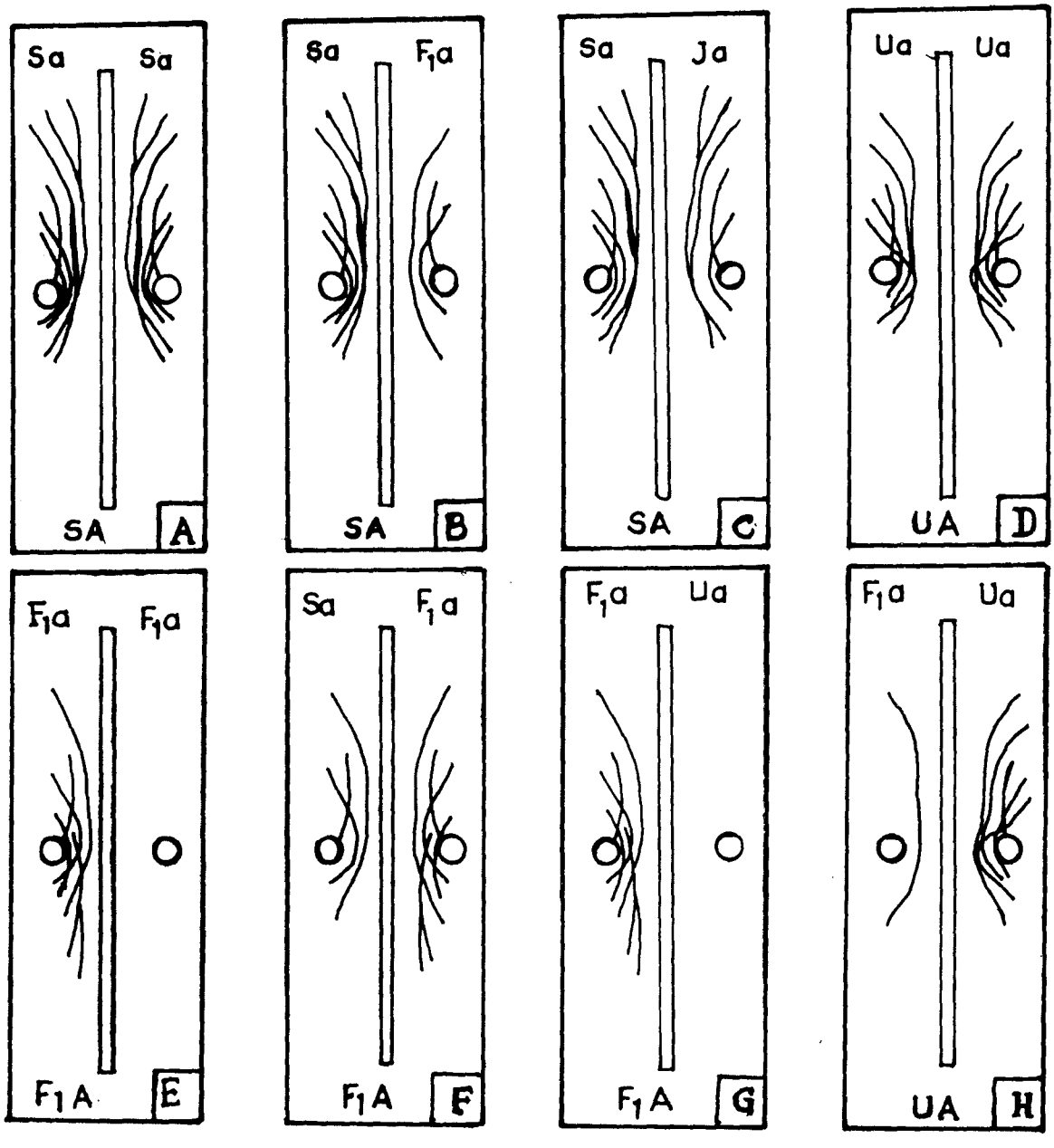
noted in homologous reaction between antigen antiserum of CV. UPSM-19 (Plate-11, Fig-B). Cross reactions between antiserum of UPSM-19(UA) and antigens of isolate Fg 1 (F_1a) developed weak precipitin bands while antigen prepared from isolate Fg 2 and two non pathogens G.cingulata (Gca) and P.theae (Pta) in cross reactions with UA failed to develop any precipitin bands (Plate-12, Fig . D).

(b) **Immunoelectrophoretic tests :**

Immunoelectrophoresis consists of a combination of electrophoresis and radial immunodiffusion in gel. By this method complex antigenic mixtures can be separated due to the additional resolving power obtained through the electrophoretic step. Results of immonodiffusion tests revealed the presence or absence of cross reactive antigens between isolates of F.graminearum and soybean cultivars. any of the cross-reactions in the immunodiffusion tests gave diffused precipitin bands which could not be clearly distinguished. It was also not clear whether precipitin reactions in all cases were due to single or several antigenic substances. Therefore, further resolution was attempted by subjecting the antigens to electrophoresis before exposing them to antisera. Details of immunoelectrophoretic technique have been described under material and methods. Antigenic comparison among soybean cultivars and the fungal isolates are shown in Table 32-35. Immunograms are presented in Fig-20(A-H).

Effectiveness of each antigen extract (Sa, Ua & F_1a) in raising antibodies (SA, UA & F_1A) was checked by homologous cross-reactions. The homologous patterns formed by Sa and Ua contained 6 and 5 precipitin lines respectively and that of F_1a cotained 6 precipitin lines. The antisera of hosts (SA and UA) and parasite(F_1A) were cross-reacted with antigens of six

IMMUNOGRAMS SHOWING IMMUNOELECTROPHORETIC PATTERNS OF RESISTANT, SUSCEPTIBLE SOYBEAN CULTIVARS AND *F. graminearum*



Central rectangular troughs contained antisera of Soymax (SA), UPSM-19 (UA), *F. graminearum* (F_1A) and the wells contained antigens of Soymax (Sa), JS-2 (Ja), UPSM-19 (Ua) and *F. graminearum* (F_1a)

Fig. 20.

Table-32 : Immunoelectrophoretic test of antiserum of CV. soymax with antigens of soybean cultivars and F.graminearum.

Antigens of soybean cultivars, pathogen and non pathogen	Code name of antigen	Precipitin lines					
		Antiserum of Soymax (SA)					
		1	2	3	4	5	6
Susceptible cultivars							
Soymax	Sa	+	+	+	+	+	+
JS-2	Ja	+	-	+	+	+	+
KU-254	Ka	+	+	-	+	-	+
Resistant cultivars							
UPSM-19	Ua	-	-	-	+	+	-
EC-55865	Ea	-	-	+	+	+	-
R-184	Ra	-	+	-	+	+	+
Isolates of F.graminearum							
Fg-1	F_1a	+	+	-	-	+	-
Fg-2	F_2a	+	+	-	-	-	-
Non-Pathogen							
G.cingulata	GC-a	-	-	-	-	-	-
P.theae	Pt-a	-	-	-	-	-	-

Precipitin line present(+) ; Precipitin line absent(-)

Table-33 : Immunoelectrophoretic test of antiserum of CV. UPSM-19 with antigens of soybean cultivars and F.graminearum

Antigens of soybean cultivars, pathogen and non pathogen	Code name of antigen	Precipitin lines				
		Antiserum of UPSM-19(UA)				
		1	2	3	4	5
Resistant cultivars						
UPSM-19	Ua	+	+	+	+	+
EC-55865	Ea	+	+	+	+	-
R-184	Ra	+	+	+	-	+
Susceptible cultivars						
Soymax	Sa	-	+	+	-	+
JS-2	Ja	+	-	+	+	+
KU-254	Ka	+	+	-	+	+
Isolates of F.graminearum						
Fg 1	F_1a	-	-	-	-	+
Fg 2	F_2a	-	-	-	-	-
Non Pathogen						
G.cingulata	GCa	-	-	-	-	-
P.theae	Pta	-	-	-	-	-

Precipitin line present (+) ; Precipitin line absent (-)

Table-34 : Immunoelectrophoretic test of antiserum of F.graminearum with antigens of soybean cultivars and F.graminearum.

Antigens of soybean cultivars, pathogen and non pathogen	Code name of antigen	Precipitin lines					
		Antiserum of F.graminearum (F_1A)					
		1	2	3	4	5	6
Isolates of F.graminearum							
Fg 1	F_1a	+	+	+	+	+	+
Fg 2	F_2a	+	+	-	+	+	-
Susceptible cultivars							
Soymax	Sa	+	+	-	-	+	-
JS-2	Ja	+	+	-	-	-	-
KU-254	Ka	-	+	-	-	+	-
Resistant cultivars							
UPSM-19	Ua	-	-	-	-	-	-
EC-55865	Ea	-	-	-	-	-	-
R-184	Ra	-	+	-	-	+	-
Non Pathogen							
G.cingulata	GCa	-	-	-	-	-	-
P.theae	Pta	-	-	-	-	-	-

Precipitin line present (+) ; Precipitin line absent (-)

Table-35 : Antigenic comparison of soybean cultivars, isolates of F.graminearum (Pathogen) and non pathogens

Antigens of host and parasite.	Code	Total number of precipitin lines		
		Antisera of host and parasite with code		
		F.graminearum (F 1A)	Soymax (SA)	UPSM-19 (UA)
Pathogen				
Isolates of F.graminearum				
Fg 1	F_1a	6	3	1
Fg 2	F_2a	4	2	0
Susceptible cultivars				
Soymax	Sa	3	6	3
JS-2	Ja	2	5	4
KU-254	Ka	2	4	4
Resistant cultivars				
UPSM-19	Ua	0	2	5
EC-55865	Ea	0	3	4
R-184	Ra	2	4	4
Non Pathogen				
G.cingulata	GCa	0	0	0
P.theae	Pta	0	0	0

soybean cultivars (Soymax, JS-2, KU-254, UPSM-19, EC-55865 and R-184) and two isolates of F.graminearum (Fg 1 and Fg 2) as well as non pathogens (G.cingulata and P.theae).

When antiserum of cv. Soymax was cross-reacted with the antigen preparations from roots of five different cultivars, it was seen that they were antigenically very close to each other. In this reaction antigens of cv. JS-2 gave rise to 5 precipitin arcs and cvs. KU-254 and R-184 exhibited four precipitin arcs while EC-55865 and UPSM-19 showed 3 and 2 precipitin acrs respectively. Both the isolates of F.graminearum were antigenically more related to cv. Soymax by sharing 3 and two common precipitin arcs by Fg 1 and Fg 2 respectively. No such precipitin lines could be observed with the non pathogens (Table-32).

Similarly antiserum of resistant soybean cultivar (UPSM-19) was cross-reacted with root antigens of five soybean cultivars. Four precipitin arcs each, were formed with antigens of EC-55865 (Ea), R-184(Ra), JS-2(Ja) and KU-254(Ka) while Soymax(Sa) exhibited 3 precipitin arcs. It is interesting to note that only one precipitin arc was observed when antigen of isolate Fg 1 (F_1a) was cross-reacted with antiserum of UPSM-19(UA) but no such precipitin was observed when antigens of isolate Fg 2(F_2a) was cross-reacted with UA. Cross-reactions involving non pathogens were formed also to be negative (Table-33).

Reciprocal cross-reactions between antisera of F.graminearum and antigens of cv.Soymax (Sa) formed 3 precipitin lines while three other cultivars JS-2, KU-254 and R-184 formed 2 precipitin lines but antigens of cv. UPSM-19 and EC-55865 failed to develop any precipitin lines.

It appears from the summary Table-35 that isolates of F.graminearum shared cross-reactive antigens among four

soybean cultivars (Soymax, JS-2, KU-254 and R-184) while no such relationship could be observed among cvs. UPSM-19 and EC-55865. However, reciprocal cross-reactions with antisera of UPSM-19 and antigen of isolate Fg 1 exhibited one common precipitin line which was not present with the antigen of isolate Fg 2. Serological relationship among the resistant and susceptible cultivars were noted by the presence of precipitin lines.

PART - IX

Detection of the cross-reactive antigen between F.graminearum and G.max by indirect enzyme linked immunosorbent assay :-

Enzyme linked immunosorbent assay (ELISA) is a common, specific and rapid method for identifying virus infections (Clark and Adams - 1977; Clark and Bas-Josheph-1984) as well as fungal diseases (Casper and Mendgen, 1979 ; Johnson et.al., 1982 ; Mohan, 1988 ; Dewey and Brasier, 1988; Mac Donald et.al., 1990 ; Harrison et.al., 1990 ; Lyons and White, 1992) and some assays have been developed to test specifically for the presence of Fusarium species (Kitagawa et.al., 1989). It has been clearly pointed out by Alba and Devay (1985) on the basis of their findings that indirect ELISA can serve as an important technique to detect CRA, to determine their properties and to investigate their possible role in host parasite interaction. Therefore, it was considered worth-while to study cross-reactive antigen between F.graminearum and soybean cultivars (Soymax, JS-2, UPSM-19 and PK-327) by using indirect ELISA since this method has been considered to be one of the most sensitive serological tests for detecting CRA at low concentrations.

Antigens were prepared from roots of four cultivars

of soybean as mentioned above, pathogen (F.graminearum) isolate(Fg 1), as well as non pathogen (G.cingulata and P.theae) and antisera were separately raised against antigen preparations from roots of Cv. Soymax and UPSM-19 and mycelia of F.graminearum (Fg 1). Indirect ELISA procedure using the above mentioned antigen and antisera preparations were performed following the method as described earlier. Antibodies produced against F.graminearum (isolate Fg 1) were titrated and compared. The antibody titre increased gradually with booster injections, reaching a maximum 8 week after the first injection. The highest reactivity in indirect ELISA was one week after the final injection. Two different dilution (1/125 and 1/250) each of three antisera preparations were tested against homologous and heterologous antigen (5 and 25 /ug protein/ml). Absorbance of all the components at 405 nm were noted in an ELISA reader. Results are given in Table 36-38.

Indirect ELISA could readily detect cross-reactive antigens in semipurified mycelial preparations at concentrations ranging 5-25 /ug protein/ml with antiserum dilutions (1/125 and 1/250). Antigenic preparation of F.graminearum (isolate Fg 1) exhibited higher absorbance value when reacted with antiserum of susceptible soybean cultivar Soymax (Table-37) than when reacted with antiserum of resistant soybean cultivar UPSM-19(Table-38). Results obtained in different assays showed that all combinations, A_{405} values for normal serum controls were below the corresponding test value. There was no cross-reactivity with P.theae and G.cingulata (non pathogens). Results are the means of three assays, using two replicates/assay.

Table-36: Enzyme linked immunosorbent assay value(A 405) of combinations of antigenic preparation of soybean cultivars, pathogen, non pathogen and antiserum of F.graminearum

Antigens of host/ pathogen/non - pathogen	Code	µg protein/ ml	Normal serum 1/125	Antiserum of F.graminearum(F_1A) 1/125	1/150
Soybean cultivars ;					
Soymax	Sa	5	0.027	1.187	0.936
		25	0.118	1.445	1.012
JS-2	Ja	5	0.031	1.072	0.879
		25	0.092	1.284	0.991
UPSM-19	Ua	5	0.172	0.713	0.556
		25	0.189	0.825	0.610
PK - 327	Pa	5	0.134	0.886	0.693
		25	0.162	1.117	0.968
Pathogen :					
F.graminearum (isolate Fg 1)	F_1a	5	0.030	1.635	1.488
		25	0.116	> 2	1.842
Non pathogen :					
G.cingulata	Gca	5	0.011	0.153	0.119
		25	0.076	0.312	0.272
P.theae	Pta	5	0.029	0.142	0.112
		25	0.052	0.308	0.265

Table-37 : Enzyme linked immunosorbent assay value (A 405) of combinations of antigenic preparation of soybean cultivars, pathogen and non pathogen and antiserum of Soymax.

Antigens of host/ pathogen/ non pathogen	Code	/ug protein/ ml.	Normal Serum 1/125	Antiserum of Soymax(SA)	
				1/125	1/250
Soybean cultivars :					
Soymax	Sa	5	0.033	1.784	1.536
		25	0.082	>2	1.882
JS-2	Ja	5	0.041	1.150	1.029
		25	0.112	1.406	1.115
UPSM-19	Ua	5	0.104	1.033	0.984
		25	0.076	1.179	1.106
PK-327	Pa	5	0.162	1.213	1.144
Pathogen :		25	0.110	1.476	1.392
F.graminearum (Isolate Fg 1)	F_1a	5	0.084	1.012	0.765
		25	0.109	1.276	0.981
Non-pathogen :					
G.cingulata	Gca	5	0.031	0.233	0.172
		25	0.084	0.309	0.226
P.theae	Pta	5	0.019	0.266	0.140
		25	0.021	0.410	0.206

Table-38 : Enzyme linked immunosorbent assasy value (A 405) of combinations of antigenic preparation of soybean cultivars, pathogen and non pathogen and antiserum of UPSM-19.

Antigens of host/ pathogen/ non pathogen	Code	/ug protein/ ml.	Normal Serum 1/125	Antiserum of UPSM-19 (UA)	
				1/125	1/250
Soybean cultivars :					
Soymax	Sa	5	0.114	1.072	0.766
		25	0.126	1.236	1.039
JS-2	Ja	5	0.131	1.042	0.846
		25	0.124	1.179	0.920
UPSM-19	Ua	5	0.102	1.816	1.704
		25	0.115	>2	1.826
PK - 327	Pa	5	0.096	1.254	1.074
		25	0.118	1.442	1.272
Pathogen :					
F. graminearum (Isolate Fg 1)	F_1a	5	0.137	0.619	0.549
		25	0.161	0.734	0.597
Non-pathogen :					
G.cingulata	Gca	5	0.102	0.226	0.172
		25	0.078	0.291	0.165
P.theae	Pta	5	0.096	0.282	0.144
		25	0.119	0.339	0.249

PART - X

Analysis of antigenic pattern after chemical induction of resistance in soybean to F.graminearum.

It is clear from the results of immunodiffusion and immunoelectrophoretic tests (Vide Part-VIII) as well as indirect enzyme linked immunosorbent assay (vide part-IX) that a serological relationship exist between isolates of F.graminearum and soybean cultivars. It is also evident from the results (vide part-V) that among the various chemicals tested sodium azide and sodium selenite reduced disease symptoms markedly in the susceptible soybean cultivar - soymax. Consequent changes in glyceollin levels were also detected (vide part-VI). Hence, it was decided to study changes in the antigenic pattern in treated roots. To determine this, antigens were extracted from roots of untreated and treated (sodium azide and sodium selenite) soybean plants (cv. Soymax) and mycelia of F.graminearum (F_1A) and cv. Soymax (SA). Results are presented in Table-39. In agar-gel double diffusion test in this case also common precipitin bands were detected when antiserum of SA was cross-reacted with antigens of F.graminearum (F_1a). Reciprocal cross reaction with the antisera of F.graminearum and antigen of cv. Soymax gave common precipitin band. An interesting observation was made when antiserum of cv. Soymax (SA) was cross reacted with the antigen preparations of healthy and treated roots of soymax. Strong precipitin bands were evident in the homologous reaction between antigens and antisera of Soymax. But antigen preparation from sodium azide treated roots developed very faint diffused band while sodium selenite treated roots exhibited weak precipitin bands in the cross reaction with antisera of Soymax(SA). Reciprocal cross reactions between antisera of F.graminearum and antigens of sodium azide treated roots of cv. Soymax failed to develop any precipitin band (Table-39).

Table-39 : Antigenic relationship between isolate of F.graminearum (Fg 1) and susceptible soybean cultivar (Soymax) before and after treatment with chemicals.

Antigens of host and parasite	Antisera of Soymax (SA)
F.graminearum (F_1a)	+
Untreated (Sa)	+
Treated (Sta)	+
Sodium Selenite	±
Sodium azide	±

Antigens of host and parasite	Antisera of F.graminearum(F_1A)
F.graminearum (F_1a)	+
Cv. Soymax	
Untreated (Sa)	+
Treated (Sta)	±
Sodium selenite	±
Sodium azide	-

Precipitin band present (+), Precipitin band absent (-)
Weak precipitin band (±)
a plant treated with 10^{-4}M Sodium azide or sodium selenite.

Table-40 : Antigenic comparison of F.graminearum (isolate Fg.1) & susceptible soybean cultivar(Soymax) before and after treatment with sodium azide and sodium selenite.

Antigens of host and parasite.	Antiserum of F.graminearum (F_1A)					
	Precipitin lines					
	1	2	3	4	5	6
F.graminearum (F_1a)	+	+	+	+	+	+
Cv. Soymax						
Untreated(Sa)	+	+	-	-	+	-
Treated (Sta) :						
Sodium selenite	-	+	-	-	+	-
Sodium azide	-	+	-	-	-	-

Antigens of host and parasite	Antiserum of Soymax (SA)					
	Precipitin lines					
	1	2	3	4	5	6
Cv. Soymax						
Untreated (Sa)	+	+	+	+	+	+
Treated (Sta)						
Sodium selenite	-	+	+	-	+	+
Sodium azide	-	+	-	-	+	+
F.graminearum (F_1a)	+	+	-	-	+	-

a Plants treated with 10^{-4} M sodium azide.
Precipitin line present (+) , Precipitin line absent (-).

To confirm this, immunoeletrophoretic test was performed. Results are presented in the Table-40. F.graminearum showed six precipitin arcs in homologous reactions. In the cross-reaction between antigens of untreated roots of susceptible cultivar (Soymax) with antiserum of F.graminearum (F_1A) three precipitin arcs developed. But when F_1A was cross reacted with the antigens of sodium azide treated roots of cv. Soymax only one precipitin arc was evident. It can be explained that untreated roots of cv. Soymax was antigenically close to F.graminearum sharing three of the six antigenic constituents of the latter, while sodium selenite treated roots of cv. Soymax shared only two of the six antigenic constituents of F.graminearum. Similarly, antigen of untreated and sodium selenite treated roots of cv. Soymax and F.graminearum were cross reacted with antiserum of Soymax (SA). Six precipitin arcs were evident in the homologous cross reactions with antigen and antisera of cv. Soymax. Sodium selenite treated roots of cv. Soymax shared only four of the six antigenic constituents of untreated roots while sodium azide treated roots shared three of the six antigenic constituent of untreated roots of cv. Soymax.

PART - XI

The tissue and cellular location of CRA, shared by soybean roots and F.graminearum :

Fluorescent antibody labelling with fluorescein isothiocyanate (FITC is known to be one of the powerful technique to determine the cell or tissue location of major cross-reactive antigen(CRA) shared by plant host and parasite. In the present study, agar-gel double diffusion, immunoelectrophoresis as well as indirect ELISA clearly indicated the presence of CRA shared by Glycine max and F.graminearum. It

was decided to determine the tissue and cellular location of CRA in soybean roots.

Antibodies indirectly labelled with FITC were used to determine location of CRA in root sections of soybean cultivars UPSM-19 and Soymax and fungal cells (F.graminearum). Detail methods of antibody staining of root sections and fungal cells have already been discussed under Material and Methods. Root sections and mycelial preparations were photographed under both Phase-Contrast and UV-fluorescent condition for comparison of treatment. These are presented in Plate 13-15.

Mycelia and conidia of F.graminearum were not autofluorescent nor did they fluoresce when treated with normal serum followed by FITC. Treatment of mycelia and conidia of F.graminearum with homologous antisera and FITC showed a general fluorescence that was more intense on young hyphae (Plate 13, Fig B) and patch like areas on the conidia. When fungal cells were reacted with antiserum to roots of Cv. Soymax and treated with FITC, only bright fluorescence was apparent on young hyphae and in patch like areas on some conidia.

Cross-sections of roots of Cvs. UPSM-19 and Soymax were cut near and above the region of root hair initiation and treated seperately with normal sera, homologous and heterologous antisera, then reacted with FITC. Root sections did not exhibit any natural autofluorescence. Sections treated first with normal serum then by FITC also did not exhibit any fluorescence. Root sections of Cv. UPSM-19 treated with homologous antiserum and then reacted with FITC developed bright fluorescence which was concentrated mainly on epidermal cells and was distributed throughout the cortex tissue (Plate 14, Fig. B) Of much significance was the

Plate-13 Fig.(A & B) Mycelia of F.graminearum (Fg 1) treated with antiserum to soybean root (cv. Soymax) and FITC - antibodies of goat specific for rabbit globulins. **(A)** photographed under phase contrast ; **(B)** Same field photographed under UV-fluorescent conditions for comparison of treatment.

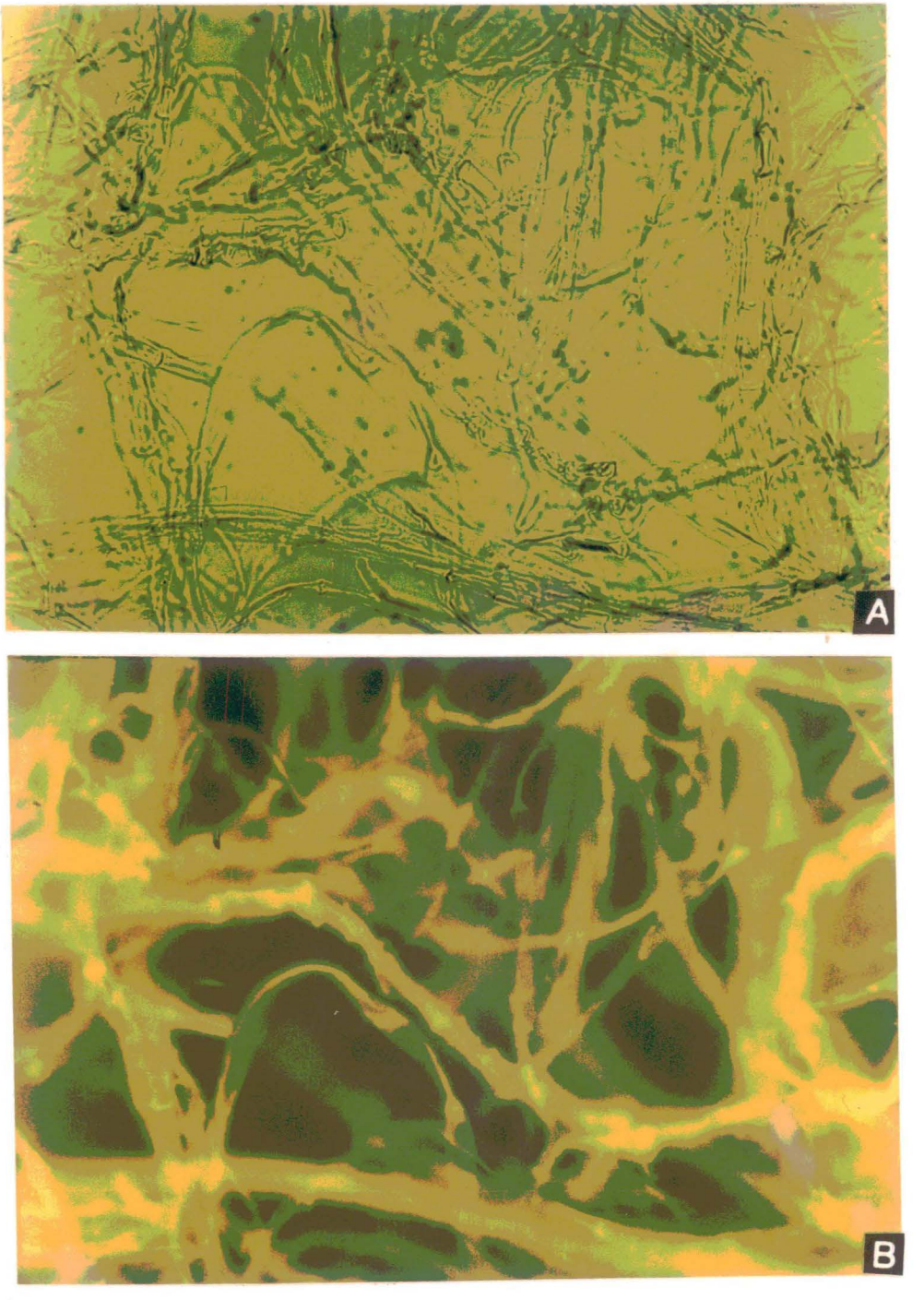


Plate- 13

Plate-14 Fig.(A-C) : Fluorescent FITC-antibody staining of Soybean root tissues (Cv-UPSM-19) for cross-reactive antigen shared with F. graminearum. **(A)** Unstained cross section of root ; **(B)** root section treated wtih antiserum to soybean root (UPSM-19) and FITC- antibodies of goat specific for rabbit globulins; **(C)** root section treated with antiserum to F.graminearum and FITC-antibodies of goat specific for rabbit globulins (Cortical tissues enlarged).

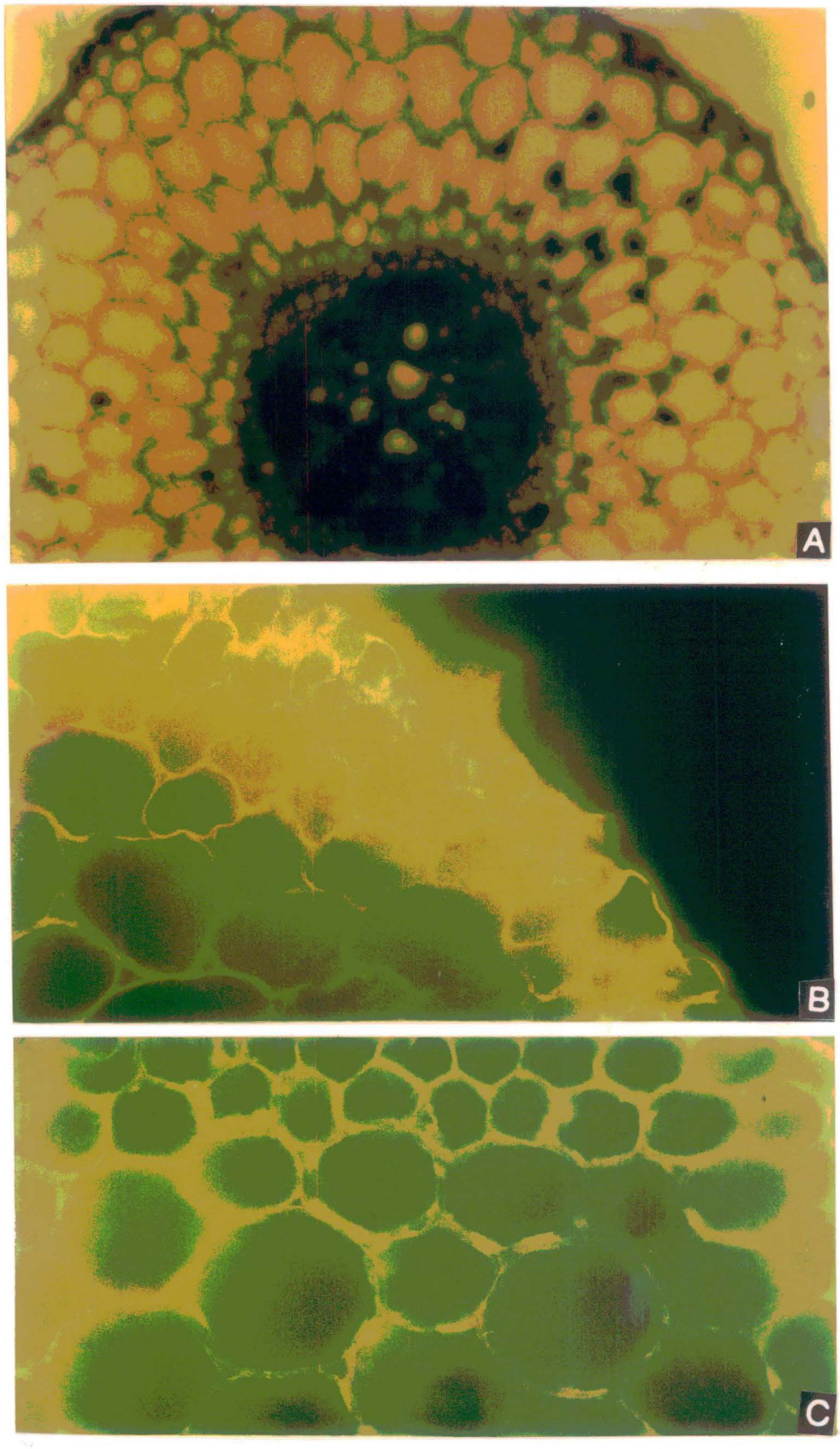


Plate - 14

Plate-15 Fig.(A-D) : Fluorescent FITC-antibody staining of soybean root tissues (cv-Soymax) for cross reactive antigen shared with F. graminearum. **(A-D)** root sections treated with antiserum to F.graminearum and FITC-antibodies of goat specific for rabbit globulins ; **(B)** cortical tissues, **(C)** Endodermis and vascular tissues and **(D)** Endodermis magnified.

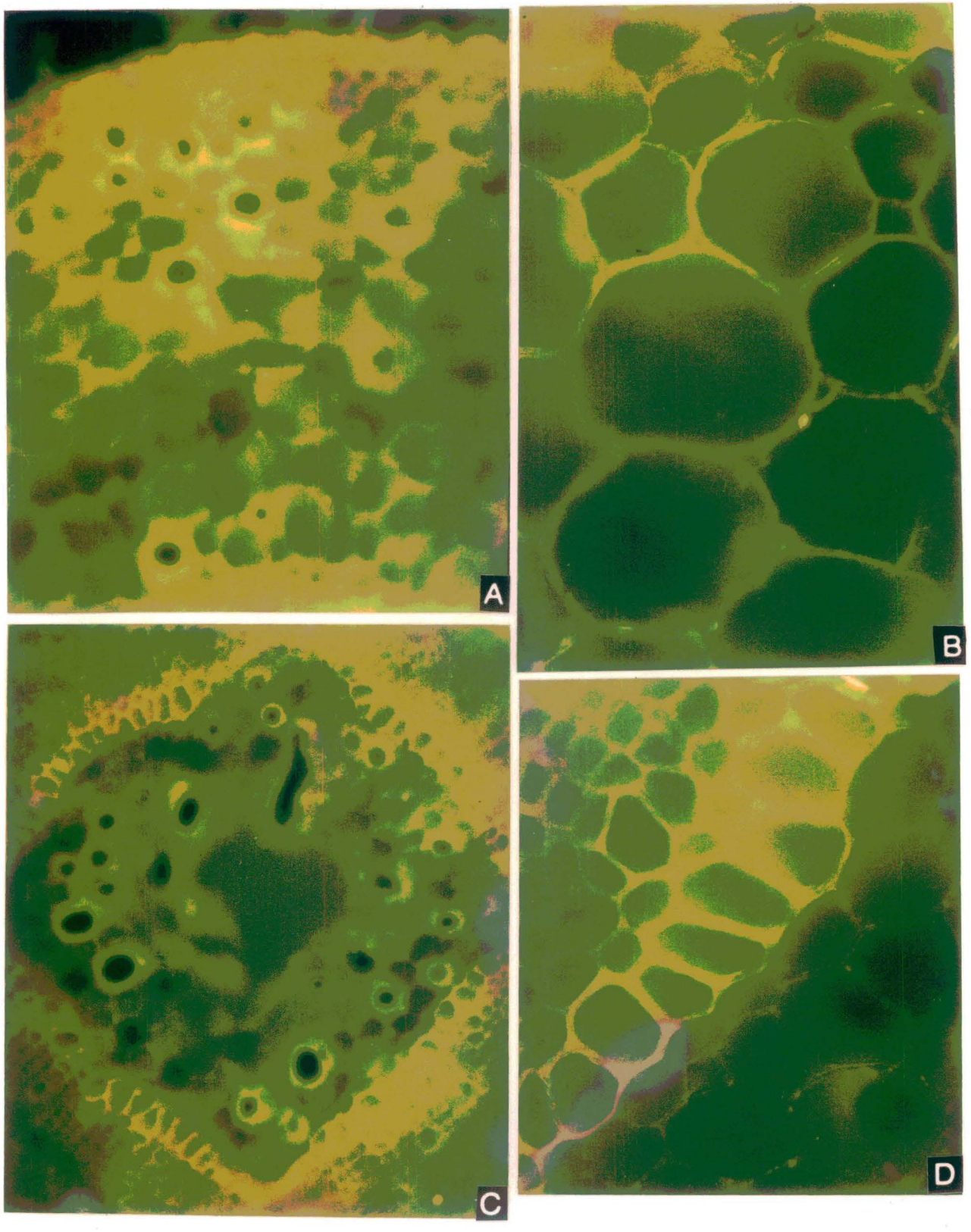


Plate- 15

strong reaction of antiserum of F.graminearum with root sections of Cv. Soymax. CRA was concentrated mainly around xylem elements, the endodermis, epidermal cells and distributed throughout the cortex tissue ; cell walls appeared to be the main cellular location of CRA (Plate 15, Fig. A-D).

DISCUSSION

Plants respond to infection by producing physical and chemical barriers which function as wall reinforcements, antibiotics or lytic enzymes. In every detailed comparative study of these responses, differences between resistance and susceptibility are quantitative rather than qualitative. Although susceptible plant possesses the machinary necessary for resistance, it is not activated in sufficient magnitude or speed to restict infection (Kuc, 1983).

In the present investigation, at the onset, pathogenicity test of F.graminearum was carried out on ten soybean cultivars. Among the tested cultivars, Soymax was found to be highly susceptible while cv. UPSM-19 was found to be resistant. Phytotoxic effect of metabolic byproducts in the culture filtrate of F.graminearum on cvs. Soymax and JS-2 could be noticed. As soybean cultivars showed differential resistance to F.graminearum, it was considered worthwhile to detect the level of phytoalexin (glyceollin) accumulation in those cultivars in response to fungal infection which appeared to be the most promising line of approach in the study of disease resistance mechanism.

The majority of studies of resistance and susceptibility in soybeans to Phytophthora megasperma f.sp. glycinea have dealt with infections of the roots and hypocotyl region. This system has attracted attention also because it has provided a model system for study of the production and role of a phytoalexin (glyceollin) in a presusmed gene-for-gene system (Yoshikawa et.al., 1978; Hahn et.al., 1985; Bhattacharyya and Ward, 1987). In another study it has been confirmed that resistant and susceptible responses can also be differentiated in leaves. However, responses of leaves were found to be greatly influenced by age and stage of development. Although Rps-gene-mediated cultivar resistance

was expressed, this was effective for a relatively short period in the development of leaves. All leaves became resistant as they aged, and very young unfolding trifoliate leaves were susceptible even in resistant cultivars. Glyceollin accumulation in leaves was associated with the development of both Rps-gene-mediated and age related resistance (Ward, 1989).

Evidence that glyceollin, the pterocarpon phytoalexin from soybeans, occurs in several isomeric forms was provided by Keen et.al. (1971) for preparations obtained from soybean hypocotyls inoculated with the pathogen Phytophthora megasperma f.sp. glycinea. Subsequently the structures of four isomers (glyceollin-I-IV) were established by Burden and Bailey (1975) and Lyne et.al. (1976). Of these, glyceollin-IV has been isolated in minor amounts only, from cotyledons treated with $CuCl_2$ and no evidence that it may play a significant role in the resistance response has been provided. Glyceollins-I-III are all inhibitory to mycelial growth and zoospore germination of P.megasperma f.sp. glycinea and have been demonstrated to accumulate in significant amount in soybean tissues (Lyne et.al., 1976 ; Kaplan et.al., 1980; Moesta and Grisebach, 1981 ; Ingham, 1982; Hahn et.al., 1985 and Bhattacharyya and Ward, 1985). The proportions of the three isomers reported by different authors have varied considerably . Glyceollin-I predominated in roots treated with $CuSO_4$ (Morandi et.al., 1984) or inoculated with P.megasperma f.sp. glycinea (Hahn et.al., 1985) or hypocotyl treated with $AgNO_3$ (Stossel and Magnalato, 1983). In cotyledons, glyceollin -I and III have been reported to occur in roughly equal amounts following treatment with $CuCl_2$ (Lyne et.al., 1976). In leaves infiltrated with bacteria or sodium iodoacetate glyceollin-III was reported to be the main constituent. Although these reports suggest that there are major differences in the ability of soybean organs to synthesize the

three isomers, the possibility remains that the differences result from a combination of different experimental conditions and the use of different eliciting agents. None of these studies has examined the relationship of the accumulation of the three isomers to resistance and susceptibility in the host-pathogen interaction.

The results of present investigation revealed that resistant cultivars (cvs. UPSM-19, EC-55865 and R-184) contained more glyceollin than the susceptible cultivars (Soymax, JS-2 and KU-254). Present results substantiate the findings of previous workers who have also presented conclusive evidence that resistant cultivars of different host species produces more phytoalexins in response to fungal infection that the susceptible ones (Gray and Klarman, 1967; Klarman, 1968; Keen et.al., 1971; Keen and Horsch, 1972; Tjamos and Smith, 1974; Johnson et.al., 1976; Keen and Littlefield, 1979 ; Fraile et.al., 1980; Obi et.al., 1980; Purkayastha et.al., 1983; Rouxel et.al., 1989).

According to Keen(1971) the speed with which a plant produces phytoalexin may be of utmost importance if fungal colonization is to be halted at an early stage. The fungitoxic phytoalexin (glyceollin-I) accumulated in soybean hypocotyls of Harasoy-63(resistant cv.), more rapidly (20-50 times faster) than in Harasoy (Susceptible cv.) after challenge inoculation with Phytophthora megasperma var. sojae. Another interesting observation was made by Tjamos and Smith(1974) who showed that the rate of phytoalexin (rishitin) accumulation was relatively low in both Verticillium infected resistant and susceptible tomato cultivars during the first 2 days after inoculation but increased sharply after third day in resistant cultivar. In the present study glyceollin accumulation increased sharply after 48h of inoculation with F.graminearum in the resistant cultivars.

Partridge and Keen (1976) stated that Kievitone (Phytoalexin) was produced more rapidly in resistant cowpea plants than in near isogenic susceptible plants. They suggested that the rate of production of Kievitone was the basis for resistance of cowpea plants to Phytophthora vignae. Significant difference in phytoalexin (B-vulgarin) level in Cercospora beticola infected resistant and susceptible cultivars of sugar beet was also noted by Johnson et.al. (1976). When bean plants were inoculated with Botrytis cinerea, the amount of phytoalexin (Phaseollin) accumulation was always higher in partially resistant cultivar than in the susceptible one. Nemistothy and Guest (1990) have shown that a resistant and susceptible cultivar of tobacco differ in the magnitude and timing of phytoalexin, phenylalanine ammonia lyase activity and ethylene responses following infection by Phytophthora nicotine var. nicotianae. Each of these responses began earlier and proceeded at a faster rate in the resistant cultivar.

The aforesaid statements indicate that phytoalexin has a role in disease resistance in plants. The differential response of susceptible and resistant soybean cultivars to F.graminearum could probably be attributed to their capacity to accumulate more glyceollin. It seemed highly interesting, therefore, to induce changes in disease reactions by the application of certain chemicals capable of inducing phytoalexin production (Bell, 1967; Cheema and Haard, 1978 ; Purkayastha et.al., 1983; Sinha, 1984; Chakraborty and Purkayastha, 1987 ; Rouxel et.al. 1989) and to find out whether these changes could be correlated with enhanced glyceollin production.

A series of experiments have been performed using 12 chemicals belonging to 3 separate groups viz. 6 metal salts (Barium chloride, Ferric chloride, Cupric chloride, Cadmium

chloride, Mercuric chloride and Silver nitrate), 2 reducing agents (Sodium selenite and Sodium sulphite) and 4 metabolic inhibitors (Sodium fluoride, Sodium molybdate, Sodium azide and Sodium malonate) with a view to alter disease reaction in susceptible soybean plants (cv. Soymax). Apart from this, the effect of different concentrations of the above chemicals on spore germination of F.graminearum as well as effect of some selected chemicals on the mycelial growth of F.graminearum were also studied. Among the tested chemicals sodium azide, sodium selenite and sodium sulphite were found to be highly effective in reducing disease intensity. Moderate effect of sodium molybdate, cupric chloride and ferric chloride were also noticed on the alteration of disease reaction. However, no correlation could be drawn between germination percentage and rate of growth of the pathogen in vitro and in vivo experiments using those chemicals.

The use of nickel chloride as foliar spray to tea plants for the control of blister blight caused by Exobasidium vexans was demonstrated by Venkataram (1961). Host sensitization as a factor in induction of resistance in rice (cv. Dharial) against Drechslera oryzae by seed treatment with phytoalexin inducers have been reported by Sinha and Hait (1982). Among the 8 chemicals tested, all produced appreciable effects ; sodium selenite, lithium sulphate, cycloheximide, cysteine and thioglycolic acid caused marked reduction in symptoms in rice seedlings when challenge inoculated at the age of 3-4 weeks. With sodium selenite and thioglycolic acid induced effect persisted upto 8 weeks after sowing. Seed treatment of wheat with dilute concentrations of nickel chloride and barium sulphate significantly induced resistance to Drechslera sorokiniana (Chakraborty and Sinha, 1984). Protection of wheat seedlings against Helminthosporium sativum by seed treatment with chemicals (known to induce phytoalexin production in other plants) was also recorded by Hait and Sinha (1986). Sodium azide was also found to be most effective

in reducing charcoal rot disease of soybean caused by Macrophomina phaseolina (Chakraborty and Purkayastha, 1987).

All the above reports as well as the results of the present study, therefore, point to the ability of certain chemicals to induce protection, which in some cases, could be due to the direct fungitoxic effects of these chemicals on the pathogen, or it could be due to the activation of certain metabolic processes within the host cells leading to the production of greater amounts of antifungal compounds (phytoalexins). Reduction in disease symptoms after the treatment either with sodium azide or sodium selenite may be due to higher accumulation of glyceollin in treated plants (vide part VI). Results revealed that treatment with sodium azide induced a high level of glyceollin (545 µg/g fresh wt.) after challenge inoculation with the pathogen in comparison to the untreated inoculated plants (256 µg/g fresh wt.).

Several previous workers have also reported that certain chemicals are capable of inducing the production of antifungal compounds. It was reported by Keen et.al., (1981) that sodium iodoacetate acts as an abiotic elicitor of glyceollin in primary leaves of cv. Harosoy soybeans and that it is associated with the resistance expression. Copper sulphate, sodium nitrate and chloram-phenicol were found to be effective in inducing capsidiol production in Capsicum annum (Watson and Brooks, 1984). Accumulation of increased levels of glyceollin following treatment with sodium azide was reported by Chakraborty and Purkayastha (1987). Rouxel et.al., (1989) reported the accumulation of phytoalexin in $CuCl_2$ and $AgNO_3$ treated leaves of Brassica juncea.

The genetic information contained in nucleic acid is expressed in the cell via protein synthesis. Several proteins

function as enzymes in the metabolic pathways which synthesize or break down cellular components. When plants are infected by pathogens, the proteins in the penetrated plant cells are changed chemically and physically. Some enzymatic proteins are also produced in penetrated cells by pathogens themselves. Thus, qualitative and quantitative changes of proteins are related to both plants and pathogens (Uritani, 1971).

In the present investigation, it was noticed that protein content increased in the infected roots of susceptible cultivars (JS-2, PK-327 and Soymax). Increased protein level was also detected after infection of susceptible bean leaves by Uromyces phaseoli. Similar findings were reported by other workers (Tomiyama, 1966; Daly-1972; Ouchi et.al., 1974). They suggested that protein configuration changes in the host may induce the hosts' accessibility to the pathogen which is related to susceptibility. The greated accumulation of protein in susceptible host after infection could be attributed to the total proteins of both host and parasite. However, it is difficult to separate the relative contribution of host and parasite to the total protein content. Sometimes protein content of the host after inoculation remains unchanged but their isozyme pattern may change. Changes in protein patterns in barley leaves after inoculation with Erysiphe graminis f.sp. hordei could be detected by polyacrylamide gel electrophoresic (PAGE) study but there was no change in protein content of mildew infected barley leaves in comparison with healthy leaves (Johnson et.al., 1976). In the present study, protein patterns of healthy and F.graminearum infected soybean roots of susceptible (Soymax and JS-2) and resistant (UPSM-19 & PK-327) cultivars of soybean were evaluated by polyacrylamide gel electrophoresis. In healthy roots of cvs.Soymax and JS-2, 14 and 15 protein bands were detected while 16 and 18 protein bands in infected roots of cvs.Soymax and JS-2 respectively could be detected. On the other hand, both healthy and infected roots of cvs.UPSM-19 and PK-327 exhibited 15 &

17 protein bands. Mycelial protein of two isolates of F.graminearum, Fg1 and Fg2 exhibited 18 and 15 protein bands respectively. Changes in protein pattern of susceptible cultivars (Soymax and JS-2) after inoculation with F.graminearum as determined by PAGE cannot be compared with the protein bands of F.graminearum because proteins of dissimilar molecular weight may be observed in the same RF by this method. The present study atleast indicates the differences in protein patterns of isolate of pathogen as well as resistant and susceptible soybean cultivars before and after inoculation with F. graminearum.

Root protein of susceptible soybean cultivars inoculated with F.graminearum exhibited 2-3 additional protein bands, in relation to their healthy control. This is in conformity with the work of Uritani and Stahmann (1961) who reported that sweet potato infected by Ceratocystis fimbriata developed new proteins both in resistant and susceptible varieties. Five new isozyme bands such as acetyl esterase, acid phosphatase, malate dehydrogenase, succinate dehydrogenase and peroxidase were detected in the susceptible line of barley after inoculation with Erysiphe graminis f.sp. hordei (Sako and Stahmann - 1972). Differential changes in soluble leaf protein of tomato after inoculation with virulent and avirulent races of Cladosporium fulvum were determined by Dewit and Bakker (1980).

The presence of cross-reactive antigen (CRA) between plant host and their parasites and the concept that these antigens might be involved in determining the degree of compatibility in such interactions have been reviewed by several authors (De Vay et.al., 1972 ; DeVay and Adler, 1976; Kalyansundaram, 1978 ; Chakraborty, 1988 ; Purkayastha, 1989 ; Purkayastha et.al., 1991). In the present study root antigens of 3 cultivars each of resistant (UPSM-19, EC-55865 and R-184) and susceptible (Soymax, JS-2 and KU-254) soybean plants and

two isolates of F.graminearum (Fg1 and Fg2) were cross reacted separately with antisera of cvs. Soymax and UPSM-19. Reciprocal cross reaction was also carried out with antisera of isolate Fg1 and antigens of both susceptible and resistant varieties. Antigens from two non pathogens of soybean, viz. Glomerella cingulata and Pestalotiopsis theae were also considered for serological comparisons. It is significant to note that in immunodiffussion test susceptible cultivars (Soymax, JS-2 and KU-254) shared the common antigens with both the isolates of F.graminearum (Fg1 and Fg2) tested. However, antigenic disparity was noticed in cross-reaction and reciprocal cross-reaction with antigens and antisera of resistant cultivars and the isolates of the pathogen. Weak precipitin band was observed in cross reactions of antiserum of resistant cv. UPSM-19 with antigens of isolate Fg1 while no such precipitation could be detected with antigens of isolate Fg2 and antiserum of cv. UPSM-19. In reciprocal cross reaction with antiserum of F.graminearum (isolate Fg1) and antigens of resistant cultivars, precipitation bands could not be detected in cvs. UPSM-19 and EC-55865 but cv. R-184 developed weak precipitin band. Antisera of soybean cultivars and antigens of non pathogens (G.cingulata and P.theae) failed to develop any precipitin band.

Several earlier studies have also implicated the importance of common antigens in host-pathogen compatibility. Presence of CRA has been demonstrated in various host-parasite combinations such as flax and Melampsora lini (Doubly et.al., 1960), cotton and Verticillium albo-atrum (Charudattan and DeVay, 1972), cotton and Fusarium oxysporum f.sp. vasinfectum (Charudattan & DeVay, 1970 ; Kalyansundaram et.al., 1975), sweet potato and Ceratocystis fimbriata (DeVay et.al., 1967), potato and Phytophthora infestans (Palmerley and Callow, 1978, Alba and DeVay, 1985), soybean and Macrophomina phaseolina (Chakraborty & Purkayastha, 1983), soybean and Colletotrichum

dematium var. truncata (Purkayastha and Banerjee, 1986), soybean and Myrothecium roridum (Ghosh and Purkayastha, 1990), jute and Colletotrichum corchori (Bhattacharyya and Purkayastha, 1985) Coffee and Hemilea vastatrix (Alba et.al., 1983), ground nut and Macrophomina Phaseolina (Purkayastha and Ghosal, 1987), Carrot and Pythium violae and Pythium sulcatum (Lyons and White, 1992) ground nut and Sclerotium rolfsii (Purkayastha & Pradhan, 1994), tea and Bipolaris carbonum (Chakraborty and Saha, 1994). Present result also support the findings of previous workers.

Conventional serological techniques have sometimes failed to detect cross reactive antigens in some host-parasite interactions such as wheat and Puccinia graminis var. tritici (Johnson, 1962), alfalfa and Corynebacterium insidiosum (Caroll et.al., 1972). The preparation and treatment of antigens are most important because most antigens are labile and easily denatured. The selection of test animal as well as the amount of antigen for immunization purpose are also important since too much material may reduce antibody formation. Moreover, a number of factors such as age of plant tissue, culture of microbes and methods of extraction of antigen have profound influence on the yield of antigenic substance and this may account for the failure to detect common antigens as suggested by DeVay and Adler (1976).

Alba et.al. (1983) showed that urediniospores of Hemilea vastatrix shared common antigenic determinant with coffee plant, in contrast to their previous conclusions made with the same host-parasite system (Alba et.al., 1973). This agreement was attributed to the low concentrations of antigenic preparations used in the earlier investigation.

Immunoelectrophoretic analysis with antigen and antisera preparation from soybean roots and F.graminearum also

substantiated the results of immunodiffusion tests. When antiserum of F.graminearum reacted with its own antigen, 6 precipitation arcs were formed of which 3 were common with cv. Soymax and 2 were common with cv. JS-2, KU-254 and R-184. No such precipitin arc was formed between antisera of F.graminearum and antigen of cv. UPSM-19 and EC-55865. However, in reciprocal cross reactions with antiserum of cv. UPSM-19 and antigen of isolate Fg1 one common precipitin arc was formed. No common antigenic relationship between host (Glycine max) and non pathogens was noticed. The present results are in agreement with the findings of Chakraborty and Purkayastha (1983), Purkayastha and Ghosal (1987). Purkayastha and his co-workers have examined various host-pathogen/non-pathogen combinations including cultivars of soybean, rice, jute, pegion pea, bean, ground nut to find out their serological relationship with some fungal pathogens as well as non pathogens following agar-gel double diffusion and immuno-electrophoretic tests. More than 50% combinations exhibited cross-reactive antigen (CRA) between host and pathogens. No such CRA could be detected between resistant host and their respective pathogens. However, at a very low concentration CRA was detected between resistant host (soybean) and Colletotrichum dematium following enzyme linked immunosorbent assay (Purkayastha & Banerjee, 1990).

Enzyme linked immunosorbent assay has proved to be one of the most sensitive serological technique in detecting CRA at very low concentration (Alba & DeVay, 1985 ; Mohan, 1988 ; Chakraborty & Saha, 1994). In the present study the presence of CRA among F.graminearum and soybean cultivars was evident in indirect ELISA using antigen and antisera of host and parasite and goat antirabbit IgG conjugate. Indirect ELISA readily detected CRA in semipurified mycelial preparation at concentrations ranging from 5-25 /ug protein/ml with antiserum dilution of 1/125 and 1/250. Antigenic preparation (25 /ug/ml)

from F.graminearum (isolate Fg1) exhibited higher absorbance value at 405 nm with antiserum of susceptible soybean cultivar (Soymax) than the reaction with antiserum of resistant cultivar (UPSM-19). Higher absorbance value was also noticed in the reciprocal cross-reactions involving antiserum of the pathogen (isolate Fg1) and antigenic preparation of cv. Soymax (25 /ug/ml) than the antigenic preparation of cv. UPSM-19. Since the indirect ELISA tests were made under the same condition and with atleast three repetitions of each combination it appears that these observed antigenic disparities as reflected in their OD. values developed as a result of antigen antibody reaction have some significance in the basic compatibility of host (Glycine max) and pathogen (F.graminearum) .

CRA was also detected in crude preparations and purified preparations from mycelia of Phytophthora infestans (races 4 and 1,2,3,4,7) with antisera of potato cvs. King Edward and Pentland Dell in concentrations lower than 50 /ug protein/ml (Alba & DeVay, 1985) using indirect ELISA. Antiserum raised against Phytophthora fragariae detected homologous soluble antigen at protein concentrations as low as 2 /ug/ml (Mohan, 1988). Indirect ELISA could also readily detect CRA in semipurified mycelial preparation of B.carbonum at concentrations ranging from 5-25 /ug/ml with antiserum dilution 1:125.In cross reaction with antiserum of susceptible tea variety (TV-18) with antigenic preparation from B.carbonum (isolate BC-1) higher absorbance value was detected than the reaction with resistant variety (TV-26) of tea (Chakraborty & Saha, 1994). Based on these findings it can be assumed that indirect ELISA may serve as an important technique to detect cross-reactive antigens, to determine their properties and to investigate their properties and to investigate their possible role in host-parasite interactions, even in those interations where conventional serological

techniques have failed to detect (Johnson, 1962 ; Caroll et.al., 1972).

The involvement of CRA in host-parasite compatibility has been discussed by several authors (DeVay et.al., 1967, DeVay and Adler, 1976 ; Chakraborty, 1988; Lyons and White, 1992) and is strongly supported by results of the present investigation. These results are also in-conformity with those of previous workers (Palmerly and Callow, 1978, Alba et.al., 1983; Alba and DeVay, 1985) who suggested that not all CRA contribute towards host-parasite compatibility but rather that only certain key CRA are important .

Changes in antigenic patterns were also detected in susceptible soybean cultivar (Soymax) after chemical induction of resistance. In this case, one common antigenic substance was missing from the uninoculated sodium selenite treated roots while two common antigenic substances were missing from the uninoculated sodium azide treated roots as evident in the immunoelectrophoretic tests. These change increased the antigenic disparity between treated roots and the parasite, and consequently the resistance of soybean plants(cv. Soymax) to F.graminearum increased to a considerable extent. In this situation sodium azide or sodium selenite appeared to act as a "Conditioner" of the plant cells which responded to infection by accumulating greater amount of phytoalexin (glyceollin).

An antigenic disparity in the susceptible soybean cultivar (Soymax) after induction of resistance by sodium azide treatment was also detected by Chakraborty & Purkayastha (1987), who discussed the changes in antigenic pattern and their involvement in induced resistance of soybean to charcoal rot disease caused by Macrophomina phaseolina. In another

immunoserological studies Ghosal and Purkayastha (1987) demonstrated the alteration in antigenic pattern in susceptible rice cultivar (Joya) after treatment with gibberellic acid which increased resistance to seed rot disease caused by Sarocladium oryzae. Similarly cloxacillin induced resistance of soybean against Colletotrichum dematium var. truncata and altered the antigenic pattern (Purkayastha and Banerjee, 1990). These findings suggests that resistance could be induced in susceptible plants by increasing antigenic disparity by suitable treatment.

In the present investigation using antibodies indirectly labelled with fluorescein isothiocyanate (FITC) the location of CRA in cross sections of soybean roots of resistant(UPSM-19) and susceptible (Soymax) cultivars and fungal cells (F.graminearum). Treatment of mycelia and conidia of F.graminearum with antiserum to roots of cv. Soymax and using indirect staining with FITC indicated that CRA was mainly present in young hyphal tips and in patch like areas on conidia. The results are also in conformity with the work of DeVay et.al. (1981a) involving treatment of conidia and mycelia of Fusarium oxysporum f.sp. vasinfectum with antisera to cotton.

Cross sections of soybean roots (cv.Soymax) treated with antisera of F.graminearum and then reacted with FITC conjugate developed bright fluorescence which was concentrated mainly around epidermal cells, the endodermis and xylem elements and was distributed throughout the cortical tissues; cell walls appeared to be the main cellular location of CRA. In roots of cv. UPSM-19 fluorescence was noticed only in the epidermal cells and cortex tissues.

The tissue and cellular location of major CRA shared by cotton and Fusarium oxysporum f.sp. vasinfectum was

determined by DeVay et.al. (1981a). Cross sections of young cotton roots with antiserum to F.oxysporum f.sp. vasinfectum and followed by an antirabbit globulin specific goat antiserum FITC conjugate exhibited strong fluorescence at the epidermis and xylem tissues indicating a general distribution of CRA determinants in roots. FITC labelled antibodies for races of P.infestans were also used to detect CRA in potato leaf sections (DeVay et.al., 1981b). In cross section of tea leaves (TV-18), cellular location of CRA shared by B.carbonum was evident mainly around epidermal cells (Chakraborty & Saha, 1994).

In the present host parasite system, the fungi F.graminearum infect and colonise the root tissues of both the resistant and susceptible cultivars but disease resistance of the host (Glycine max) is not expressed until the parasite invades the vascular tissues. Rather than search for a role for CRA in interactions of host and parasite within the realm of gene-for-gene relationships, several studies have provided evidence that the time of CRA involvement in basic compatibility phenomena is probably during the early cellular interactions of host and parasite when an exchange of metabolites may occur (Andrews, 1975) and induce a compatible or an incompatible interaction. A dynamic role is visualised for CRA after passive recognition events have occurred, which also may involve CRA and a host lectin (Dazzo and Brill, 1977), but prior to specific gene effects. Subsequent studies of Dazzo and Brill (1979) confirmed by Bhagwat and Thomas (1980), described a recognition phenomenon dependent primarily on exopolysaccharide of Rhizobium trifoli and a polysaccharide on the surface of root hairs of Trifolium repens having similar cross-reactive antigenic determinants and reactivity with trifoliin, a lectin produced by the clover roots. However, following recognition, intracellular compatibility of nodulating Rhizobium species with host roots may also involve

CRA common to legume hosts and the bacteria. In a comparison of 8 legumes and 8 non leguminous plant species, Charudattan and Hubbell (1973) found that all 3 species of Rhizobium tested shared CRA with the legumes but not with the other plant species.

DeVay and Adler (1976) suggested that common antigens are constitutive components of the cells of both host and parasite. Selection of parasite by host or vice versa may be controlled to some degree by fortuitous homologies of their genomes. Where there exist similarities for synthesis of cortical cell component, a compatible relationship would result, where as lack of homology would either repress metabolic processes or trigger the formation of metabolites which are toxic to cells of both host and parasite. If common antigens or cross reactive antigens (CRA) have a functional role other than in recognition phenomena, it probably will be found in the infection process and be subject to the over-riding effects of substances such as phytoalexins or other inhibitory substance already present in host tissues or induced by parasitic micro-organisms (DeVay et.al., 1981a). Physiological function for CRA in host-parasite interaction is not clear. However, it can be stated that CRA may form a continuum between cells of host and parasite that favours the progressive growth and establishment of the parasite.

SUMMARY

1. A brief review of literature pertaining to the present lines of investigation has been presented which mainly deals with (a) evaluation of the role of phytoalexins in plant disease resistance, (b) plant disease alteration by chemical treatment and (c) serological relationship between host and parasite.

2. Experimental procedure followed and the materials used in this investigation have been described in detail.

3. Pathogenicity of F.graminearum was tested on ten cultivars of soybean (Soymax, KU-254, EC-2575, PK-327, JS-2, EC-95287, Pusa-16, EC-55865, UPSM-19 and R-184). Of the ten cultivars, cvs. Soymax and JS-2 were found to be highly susceptible while UPSM-19 and Pusa-16 were resistant to F. graminearum.

4. The optimum pH, temperature and incubation time required for maximum growth of F.graminearum were determined. The fungus grew at a wide range of pH(5.5-7.0) and temperature (20°C-40°C), the optimum pH and temperature being 6.5 and 30°C respectively. Maximum growth was observed after 15 days of incubation and then rate of growth declined.

5. Phytotoxic effect of metabolic by products in the culture filtrate of F.graminearum on soybean plants (cvs. Soymax and JS-2) was determined. Toxic principle was found to be partially thermolabile and non dialysable.

6. Glyceollin accumulation in the roots of six soybean cultivars (UPSM-19, JS-2, Soymax, KU-254, R-184 and EC-55865) after 24, 48, 72 and 96h of inoculation with F.graminearum were detected. Antifungal activity of glyceollin was determined by "on the Chromatogram inhibition assay" using Bipolaris carbonum as the test organism. Results of both

radial mycelial growth and spore germination tests also confirmed the antifungal nature of glyceollin. The relative antifungal activity of glyceollin was also compared against F.graminearum, F.oxysporum, f.solani, Dreschlera oryzae, Glomerella cingulata, Pestalotiopsis theae and Bipolaris carbonum. UV-spectrophotometric analysis of glyceollin revealed absorptioin peak at 286 nm which was identical with authentic glyceollin.

7. Highest accumulation of glyceollin after 48h of inoculation was noticed. Resistant cultivars (UPSM-19, EC-55865 and R-184) contained more glyceollin (705-896 /ug/g fresh weight of roots) than susceptible cvs. Soymax, JS-2 and KU-254 (285-652 /ug/g fresh weight of roots).

8. A series of experiments were performed in order to study the effects of nine chemicals of three separate groups viz. (a) metal salts (cupric chloride, ferric chloride, mercuric chloride and silver nitrate); (b) reducing agents (sodium selenite and sodium sulphite) and (c) metabolic inhibitors (sodium azide, sodium malonate and sodium molybdate) on disease development of susceptible cultivar (Soymax). Each chemical was also tested for its fungitoxic effect if any on F.graminearum. Sodium selenite and chlorides of mercury, copper and iron totally inhibited spore germination. Cadmium chloride, silver nitrate, sodium sulphite, sodium azide, sodium malonate, sodium fluoride and sodium molybdate also markedly inhibited the spore germination and germ tube growth. Pronounced protective effects were recorded with three chemicals (viz. sodium azide, sodium selenite and sodium sulphite) as evident from the reductions in disease index after 30 days of inoculation with F.graminearum.

9. Susceptible plants (cv. Soymax) treated with sodium azide (10^{-4}M) followed by inoculation with F.graminearum produced high level of glyceollin (545 /ug/g fresh wt. of roots) in relatioin to untreated inoculated plants (256 /ug/g fresh wt. of roots). Production of glyceollin was maximum in sodium azide treated plants inoculated with F.graminearum than sodium selenite treated inoculated plants.

10. Total soluble protein content of healthy and F.graminearum inoculated roots of five cultivars were estimated. Protein contents increased in the susceptible cultivars (JS-2, PK-327 and Soymax) more than resistant cultivars (UPSM-19 and Pusa-16) after 48h of inoculation with F.graminearum.

11. Protein patterns of healthy and infected (with F.graminearum) soybean roots of cvs. Soymax, JS-2, PK-327 and UPSM-19 as well as the mycelia of two isolates of F.graminearum have been evaluated by polyacrylamide gel electrophoresis. Mycelia of two isolates of F.graminearum Fg1 and Fg2 were exhibited 18 and 15 protein bands respectively. Infected roots of cvs. Soymax and JS-2 showed 16 and 18 protein bands while healthy roots exhibited 14 and 15 bands respectively.

12. Plant antigens were prepared from soybean roots of six cultivars (Soymax, JS-2, KU-254, UPSM-19, EC-55865 and R-184). Fungal antigens were prepared from two isolates of F.graminearum (Fg1 and Fg2) and two non pathogens of soybean (viz. G.cingulata, and P.theae). Rabbit antisera were raised against cvs. Soymax (SA), UPSM-19(UA) and isolate Fg1 of F.graminearum (F_1A).

13. In agar gel double diffusion test strong precipitin reactions occurred when antiserum of F.graminearum was

reaction against its homologous antigen (isolate Fg1) but weak precipitation reaction was observed with antigen preparation of isolate Fg2 of F.graminearum.

14. Cross reaction between antiserum of F.graminearum and antigens of susceptible cultivars (Sa, Ja & Ka) developed common precipitin band but no precipitin band was observed with the antigen of resistant cultivar (UPSM-19).

15. Reciprocal cross reaction between antiserum of cv. UPSM-19 (UA) and antigens of F.graminearum isolate Fg1 developed weak precipitin band but no precipitin band could be detected with isolate Fg2. Whereas common precipitin bands were observed in the reciprocal cross reaction between antiserum of cv. Soymax (SA) and antigens of isolates of F.graminearum. Absence of cross reactive antigens were also noted between antisera of soybean cultivars (SA and UA) and antigens of non pathogens of soybean (G. cingulata and P.theae).

16. Effectiveness of each antigen extract of cvs. Soymax(Sa), UPSM-19 (UA) and isolate Fg1 (F_1a) in raising antibodies SA, UA, and F_1A respectively was checked by homologous cross reactions in immunoelectrophoretic test. The homologous patterns formed by cvs. Soymax, UPSM-19 and F. graminearum contained 6, 5 and 6 precipitin lines respectively.

17. In cross reaction with antiserum of Soymax and antigen preparations of roots of five other different cultivars JS-2 gave rise to 5 precipitin arcs and cvs. KU-254 and R-184 exhibited 4 precipitin arcs while cvs. EC-55865 and UPSM-19 showed 3 and 2 arcs respectively. Both the isolates of F.graminearum were antigenically related to cv. Soymax. In

this case, isolate Fg1 and Fg2 shared 3 and 2 common precipitin arcs respectively.

18. In cross reactions with antiserum of UPSM-19 and antigens of soybean cultivars, 4 precipitin arcs each were formed with cvs. EC-55865, JS-2, R-184 and KU-254 while Soymax exhibited 3 precipitin arcs. Antigen prepared from isolate Fg1 developed only one precipitin arc but no such precipitation was observed with isolate Fg2 in cross reaction with antiserum of UPSM-19.

19. Reciprocal cross reactions between antisera of F.graminearum and antigens of cv. Soymax formed 3 precipitin lines while JS-2, KU-254 and R-184 formed 2 precipitin lines only, but antigens of cvs. UPSM-19 and EC-55865 failed to develop any precipitin line.

20. Cross reactive antigens (CRA) in semipurified mycelial preparation from F.graminearum (isolate Fg1) at concentrations ranging from 5-25 µg/ml with antiserum dilutions (1:125 and 1:250) have been detected by indirect immunosorbent assay (A_{405}). CRA were also detected between F.graminearum and cv. Soymax. Antigenic preparations from F.graminearum exhibited higher absorbance value when reacted with antiserum of soymax than when reacted with antiserum of resistant soybean cultivar UPSM-19. There was no cross reactivity with P.theae and G.cingulata (non pathogens).

21. When antigens obtained from F.graminearum (Fg1) and susceptible cultivar (Soymax) before and after treatment with sodium selenite and sodium azide (10^{-4}M) were cross reacted separately with the antisera of Soymax roots developed very faint diffused band in agar-gel double diffusion test.

22. In immunoelectrophoretic tests, antigens of untreated healthy roots of Soymax exhibited 6 precipitin arcs

in homologous reactions while sodium selenite and sodium azide treated roots of Soymax developed 4 and 3 precipitin lines respectively with antiserum of Soymax. Reciprocal cross reaction between antiserum of F.graminearum and antigens of sodium selenite and sodium azide treated roots of Soymax developed 2 and 1 precipitin arc respectively, while untreated Soymax developed 3 precipitin lines.

23. Antibodies indirectly labelled with fluorescein isothiocyanate (FITC) were used to determine the location of CRA in sections of roots of Soymax and fungal cells.

24. Mycelia and conidia of F.graminearum were not autofluorescent nor did they fluores when treated with normal serum followed by FITC. Treatment of mycelia and conidia of F.graminearum with homologous antisera and FITC showed a general fluorescence that was more intense on young hyphae and patch like areas on the conidia.

25. When fungal cells were reacted with antiserum to roots of cv.Soymax and treated with FITC, fluorescence was apparent on young hyphae.

26. Root sections did not exhibited any natural autofluorescence. Sections treated first with normal serum then by FITC also did not exhibited any fluorescence.

27. Root sections of cv. UPSM-19 treated with homologous antiserum and then reacted with FITC developed bright fluorescence which was concentrated mainly on epidermal cells and was distributed throughout the cortical tissue.

28. Strong reaction was evident with root sections of cv. Soymax and antiserum of F. graminearum CRA was

concentrated mainly around xylem elements, the endodermis, epidermal cells and distributed throughout the cortex tissue; cell walls appeared to be the main cellular location of CRA.

29. The implications of the results embodied in Part I-XI have also been discussed.

REFERENCES

Abd-El-Rehim, M.A. and Hassan, M. (1970). A serodiagnostic method for the differentiation between resistant and susceptible Egyptian cotton varieties to infection with Fusarium oxysporum. Phytopath.Z. 68 : 180.

Abd-El-Rehim, M.A., Ibrahim, I.A. and El-Namlah, E.M.(1971a). Serodiagnostic studies on Citrus spp. with respect to their resistance to Phytophthora citrophthora Alex. J.Agr. Res. 19 : 145.

Abd-El-Rehim, M.A., Ibrahim, I.A. ; Michail, S.H. and Fadel, F.M.(1971b). Serological and immunoelectrophoretical studies on resistance and susceptible watermelon varieties to Fusarium semitectum Berk. Ann. Rev. Phytopath.Z. 71 :49

Abbott, L.K. (1973). Taxonomy and host specificity of Ophiobolus graminies Sacc; an application of electrophoretic and serological techniques, Ph.D. Thesis, Monash Univ., Clayton, Victoria, Australia, p.200.

Abrarov, A.H. ; Ibragimov, S.I. and Kariev, U. (1973). Effect of kinetin and indole acetic acid on cotton plants infected with Verticillium wilt. Fisiol. Rast., 20 :949.

Agarwal, D.K. and Sarbhoy, A.K. (1978). Physiological Studies on four species of Fusarium pathogenic to soybean. Indian Phytopathol, 31 : 24.

Alba, A.P.C. and DeVay, J.E. (1985). Detection of cross reactive antigens between Phytophthora infestans (mont). de Bary and Solanum species by indirect enzyme-linked immunosorbent assay, Phytopath Z., 112 : 97.

Alba, A.P.C. ; Guzzo, S.D. ; Mahlow, M.F.P. and Moraes, W.B.C (1983). Common antigens in extracts of Hemileia vastarix Berk, et. Br. urediniospores and Coffea arabica L. leaves and roots, Fitopatho 8 :473.

Anomymous (1980). The United States Pharmaxopeia XX ; United States Pharmacopeial Convention Inc., 12601- Twinbrook Parkway, Rockville, Md. 20852 ; p.980.

Avazkhodjaev, M.H. ; Zeltser, S.S ; Nuritdinova, X.V. and Dani, Raviprakash. G.(1995). Phytoalexin as a factor in the wilt Resistance of Cotton. In Handbook of Phytoalexin Metabolism and Action. (Ed. by M. Daniel and R. P. Purkayastha) Marcel Dekker, Inc. New York. pp.624.

Bailey, J.A. and Deverall, B.J. (1983). The dynamics of host defence, Academic Press, Sydney, p.233.

Bell, A.A. (1967) Formation of gossypol in infected and chemically irritated tissues of Gossypium species. Phytopathology, 57 : 759.

Bhagwat, A.A. and Thomas, J.(1980). Dual binding sites for peanut lectin on Rhizobia. Journal of General Microbiology, 117 : 119.

Bhattacharyya, B. and Purkayastha, R.P.(1985). Occurence of common antigens in jute and Colletotrichum corchori, Current Science, 54(5), 251.

Bhattacharyya, M.K. and Ward, E.W.B.(1985). Differential sensitivity of Phytophthora megasperma f.sp.glycinea isolates to glyceollin isomers. Physiological Plant Pathology, 27 : 299 .

Bhattacharyya, M.K. and Ward, E.W.B. (1986). Resistance, susceptibility and accumulation of glyceollins I-III in soybean organs inoculated with Phytophthora megasperma f.sp. glycinea. Physiological and Molecular Plant Pathology. 29 :227.

Bhattacharyya, M.K. and Ward, E.W.B.(1987). Biosynthesis and metabolism of glyceollin I in soybean hypocotyls following wounding or inoculation with Phytophthora megasperma f.sp. glycinea. Physiological and Molecular Plant Pathology, 31 : 387.

Bier, R.C. and Oertelli, E.H. (1983). Psoralene and other linear furocourmarins and phytoalexins in celery. Phytochemistry, 22 : 25295.

Bojarezuk, J. and Ruszkowski, M. (1972). The effect chlorocholine chloride (C.C.C) on infection of winter wheat and rye by eyespot (Cercosporella herpotrichoides Fron.) Biubtyn Instytutu Hodowlie Aklinatyzacji Roslin. 3 : 77.

Bridge, M.A. and Klarman, W.L. (1973). Soybean phytoalexin, hydroxyphaseollin induced by ultra violet irradiation. Phytopathology, 63 : 606.

Burden, R.S. and Bailey, J.A. (1975). The structure of the phytoalexin from soybean, Phytochemistry, 14 : 1389.

Carlos Montero, J. (1979). Predisposition of soybean (Glycine max (L. Merril.) to blight caused by Sclerotium rolfsii Sacc. Fitopatologia, 14 : 54.

Carroll, R.B. ; Lukezic, L. and Levine, R.G. (1972). Absence of common antigen relationship between Corynebacterium insidiosum and Medicago sativa as a factor in disease disease development ; Phytopathology, 62 : 1351.

Cartwright, D.Langcake, P.Pryee, R.J., Leworthy, D.P. and Ride, J.P. (1977). Chemical activation of host defence mechanism as basis for crop protection. Nature, 267 : 54.

Cartwright, D.W., Langcake, P. and Ride, J. P. (1980). Phytoalexin production in rice and its enhancement by a dichlorocyclopropane fungicide. Physiological Plant Pathology, 17 : 259.

Casper, R. and Mendgen, K. (1979). Quantitative serological estimation of hyperparasite : detection of Verticillium lecanii in yellow rust infected wheat leaves by ELISA. Phytopathologische Zeitschrift, 94 : 89.

Chakraborty, B.N. (1988). Antigenic disparity. In Experimental and conceptual plant pathology (Ed. R.S.Singh U.S.Singh, W.M.Hess and D.J.Webar) p.477. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi.

Chakraborty, B.N. ; Chakraborty, U. ; Das, R. ; Basu, P. and Saha, A. (1994a). Serological relationship between Glomerella cingulata (Stoneman) Spaulid and Schrenk and Camellia sinensis (L.) O. Kuntze, Proc. International Symposium on plantation crops. (PLACROSYM - XI) p.26.

Chakraborty, B.N. ; Chakraborty, U., Saha, A.; Basu, P. and Das, R. (1994b). Detection of cross reactive antigens and protein changes in tea leaves during infection with Pestalopsis theae. Proceedings of the Annual meeting of Indian Phytopathological Society, p.37.

Chakraborty, B.N., Chakraborty, U. and Saha, A. (1995). Defense Strategies of tea (Camellia sinensis) against fungal pathogens. In Handbook of Phytoalexin Metabolism and Action. (Ed. N.Daniel and R.P.Purkayastha) Marcel dekker, Inc. New York, p. 485.

Chakraborty, B.N. ; Chakraborty, U. ; Saha, A. ; Basu, P. and Das, R. (1994c). Detection of cross-reactive antigens and protein changes in tea leaves during infection with Pestalotiopsis theae. 46th Annual meeting of Indian Phytopathological Society and National Symposium on 'Ultrastructure, cytology, sexuality and variability in Plant Pathogens'. Tamilnadu Agricultural University, Coimbatore, pp. 37.

Chakraborty, B.N. and Purkayastha, R.P. (1981). Effect of some growth substances on charcoal-rot resistant and susceptible cultivars of soybean (Glycine max (L.) Merril) Bull.Botan.Soc.Bengal, 35 : 103.

Chakraborty, B.N. and Purkayastha, R.P. (1983). Serological relationship between Macrophomina phaseolina and soybean cultivars. Physiological Plant Pathology, 23 : 197.

Chakraborty, B.N. and Purkayastha, R.P.(1987). Alteration of glyceollin synthesis and antigenic patterns after chemical induction of resistance in soybean to Macrophomina phaseolina. Can. J. Microbiol. 33 : 835.

Chakraborty, B.N. and Saha, A. (1994). Detection and cellular location of cross-reactive antigens shared by Camellia sinensis and bipolaris carbonum. Physiological and Molecular Plant Pathology 43 : 403.

Chakraborty, B.N. and Shil, B.C. (1989). Suppression of root rotofSoybean by organic amendments. Indian Botanical Contactor, 6 : 91.

Chakraborty, D. and Sinha, A.K. (1984). Similarity between the chemically and biologically induced resistance in wheat seedlings to Drechslera sorokiniana, 91 : 59.

Chakraborty, U. and Chakraborty, B.N.(1989). Interaction of Rhizobium leguminosarum and Fusarium solani f.sp. pisi on pea affecting disease development and phytoalexin production. Canadian Journal of Botany, 67 : 1698.

Chakraborty, U.; Chakraborty, B.N. and Purkayastha, R.P. (1989). Application of growth substances and mineral nutrition affecting disease development and glyceollin production of soybean. Folia Microbiol. 34 : 490.

Chalutz, E. and Stahmann, M.A. (1969). Induction of pisatin by ethylene. Phytopathology, 59 : 1972.

Charudattan, R. and DeVay J.E. (1972). Common antigens among varieties of Gossypium hirsutum and isolates of Fusarium and Verticillium species. Phytopathology, 62(2) : 230.

Charudattan, R. and DeVay, J.E. (1981). Purification and partial characterization of an antigen from Fusarium oxysporum f.sp. vasinfectum that cross-reacts with antiserum to cotton (Gossypium hirsutum) root antigens.Physiological Plant Pathology, 18 : 289.

Charudattan and Hubbell, D.H. (1973). The presence and possible significance of cross-reactive antigens in Rhizobium legume associations, Antonic Van Leeuwenhock, 39 : 619.

Cheema, A.S. and Haard, N.F. (1978). Induction of rishitin and lubimin in potato tuber disease by non-specific elicitors and the influence of storage conditions. Physiological Plant Pathology, 13 : 233.

Clark, M.F. (1981). Immunosorbent assays in plant pathology, Annual Review of Phytopathology, 19 : 83.

Clark, M.F. and Adams, A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Microbiology, 34 : 475.

Clark, M.F. and Bar-Joseph, M. (1984). Enzyme immunosorbent assay in plant virology. In Methods in Virology (Ed. by Maramoirasch, K. and Koprowski, H. Vol.VII, pp. 51. Academic Press, London.

Clausen, J. (1969). Immunochemical techniques for the identification and estimation of macromolecules. In Laboratory techniques in Biochemistry and Molecular Biology, Vol.I. (Ed.) T.S.Work and E.Work, North Holland Publishing Company , p.399.

Crosier, W.F. and Yountburg, W.L. (1967). Reduction of common bunt in Winter wheat by 2-chloroethyl trimethyl ammonium chloride. Phytopathology, 57 : 339 (Abstr.)

Cruickshank, I.A.M. (1963). Phytoalexins. Ann. Rev. Phytopathol. 1 : 351.

Cruickshank, I.A.M.(1977). A review of the role of phytoalexins in disease resistance mechanisms. In Natural products and protection of plants Ed. Marini-Bettolo, G.B. pp.509.

Cruickshank, I.A.M. (1980). Defense triggered by the inveder chemical defenses. In Plant Disease, an advanced treatise. Vol.V, Eds. Horsfall, J.G. and Cowling, E.B., pp. 247, Academic Press, Inc.

Daly, J.M. (1972). The use of near isogenic lines in biochemical studies of the resistance of wheat stem rust. Phytopathology, 62 : 392.

Daly, J.M. and Deverall, B.J. (1963). Metabolism of indole acetic acid in rust diseases V. Factors influencing rates of decarboxylation. Plant Physiol., 38 : 741.

Damian, R.T. (1964). Molecular mimicry : antigen sharing by parasite and host and its consequences. Amer, Natur., 98 : 129.

Damian, R.T.(1979). Molecular mimicry in biological adaptation. Host-parasite Interfaces, (Ed. B.B.Nickol), pp.103. Academic Press, Inc., New York.

Darozhkin, M.A. and Imanyuk, U.R. (1976). Phytoalexins and resistance of tomato to early dry spot. Vestsi Akademii Navuk BSSR, Biyalagi Chnykh Navuk, 4 : 60.

Darvill, A.G. and Albersheim, P. (1984). Phytoalexins and their elicitors - defense against microbial infection in plants. Annu. Rev. Plant. Physiol. 35 : 243.

Davis, B.J. (1964). Method and application to human serum proteins. Annals. N.Y. Acad. Sci., 121 : 404.

Dazzo, F.B. and Brill, W.J. (1977). Receptor site on clover and alfalfa roots for Rhizobium. Applied and Environmental Microbiology, 33 : 132.

Dazzo, F.B. and Brill, W.J. (1979). Bacterial polysaccharide which binds Rhizobium trifollii to clover root hairs. Journal of Bacteriology, 137 : 1362.

Devay, J.E. and Adler, H.E. (1976). Antigens common to host and parasites, Annual Review of Microbiology, 30 : 147.

DeVay, J.E., Charudattan, R. and Wimalajeewa, D.L.S. (1972). Common antigenic determinates as possible regulator of host pathogen compatibility. The American Naturalist., 106(948) : 185.

DeVay, J.E., Schanthrost, W.C. and Foda, M.S.(1967). Common antigens and host parasite interactions p.313. In C.J. Microcha and I. Uritani Ed. The dynamic role of molecular constituents in plant parasite interaction. Bruce, Minneapolis. 373 p.

DeVay, J.E., Wakeman, R.J. ; Kavanagh, J.A. and Charudattan, R.(1981a). The tissue and cellular location of a major cross-reactive antigen shared by cotton and soil borne fungal parasites. Physiological Plant Pathology, 18:59.

DeVay, U.E., Wilson, R.J., Wakeman and Kavanagh, J.A. (1981b). Occurrence of common antigenic determinants between potatoes and Phytophthora infestans in relation to host-parasite compatibility. In D.C.Erwin, S.Bartniki - Garcia, p. Tsao (ed.). Proc. Inter Symp. on Phytophthora, Its Biology, Ecology, and Pathology, 43. Dept. of Plant Pathology, University of California, Riverside.

Deverall, B.J. (1967). Biochemical changes in infection droplets containing spores of Botrytis spp. incubated in the seed cavities of pods of bean (Vicia faba L.) Ann. Appl. Biol. 59 : 375.

Deverall, B.J. (1972). Phytoalexins. In Phytochemical Ecology, Ed. Harborne, J.B., p.217. Academic Press, London

Deverall, B.J. (1976). Current perspectives in research on phytoalexins. In Biochemical aspects of plant-parasite relationships. Ed. Friend, J. and Threlfall, D.R., Phytochemical Society Symposia Series, Vol.13, Academic Press, London, p.208.

Deverall, B.J.(1977). Defence mechanisms of plants. Cambridge University Press, Cambridge, London.

Deverall, B.J. and Vessey, J. (1969). Role of a phytoalexin in controlling lesion development in leaves of Visica faba after infection by Botrytis spp. Ann. Appl. Biol. 63:449.

Dewey, F.M. and Brasier, C.M. (1988). Development of ELISA for Ophiostoma ulmi using antigen coated wells. Plant Pathology, 37 : 28.

Dewit, P.J.G.M. and Bakker, J. (1980). Differential changes in soluble tomato leaf proteins after inoculation with virulent and avirulent races of Cladosporium fulvum (Syn. Fulvia fulva). Physiol Plant Pathol, 17 : 121.

Dineen, J.K. (1963). Antigenic relationship between host and parasite. Nature, London, 197 : 471.

Doubly, J.A., Flor, H.H. and Clagett, C.O. (1960). Break through on plant disease. Agar.Res. (Washington), 8:3.

Ebel, J. and Grisebach, H. (1988). Defense strategies of soybean against the fungus Phytophthora megasperma f.sp. glycinea : molecular analysis, Trends in Biochemical Sciences, 13 : 23.

Elgersma, D.M. and Liem, J.I. (1989). Accumulation of phytoalexins in susceptible and resistant near-isogenic lines of tomato infected with Verticillium albo-atrum or Fusarium oxysporum f.sp. lycopersici. Physiological and Molecular Plant Pathology, 34,:545.

Elnaghy, M.A. and Heitefuss, R. (1976). Permeability changes and production of antifungal compounds in Phaseolus vulgaris infected with Uromyces phaseoli II. Role of phytoalexins Physiol. Plant Pathol., 8 : 269.

Erwin, D.C. ; Tsai , S.D. and Khan, R.A. (1979). Growth retradants mitigate Verticillium wilt and increase yield of cotton. California Agriculture, 33 : 8.

Fraile, A. ; Garcia, A.F. and Sagasta, E.M. (1980). Phytoalexin accumulation in bean (Phaseolus vulgaris L.) after infection with Botrytis cinerea and treated with mercuric chloride. Physiol. Plant Pathol., 16 : 9.

Frank, J.A. and Paxton, J.D. (1970). Time sequence for phytoalexin production in Harasoy and Harosay 63 soybeans, Phytopathology, 60 : 315.

Frank, J.A. and Paxton, J.D. (1971). An inducer of soybean phytoalexin and its role in the resistance of soybean to Phytophthora rot. Phytopathology 61 : 954.

Furrer, O.J. and Staulfer, W. (1972). Results of nitrogen fertilization and C.C.C. experiments on spring wheat Schweizersche Landwirtschaftliche Forschung, 11 : 273.

Ghosal, A. and Purkayastha, R.P. (1984). Elicitation of momilactone by gibberellin in rice, Current Science, 53 : 506.

Ghosal, A. and Purkayastha, R.P. (1987). Biochemical response of rice (Oryza sativa L.) leaves to some abiotic elicitors of phytoalexin. Indian Journal of Experimental Biology, 25 : 395.

Ghosh, S. and Purkayastha, R.P. (1990). Analysis of host parasite cross-reactive antigens in relation to Myrothecium infection of soybean. Indian Journal of Experimental Biology, 28 : 1.

Gray, G. and Klarman, W.L. (1967). Comparison of phytoalexin produced by two soybean varieties differing by a single gene. Phytopathology, 57 : 645 (Abstr.).

Gray, G. ; Klarman, W.L. and Bridge, M.L. (1968). Relative quantities of antifungal metabolites produced in resistant and susceptible soybean plants inoculated with Phytophthora megasperma var. Sojae and closely related non-pathogenic fungi. Canad. J. Bot. 46 : 285.

Green, A.A. and Hughes, W.L. (1955). Protein fractionation on the basis of solubility in aquous solutions of salts and organic solvents. In Methods in Enzymology. Vol.I(Ed.) S.P. Academic Press, New York, p.67.

Hadwiger, L.A.; Sander, C. ; Eddywean, J. and Ralston, J. (1976). Sodium azide induced mutants of peas that accumulate pisatin. Phytopathology, 66 : 629.

Hahn, M.G., Bouhoff, A. and Grisebach, H. (1985). Quantitative localization of the phytoalexin glyceollin I in relation to fungal hyphae in soybean roots infected with Phytophthora megasperma f.sp. glycinea. Plant Physiology.

Hait, G.N. and Sinha, A.K.(1986). Protection of wheat seedlings from Helminthosporium infected by seed treatment with chemicals. J.Phytopathology 115,: 97.

Hale, M.G., Roane, C.W. and Huang, M.R.C. (1962). Effects of growth regulators on size and number of leaf spots and on O_2 uptake and extension growth of coleoptile sections of corn inbred lines K41 and K44. Phytopathology, 52 : 185.

Halverson, L.J. and Stacey, G. (1986). Signal exchange in plant-microbe interactions. Microbiological reviews, June 1986, p.193.

Harborne, J.B. and Ingham, J.L. (1978). Biochemical aspects of the co-evolution of higher plants with their fungal parasites. In Biochemical aspects of Plant and Animal Coevolution. Harborne, J.B. Ed. pp.343. Academic Press, New York.

Hardham, A.R. ; Suzaki, E. and Perkin, J.L. (1986). Monoclonal antibodies to isolate, species and genus specific components of the surface of Zoospores and cysts of the fungus Phytophthora cinnamoni. Can. J. Bot.,64:311.

Hare, R.C.(1966). Physiology of resistance to fungal disease in plants. Bot. Rev., 32 : 95.

Harrison, J.G. ; Barker, H., Lowe, R. and Rees, E.A. (1990). Estimation of amounts of Phytophthora infestans mycelium in leaf tissue by enzyme-linked immunosorbent assay. Plant Pathology, 39, : 274.

Heide, M. and Swedegard-Peterson, V. (1985). Common antigens between barley and barley powdery mildew and their relation to resistance and susceptibility ; Canad. J. Pl. Pathol, 7 : 341.

Heitefuss, R. ; Buchanan-Davidson, D.J. ; Stahmann, M.A. and Walker, J.C. (1960). Electrophoretic and Immunochemical studies proteins in cabbage infected with Fusarium oxysporum f. conglutinans Phytopathology, 50 : 198.

Ingham, J.L. (1972). Phytoalexins and other natural products as factors in plant disease resistance. Bot. Rev., 38:343.

Ingham, J.L. (1973). Disease resistance in higher plants. The concept of pre-infectional and post-infectional resistance Phytopathol. Z. 78 : 314.

Ingham, J.L.(1982). Phytoalexins from the Leguminosae. In Phytoalexins, Ed. by J.A. Bailey & J.W. Mansfield. pp.21-80. John Wiley & Sons, New York.

Irvang, H.R. and Kuc, J.A. (1990). Local and systemic induction of peroxidase, chitinase and resistance in cucumber plant by K_2HPO_4. Physiology and Molecular Plant Pathology, 37 : 355.

Johnson, G. ; Maag, D.D. ; Johnson, D.K. and Thomas, R.D. (1976). The possible role of phytoalexins in the resistance of sugarbeet (Beta vulgaris) to Cercospora beticola, Physiol. Plant Pathol. 8 : 225.

Johnson, R. (1962). Serological studies on wheat and wheat stem rust, p.57 M.S. Thesis, University of Saskatchewan, Saskatoon, Sask, Canada.

Johnson, M.C.; Pirone, T.P. ; Siegel, M.R. and Varney, D.R. (1982). Detection of Epichloe typhina in tall fescue by means of enzume-linked immunosorbent assay. Phytopathology 72 : 647.

Jones, D.R. ; Unwin, C.H. and Ward., E.W.B. (1975). The significance of capsidiol induction in pepper fruit during an incompatible interaction with Phytophthora infestans. Phytopathology, 65 : 1286.

Joosten, M.H.A.J. and DeWit, P.J.G.M.(1988). Isolation, purification and preliminary characterization of a protein specific for compatible Cladosporium fulvum (Syn. Fulvia Fulva) tomato interactions. Physiological and Molecular Plant Pathology, 33 : 241.

Joshi, L.M.(1965). Effect of maleic hydrazide on rusts of wheat and barley. Indian Phytopath. 18 : 303.

Kallmann, A., Rouxel, T. and Bousquet, J.F. (1989). Applications to the determination of phytoalexins from Brassica spp. by high performance liquid chromatography. Journal of Chromatography, 473 : 293.

Kalyanasundaram, R. ; Lakshminarasimhan, C. and Venkataraman, S.(1975). Common antigens in host-parasite relationship. Current Science, 44 : 55.

Kalyansundaram, R., Lakshminarasimhan, C. and Venkataraman, S. (1978). Antigenic relationship between host and parasite in Fusarium wilt of cotton. In : T.S.Sadasivan, C.V. Suramanian, R. Kalyansundaram, L. Sarswathi-Devi(ed.) 348. The University of Madras, India.

Kaplan, D.T.; Keen, N.T. & Thomason, I.J. (1980). Studies on the mode of action of glyceollin in soybean incompatibility to root knot nematode, Meloidogyne incognita, Physiologucal Plan Pathology, 16 : 319.

Keen, N.T. (1971). Hydroxyphaseollin production by soybeans resistant and susceptible to Phytophthora megasperma var. Sojae. Physiol. Plant. Pathol., 1 : 265.

Keen, N.T. (1981). Evaluation of role of Phytoalexins, In Plant disease control. Ed. Staples, R.C.John Wiley & Son, Inc. pp.155.

Keen, N.T.(1982). Mechanisms conferring specific recognition in gene for gene plant-parasite systems. In Active Defense mechanisms in plants Ed. Wood, R.K.S. pp. 67. Plenum Publishing Corporation.

Keen, N.T. (1990). Gene for gene complementarity in plant pathogen interactions. Ann. Rev. Genetics, 24 : 447.

Keen, N.T. and Bruegger, (1977). Phytoalexins and chemicals that elicit their production in plants. In Host Plant Resistant to Pests. Ed. Hedin, P.Am. Chem. Soc. Symp. Ser. 62 : 1.

Keen, N.T. ; Ersek, T. ; Long, M.; Bruegger, B. and Holliday, M. (1981). Inhibition of the hypersensitive reaction of soybean leaves to incompatible Pseudomonas spp. by blasticidin, S., streptomycin or elevated temperature. Physiol. Plant. Pathol. 18 : 325.

Keen, N.T. and Horsch, R. (1972). Hydroxyphaseollin production by various soybean tissues, a warning against use of "unnatural" host-parasite systems. Phytopathology, 62:439.

Keen, N.T. and Littlefield, L.J. (1979). The possible association of phytoalexins with resistance gene expression in flax to Melampsora lini. Physiol. Plant Pathol., 14:265.

Keen, N.T. ; Sims, J.J.; Erwin, D.C. ; Rice, E. and Partridge J.E. (1971). 6a-Hydroxyphaseollin : an antifungal chemical induced in soybean hypocotyls by Phytophthora megasperma var. sajae. Phytopathology, 61:1084.

Khan, F.Z., and Milton, J.M. (1978). Phytoalexin production and the resistance of lucerne (Medicago sativa L.) to Verticillium albo-atrum. Physiol. Plant. Pathol., 13:251.

Kitagawa, T. Sakamoto, K. ; and Ogura, H. (1989). Novel enzyme immunoassays for specific detection of Fusarium oxysporum f.sp. cucumerinum and for general detection of various Fusarium species. Phytopathology, 79 : 162.

Klarman, W.L., and Gerdemann, J.W. (1963). Resistance of soybean to three Phytophthora species due the production of a phytoalexin. Phytopathology, 53 : 1317.

Klarman, W.L. (1968). The importance of phytoalexins in determining resistance of soybeans to three isolates Phytophthora. Netherlands J. Plant Path., Suppl. 1 : 171.

Koening, R. and Paul, H.L. (1982). Variants of ELISA in plant virus diagnosis. J. Virol, Method, 5 : 113.

Kuc, J. (1966). Resistance of plants to infectious agents, Annual Review of Microbiology, 20 : 337.

Kuc, J.(1972). Phytoalexins. Annual Review of Phytopathology, 10 : 207.

Kuc, J. (1976). Phytoalexins, In Physiological Plant Pathology, Eds. Heitefuss, R. and Willams, P.H. pp.632. Springer, Verlag, Barlin.

Kuc, J. (1983). Induced systemic resistance of plants to disease caused by fungi and bacteria. In The Dynamics of Host Defence, Ed. by J.A. Bailey & B.J. Deverall, pp. 191-221. Academic Press, Sydney.

Kuc, J. ; Currier, W.W. and Shih, M.J. (1976). Terpenoid phytoalexins. In Biochemical aspects of plant parasite relationships (Ed.) J. Friend and D.R. Threlfall. Academic Press, London, p.225.

Lamb, C.J.Lawton, M.A., Dron, M. and Dixon, A. (1989). Signals and transduction mechanisms for activation of plant defenses against microbial attack. Cell, 56 : 215.

Letcher, R.; Widdowson, B. ; Deverall, B.J. and Mansfield, J. (1970). Identification and activity of wyerone acid as a phytoalexin in broad bean (Vicia faba) after infection by Botrytis. Phytochemistry, 9 : 249.

Linfield, C.A. (1993). A rapid serological test for detecting Fusarium oxysporum f.sp. narcissi in Narcissus, Ann. appl. Biol., 123 : 685.

Long, M. ; Barton - Willis, P., Staskawicz, B.J., Dahlbeck, D. and Keen, N.T. (1985). Further studies on the Relationship between glyceollin Accumulation and the Resistance of Soybean leaves to Pseudomonas syringae pv. glycinea. Phytopathology, 75 : 235.

Lowry, O.H.; Rasebrough, N.J. ; Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193 : 265.

Lyne, R.L. and Mulheirn, L.J. (1978). Minor pterocarpinoids of soybean. Tetrahedron letters, 34 : 3127.

Lyne, R.L., Mulheirn, L.J. and Leworthy, D.P. (1976). New pterocarpinoid phytoalexins of soybeans. Journal of Chemical Society, Chemical Communications, p. 497.

Lyons, N.F. and White, J.G.(1992). Detection of Pythium violae and Pythium sulcatum in carrots with cavity spot using competition ELISA. Annals of Applied Biology,120:235

MacDonald, J.D.; Stites, J.Kabashima, J.(1990). Comparison of serological and culture plate methods for detecting species of Phytophthora, Pythium and Rhizoctonia in ornamental plants. Plant disease, 74 : 655.

Mackie, A.J.Roberts, A.M., Green, J.R. and Gallow, J.A.(1993) Glycoproteins recognised by antibodies UB7, UB8 and UB10 are expressed early in the development of pea powdery mildew haustoria. Physiological and Molecular Plant Pathology. 43,:135.

Madamanchi, N.R. and Kuc, J.(1991). Induced systemic resistance in plants. In the fungal spore and disease initiation in plants and animals. (Ed. by G.T. Cole and H.C. Hoch). Plenum Publishing Corporation, P. 347.

Mandal, N.C. and Sinha, A.K.(1992). An alternative approach for the chemical control of Fusarium wilt of tomato.Indian Phytopathology, 45(2) : 194.

Massala, R., Legrand, M. & Fritig, B.(1987). Comparative effects of two competitive inhibitors of phenylalanine ammonia lyase on the hypersensitive resistance of tobacco to tobacco mosaic virus. Plant Physiology and Biochemistry 25 : 217.

Mathur, A.K. and Bhatnagar, G.C. (1991). Management of barley stripe with mineral elements. Indian Phytopathology, 44 : 382.

Mazarei, M., and Kerr, A. (1990). Distinguishing pathovars of Pseudomonas syringae on peas : nutritional, pathogenicity and serological tests. Plant Pathology, 39 : 278.

Merz, W.G., Burrell, R.G. and Gallegly, M.E. (1969). A serological comparison of six heterothallic species of Phytophthora. Phytopathology, 59 : 367.

Metlitskii, L.V.(1975). Phytoalexins and their protective role in plant resistance to parasitic fungi. In Immunitet S-Kh-rast. K. boleznyam 1 Vreditelyam, Moscow, USSR Kolos p.113.

Metlitskii, L.V.(1976). Phyto-immunity molecular mechanisms. Transl. from 31st Ann. Bakh. Symp., Moscow "Nauka" USSR p.1.

Moesta, P. and Grisebach, H. (1980). Effects of biotic and abiotic elicitors on Phytoalexin metabolism in soybean. Nature., 286 : 710.

Moesta, P. and Grisebach, H. (1981). Investigation of the mechanism of glyceollin accumulation in soybean infected by Phytophthora megasperma f.sp. glycinea. Archives of Biochemistry and Biophysics, 212 : 462.

Mohan , S.B. (1988). Evaluation of antisera raised against Phytophthora fragarie for detecting the red core disease of strawberries by enzyme-linked immunosorbent assay (ELISA), Plant Pathology, 27 : 206.

Mohan, S.B.(1989). Cross-reactivity of antiserum raised against Phytophthora fragarie with other Phytophthora species and its evaluation as a genus-detecting antiserum. Plant Pathology, 38 : 352.

Morandi, D.; Bailey, J.A. & Gianinazzi-Pearson, V.(1984). Isoflavonoid accumulation in soybean roots infected with vesicular - arbuscular mycorrhizal fungi. Physiological Plant Pathology, 24 : 357.

Muller, K.O. and Borger, H.(1940). Experimentalle Untersuchungen iiber due Phytophthora resistenz der kartoffel. Arab. Biol. Reichsanst. Landu. Forstwirtsch. Berlin-Dahlem 23 : 189.

Murch, R.S. and Paxton, J.D. (1980). Rhizosphere salinity and phytoalexin accumulation in soybean. Plant and Soil, 54 : 163.

Natalina, O.B. and Svetvov, V.G. (1972). Effectiveness of chlorocholin chlorides, against grey rot of grapevine, Khimiya V. Selskom Khozyaistve, 10 : 34.

Nemestothy, G.S. and Guest, D.I. (1990). Phytoalexin accumulation, phenylalanine ammonia lyase activity and ethylene biosynthesis in fosetyl-Al treated resistant and susceptible tobacco cultivars infected with Phytophthora nicotianae var. nicotianae. Physiological and Molecular Pathology, 37 : 207.

Nene et.al. (1981). Chickpea diseases, resistance-screening techniques, ICRISAT, Information Bulletin No. 10.

Obi, I.U.; Hooker, A.L. and Lim. S.M. (1980). Phytoalexin production by corn plants with different genetic backgrounds having chlorotic-lesion resistance against Helminthosporium turcicum. Zeitschrift fur Pflanzenkrankheiten and Pflanzenschutz (Journal of Plant Diseases and Protection), 87 : 244.

Oku, H. (1960). Biochemical studies on Cochliobolus miyabeanus.VI On the breakdown of disease resistance in rice by reducing agents. Ann. Phytopath. Soc. Japan, 25 : 92.

Oku, H. and Nakanishi, T. (1962). Relation of phytoalexin-like antifungal substance to resistance of rice plant against Helminthosporium leaf spot disease. Ann. Rep. Takamine Lab., 14 : 120.

Oswald, T.H. and Wyllie, T.D. (1973). Effects of growth regulator treatments on severity of charcoal rot disease of soybean. Plant. dis. reporter, 57 : 789.

Ouchi, S. (1983). Induction of resistance or susceptibility. Ann. Rev. Phytopathol., 21 : 289.

Ouchi, S.; Oku, H. ; Hibino, C. and Akiyama, I.(1974). Induction of accessibility and resistance in leaves of barley by some races of Erysiphe graminis. Phytopathol. Z, 79:24.

Ouchterlony, O. (1967). Immunodiffusion and immunoelectrophoresis. In Handbook of Experimental Immunology. (Ed.) D.M. Weir. Black Scientific Publications, Oxford and Edinburgh, p.655.

Palmerley, R.A. and Callow, J.A. (1978). Common antigens in extracts of Phytophthora infestans and Potatoes. Physiol. Plant. Pathol. 12 : 241.

Paradies, I., Konze, J. & Elstner, E.F. (1980). Ethylene : Indicator but not inducer of phytoalexin synthesis in soybean. Plant Physiology, 66 : 1106.

Partridge, J.E. and Keen, N.T. (1976). Association of the phytoalexin Kievitone with single gene resistance of cowpeas to Phytophthora vignae. Phytopathology, 66 : 426.

Paxton, J. (1995). Soybean Phytoalexins, Nature, Elicitation, Mode of action and Role. In Hand book of Phytoalexin Metabolism and Action. (Ed. by M.Daniel and R.P. Purkayastha). Marcel Dekker, Inc. New York, pp.624.

Paxton J.D.(1988). Phytoalexins in plant parasite interactions. In Experimental and Conceptual Plant Pathology Eds. Singh, R.S., Singh, V.S., Hess, W.M.Weber, D.J. pp.537 Oxford IBH. Publishing Co. Pvt. Ltd. New Delhi.

Peterson, L.J. ; DeVay, J.E. and Houston, B.R.(1961). The effect of gibberellic acid on the parasitism of Rhizoctonia solani on beans and cotton. Phytopathology 51 : 67(Abstr.)

Peterson, L.J. ; DeVay, J.E. and Houston, B.R.(1963). Effect of gibberellic acid on development of hypocotyl lesions caused by Rhizoctonia solani on red kidley bean. Phytopathology 53 : 630.

Protsenko, M.A. and Ladyzheskaya, E.P. (1989). Detection of an antigen common to potato late blight fungus and its host plant by double immunodiffusion in gel; Appl.Biochem. Microbiol. 24 : 576.

Purkayastha, R.P. (1973). Phytoalexins relation to plant disease control Sci. and Cult., 42 : 586.

Purkayastha, R.P. (1976). Induction of phytoalexins in relation to plant disease control. Sci. and Cult. 42 : 586.

Purkayastha, R. P.(1985). Phytoalexins. In Frontiers in Applied Microbiology Eds. Mukerjee, Pathak, N.C. and Singh, V.P., pp.363. Print House (India). Lucknow.

Purkayastha, R.P. (1986). Elicitors and elicitation of phytoalexins. In vistas in plant Pathology. Eds. Verma A. and Verma, J.P. pp. 25, Malhotra Publishing House, New Delhi, India.

Purkayastha, R.P.(1989). Specificity and disease resistance in plants. Presidential address, Section Botany, 76th Session of the Indian Science Congress, Madurai.

Purkayastha, R.P.(1994). Phyto-immunology an emerging discipline of Plant Science. Everyman's Science, 29(2) : 41.

Purkayastha, R.P. and Banerjee, R.(1986). Immunological studies on anthroacnose disease of soybean. International Journal of Tropical Plant disease, 4(1) : 77.

Purkayastha, R.P. and Banerjee, R.(1990). Immunoserological studies on cloxacillin-induced resistance of soybean against anthracnose. (Zeitschrift fur Pflanzen Krankhiiten und Pflenzenschutx) Journal of Plant disease and Protection , 97(4) : 349.

Purkayastha, R.P. and Chakraborty, B.N. (1983).Immunoelectrophoretic analysis of plant antigens in relation to biosynthesis of phytoalexin and disease resistance of soybean (Tropical Plant Science Research, 1(1) : 89.

Purkayastha, R.P. and Chattopadhyay, M. (1975). Studies on the resistance of rice cultivars to Helminthosporiase Indian Phytopath, 28 : 33.

Purkayastha, R.P. and Deverall, B.J.(1964). A phytoalexin type of reaction in the Botrytis-infection leaves of bean (Vicia faba L.) Nature, 201 : 938.

Purkayastha, R.P. and Deverall, B.J.(1965). The growth of Botrytis fabae and B. cinerea into leaves of bean (Vicia faba L.) Ann. Appl. Biol. 56 : 139.

Purkayastha, R.P. ; Ghosal, A., Garai, M. and Ghosh, S. (1991). Cross reactive antigens as determinants of susceptibility of pigeon pea cultivars to Fusarial wilt ; In Botanical Researches in India ed. N.C.Aery, B.L.Chaudhury. Himanshu Publications, Udaipur, India. 508.

Purkayastha, R.P. and Ghosal, A. (1985). Analysis of cross-reactive antigens of Acrocylindrium oryzae and rice in relation to sheath rot disease. Physiological Plant Pathology, 27 : 245.

Purkayastha, R.P. and Ghosal, A.(1987). Immunoserological studies on root rot of ground nut (Arachis hypogea L.). Canadian Journal of Microbiology, 33 : 647.

Purkayastha, R.P., Ghosal, A. and Biswas, S.(1983). Production of momilactone associated with resistance of rice cultivars to sheath rot disease,. Current Science 52(3):131

Purkayastha, R.P. and Ghosh, S. (1983). Elicilation and inhibition of Phytoalexin Biosynthesis in Myrothecium infected Soybean. Indian Journal of Experimental Biology, 21 : 216.

Purkayastha, R.P. and Mallik, F. and Chakraborty, R. (1972). Responses of Healthy and Macrophomina infected jute plants to growth substances. Indian Phytopath, 25 : 366.

Purkayastha, R.P., Menon, U. and Chakraborty, B.N.(1981). Rhizobium-Macrophomina. Interaction Affecting Phytoalexin Production and Disease Resistance of Soybean. Indian Journal of Experimental Biology, 19 : 462.

Purkayastha, R.P. and Mukhopadhyay, R.(1976). Level of amino acids and postinfectional formation of antifungal substances in relation to susceptibility of rice plants against Helminthosporium oryzae at different nitrogen supply. Z.Pflanzenkr. Pflanzenschutz., 83 : 221.

Purkayastha, R.P. and Pradhan, S. (1994). Immunological approach to study the etiology of Sclerotium rot disease of ground nut. Proc. Indian. Natn. Sci. Acad., B60, No.2 pp.157.

Purkayastha, R.P. and Ray, C. (1975). The detection of phytoalexin in jute leaves after infection by Colletotrichum corchorium and their possible role in lesion development. Physiol. Plant. Pathol., 6 : 265.

Purkayastha, R.P. and Ray, C. (1977). Effected of foliar application of plant hormones and mineral nutrition of host on the development of anthracnose disease of jute. Zeitschrift fur Pflanzenkran kheiten and Pflanzenschutz. (Journal of Plant disease and protection). 84 : 193.

Ramarao, P. and Isaac, I. (1980). Effect of foliar application of antibiotic and gibberellic acid on the rhizosphere microflora of pea (Pisum sativum) infected with Verticillium dahliae. Foiia Microbiol., 25 : 357.

Rivera, J.C. and Mavrich, E. (1978). Control of Botrytis cinerea. use of gibberellin to lengthen bunches of Pinot gris at Mendoza (Argentina) Progris Agriole et. Viticole, 95 : 9.

Roberts, A.M., Mackie, A.J., Hathway, V., Gallow, J.A. and Green, J.R.(1993). Molecular differentiation in the extra-haustorial membrane of pea powdery mildew haustoria at early and late stages of development. Physiological and Molecular Plant Pathology, 43 : 147.

Rouxel, T., Sarniguet, A., Kallmann and Bousquet, J.F.(1989). Accumulation of Phytoalexin Brassica spp. in relation to hypersensitive reaction to Leptosphaeria maculans. Physiological and Molecular Plant Pathology, 34 : 507.

Sad, A.T. and Rashid, K.(1973). The effects of growth regulators on the incidence and severity of internal crocolate spot disease of potatoes. 2nd Int. Cong. of Plant Pathology. 344 (Abstr.).

Sako, N. and Stahmann, M.A. (1972). Multiple molecular forms of enzymes in barley leaves infected with Erysiphe graminis f.sp. hordii. Physiol. Plant. Pathol., 2 : 217.

Samborski, D.J., Person, C. and Forsyth, F.R. (1960). Differential effects of maleic hydrazide on the growth of leaf and stem rusts of wheat. Canadian Journal of Botany, 38 : 1.

Sarkar, M.L. and Sinha, A.K. (1991). Control of sheath blight of rice by unconventional chemicals. Indian Phytopathology 44 : 379.

Sengupta, D. ; Datta -Roy, K. ; Banerjee, K. and Ghosh, A.C. (1989). Identification of some antigenically related outer membrane proteins or strains of Vibrio cholerae 01 and non-01 serovars involved in intestinal adhesion and the protective role of antibodies to them. J. Med. Microbiol, 29 : 33.

Sequeira, L. (1963). Mechanisms of induced resistance in plants. Annual Review of Microbiology, 37 : 51.

Sharma, J.K. (1973). Effect of certain chemicals in inducing resistance to Anthracnose of sorghum caused by Colletotrichum graminicola. 2nd Int. Cong. of Plant Pathology, p. 957 (Abstr.).

Sinha, A.K. (1984). A new concept of plant disease control. Science and Culture, 50 : 181.

Sinha, A.K. and Das, N.C. (1972). Induced resistance in rice plants to Helminthosporium oryzae. Physiological plant Pathology. 2 : 401.

Sinha, A.K. and Hait, G.N. (1982). Host sensitization as factor in induction of resistance in rice against Drechslera by seed treatment with Phytoalexin inducers. Transaction of British Mycological Society, 79 : 213.

Sinha, A.K. and Wood, R.K.S. (1967). The effect of growth substances on Verticillium wilt of tomato plants. Ann.App. Biol., 60 : 117.

Smith, C.J. and Milton, J.M. (1992). Phytoalexin accumulation in the forage crop, Medicago sativa as defence against the soil borne wilt pathogen Verticillium alboatrum. Indian Phytopathology, 45 : 81.

Stoessel, A. ; Ward, E.W.B. and Stothers, J.B. (1977). Biosynthetic relationship of sesquiterpenoidal stress compounds from the Solanaceae. Acs. Symp. Ser. No. 62 : 61.

Stoessel, P. and Magnalato, D. (1983). Phytoalexins in Phaseolus vulgaris and Glycine max induced by chemical treatment, microbiol contanuation and fungal infections, Experentia, 39 : 153.

Tahori, A.S. ; Zeidler, G. and Halevy, A.H.(1965). Effect of some plant growth retarding compounds on three fungal diseases and one viral disease. Plant Disease Reporter, 49 : 775.

Tegtmeir, K.J. and VanEtten, H.D. (1982). The role of pisatin tolerance and degradation in the virulence of Nectria haematococca on peas : A genetic analysis, Phytopathology, 79 : 608.

Tomiyama, K. (1966). Double infection by an incompatible race of Phytophthora infestans of a potato plant cell which has been previously infected by a compatible race. Ann.Phytopathol. Soc. Japan, 32 : 181.

Uehara, K. (1965). Phytoalexins. Ann. Phytopathol. Soc.Japan, 31 : 334.

Uritani, I. (1971). Protein changes in diseased plants. Ann. Rev. Phytopathol., 9 : 211.

Uritani, I. (1976). Protein Metabolism. In Encyclopedia of Plant Physiology, Vol.4. Physiological Plant Pathology, Ed. Heitefuss, R. and Williams, P.H., pp.509. Springer-Verlag, Barlin, Heidel-berg, New York.

Uritani, J. and Stahmann, M.A. (1961). The relationship between antigenic compounds produced by sweet potato in response to black rot infection and the magnitude of disease resistance. Agar. Biol. Chem., 25 : 479.

Van Etten, H.D., Mathews, D.E. and Mathews, P.S. (1989). Phytoalexin detoxification importance for pathogenicity and Practical implications. Ann. Rev. Phytopathol., 27: 143.

VanEtten, H.D., Mathews, D.E. and Smith, D.A. (1982). Metabolism of phytoalexins. In Phytoalexinsm a Bailey, J.A. and Mansfield J.W. pp.21, Glasgow, London : Blackie.

Van Etten, H.D. and Purppke, S.G.(1976). Isoflavonoid phytoalexins. In Biochemical aspects of plant-parasite relationships, Ed. Friend, J. and Threfall, D.R. Ann. Proc. Phytochem.Soc., 13 : 239.

Venkataram, C.S. (1961). Application of nickel chloride to plants (Camellia sinensis) and control of blishter blight. Current. Science, 30 : 57.

Vidyasekaran, P. (1976). Role of auxins in leaf spot incidence in ragi incited by Helminthosporium tetramera. Indian Phytopath., 27 : 517.

Volken, P. (1972). Some aspects of the host parasite relationship under the influence of IAA and GA treatments. Phytopathol. S., 75 : 163.

Von gross, D. (1977). Phytoalexine und verwandte Pflanzenstoffe. Fortschr. Chem. Org. Naturst., 34 : 188.

Ward, E.W.B. (1986). Biochemical mechanisms involved in resistance of plants to fungi. In Biology and Molecular Biology of Plant Pathogen Interactions. Ed. Bailey, J. pp. 107. Springer Verlag, Barlin, Heidelberg.

Wood, R.K.S. (1982). Active defense mechanisms in plants, Plenum, New York.

Ward, E.W.B. (1989). Susceptibility of immature soybean leaves to Phytophthora species. Physiological and Molecular Plant Pathology, 34,: 393.

Watson, D.G. and Brooks, C.J.W. (1984). Formation of Capsidiol in Capsicum annum fruits in response to non-specific elicitors. Physiological Plant Pathology, 24 : 331.

Wilcoxson, R.D. and Sudia, T.W. (1960). The influence of gibberellic acid on seedling blight of corn. Plant disease Reporter, 44 : 312.

Wimalajeewa, D.L.S. and DeVay, J.E. (1971). The occurrence and characterization of a common antigen relationship between Ustilago maydis and Zea mays. Physiol. Plant. Pathol., 1 : 523.

Wyllie, T.D. and DeVay, J.E. (1970). Immunological comparison of isolates of Verticillium albo-atrum and V.nigrescens pathogenic to cotton. Phytopathology, 60 : 1682.

Yoshikawa, M., Yamauchi, K. & Masago, H. (1978). Glyceollin : its role in restricting fungal growth in resistant soybean hypocotyls infected with Phytophthora megasperma var. sojae.Physiological Plant Pathology, 12 : 73.

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