S. L. J. Tea Sci. 64 (1/2), 5-11, 1996. *Printed in Sri Lanka.*

A PRELIMINARY STUDY OF THE PHYTOALEXINS PRODUCED IN THE TEA LEAF IN RELATION TO THE BLISTER BLIGHT LEAF DISEASE CAUSED BY *EXOBASIDIUM VEXANS* MASSEE OF TEA

S. M. Nagahaulla, N. L. Herath and P. A. N. Punyasiri
(Tea Research Institute of Sri Lanka, Talawakele, Sri Lanka)

Leaf tissues obtained from *Exobasidium* infected leaves of different stages such as translucent spots (TS), chlorotic areas (CA) and mature blisters (MB) and healthy leaf tissues (HL) were used in this study, in order to determine the presence of antifungal compounds such as phytoalexins.

Attempts were made to identify these chemicals by means of the thin layer chromatographic technique.

Spore germination of the *E. vexans* was inhibited in the media prepared using extraction of translucent spots and healthy tissues, while extracts prepared in 95% ethanol in the presence and absense of polyvinylpyrrolidone, indicated the presence of antifungal zones when spotted in the TLC plate.

It was seen that antifungal compounds are produced in the tea leaf as a result of the infection of the blister blight pathogen, and these may contain polyphenolic compounds which do not absorb UV radiation at 254 – 366 nm.

INTRODUCTION

The mechanism of disease resistance is two fold. It involves physical and chemical barriers which keep out infective agents from the host. This response mechanism leads to the inhibition of the development of penetrated organisms. Phytoalexins are a group of antifungal or antimicrobial compounds involved in this process (Bailey and Deverall, 1971).

Phytoalexins are produced by plants in response to infection. Their existence was postulated by Muller and Borger (1941) long before any chemical entity was detected as a phytoalexin. These compounds are also defined as antibiotics (Muller, 1958) and known to occur at micro-sites within the plant tissues where they might contact a parasitic bacterium or fungus (Kiraly, Barana and Ersek, 1972). They are synthesized as a result of the stimulation of specific metabolic pathways by some process which occur during infection.

Exobasidium vexans Massee which causes the blister blight disease of tea is an obligate parasite. The relationship that exists between the pathogen and the host is

biotrophic, which can last for several days. During this period phytoalexins are not produced. They can be detected only after infected tissues have collapsed (Bailey and Deverall, 1971). Their concentration usually remain low resulting in a spreading lesion rather than a restricted one. However under certain circumstances they are produced in greater quantities resulting in restricted lesions. This is called the hypersensitivity reaction (Bailey, Rowell and Arnold, 1980).

The tea leaf also allows the pathogen to grow only in a limited circular area in the infected leaf probably due to the resistance mechanism of the phytoalexins produced during infection.

This study was undertaken to detect the location of phytoalexins and to identify them.

MATERIALS AND METHODS

In these experiments the following stages of the infected leaves were used:

1. Healthy tissue (HL)
2. Translucent spots (TS)
3. Mature blisters (MB)
4. Chlorotic areas (CA)

Experiment 1.

Testing the germination of the blister blight spores in different media

Four media were prepared using the above extracted tissues and the germination of blister blight spores in each of these media were observed.

The medium included the following materials.

Distilled water	150ml
Dextrose (Oxoid Ltd. Basingstoke, Hants., England)	1g
Agar (Oxoid Ltd. Basingstoke, Hants., England)	3g
Leaf material	1g

Experiment 2.

Bioassay of the antifungal component

Mature blisters, translucent spots, healthy leaf tissues and chlorotic areas were taken separately and stored in the deep freezer (–7°C) for two days. Two of each tissue was macerated in 95% ethanol adding 2 ml amounts gradually and filtered through a Whatmann No. 1 filter paper and washed the residue twice with 10 ml portions of the same solvent and combined the washings. The original filtrates were concentrated

in a rotary evaporator at 45°C to a volume of 1 ml.

Aliquots (110µl) of this crude concentrate were spotted on a thin layer silica gel chromatogram (70–230 mesh ASTM Art. 7734 Kieselgel 60). The mobile phase was chloroform:methanol 9:1 (v/v).

The developed chromatogram was dried and examined under UV illumination between 254 nm and 366 nm.

The dried chromatogram was sprayed with a spore suspension of the fungus *Cladosporium fulvum*, which was prepared in Czepakdox liquid medium and incubated in a humid chamber at room temperature (22°C).

Experiment 3.

Identification of the antifungal compounds

Mature blister tissues, translucent spots, healthy leaf tissues and chlorotic areas surrounding the blister were extracted in 95% ethanol in the presence and absence of polyvinylpyrrolidone (Polyclar–AT).

Polyclar–AT (5g) was added to 2g portions of the above mentioned tissues in 95% ethanol and macerated according to the procedure in experiment 2; the developed chromatogram was dried and examined under UV illumination at the same ranges as the bioassaying of the antifungal component.

RESULTS AND DISCUSSION

Experiment 1.

TABLE 1 – *Spore germination in the various types of tissues*

Type of tissue	*Spore germination*
HL	No germination
TS	No germination
MB	Germination observed
CA	No germination

The blister blight spores had germinated in the media prepared using mature blister tissues. No germination was noted in the other media prepared using HL, TS or CA tissues. This may be due to a fungal toxic substances or an fungal inhibitory compound/s present in the HL, TS and chlorotic areas as these media inhibited the germination of the blister blight spores.

It is probable that the healthy leaf tissues (HL), chlorotic areas (CA) and translucent spots would have contained spore germination inhibitory compounds.

It was indicated by Bailey and Deverall (1971) that plant phenolic compounds reduce the spread of lesions by retarding the germination of the fungal spores.

Mature blister tissues had not inhibited the germination of spores as this is the tissue where blister had successfully grown. This indicates that they may not contain fungal inhibitory compounds specific to the *Exobasidium* fungus.

Experiment 2.

When the thin layer chromatogram was examined under UV light between 254–366 nm, there were visible red colour spots in the zone where the healthy leaf extract was spotted. However the colour spots do not overlap with the inhibitory zones on the chromatogram.

Germination of the *Cladosporium fulvum* spores were observed on the chromatogram after five days and it showed distinct areas where fungal growth was not visible (Fig. 1). When the experiment was replicated it could be confirmed that these inhibitory zones tended to become larger with increasing concentration of the spots on the plate.

Rf values of these zones were noted down.

TABLE 2 – *Rf values (cm)*

Spots	*Upper limit*	*Lower limit*	*Average*
HL			
1.	14.1	11.1	12.6
2.	14.0	10.7	12.4
3.	13.2	8.1	10.7
TS			
4.	13.8	11.1	12.5
5.	14.0	11.3	12.7
6.	13.8	11.0	12.4
MB			
7.	14.0	11.3	12.7
8.	13.8	11.3	12.6
9.	10.9	13.5	12.2

It is clearly noted in this experiment that these extractions contain fungal inhibitory compounds, which resulted in the inhibition of spore germination of the fungus *Cladosporium fulvum.*

S. L. J. Tea Sci. **64** (1/2), 5-11, 1996. *Printed in Sri Lanka.*

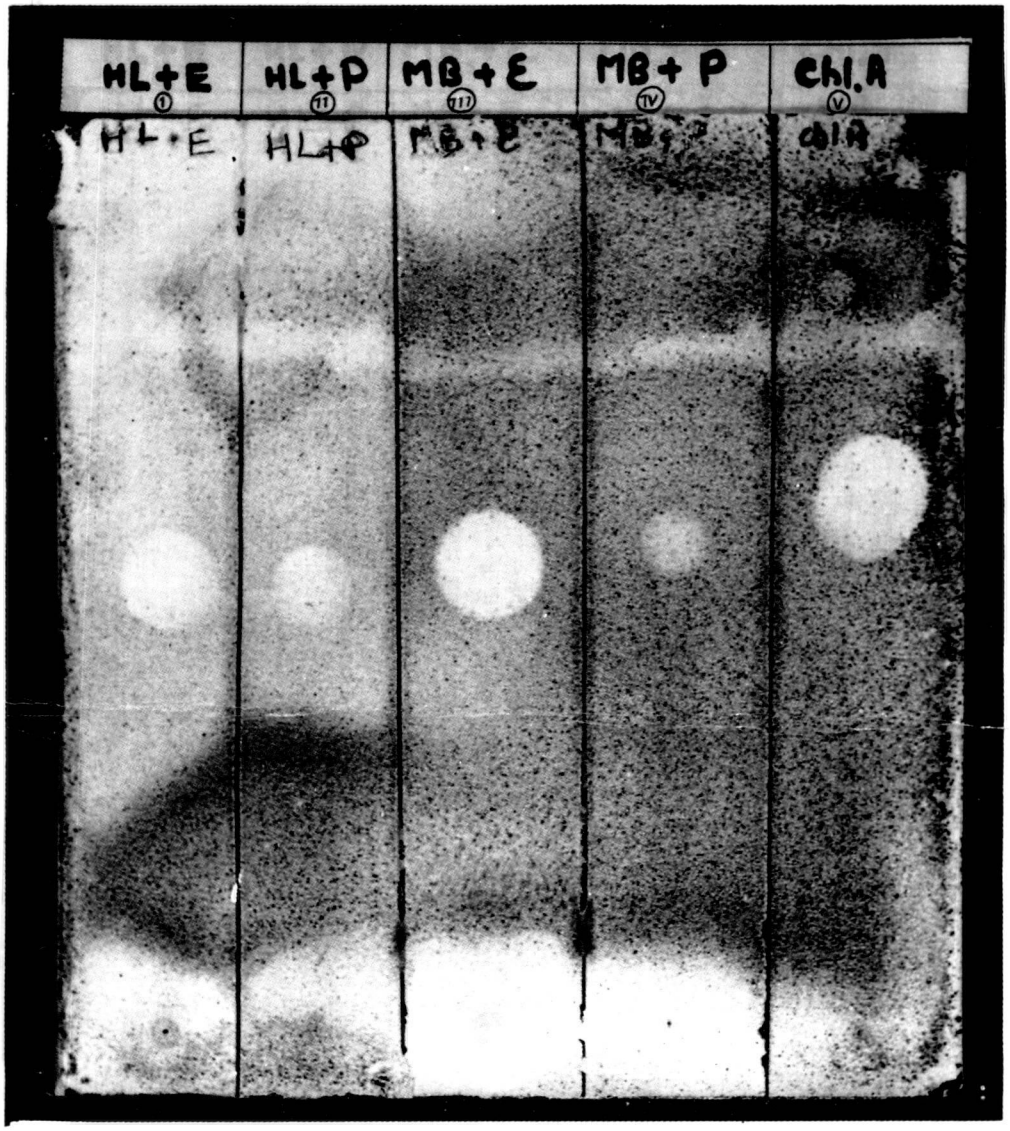

Fig. 1 – *Illustration of fungal inhibitory zones of different leaf extracts on thin layer chromatogram.*

S. M. Nagahaulla, N. L. Herath and P. A. N. Punyasiri

When the same procedure was adopted using acetone as the solvent, UV illumination indicated the presence of red colour spots at the zones of both TS and HL.

In these bioassays the fungus *Cladosporium fulvum* was used which caused the leaf mould of tomato as this fungus is often used as a standard in similar experiments (Afek and Sztejnberg, 1988). Further it shows rapid growth in favourable conditions and has a distinct colour of olive green which can be clearly detected against the white background of the chromatogram.

The bioassay indicated the presence of antimicrobial compounds in healthy leaf tissues, translucent spots and the chlorotic areas. Chlorotic areas are the material taken from the surrounding layer of the blister where phytoalexins are likely to be accumulated and it also showed the highest level of antifungal component with the biggest diameter (Table 3).

Examination under UV illumination revealed that these compounds do not absorb UV light at 366 nm since the red colour spots do not overlap with the inhibitory zones in the chromatogram.

Healthy leaf tissues showed formation of phytoalexins probably due to the cell injury that occurred during the extraction procedure, as wounding can induce secretion of the enzymes responsible for the metabolism of phytoalexins (Muller, 1958). It is known that the tea leaf tissues contain material capable of initiating synthesis of appropriate enzymes which remain inactive until the cells are injured and release material by membrane dysfunction (Bailey and Deverall, 1971).

Experiment 3.

Inhibitory zones were observed in HL, MB and chlorotic areas with and without polyclar (Fig. 1). Chlorotic areas produced the largest inhibitory zones while with polyclar the areas tended to become smaller. The role of polyvinylpyrrolidone is to bind polyphenolic compounds.

The Rf values and the diameters of the inhibitory zones were noted.

It could be noted that the higher percentage of the antifungal compounds probably contains polyphenols as diameters of the inhibitory zones decreased with the adding of polyclar (Table 3).

TABLE 3 – *Rf values (cm)*

Extract	*Rf values*	*Diameter of the inhibitory zone (cm)*
HL+Et	9.2	2.0
HL+P	9.2	1.8
MB+Et	9.5	2.4
MB+P	9.8	1.4
CA+Et	11.1	2.5

Solvent front is 16.3 cm

HL–Healthy tissues, Et–Ethanol, P–Polyvinylpyrrolidone
MB–Mature blister tissues, CA–Chlorotic areas.

The resistance resulting in the host-pathogen interaction of *Exobasidium vexans* and the tea leaf can be attributed mainly towards the phytoalexins produced in the tea leaf in response to the infection.

The other antimicrobial compounds produced also may respond in a similar way together with the phytoalexins as defense mechanism against the pathogen.

However it was evident in some tea clones that these lesions are restricted to small areas whereas in other leaves they seem to grow all over the leaf as large patches. This is probably due to the genetic variability of the tea clones, which can be expected to produce different quantities of phytoalexins. Therefore a relationship can be easily established between the phytoalexins production and the resistance of the tea plant *Camellia sinensis* to the blister blight pathogen.

These findings would help the plant breeder too in differentiating the clonal characters.

Identification attempted in this study suggested that these compounds could be of polyphenolic, non polyphenolic or both and probably not absorbing UV radiation at the tested range 254 – 366 nm.

Taking above information into consideration and with proper identification of the respective compounds involved it is probable that a suitable systemic fungicide could be developed against the blister blight disease of tea.

REFERENCES

AFEK, U. and SZTEJNBERG, A. (1988). Accumulation of scoparone, a phytoalexin associated with resistance of citrus to *Phytopthora citropthora. Phytopath.* **78,** 1678–1682.

BAILEY, J. A. and DEVERALL, B. J. (1971). Formation and activity of Phaseolin in the interaction bean hypocotyls *(Phaseolus vulgaris)* and physiological races of *Colletotrichum lindamuthianum. Physiol. Plant Path.* **1,** 435–449.

BAILEY, J. A., ROWELL, P. M. and ARNOLD, G. M. (1980). The temporal relationships between host cell death, phytoalexin accumulation and fungal inhibition during hypersensitive reactions of *Phaselous vulgaris* to *Colletotrichum lindemuthianum. Physiol. Plant Path.* **17,** 329–339.

DEVERALL, B. J. and VESSEY, J. C. (1969). Role of phytoalexins in controlling lesion development in leaves of *Vicia faba* after infection of Botrytis spps, *Ann. appl. Biol.* **63,** 449–458.

FRIEND, J. and THREFALL, D. R. (1975). Biochemical aspects of plant-parasite relationships; Proc. Phytochem. Soc. Symp. University of Hull, England. Academic press, London, pp 1–35.

GOODWING, R. H. (1939). Germination inhibitors in plant material. *Amer. J. Bot.* **26,** 449–458.

KIRALY, Z., BARANA, B. and ERSEK, T. (1972). Hypersensitivity as consequence not cause of plant resistance to infection. *Nature.* Lond. **239,** 456–458.

MULLER, K. O., and BORGER, H. (1941). Experimentelle untersuchungenuber die Phytopthora Resistenze derkartoffel. Arb. biol. Anst. (Reichaust) Berl. **23,** 189–231.

MULLER, K. O. (1958). Studies on phytoalexins, the formation and immunological significance of phytoalexins produced by *Phaselous vulgaris* in response to infections with *Sclerotinia fructicola* and *Phytopthora infestans. Aust. J. biol. sci.* **2,** 275–300.