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Biochemical changes in the *Lawsonia inermis* L. infected with *Asterina lawsoniae* Henn. & Nyn.

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Abstract

Lawsonia inermis was infected with *Asterina lawsoniae*. This disease is common throughout the Southern Western Ghats of peninsular India. Taxonomic details of the fungus is provided. Infected plants revealed reduction in the total chlorophyll, chlorophyll 'a', chlorophyll 'b', soluble sugar, starch, protein and total amino acids. There is an increase in the proline and total phenol contents. Amylase and protease enzyme activities increased in the infected leaves while peroxidase was reduced.

Keywords – amino acids – amylase – black mildew – chlorophyll – enzyme activities – foliicolous fungi – phenol – protease – protein – soluble sugar – starch

Introduction

The genus *Lawsonia* has one species, *L. inermis* (commonly called Henna, Mehndi, Shudi, Madurang, Mendi, Manghati, Madayantika and Goranti) (Sastri 1962, Gupta 2003), having different synonyms as *alba* and *spinosa* belonging to the family Lythraceae. It is a biennial dicotyledonous herbaceous shrub. The henna plant is native to tropical and subtropical regions of Africa, southern Asia, and northern Australia in semi-arid zones.

Henna is commercially cultivated in Afghanistan, Algeria, Bangladesh, Egypt, India, Iran, Iraq, Libya, Morocco, Pakistan, Saudi Arabia, Somalia, Sudan Tunisia, Turkey, UAE and Yemen. Henna has been used since the Bronze Age to dye skin (including body art), hair, fingernails, leather, silk and wool. In several parts of the world it is traditionally used in various festivals and celebrations. Henna also acts as an anti-fungal and a preservative for leather and cloth. Henna flowers have been used to create perfume since ancient times, and henna perfume is experiencing resurgence. Henna is bitter and astringent in taste, pungent in the post digestive effect and has cold potency. The seeds are anti-diarrhea and liver stimulant. The flowers are beneficial to the heart: induce the sleep and promote the intelligence. The leaves are diuretic, pain killer, reduce edema, cleanse and heal the wounds. Flower oil contains alpha- and beta-ionone, the latter being the main component. Astringent roots are ground and rubbed on the heads of children to treat boils and eye diseases. The importance and utilization of this plant in India is legendary. Such an important and interesting plant is subjected for several diseases including the disease caused by *Asterina lawsoniae*.

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Materials & Methods

This is a quite prevalent disease in the southern Western Ghats, Initially appears as a small, black specks on the young and sprouting leaves, persists in its anamorph state during rainy season, forms teleomorph during winter. The black, ectophytic colonies cover both surfaces of the leaves, petioles and often tender stem portions. The infected plants during winter can be easily identified, even from a distance, by their black appearance. For the microscopic study, colonies were mounted in their natural condition (Hosagoudar & Kapoor 1985).

To know the effect of this ectophytic fungus on the host, biochemical tests were conducted. In case of infected leaves, colonies were removed by nail polish technique (Hosagoudar & Kapoor 1985). Different standard methods were used to quantify the ingredients in the infected and healthy leaves *viz*., soluble sugars and starch (Mc. Cready et al 1950), protein (Lowry *et al* 1951), amino acid (Plummer 1977), proline (Bates *et al* 1973), phenols (Swain & Hillis 1959), chlorophyll (Arnon 1949), peroxidase (George 1953, Maehly 1954), amylase (Jones 1968) and protease (Snell & Snell 1971).

Results

Asterina lawsoniae Henn. & Nyn., *Monsumia* 1:159, 1899; Hansf., *Proc. Linn. Soc.* London 160: 145, 1949; Patil & Thite, *J. Shivaji Univ.* 17: 152, 1977; Hosag. & Abraham, *J. Econ. Taxon. Bot.* 4: 572, 2000; Hosag., C.K. Biju& Abraham, *J. Econ.Taxon. Bot.* 25: 305, 2001; Hosag., *Zoos' Print J.* 18: 1283, 2003; 21: 2328, 2006; Hosag., H. Biju & Appaiah, *J. Mycopathol. Res.* 44: 8, 2006; Thaung, *Australian Mycol.* 25: 8, 2006.

Colonies amphigenous, caulicolous, cover the softer portion of the stem and the total coverage of the black colonies on the plant can be noticed even from a distance during winter. Hyphae flexuous, branching irregular at acute to wide angles, loosely to closely reticulate, cells 11 –21 µm long and 3 –5 µm wide. Appressoria alternate, scattered, sessile, unicellular, ovate, mostly globose, rarely entire, mostly 1-3-sublobate to lobate, 4 –9 µm long and 4 –7 µm wide. Thyriothecia scattered, orbicular, up to 120 µm in diameter, margin crenate, stellately dehisced at the centre; asci few to many, octosporous, ovate to globose, 21 –30 µm in diameter; ascospores conglobate, brown, uniseptate, constricted at the septum, 12 –18 µm long and 5 –9 µm wide, wall smooth. Pycnothyria similar to thyriothecia, mixed with thyriothecia, orbicular, smaller; pycniothyriospores pyriform, brown, unicellular, 9 –13 µm long and 4 –9 µm wide.

The fungus is always associated with the host plant throughout the winter season. It is very common in the Southern Western Ghats in Kerala.

Table 1 Biochemical effect of infection on *Lawsonia inermis* L.

Primary metabolites mg g^{-1} F.W.	**Healthy leaf**	**Infected leaf**	**Percentage of increase/ decrease over healthy leaves**
Total chlorophyll	1.86± 01	1.19 ± 0.2	-35.9
Chlorophyll 'a'	0.48 ±0.03	0.28 ±0.05	-41.5
Chlorophyll 'b'	1.28 ±0.02	0.71 ±0.04	-44.6
Soluble Sugar	44.34±0.16	33.43 ± 0.05	-27.7
Starch	0.121 ±0.02	0.040 ± 0.02	-66.9
Protein	2.23 ± 0.20	1.65 ±0.07	-26.1
Total amino acid	5.22 ±0.25	3.756 ±0.9	-28.1
Praline	0.64 ± 0.05	0.75 ± 0.02	+ 17.7
Total phenol	14.13 ±2.0	15.06 ±0.6	+ 6.6

Table 2 Enzyme activities in healthy and infected leaves

Enzymes (specific activity)	Healthy leaf	Infected leaf	Percentage increase/ decrease over healthy leaves
Peroxidase	9. 18 ±0.2	6. 14 ±0.5	-33.2
Amylase	6.77 ±0.61	13.44 ±2 8	+ 98.6
Protease	0.26 ±0.31	0.70 ±0.05	+ 170.2

Discussion

Fungi which parasitize plants would appear to gain two main advantages by doing so they avoid competition for food and to certain extent they are protected from those changes in environmental conditions which are adverse to their mycelial growth. Success in parasitism may be judged by the extent to which these advantages are enjoyed. Black mildews are mostly ectoparasites and do not cause serious pathogenic effects on the host plants. However, the black colonies of these fungi increase the temperature in the infected parts and cause physiological imbalance. They decrease the photosynthetic efficiency of the plants, affect the hormonal and phenolic compound level and in short affect the efficiency of the plants.

Schmiedekhencht (1970) showed that black colonies absorb more light and there will be an increase in the temperature by 1°C to 1.5°C. Probably this would be compensated by the plants by transpiring more water. Since black colonies prevent entry of light to the leaf surface, there is a reduction in the chlorophyll content (Table 1). This may be due to the lack of light for the chlorophyll synthesis or increased proteinase in the infected leaves, would have caused the proteolysis to degenerate chlorophyll apparatus. (Kumar & Singh 1996, Hosagoudar et al 1997). Reduction in carbohydrate content (starch and soluble sugars) in the infected leaves can be attributed to the reduced rate of photosynthesis due to the reduction of light harvesting pigment (Hosagoudar et al 1997). On the other hand, increase in phenol may be due to an enhanced synthesis of phenolic compounds or hydrolysis of phenolic glycosides by fungal glycosidases to yield free phenols (Sharma et al 1983). An increase in the proline content in the infected leaves indicate that this fungal infection is causes stress to the plants. The enzymes involved in amino acids and amide synthesis are activated as a result of infection (Rudolph 1963), which lead to decrease in amino acids content in the infected leaves. The decrease in the peroxidase enzyme activity in the infected leaves may be due to the impaired synthesis of chlorophyll. The decrease in the peroxidase enzyme activity in the infected leaves may be due to the impaired synthesis of chlorophyll. The enzymes involved in the hydrolysis of starch and protein are found higher in the infected plants and it would have been activated by the fungus for utilization of the complex molecules. The decreased content of starch and protein in the infected leaves are also in co-ordination with the increase in protease and amylase enzymes in the infected leaves.

Infection of *Asterina lawsoniae* on *Lawsonia inermis* lead in the reduction of the primary metabolites like starch, sugar and protein can be due to the reduced synthesis or due to the utilization by the fungus for its growth and has been authenticated in the reduction of photosynthetic pigments as well as promotion in the hydrolysing enzymes viz., amylase and protease.

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