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Research article

VARIATION IN PIGMENT PRODUCTION BY *Cercospora personata*

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ABSTRACT: Pigments from two different isolates of Cercospora personata CP_3 & CP_4 were proved to be pathogenic, toxigenic and photosensitive. Spectral characters of the pigments were studied and correlated with their phytotoxicity to groundnut leaves. The red pigment from CP_4 isolates was more toxic, caused highest degree of necrosis, and was identified as cercosporin. Further cytotoxic functions of cercosporin were also studied in an attempt to understand the major cause of hepatic cancers in communities at high risk.

Keywords: Phytotoxicity, aflatoxin, photosensitizers, cytotoxicity and lymphocytes.

INTRODUCTION

Groundnut (Arachis hypogea L) is one of the most common and economic crop in major parts of the world. It became a cash crop for many agricultural communities. (Nutsugah et al., 2007) Cercospora spp. are a group of fungal plant pathogens that cause serious leaf spot diseases on a large number of host plants worldwide. The success of this group of fungi as pathogen is attributed to their production of a toxin, cercopsporin, which is considered to be a primary pathogenecity factor. Cercospora arachidicola Hori and Cercosporidium personatum Berk and Curt causing early and late leaf spot of groundnut resulting in 50-80% yield loss (Waliyar 1991). The disease causes leaf necrosis and defoliation and affects crop carbon balance in ways that reduce yield. The sudden appearance of necrotic area in the leaves of sugar beat following infection with Cercospora beticola Sacc, has been attributed to the action of toxin. Evidence for this possibility was first reported by Schlosser (1971). Cercosporin, which was first isolated in 1957 by Kuyama and Tamura belongs to a unique group of molecules known as photosensitizers (Compounds that require light for cellular toxicity). Several lines of evidence indicate that cercosporin plays a crucial role in the ability of Cercospora species to infect host plants. Many members of the genus, Cercospora produce cercosporin and the same can also be extracted from infected tissue (Fajola, 1978). Further investigations suggested that this pigment was a pyrelene derivative having an extended quinone system. Venkataramani (1967) reported the isolation of cercosporin from Cercospora personata which was found to be toxic to groundnut leaves. The chemical structure of the compound was investigated by Lousberg et al., 1971 by spectroscropic means. Additional chemical evidence in support of chemical structure came from the independent work of Yamazaki and Ogawa 1972. Ogawa also presented the data on stereo chemistry of the compound. Ramanujam and swamy (1984) carried out a detailed study on production of Dothistromin a phytotoxic pigment from Cercospora personata. Stoessel. A. (1984) identified dothistomin a phytotoxic pigment from Cercospora arachidicola. The present study deals with the screening of two Cercospora isolates CP_3 and CP_4 and two CP_3 variants CP_3BV and CP_3OV for production of cercosporin. Further experiments were done to study the spectral characters of the pigments along with phytotoxic and cytotoxic functions of cercosporin. Finally, the antimicrobial activity of cercosporin and the two other compounds dothistromin and averufin was also tested. Different experiments and studies on these pigments especially cercosporin of Cercospora were focussed on characterization, purification and phytotoxic properties. As these pigments are the major contaminants of the animal fodder and as the animals being fed with the same, there is always a high risk of entry of these pigments and toxins through the animal body into milk and there by reaching the human body. Unfortunately sufficient data is not available on cytotoxic functions of these pigments. In the present study, we aimed at exploring the cytotoxic properties of these pigments in order to know the possible cause of high rate of incidence of hepatic cancers in local populations consuming such contaminated milk.

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MATERIALS AND METHODS

Fungal strains

A virulent, monoconidial isolate (CP_4) of Cercospora personata, isolated in 2004(Prof. B. Venkatappa, S.K. University, Anantapur.) from naturally infected lesions on groundnut leaves of Anantapur district and CP_3 (Narayana swamy, 1984, University of Madras.) comparatively a less virulent isolate were used throughout the investigation. The cultures were maintained on PDA slants. Prolonged incubations resulted in the production of two variant strains CP_3OV and CP_3BV of the parent isolate CP_3.

Purification and characterization of Pigments:

The cultures were grown on PDA petri plates overlaid with cellophane discs. Crude extracts from the PDA grown isolates were prepared by air drying. Air dried cultures were ground and their components extracted with ethyl acetate. About 75g of mycelial tissue extracted twice with 50ml ethyl acetate. Dried residue was dissolved in 20ml chloroform. Pigments were crystallized directly from the chloroform layer. Spectral grade solvents were used for purification of pigments. Column chromatography was performed with acme silica gel. The purity of products was checked by thin layer chromatography and infrared spectral data was used for the purpose of functional group elucidation.

Host Plants and inoculation:

Commercial varieties of groundnut, TMV_2 and K_6 highly susceptible to tikka leaf spot disease were employed in the study. The seeds were obtained from crop specialist, ICRISAT, Hyderabad, India.

Plants were grown in a green house in earthenware pots (15 cm diam) filled with clay, red soil mixture (2:1) and were watered on alternate days. The temperature in the green house ranged between 28-33^{o}C and relative humidity was 50-80%. Light intensity at plant height was maintained at 5-6000lux. Supplemented light was provided during winter months using banks of incandescent and mercury lamps (Philips, India) from 8.00 a.m. to 6.00 p.m. Sporulating cultures of CP_4 were flooded with sterile distilled water and the conidia were dislodged by gently brushing the culture surface. The conidial suspension was filtered through cheese cloth layers to remove large mycelial clumps. The conidial suspension was once centrifuged at 3000 rpm for 5 min in sterile water and resuspended in distilled water to give a concentration of 2 x10^{5} spores/ml. A drop of Tween 20 was added to the conidial suspension to avoid clumping of conidia. The abaxial surfaces of the third leaves (from below) of 15 day old plants were sprayed with the spore suspension using a glass sprayer, till the leaves were completely wet. The inoculated plants were arranged in plastic sheet-lined shallow trays containing water and covered with polythene hoods (75 x 30 cm). This set up ensured high humid conditions (90-100%) conducive for infection. After 36h, the plants were removed to the green house benches. Plants sprayed with water plus wetting agent and incubated similarly served as control. Symptoms appeared as circular brown specs by the 10^{th} day after inoculation. These turned brown to tan (15-20 days) and later became black and sporulating (20-25 days.). Spots grow in size progressively.

Thin Layer chromatography (TLC)

Separation of pigments from mycelium and infected tissues was carried out on thin layer silica gel G plates. Thin-layers (500 µ) were prepared by layering silica gel (SRL) on 20x 20 cm or 10 x 20 cm glass plates using Dosage Brinkmann applicator. The plates were activated overnight at 100^{o}C and developed in ethylacetate : benzene (2:3v/v) solvent system.

Spectral analysis:

a) The sprayed chromatograms were viewed under long wave ultraviolet light (356 nm) for fluorescence or absorption.

b) Absorption spectra were recorded in the Pyo Unicam SP-800-B spectrophotometer using appropriate blanks.

c) Infra red spectrum was recorded using KBr discs in a Parkin-Elmor infra-red spectrophotometer.

Leaf puncture assay

Leaflets were injured on their abaxial side using the blunt tip of a micropipette (1mm) at equidistant places. A micro drop of the test solution (10-20 µl) was placed on each wound. Leaves were placed in the Petri plates converted into moisture chambers by lining the base with wet filter paper discs. After incubation at 27 ± 1^{0}C under alternating light/dark cycle (12h : 12h) for 72-96 h, the leaves were examined for visible symptoms.

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Measurement of toxicity of compounds (Dothistromin Averufin and Cercosporin)

Toxicity of the compounds was measured on bacterial (B.subtilis) and fungal cultures (R.nigricans and A.flavus). lawn cultures of the bacteria were prepared on Nutrient agar where in fungi were inoculated as agar plugs of the cultures upside down on Malt agar. What man no.1 filter paper discs were soaked in crude culture extracts, 1 mM cercosporin and solutions of the compounds allowed to dry and placed in the nutrient agar and on malt agar 1 cm away from fungal mycelial plugs. Incubated in dark and under constant light. Fungal inhibition was noted 2 days after treatment and bacterial inhibition as clearing in the lawn around discs 2 days after incubation.

Measurement of Cytotoxic effect of cercosporin

Haemolysis

This was assayed by the method of Boyum (1968). For experiments in an inert atmosphere the red blood cell suspension (400 ml) was placed in a test tube (15 ml) which was closed with a rubber septum and then purged with oxygen free N_2 admitted and voided by means of hypodermic needles for 45 minutes. The needles were removed and cercosporin solution (10 ml) was injected through the septum in a dark room. Experiments in the presence of air were done similarly in open tubes not purified with N_2. The blood was taken from healthy staff from our laboratory. It was stabilized by the addition of heparin and stored at 4^0 C in the dark until used. Peripheral Blood Lymphocytes were isolated from fresh heparinised venous blood from healthy persons. 10 ml of venous blood was drawn from the donor after obtaining his consent using 10 ml vacuette tubes, coated with heparin. Blood was then diluted with equal volume of RPMI 1640 (Rosewell Park Memorial Institute 1640) medium and carefully layered over lymphoprep (Sigma, USA). After centrifugation at 1800 rpm, at 25^0 C the whole lymphocyte cell layer seen at the interface was carefully transferred to a tube containing 5 ml of RPMI 1640 medium. The cells were thoroughly mixed with medium and washed by centrifugation for 10 minutes. The procedure of washing with RPMI 1640 medium was repeated thrice and the final suspension was made in 1 to 5 ml of RPMI 1640 medium. Peripheral blood lymphocytes are used for measuring the cycotoxicity of cercosporin. PBL ($1x10^6$) in RPMI 1640 medium were incubated for two hours at 37^0 C under air with cercosporin as log concentrations, 50 µl, 150 µl and 200 µl. Control subjects were tested as lymphocytes pre incubated with RPMI 1640 medium alone. The plasma membrane of a viable cell does not permit the entry of non-electrolyte dye substance. This phenomenon is used to distinguish dead from living lymphocytes. Percentage of viability was calculated as follows: (Trypan blue dye exclusion test)

$$\% \text{ of viable cells} = \frac{\text{No. of viable cells}}{\text{No. of viable cells} + \text{No. of dead cells}} \times 100$$

RESULTS

Pigments produced by C.personata

The single spore isolate CP_3, its variants orange (CP_3OV) brown (CP_3 BV) and the new virulent isolate CP_4, were used to study pigment production. After 4 weeks of growth under blue light, they were harvested, extracted twice with ethyl acetate and the pigments were purified. The pigments were separated into light yellow and orange red bands .A small deep red band in addition to yellow and orange red bands were collected adding an equal volume of 2% HCl to it. The mixture was allowed to stand at 4^oC for 2-3 days when crystals appeared.

TLC separation of pigments

The isolates were grown on PDA. The mycelium was extracted with ethyl acetate, the extracts were concentrated, chromatographed and developed in solvents. The isolates produced dothistromin and averufin (Table 1). Cercosporin pigment was identified only from CP_4 isolate. (Fig 1A & 1B). When infected leaf tissue extracts were subjected to TLC, only small quantity of cercosporin was detected from CP_4 infected leaves but not from CP_3 infected ones. Mobility of CP_3 and CP_4 pigments in different solvents is presented in table 2. In the process, the yellow pigment emerging first from the silica gel column was discarded (this was later identified as averufin) and only the red pigment emerging later was isolated and used. This pigment has been referred to as 'CP_4 pigment.' It was found out later that the CP_4 pigment was a mixture of dothistromin and one of it's derivatives.

Comparison of CP3 pigment with Cercosporin

Chemical tests

The CP_3 pigment and cercosporin were subjected to chemical tests. On reduction with Zinc dust in glacial acetic acid CP_3 pigment became purple and cercosporin gave yellowish green colour. With the addition of 0.1 N NaOH yielding clear green colour. Cercosporin gave red colour with ferric chloride whereas CP_3 pigment gave reddish brown. Addition of concentrated sulfuric acid to CP_3 pigment gave violet colour and cercosporin gave purple (Table 3.) Cercosporin when dissolved in dilute alkali yielded a green colour (Characteristic feature) and in a reduced state, it gave bright yellow colour with intense green fluorescence.

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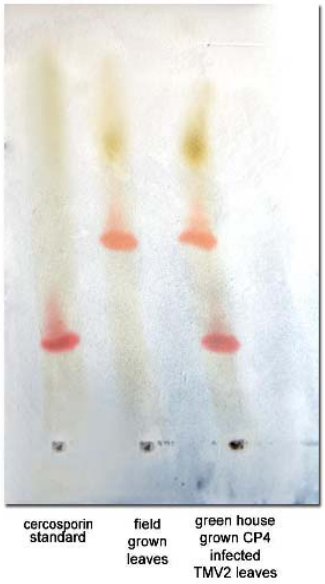
Ethylacetate extracts of CP3 and CP4 isolates grown on PDA were concentrated, chromato graphed and devoloped (Ethylacetate : Benzene 2 : 3 v/v)

Figure-1A: TLC showing pigment isolation from CP3 and CP4 culture filtrates



Ethylacetate extracts of field grown infected leaves and green house grown CP4 infectedTMV2 leaves were chromatographed and devoloped (Ethylacetate : Benzene 2 : 3 v/v)

Figure-1B: TLC showing pigment isolation from CP3 and CP4 culture filtrates

Table-1: Variability of isolates of C.personata to produce pigments

Isolate	Pigment
CP_3	Dothistromin Averufin
CP_3OV	Dothistromin Averufin
CP_3BV	Dothistromin Averufin
CP_4	Dothistromin Cercosporin Averufin

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Table-2: Mobility of CP_3, CP_4 pigments in different solvent systems

Solvent system	CP_3 Pigment	CP_4 Pigment
Benzene: acetic acid: Water (2:2:1v/v)	0.71	0.60
Ethyl acetate : Methanol (4:1 v/v)	0.84	0.64
Ethyl acetate: benzene (2:3v/v)	0.83	**0.13**

Table-3: Comparison of reactions of CP_3 and CP_4 pigments with chemical reagents

Test	Reaction with CP_3	Reaction with CP_4 pigment (Cercosporin)
Reduction with Zinc dust in glacial acetic acid	Purple	Yellowish green
Addition of 0.1N NaOH	Purple	Green
Addition of methanolic ferric chloride	Reddish brown	Red
Addition of conc: sulfuric acid	Violet	Purple

Spectral Characters of CP_4 pigments: dothistromin, cercosporin and averufin

Ultraviolet spectra of CP_3, CP_4 pigments:
The absorption spectra of CP_3 pigment in methanol is shown in Fig.2 (λ max) 528, 467, 283, 240, 220 and 213 mm. The absorption sepctra of CP_4 pigment (cercosporin) in methanol were (λ max) 565, 471, 322, 270 and 222 mm (Fig. 3).

Absorption spectra of averufin:
The absorption spectra of averufin in methanol (λ max) 451, 323, 294, 266, 225 nm (Fig.4) matched with averufin (Stoessl,1984).

Infra red spectrum of dothistromin and cercosporin
The infra red spectra of dothistromin from CP_3 and CP_4 isolates is presented in Fig.5, and contains five hydroxyl groups.
The identity of the deep red substance with cercosporin is further supporetd by infra red spectrum (KBr window) were 3,400, 2,940, 1,623, 1,585 1,554, 1,455, 1,428, 1,395, 1,348, 1,315, 1,268, 1,223, 1,170, 1,145, 1,133, 1,075, 1,055, 1,017, 978, 938, 921, and 850 cm^{-1}.

The infra red spectrum of cercosporin shows a quinone carbonyl 1,169 cm^{-1}(Fig.6). Whenever quinone system was present in one nucleus it showed a maximum at 1,600-1,680cm^{-1}. The cercosporin from CP_4 isolate displayed maximum at 1,635 cm^{-1} indicating the quinone system is extended through more than one ring. On the basis of infra red spectral data, it is reasonable to conclude that cercosporin is a polyhydroxy derivative of a polycyclic quinone, and has an extended quinone system. (Table 4).

Table-4: Spectral characters of Cercosporin, Dothistromin and Averufin

Substance	Molecular formula	m/e molecular wt.	(λ max)
Cersosporin (CP_4)	$C_{29}H_{26}O_{10}$	534	222, 270, 322, 471, 565
Dothistromin (CP_3 OV, CP_3BV)	$C_{18}H_{12}O_9$	372	233, 280, 480, 490, 509, 524
Averufin (CP_3 OV, CP_3BV)	$C_{20}H_{16}O_7$	368	225, 266, 294, 323, 451

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The striking colour change (red to green) up on the addition of 0.1N NaOH confirmed the conversion of quinones to deep red pigment. It is noteworthy that very few quinones gave green colour in alkaline solutions. The band at 1,623 cm^{-1} in the IR spectrum of red pigment is consistent with an extended quinone. The results indicate that the red pigment contains two quinoid carbonyls, two phenolic hydroxyl and two alcohol hydroxyl groups. From UV and IR data of red pigment, it is reasonable to conclude that this substance is the same as cercosporin.

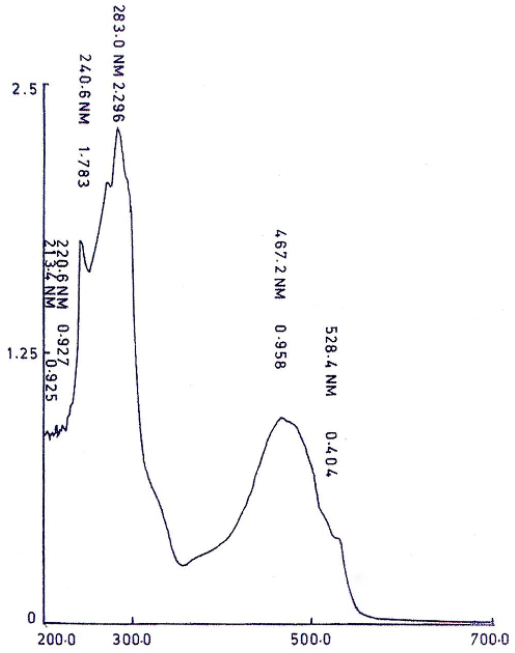

Figure-2: Ultraviolet Spectrum of CP3 Pigment

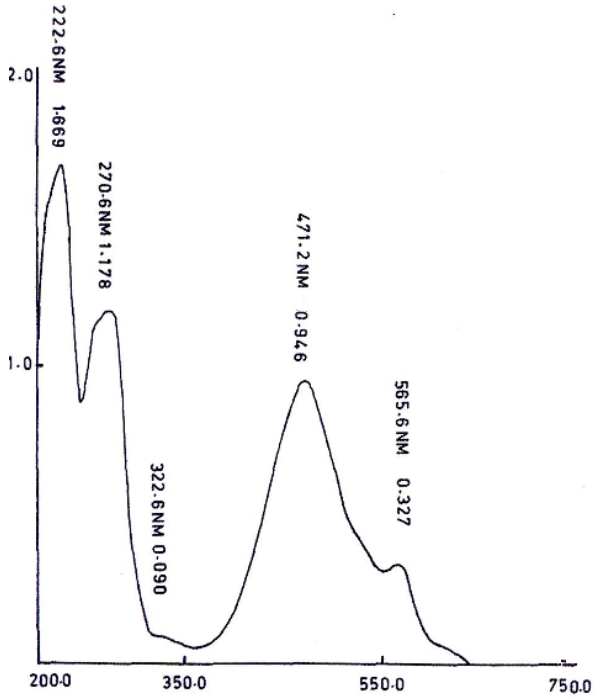

Figure-3: Ultraviolet Spectrum of CP4 Pigment (Cercosponin)

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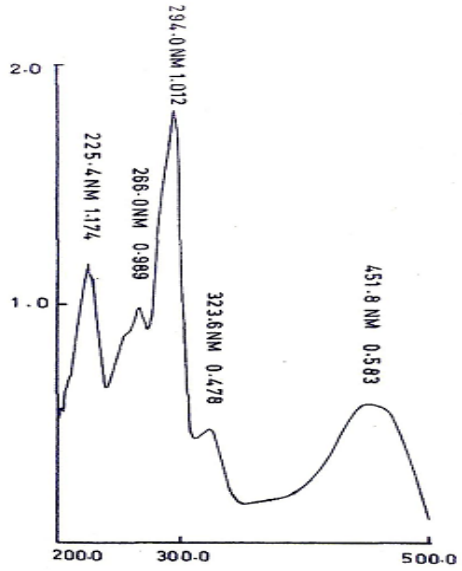

Figure-4: Ultraviolet spectrum of averufin

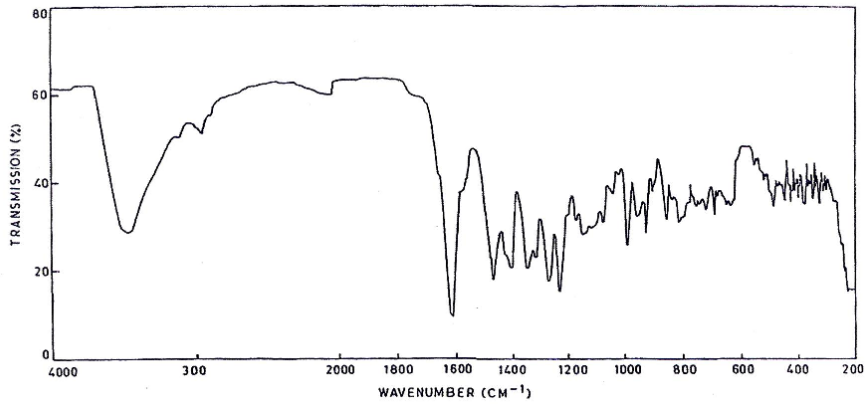

Figure-5: Infrared Spectrum of dothistromin

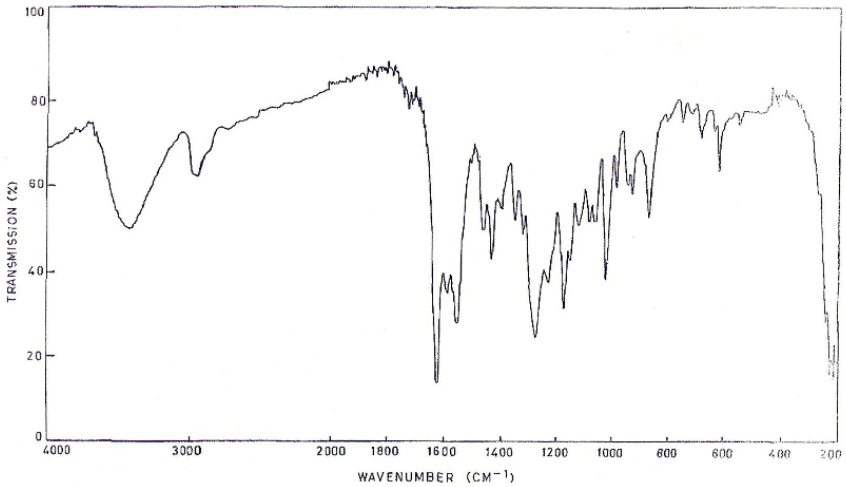

Figure-6: Infrared spectrum of Cercosponin

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Toxicity measurement of the Cercosporin, Dothistromin and Averufin:

Both dothistromin and averufin exhibited toxicity to microorganisms tested. (table.5) A.flavus and B.cereus were inhibited by dothistromin and averufin in both light and dark. Neither organism was affected by the crude culture extracts. Presumably, the two compounds were not as concentrated in crude culture extracts and therefore did not inhibit growth even though the extract contained the compounds. Growth of R.nigricans was inhibited by the culture extract in both light and dark but not by either dothistromin or averufin. Cercosporin exhibited light dependent inhibition of A.flavus but not of R.nigricans, as expected. Cercosporin also did not inhibit growth of B.cereus**.**

Table-5: Action of Crude culture filtrates, Dothistromin, Averufin and Cercosporin in the light and the dark

Microorganism	Crude extract	Dothistromin	Averufin	Cercosporin
Rhizopus nigricans	L/D	……	…….	…….
Aspergillus niger	……	L/D	L/D	L
Bacillus cereus	.....	L/D	L/D	…….

L = inhibition in light D = inhibition in dark **.....** = no inhibition

Haemolysis

Cercosporin has broad spectrum toxicity (Stoessl et al, 1990) and is produced during early growth stages of the fungus. The toxicity of cercospoin to plant host tissue, and indeed to most other cell types, is dependent on the presence of light. (Daub & Ehrenshaft 2000). The broad toxicity of cercosporin to many cell types presumably reflects its action as a producer of oxygen radicals. Cercosporin, causes lipid peroxidation and breakdown of photosynthetic pigments and exhibits higher rates of toxicity against RBC and lymphocytes. Haemolysis of human blood cells was observed only in the presence of air and is concentration dependent. (Table 6).

Table 6: Haemolysis of Human Red Blood Corpuscles by Cercosporin

Time	%Haemolysisa			
	N_2		Air	
	D[b]	C[c]	D	C
0	9	4	9	3
0.5	11	-	12	-
1.0	14	8	30	5
11.5	15	-	81	-
2.0	14	4	100	3

a * Referred to complete haemolysis caused by 10% Trition X-100 (10 µl – 0.4 ml red blood cell suspension) as estimated by absorbance A at 540 nm. [b] 2.5×10^{-4} M cercosporin in 1% DMSO. [c]Control 1% DMSO.

Toxic effect of cercosporin on PBL's

The difference in the peripheral blood lymphocytes death between control and cercosporin treated groups are markedly increased (Table 7), suggesting the test system does have sensitivity. In vitro cytotoxicity assay utilized in this project has been used to determine the ability of different human populations sensitive to fungal pigment cercosporin. This lymphocyte bioassay is very useful to assess cell defense mechanisms against toxic metabolites formed from carcinogenic fungal toxins. It has been suggested by Sheer et al (1988) that in vitro cytotoxic assays could be used prospectively to individuals sensitive to drugs and toxicants and the results obtained with cercosporin in the present project are consistent with Sheer's hypothesis. The in vitro cytotoxicity assay which has been described shows a high degree of biological, toxicological and immunological specificity and does help in differentiating immune and immuno suppressive subjects.

Table 7: The effect of Pre-incubation with Cercosporin on Lymphocyte Cytotoxicity

Cercosporin (conc.µl)	%Cell death
50	14.8±1.0
150	58.6±1.6
200	84.2±2.8
RPMI 1640[a]	4.1±0.7

[a]Control, lymphocytes pre-incubated with RPMI 1640 medium. Results are expressed as mean ± S.E. mean $P < 0.05$.

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DISCUSSION

Cercospora personata infection was established on detached leaves of susceptible (TMV_2) and resistant (K_6) groundnut cultivars. Development of symptoms has been similar to that occurring in intact plant leaves, although detached leaf technique does not require any additional supply of nutrients. But in the case of biotrophic fungi like groundnut rust, Puccinia arachidis, the maintenance of the rust culture on rooted leaves required nutrient solution. (Subramanyam et al., 1981). C. personata grew very slowly. The first visible symptoms of disease are the appearance of brown lesions 10- 12 days after inoculation. These lesions continue to expand for 3-4 weeks before reaching the final size 3-5 mm on TMV_2 variety. A few isolated spots produced on some resistant varieties like K_6 are very minute. Characteristically leaf spot damage occurs primarily on older leaves. The influence of timing and degree of defoliation on groundnut crop is very crucial. Symptom development and defoliation studies with TMV_2 groundnut plant revealed that the pod quality and quantity declined compared to the control plants, which had more foliage than defoliated plant. Field studies were also made to understand the infection and symptom development. Susceptible cultivar (TMV_2) shows larger amount of spots and defoliation than resistant (K_6) cultivar. Restriction of pathogen by resistant host plants involves several factors and mechanisms. One important mechanism is 'prohibition concept' forwarded by Mahadevan (1970). Prohibitins may act in different ways in the plants, like inhibition of spore germination and inhibition of function of microbial toxins. Indeed Kalaichevan (1980) found the presence of phenolic prohibitin in groundnut cultivars which strongly inhibited a variety of fungi and bacteria. Detached rooted leaf technique is very useful in evaluating cultivar reactions, disease management programmes under uniform controlled environmental conditions by using known amount of spores.

From the original CP_3 isolate of C. personata, two variants were obtained, one producing orange pigment (CP_3OV) and the other brown pigment (CP_3BV). These two variants differed from the original isolate in the production of conidia and in the morphology and size of the conidium. The growth also differed between the isolates.CP_3 and the new isolate CP_4 showed moderate and high virulence where as the two variants were less virulent. The production of toxin also differed among the four strains. CP_4 new isolate produced cercosporin, dothistromin and averufin where as CP_3 and it's variants produced dothistromin and averufin. Clearly production of cercosporin varies from strain to strain. Similar observations between strains, in pigment production have been reported in C. Beticola (Assante et al.,1977b, Daub & Ehrenshaft 2000) as wellas in C. apii and C.zebrine (Lynch and Geoghegon, 1977; Assante et al., 1977b). Cercosporin can be isolated from lesions on infected plants.(Upchurch et al,1991). When attempts were made to extract cercosporin from field infected leaf tissue it was detected neither in initial stage of infection nor in the later stages of development but large amount is detected in glass house CP_4 infected groundnut leaves. Indicating that not all strains of Cercospora produce cercosporin. Present methods allowed us to detect even low concentrations of cercosporin, including loss factor during extraction. We were able to extract cercosporin at a concentration of 1 to 2µg/g of whole groundnut leaves (K_6 cultivar) inoculated with C.personata (CP_4). This value agrees with Fajola (1978), who reported extraction between 1 to 10 µg of cercosporin from 1gram of infected tissue. Results are agreeing with Stoessl. We have not detected cercosporin from infected tissue with isolate (CP_3). Stoessl (1984), did not detect in vitro production of cercosporin in an isolate of C.arachidicola. Dothistromin and averufin were produced in large quantities Unfortunately little is known about the role of these two toxins in development. It appears that some factor other than cercosporin must be responsible for the necrosis. The crude culture extracts of C.personata isolates were phytotoxic and absorption maxima of two C.personata pigments were different from cercosporin of CP_4 isolate. Although the red pigment in methanol and other organic solvent was stable on exposure to light, it was photosensitive in dilute alkaline solution. The colour change (red to green) on addition of the dilute alkali confirmed the presence of phenolic hydroxyl quinone in deep red pigment from CP_4 isolate. The band at 1,623 cm^{-1} in the IR spectra of red pigment is consistent with an extended quinone system (Johnson et al., 1961). The results indicate that the red pigment contains two quinine carboxyl, two phenolic hydroxyl and two alcohol hydroxyl groups, from UV and IR spectrometric data of the red pigment. The red pigment produced by C.personata is a naturally occurring dihydroxy-perylenequinone, is unusual in having a methylenedioxy-group present in a seven membered ring bridging a biphenyl system, which may act as photoreceptor molecule in fungus. Many of the best characterized photoresponse spectra have maximum response to the 380-480 nm region. The upper limit being about 520nm (Carlile,1970). Cercosporin was characterized based on absorption spectrum of purified red fraction in methanolic solution, change of colour from red to green under alkaline conditions, red colour with ferric chloride, ready solubility in chloroform, ethylacetate and acetone, insolubility in water and petroleum ether with Rf value is 0.13 with ethylacetate : benzene (2:3)(v/v) as eluent. Dothistromin is another substance isolated from all strains of C. personata. While characterizing the dothistromin, IR spectrum of dothistromin were almost identical to that of averantin from A.parasiticus (Bennett et al.,1980).

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All spectral characters of dothistromin are closely related to averantin which is an aflatoxin B_1 precursor. Aflatoxins are a group of toxic carcinogenic secondary metabolites produced by certain strains of Aspergillus flavus and A. Parasiticus (Turner and Aldridge, 1983). Averufin and versicolorins have long been regarded as mycotoxins (Cole and Cox, 1981). Dothistromin has considerable importance as a phytotoxin implicated in Dothistroma pini (Shain and Franich, 1981) and in C. personata (Ramanujam and Swamy, 1984). The infected groundnut leaves contained dothistromin as a major metabolite. Even though dothistromin earlier isolated from Cercospora smilacis has been referred to as a phytotoxin (Assante et al., 1977a), there is no information about the nature of it's phytotoxicity. The antimicrobial activity of cercosporin and dothistromin indicated that they are toxic to both fungi and bacteria. The bacteria which were able to degrade cercosporin were resistant to the action of cercosporin where as the non cercosporin degrading isolates were inhibited by cercosporin. This is because the cercosporin degrading bacteria convert cercosporin into a non toxic product, so able to overcome the action of cercosporin. Perhaps antimicrobial activities of cercosporin and dothistromin has an important character acting like the classical antibiotics and could minimize the competition from other microflora occupying the same site. The results obtained with dothistromin in the present study are consistent with (Shear et al 1988). In vitro cytotoxicity assay utilized in this study has been used to determine the ability of different human populations sensitive to fungal toxin dothistromin. Lymphocyte bioassay is very useful to assess cell defense mechanisms against toxic metabolites formed from carcinogenic fungal toxins of C.personata. It has been suggested by Shear et al (1988) that in vitro cytotoxicity assays could be used prospectively to individuals sensitive to drugs and toxicants and the results obtained with dothistromin and cercosporin in the present study are consistent with Shear's hypothesis. The in vitro cytotoxicity assay which has been described shows a high degree of biological, toxicological and physiological specificity. The production of such a generalised toxic compound as cercosporin may help to explain the almost universal pathogenecity of the genus. Like other perylenequinones, (Hypocrellins-Hudson 1994) cercosporin is photodynamically active. This photodynamic nature is linked with the photosensitive activity of the cercosporin. The above investigations collectively provide a compelling rationale for the development of cercosporin as photodynamic therapy photosensitizer. It is note worthy that photoactivated hypocrellins can selectively inhibit the growth of human colorectal carcinoma cells. Thus on the similar lines the molecular mechanisms of apoptosis induced by photoactivated cercosporin are worth further investigation.

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