EFFECT OF URBAN ENVIRONMENT ON LEAF SPOT DISEASES OF MEDICINAL PLANTS.

Rudranil De, Debapriya Ghatak, Shreya Kar, Martha Sinha, Dr. Mahashweta Mitra Ghosh

Department of Microbiology, St. Xavier's College, Kolkata

****email-id- rudierules@gmail.com***

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Abstract:

In the present day, medicinal plants are grown by different nurseries but because of high amount of suspended particulate matter (SPM), they get mechanically damaged paving the pathway for different airborne fungal pathogens. Three such medicinal plants were collected from a nursery in Batanagar, south 24 pgs; West Bengal (in the vicinity of a mega construction area) and they were all suffering from Leaf spot diseases. The respective pathogens were isolated from these plants namely, *Adhatoda vasica, Ocimum sanctum* and *Asteracantha longifolia* and the fungal pathogens were *Rhizoctonia* spp, *Fusarium oxysporium, Sclerotinia* spp and *Fusarium* spp along with protozoan cysts found adjacent to the spores-Monocystis, respectively. The infective structures of these organisms were observed. For *Rhizoctonia* spp subterminal or apically positioned spores which are oval or round in outline. For *Fusarium oxysporium* branched mycelia with distinct spores were observed. Microconidia distinct but sickle shaped structure could not be identified. Whereas a thick walled scelocium was observed for *Sclerotinia* spp. *Adhatoda vasica seems* to be worstly affected and the SPM level has increased from 1.53 mg to 3.5 mg or by 56.28%. Whereas, *Ocimum sanctum* and *Asteracantha longifolia* are accounted for an increase in infection by 55% (from 2.16 mg to 4.8 mg) and 63.88% (from 1.3 mg to 3.6 mg) respectively. On analysis of this particulate matter deposited on the infected leaves, it was found that the metals chromium and lead were present with the content of 0.3ppm and 0.9ppm respectively. Hence, it can be correlated that Chromium and lead particulate emitted from the constitutional area in vicinity has resulted in the increase incidence of Leaf spot disease in *Adhatoda vasica, Ocimum sanctum and Asteracantha longifolia.* So, this definitely suggests that increased air-borne pollutants are possible predisposition factors of fungal Leaf-spot diseases.

INTRODUCTION:

Medicinal plants traditionally occupied an important position in rural and urban lives of India and are considered as one of the most important sources of medicines since the dawn of human civilization. The medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice. *Adhatoda vasica (*popularly known in India as vasaka or Malabar nut), , *Ocimum sanctum* (popularly known in India as tulsi) and *Asteracantha longifolia (*popularly known as kulekhara) are important medicinal plant in traditional as well as modern systems of medicines. The leaves are used, either alone or in combination with other drugs, for preparation of expectorants (in case of *Adhatoda vasica*, Singh *et al.*, 1996; Jain & DeFilipps, 1991), antioxidants (in case of *Ocimum basilicum*, Seung-Joo Lee , Katumi Umano , Takayuki Shibamoto , Kwang-Geun Lee , 2004) and in treatment of blood related diseases in humans (in case of *Asteracantha longifolia).* The shrub *Adhatoda vasica,* habitating the plains of India, has its leaves, roots, flowers and stem bark in use in medicinal applications.The leaves of the plant contain two major alkaloids called vasicine, and vasicinone(Pandita, K,1983). The active alkaloid vasicine and its autooxidation product vasicinone have shown bronchodilator and antihistaminic effects(Amin, A.H. and D.R Mehta, 1959). The leaf extract has been used for the treatment of bronchitis and asthma for many centuries. It relieves cough and breathlessness (Gogate, V.M., 1982). It is also prescribed commonly for bleeding due to idiopathic thrombocytopenic purpura, local bleeding due to peptic ulcer, piles, menorrhagia etc. Its local use gives relief in pyorrhoea and in bleeding gums (Doshi, J.J. et al., 1983). *Asteracantha longifolia* is a robust, erect, annual herb which inhabits in india in tropical and sub-tropical regions. Roots are sweet, sour, bitter, refrigerant, diuretic, anti-inflammatory, analgesic, haemopoictic, hepatoprotective and tonic. It is useful in inflammations, hyperdipsia, strangury, jaundice and vesical calculi. It is also used in flatulence and dysentery. Leaves are haemopoictic, hepatoprotective, anti-inflammatory, antioxidant, analgesic, antidiabetic, stomachic, ophthalmic, diuretic and liver tonic. It is used in hepatic obstruction, jaundice, arthritis, rheumatism and diseases of urinogenital tract. It is useful in flatulence and other stomach related diseases. It is useful in anemia and for treating blood diseases. , *Ocimum sanctum* is a small herb seen throughout India, have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, skin diseases, arthritis, painful eye diseases, chronic fever, insect bite, etc. has also been suggested to

possessantifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, adaptogenic and diaphoretic actions (P. Prakash and Neelu Gupta, 2005)

These plants have immense importance and are used as constituents of many ayurvedic drugs. But the growing incidence of environmental pollution and other factors has resulted in assisting the fungal diseases in these plants. And it is observed that with increase in pollution and increased suspended particulate matter (SPM) rate the proportion of metal detected in these plants increases.

2. MATERIALS AND METHODS:

2.1: Materials:

Source:

- Infected and healthy leaves of *Adhatoda vasica, Ocimum sanctum* and *Asteracantha longifolia*.

Media:

- Potato dextrose agar (Potato infusion 200 g, Dextrose 20 g, Agar 20 g, Distilled water 1 litre).

Glass wares:

- Test tubes.
- Petri plates.
- Forceps.
- Watch Glass.
- Conical Flasks.

Chemicals

HCl, Lacto Phenol Cotton Blue.

2.2: Procedure:

Infected leaves collection and characterization

infected plant leaves were taken and the infected portion i.e., the spotted portions were isolated by using a clear sharp scalpel and inoculated in potato dextrose agar slants and incubated for 48 hours at room temperature. The fungus grown was then taken from the slants and they were stained with lacto phenol cotton blue in order to characterize them.

SPM calculation

For calculating the percentage increase in SPM (suspended particulate matter), the healthy leaves from each of the plants were taken and their initial weight was measured. Then these leaves were washed with distilled water and their weights were again measured.

The difference in the final and the initial weights give the mean suspended particulate matter on these leaves. Similar procedure was followed for fungal infected leaves and again the spm value was recorded. The difference in spm value in fungal infected leaves and healthy leaves gave us the percentage increase in spm in the infected leaves.

Acid hydrolysis

2 ml of the the leaf wash discarded water is taken with 3 ml of 7% acid and boiled and when the hydrolyzed mixture got reduced to about 1 ml volume made upto 10 ml with distilled water.

Then the hydrolysis product was subjected for metal detection.

3. RESULTS:

Table 3.1 - Isolation of fungal pathogen and characterization.

Name of the plant	**Percentage infection on leaf sample**	**Characteristics of fungal colonies**	**Organism identified**
Adhatoda vasica	45%	The mycelia is grey black in colour with hyaline portion, spores are oval or round in outline and subterminal or apical in position.	*Rhizoctonia* spp
Ocimum sanctum	60%	Branched mycelia with simple septation. Mycelia dark brownish-green in colour, spore forms is distinct. Microconidia distinct but sickle shaped structure could not be identified.	*Fusarium oxysporium*
Asteracantha longifolia	30%	(i)Thick walled sclerocium present. Broadly spindle shaped or oval spores liberated, grey in colour without septation. It clearly indicates that the organism can sustain better in comparison to its competitive species.	(i)*Sclerotinia* spp (ii)*Fusarium* spp And protozoan cysts were found adjacent to the spores-Monocystis.

Table 3.2 – SPM Count and metal detection.

(i)For healthy leaf

Plant leaf sample	**Weight (in grams)**		**Difference in weight (in grams)**	**Mean SPM**	**Metal detected**
	Before surface wash	**After surface wash**			
Adhatoda vasica	0.0248 0.0294 0.0292	0.0243 0.0264 0.0281	0.0005 0.0030 0.0011	0.00153 g Or 1.53 mg	No metal Detected
Ocimum sanctum	0.0105 0.0100 0.0112	0.0085 0.0070 0.0097	0.0020 0.0030 0.0015	0.00216 g Or 2.16 mg	
Asteracantha longifolia	0.0326 0.0345 0.0264	0.0315 0.0337 0.0244	0.0011 0.0008 0.0020	0.0013 g Or 1.3 mg	

(ii)For diseased leaf

Plant leaf sample	**Weight (in grams)**		**Difference in weight (in grams)**	**Mean SPM**	**Metal detected**
	Before surface wash	**After surface wash**			
Adhatoda vasica	0.0202 0.0206	0.0178 0.0160	0.0024 0.0046	0.0035 g Or 3.5 mg	(i)Lead (ii)Chromium
Ocimum sanctum	0.0137 0.0150	0.0076 0.0115	0.0061 0.0035	0.0048 g Or 4.8 mg	
Asteracantha longifolia	0.0302 0.0321	0.0271 0.0280	0.0031 0.0041	0.0036 g Or 3.6 mg	

Column chart 3.3- Correlation in healthy and diseased plant spm.

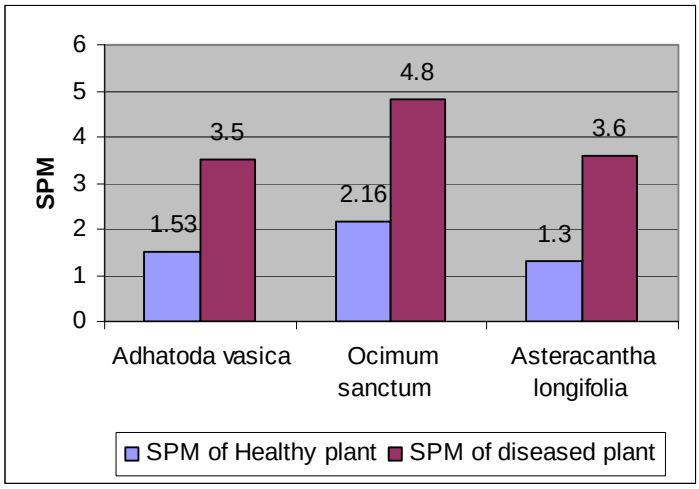

Table 3.4- Concentration of metals detected in the infected leaves in polluted environment.

Courtesy: SGS India Pvt. ltd. Report no. : CA:GL:2120002476 Date:22.02.2011

Leaf surface extract acid hydrolysis was done for metal detection. 10^{-1} fold dilution was done in 10 ml sample

Metal found	**Concentration in 10^{-1} dilution**	**Actual concentration**
Lead	0.09 ppm	0.9 ppm
Chromium	0.03 ppm	0.3 ppm

Table 3.5- Percentage increase in SPM and proportion of metals detected.

Plant leaf sample	**Percentage increase in SPM**	**Proportions of metals detected (Cr:Pb)**
Adhatoda vasica	56.28	In 3.5 mg, 1:3
Ocimum sanctum	55	In 4.8 mg, 9:20
Asteracantha longifolia	63.88	In 3.6 mg, 17:50

4. DISCUSSION:

It has been surveyed that our city roadsides have quite a good collection of a variety of economically important traditional medicinal plants. We chose three of age old famous

medicinal plants common in the domestic uses of the eastern part of India, namely, vasaka, kulekhara and tulsi. But at present day of increasing air-water-soil pollution those precious plant populations in urban territories are under threat. Our study revealed that those three important medicinal plants are getting infected by the fungal leaf spot diseases as a result of the environmental hazards at Batanagar (south 24 prgns, West Bengal). Reckless cutting of trees, filling up of pond, harmful materials used for construction increased the air contamination and the elevated levels of metal toxicity have put the community of roadside vegetations, including those having high economic values such as the medicinal plants, at stake. Eighteen species of endophytic fungi were isolated from bark, stem and leaf segments of five medicinal plant species. The highest species richness as well as frequency of colonization of endophytic fungi was found in the leaf segments, rather than the stem and bark segments, of the host plant species(N. S. Raviraja Dr. et al. 2005). In many recent studies medicinal plant infection is the topic of interest. In quite a few cases, plants were examined in situ for disease symptoms as well as samples being collected for laboratory analyses. Fungi were identified using morphological characteristics, and where necessary with molecular techniques. The survey resulted in a range of fungi identified from the target plants. Common diseases on all the three plants were leaf spots, leaf blight and leaf rust causes harmful effects on medicinal value of the plant parts (S.P. Chavan and S.L. Korekar, 2011). We, in our study also isolated foliage fungal species such as *Rhizoctonia* spp, *Fusarium oxysporium, Sclerotinia* spp from the leaves of medicinal plants namely Vasaka, Tulsi and Kulekhara respectively. Repeated observation showed, with respect to the percentage of infection Tulsi (*Ocimum sanctum)* is mostly affected (60%) but taking both the data of SPM increaseand percentage of infection together Vasaka (*Adhatoda vasica)* seemed to be worstly affected which showed 56.28% increase in SPM and 45% of infection area on sample. Correlating SPM counts in healthy and diseased plants we found significant increase in SPM values in diseased ones suggesting the probable adverse effect of airborne pollution present in the environment.

In metal detection study two potentially toxic metal like lead(Pb) and cromium (Cr) was detected with significant amount (0.9 and 0.3 ppm respectively). Looking for correlation between the percentage of SPM increase and metal toxicity it was found that worstly affected *Adhatoda* was bearing high percentage of lead metal which also in turn correlates with the high percentage of infection. So, it can be suggested that high percentage of metal toxicity favoured the onset and/or increase in fungal infection which clearly reveals the extent of hazards caused by environmental pollution.

5. CONCLUSION:

Thus it can be concluded that the percentage increase in SPM, and the increase in the proportion of metal over the leaves is a potential reason behind the increased fungal infection in roadside medicinal plants.

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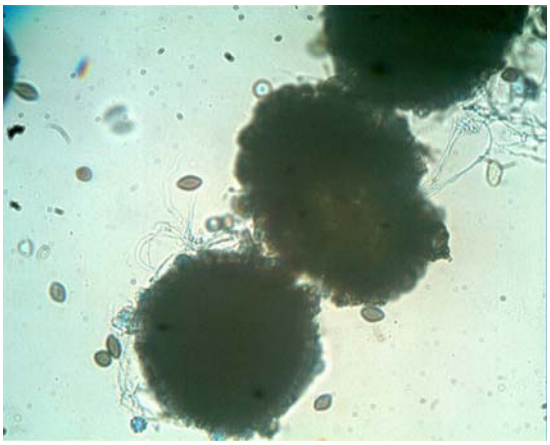
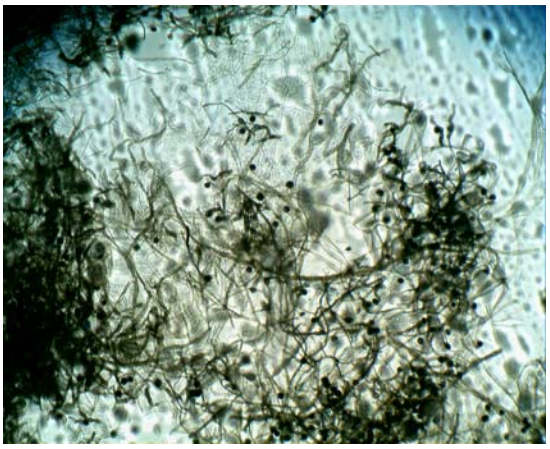
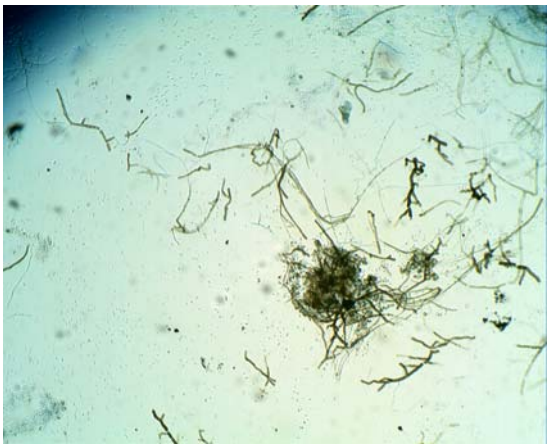
PHOTOGRAPHS OF THE FUNGUS IDENTIFIED FROM EACH INFECTED LEAF:



Sclerotinia spp in *Adhatoda vasica*



Fusarium spp in *Ocimum sanctum*



Rhizoctonia spp in *Asteracantha longifolia*