# POST-INFECTIONAL COMPOUNDS AND PHYTOALEXIN RESPONSE IN *SYZYGIUM CUMINI* (L) SKEELS

# CHAPTER - VI

## POST INFECTIONAL COMPOUNDS AND PHYTOALEXIN RESPONSE IN SYZYGIUM CUMINI (L.) SKEELS

Syzygium cumini is an evergreen tree found all over India. It is found in forests upto an elevation of 6000 ft. It is also cultivated in gardens for their edible fruits and also planted as avenue trees.

S. cumini is a large tree, 10-15 m tall with dark stem and shining green foliage in a dense crown. The bark is smooth and light grey to brown in color. Leaves are leathery, smooth, 6-14 cm long, elliptic-oblong or elliptic lanceolate. The trees shed their leaves during January and February. Flowers 0.4-0.8 cm across, pale greenish white, fragrant, crowded in short racemes arising below the leaves, rarely in the axils of the leaves. The blooming period is from March to May. Fruits are produced in the month of June and July. Fruits 1-2 cm across, ovate or oblong, glabrous, purplish when young, almost black when ripe. Seed usually one, ellipsoidal, oblong, white or pinkish.

The wood of S.cumini is hard and durable and is used as fuel and also for making agricultural implements. The bark is astringent and it is used in the form of decoction, for

sore throat, bronchitis, asthma, ulcers and dysentry. It is also given for purifying blood. The fresh juice of the bark is used to cure diarrhoea. The seeds are used against diabetes. The fruit juice is also hypoglycaemic but the effect of preparation from seeds is more marked.

Bhargawa et al.,(1974) reported betulinic acid, friedelin, epifriedelanol, β-sitosterol-D-glucoside, kaempferol-3-D-glucoside, kaempferol, quercetin, gallo- and ellagitannins in the stem bark of S.cumini. The bark also contains gallic acids, resins, starch and proteins. Flowers of S.cumini contain acetyloleanolic acid, ellagic acid and flavonoids such as kaempferol, quercetin, myricetin, isoquercetin, myricetin-3-L-arbanioside, quercetin-3-D-galactoside and dihydromyricetin (Nair and Subramanian, 1962; Subramanian and Nair, 1972).

Rao and Joseph (1971) have reported that essential oil from S. cumini inhibited the growth of certain phytopathogenic fungi. Tannins from S.cumini inhibited the growth of two pathogenic fungi, Colletotrichum falcatum and Pyricularia oryzae (Janardhanan et al., 1963).

Leaf spot disease in S.cumini was found to occur in the months of November and December. In the present investigation, healthy and infected leaves of S.cumini were analysed for post infectional compounds. In addition phytoalexin response of the plant is studied using a pathogenic and a nonpathogenic fungus.

## MATERIALS AND METHODS

Healthy and infected leaves of S.cumini were collected from cultivated trees in Baroda, Gujarat State.

The procedures followed for the isolation and culture of the pathogenic fungus, pathogenicity tests, extraction, isolation and identification of compounds, drop diffusate technique and facilitated diffusion technique have been described in Chapter-II.

## BIOASSAY TESTS

Assay of mycelial growth: The antifungal activity of the diffusates i.e. control (A) and treated (B) were found out by bioassay tests. The diffusates were made upto 100 ml (Stock solutions: A and B). 1 and 5 ml of the diffusate from the stock (B) was considered as two different test solutions were added to two different petriplates containing PDA. In the case of control, only one solution i.e., 5 ml of the stock solution (A) was added to the medium. Three replicates were maintained in each case. The centre of each petriplate was inoculated with an agar plug of the test fungus cut from the margin of a parent colony growing on PDA. The assay plates were incubated at 25° ± 2°C for a period of 6 days. Measurement of the mycelial growth was taken every 2 days. Mycelial growth was calculated by measuring perpendicular diameter of each of the three replicates

colonies and subtracting the diameter of the mycelial plugs used to inoculate the plates. The assay plates of the treated were compared with that of the control.

Assay for spore germination and germ tube growth: The test solutions were prepared as mentioned above. The procedure followed for the bioassay of spore germination and germ tube elongation have been described in Chapter-II.

RESULTS

Aspergillus niger van Tieghem was the fungus isolated from the diseased spots of infected leaves. Pathogenicity tests were confirmed A.niger to be pathogenic on leaves of S.cumini (Fig.29).

The disease symptoms appeared as small yellowish spots, 5 to 6 days after artificial inoculation. The lesions due to the disease was more towards the base and tip of the leaf. The spots which were small in the beginning grew in size covering a large portion of the leaf blade. These areas were light yellow to greyish in colour. The diseased lesions were produced on both the surfaces of the leaves but were more prominent on the upper surface.

The distribution of saponins, tannins, proanthocyanidins and iridoids in healthy and infected leaves of S.cumini is presented in Table X. Alkaloids were absent, while saponins,

tannins, proanthocyanidins and iridoids were present in both healthy and infected leaves of S.cumini.

The distribution of various phenolics in healthy and infected leaves of S.cumini is presented in Table XI. Both the healthy and infected leaves of S.cumini contained the same flavnoids, 3'-OMe quercetin and myricetin. Vanillic, gallic, p-hydroxybenzoic and syringic acids were present in both types of leaves. p-Coumaric acid which was present in healthy leaves is conspicuously absent in the infected leaves which contained a new phenolic acid, gentisic acid instead. The concentration of the phenolic acids were more in the infected leaves.

PHYTOALEXIN RESPONSE

When phytoalexin response was induced in the leaves of S.cumini with spores of the pathogenic fungus a visibly coloured compound (pinkish brown : $\lambda \frac{Max}{MeOH}$ 274, 318, 332, 400 nm; Rf = 0.34 )was obtained in TLC with toluene : Ethyl formate : formic acid (5:4:1) (Figs.30 and 31). The pinkish color with 10% sodium carbonate and the absorption spectrum indicate that this compound could be quinone. The compound was conspicuously absent from the chromatograms of the control experiment.

There was no qualitative and quantitative differences between the diffusates (control and treated), when the leaves

Fig.29. Diseased leaves of Syzygium cumini (L.) Skeels

Fig.30 A visible photograph of a thin layer chromatogram showing compounds of the diffusates from leaves of Syzygium cumini treated with Aspergillus niger. Control (left) and treated (right).

FIG . 29

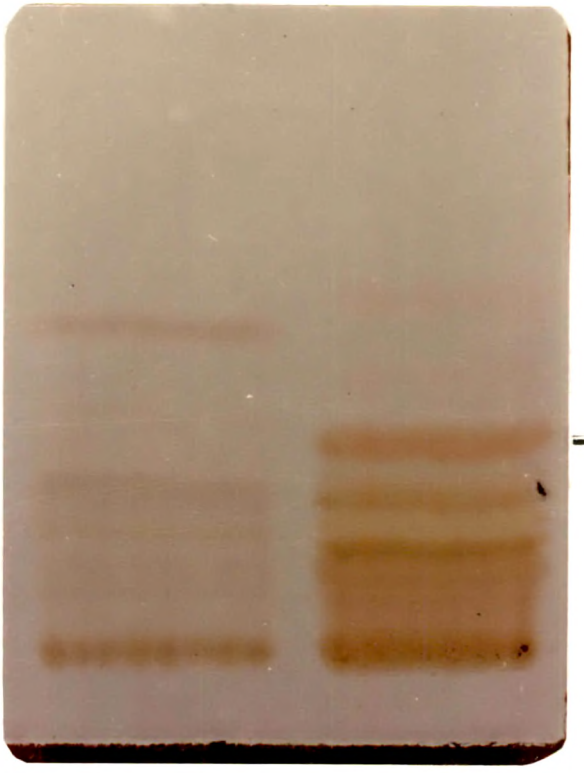


FIG . 30

were exposed to spores of the non-pathogen Fusarium solani (Mart.) Sacc. Facilitated diffusion technique employed with both the pathogen and the non pathogen also yielded similar results.

BIOASSAY TESTS

The diffusate from the treated leaves containing the phytoalexin, inhibited the mycelial growth of the pathogen A.niger at 1 ml and 5 ml dilution (Fig. 32). A maximum of 25 per cent inhibition of the mycelial growth over control was noted at 5 ml dilution of the diffusate (Fig.33). This diffusate also inhibited the spore germination and germ tube elongation of A.niger (Fig.34). A maximum of 87 per cent inhibition of spore germination and 48 per cent inhibition of germ tube elongation was seen at 5 ml dilution.

DISCUSSION

Production of gentisic acid and increase in concentration of phenolic acids is due to the increasing production of phenolic acids at the site of infection, triggered off by the interaction between the invading fungus and the cells of the host plant. The absence of p-coumaric acid in the infected leaves may be due to the degradation of the acid by the pathogen.

The presence of a quinone in the diffusates of leaves treated with spores and its absence in control indicate this

Fig. 31. A thin layer chromatogram (under UV light) showing compounds of diffusates from leaves of Syzygium cumini treated with Aspergillus niger. Control (left) and treated (right).

Fig.32. Antifungal activity of the diffusate (treated) from leaves of Syzygium cumini. Control (left) and treatments : 1 and 5 ml dilutions (right)

FIG .31

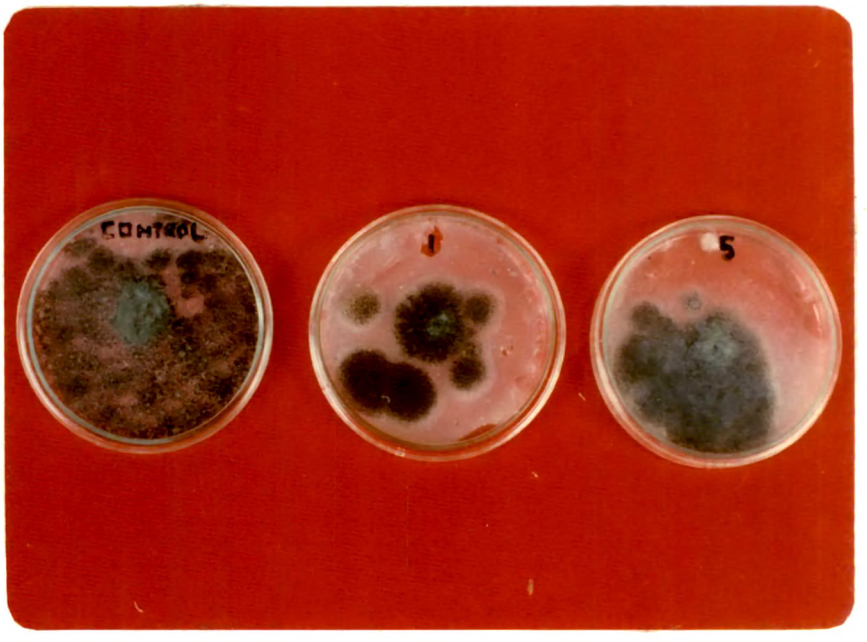


FIG .32

Fig.33. The effect of the diffusate (treated) from leaves of Syzygium cumini on the mycelial growth of Aspergillus niger as observed after 6 days.

Fig.34. The effect of diffusate (treated) from leaves of Syzygium cumini on the spore germination (•——•) and germ tube elongation ( X-·--X) of Aspergillus niger after a incubation period of 24 hrs at 25° ± 2°C

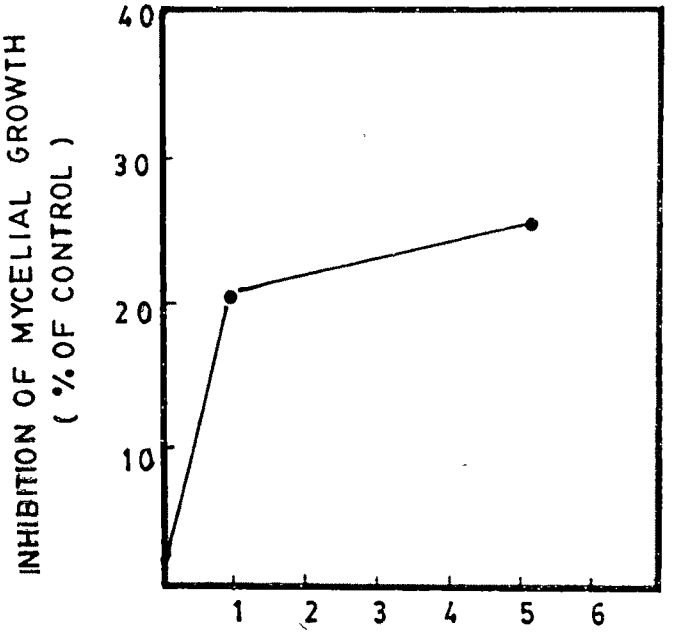
INHIBITION OF MYCELIAL GROWTH
( % OF CONTROL )
40
30
20
10
1
2
3
4
5
6
DILUTION

FIG. 33

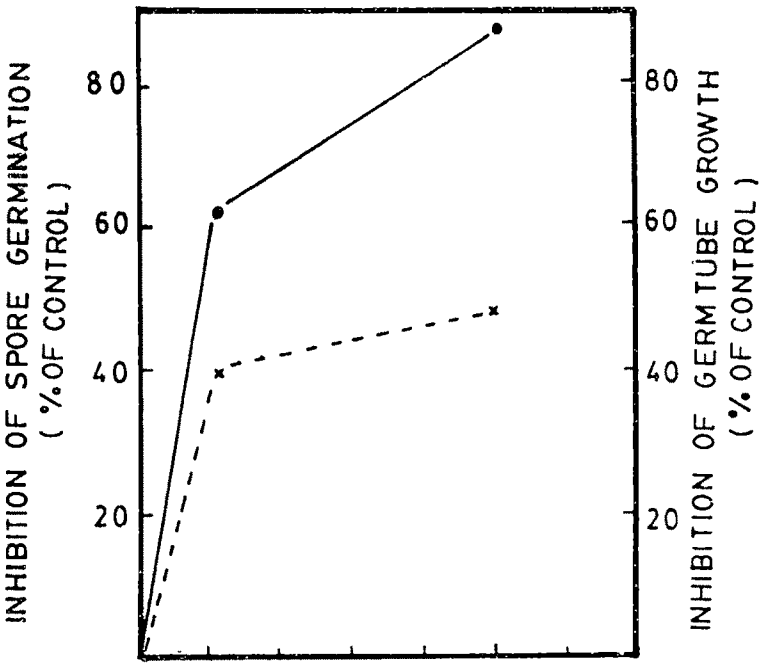
INHIBITION OF SPORE GERMINATION
( % OF CONTROL )
INHIBITION OF GERM TUBE GROWTH
( % OF CONTROL )
80
60
40
20
1
2
3
4
5
6
DILUTION

FIG. 34

compound to be a phytoalexin. It is well known that phenolic compounds and their highly oxidised derivatives such as quinones are accumulated in plants in response to infection and injury (Farkas and Kiraly, 1962). Quinones exhibit more toxicity to microorganisms than their reduced forms. The diverse biological activities of quinones have been attributed to the ability of these compounds to react with precipitate and then inactivate certain biologically important substances such as proteins, enzymes and nucleic acids (Mason and Paterson, 1965). Quinones also interfere with enzyme activity by binding metal cofactors, by reacting with sulphydral groups or substrates (Kosuge,1969).

The quinone produced in the infection droplets was proved to be antifungal in nature when it inhibited the mycelial growth, spore germination and germ tube growth of A.niger. Since the quinone exhibits antifungal activity and the evidence that it was produced as a result of elicitation proves its phytoalexin nature. Quinonoid phytoalexins such as Benzoquinone and its dihydro-derivative, hydroquinone are reported from barley (Evans and Pluck,1978) while anthraquinones are located in cell and tissue cultures of Cinchona sp. (Wijnsma et al., 1985).

Table - X : Distribution of Saponins, Tannins, Proanthocyanidins and Iridoids in healthy and infected leaves of Syzygium cumini.

| Leaves | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Healthy | + | + | + | + | + |
| Infected | + | + | + | + | + |

1 Saponins 2 Tannins 3 Proanthocyanidins

4 Iridoids 5 Alkaloids

Table XI : Pre-infectional and post-infectional phenols of Syzygium cumini.

| Pre-infectional compounds | Post-infectional compounds | Drop diffusate technique | |
|---|---|---|---|
| | | Control | Treated |
| Quercetin 3'-OMe | Quercetin 3'-OMe | | |
| Myricetin | Myricetin | | |
| Vanillic acid | Vanillic acid | | |
| Gallic acid | Gallic acid | | |
| p-hydroxybenzoic acid | p-hydroxybenzoic acid | | |
| Syringic acid | Syringic acid | | |
| p-coumaric acid | Gentisic acid | - | Quinone |