Asian Journal of Agriculture & Life Sciences Vol. 2(4),October 2017: 1-5

Website: www.crsdindia.com/ajals.html e-ISSN: 2455-6149

ORIGINAL ARTICLE

Isolation and characterization of fungal pathogens of mulberry leaves

Rajesh Kumar and Jamil Ahmad

Department of Zoology, Gandhi Faiz-e-Aam College, Shahjahanpur, U.P.

Email: rajesh.seri@yahoo.in

Received: 2nd June 2017, Revised: 21st July 2017, Accepted: 2nd August 2017

ABSTRACT

Mulberry leaf spot disease is common in the tropical region. It reduces the quality of leaf, which directly affects the suitability for silkworm rearing. The study was conducted to isolate and characterize the fungal pathogens of mulberry plants. There were two isolates of pathogenic fungi causing leaf spot, isolated from the diseased leaves of mulberry collected from different mulberry gardens of Uttar Pradesh. The findings revealed that two pathogens i.e., Pseudocercospora mori *and* Cercospora moricola *were found to be associated with the concentric leaf spot of the mulberry plants. The infections in the mulberry plants began early to the rainy and winter seasons in the form of patches and later caused severe defoliation at every experimental site.*

Key words: *Fungal infection, Leaf spot, Mulberry, Silkworm*

INTRODUCTION

Sericulture is known as an agro based cottage industry. It plays an important role in improving the rural economy because it possesses high employment and income generation capability with minimum investment (Hiwara, 2001). Mulberry leaves are the source of nutrition (Proteins, carbohydrates, vitamins, and minerals). It is reported that about 70% of the silk protein produced by silkworm are directly derived from the protein of mulberry leaves (Narayan *et al.* 1967 and Krishnaswami *et al.* 1970). A mulberry plant belongs to genus *Morus* and family Moraceae. It is widely distributed in Asia, Europe, and Africa on a wide range of climatic conditions that vary from temperature to tropical areas (Yoshida *et al*., 2002 and Tang *et al.,* 2005). Four species of mulberry plants namely, *Morus alba*, *M. indica*, *M*. *serreta* and *M*. *laevigata* are raised as main food plant of silkworm in India. Mulberry is affected by a number of diseases (Reddy *et al*., 2009). Among the various diseases, leaf spot is the most devastating for mulberry cultivation. The leaf spot caused by *Cercospora moricola* Cook decreases the leaf production. It is a major disease of mulberry (*Morus spp*.) occurring both in temperate and tropical regions. Both diseases damage the quality and quantity of mulberry leaf and cause annual leaf loss of about 20-25 % due to defoliation and destruction of leaf area (Sukumar and Ramalingam, 1989), and make the leaves less nutritive (Siddaramaiah and Hegde, 1990).

The nutrients of mulberry leaves such as chlorophyll and carbohydrates were decreased due to fungal infection (Ghose *et al.,* 2003 and Tang *et al.,* 2005). Many fungicides are reported to be effecting in combating both the diseases (Munshi *et al.,* 1987; Munshi *et al.,* 1994; Philip *et al,*. 1994 and Tanki *et al*., 2005). The affected leaves of *Cercospora moricola* become yellowish and wither as the diseases become severe (Baijewu, *et al.,* 2005). The foliar disease leaf spot (*Cercospora moricola)* deteriorates the nutritional value of the leaves reduces the leaf yield and make the leave unsuitable for silkworm larvae (Jayarajan, 1986 and Sharma *et al.,* 1993). Sengupta *et al.,* (1991) reported that the *Cercospora moricola* and *Cercosporaella mori* cause major epidemic disease of mulberry plants and reduced leaf yield between 10-20% during rainy and winter seasons. During the growth period of the mulberry plants in rainy season, fungal pathogen, *Cercospora moricola* comes in contact with mulberry leaves under existing environmental conditions and causes disease with interfering the normal physiological function of the mulberry leaf. The net result is the degradation of quality leaves. This paper describes the symptoms of the disease, and identification of the pathogen.

MATERIAL AND METHOD

Systemic surveys were conducted in different sericulture farms during 2014-15 and 2015-16. The infected leaves were collected from Government / Private sericulture farms in different Districts of Uttar Pradesh. The samples were categorized as wet and dry. The dry samples have been put in polyethylene bags and tagged with date and place of collection and sealed. Dry samples were kept in low-temperature in incubator. The leaf samples were washed with running water for 15-20 times as recommended by Yoshida and Shirata (1999). The samples were immersed in 5% sodium hypo chloride solution for about 3 minutes for surface disinfection rinsed with sterilized water, dried under the aseptic condition for five minute and cut into small pieces (3-5mm) (Fig. 1).

Fig. 1: Black patches showing leaf spot on mulberry leaves

The pieces were placed on plates containing2%Potato Dextrose Agar (High media) at 5.6 ± 0.2 pH supplemented with 8 mg/l of streptomycin sulphate and 30 mg/l of penicillin(Baird *et al*. 1991).The plates have incubated at 26 ± 2.00 ºC for 5-7 days (Fig 2). The isolated fungal species were identified based on morphological and culture characteristics (Booth 1977). The fungi grown from the leaf pieces have been subcultured with potato dextrose agar (Fig. 3). The morphological identification was carried out under the Phase Contrast Microscope.

Fig. 2: Culture of fungal pathogens in petri plates **Fig. 3:** Subculture of fungal pathogen in test tube

SLIDE PREPARATION

The material was spread over the slide by a needle and stained with cotton blue (for hyaline spores) and mounted in lacto phenol and studied under the phase-contrast microscope at different magnification (10x-100x). Slides were prepared from fungal culture; a small piece from the periphery of the fungal colony will be taken aseptically with the help of a needle and put on a slide, mycelium spread with a needle, stained with cotton blue and mounted in lacto phenol. Measurements of fungi will be recorded and photographs will be taken by Phase contrast microscope. The choice of media depends largely on the nature of the tissue involved in the

isolation exercise. Low nutrient media such as CLA, WA or quarter-strength PDA are suitable for isolation from larger roots or stem bases. Antibiotics can be added to these media if bacteria interfere with the recovery of fungi. Carbohydrate rich media are generally avoided in isolation studies because they favor fast-growing saprophytes such as the mucoraceous fungi and *Trichoderma*. In addition, some pathogens degenerate rapidly to a virulent form of these media. Richer media such as PDA may be more appropriate for the isolation of slow-growing fungi or isolation from very fine roots.

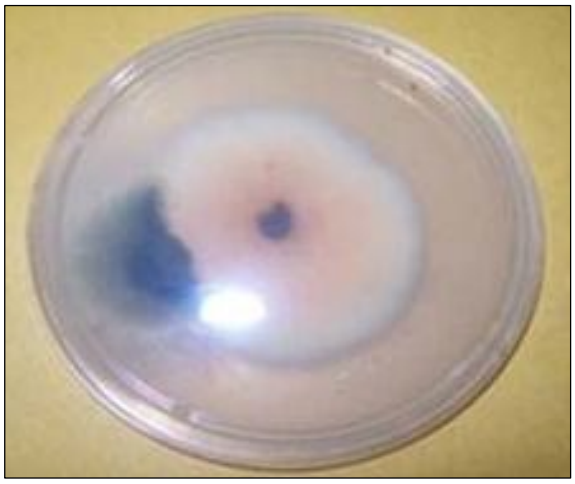
For isolation of fungi, diseased specimens will be surface sterilized either by 0.1% Mercuric chloride or Hydrogen peroxide, for 1-10 seconds then rinsed with distilled water. 2% potato dextrose agar (PDA) medium will be autoclaved (Astell CE 0353, England) at 121^{o}C 15lb/inch2 for 15 minutes and will be poured into sterilized Petri plates. Samples will be observed under dissecting microscope to select infected parts and a small piece will be taken by sterilized needle/scalpel and transferred under aseptic conditions to the sterilized Petri plates containing 15 ml 2% PDA. Inoculated Petri plates will be kept in an incubator set at 25-30°C for few days for fungal growth and germination. The plates will be observed regularly for pure fungal growth and if it will be contaminated, the process will be repeated till the pure culture of fungus will be obtained.

RESULT AND DISCUSSION

The findings observed that the leaf spot occurs throughout the year with high severity during winter months (November- January) the disease is caused by C*ercospora mori*cola and *Pseudocercospora mori* a fungal pathogen (Fig.4 and Fig 5). It can be easily distinguished from healthy mature mulberry leaves by physical observation. The mycelium is in the form of branched, septets interwoven with a large number of conidiophores. Mycelia may be external or internal. The conidiophores are brown in color, branched septets having chromonema. It may vary in size from 100-200µm in length. They develop in the group from external or internal mycelia. Each conidium measures 50-70µm in length and 2-5µm in breadth. The pathogens appear gray in color which later brown colored spots on the ventral surface of the leaves. Spot coalesce in the later stages covering all over the ventral surface. The leaves become darkening and premature fall from plants.

Fig. 4: Growth of *Cercospora moricola*

Fig. 5: Growth of *Pseudocercospora mori*



In the case of *Cercospora moricola* the leaves appear of black irregular necrotic spots with yellow hollow margin. The falling of necrotic area from shoot holes on the leaves. The spots coalesce in the later stage and leaves are premature fall. The pathogen produces a compact mass of interwoven cushion like hyphae bearing conidia on the conidiophores. The rapid spread of the disease at the peak of the rainy season because due to the humid condition prevailing at such period which usually supports theprofuse growth of the fungal mycelium. At the peak of the rainy season the leaf spot enlarges rapidly and coalesce leading to extensive blighting and defoliation. Our results correlate with many researchers. Peris *et al*. (2012) reported that the occurrence of leaf spot

disease showed black irregular spots on the surface of the leaves, which eventually enlarged and left large holes with a yellow surrounding. The diseases appear small to medium sized brown irregular spots appear on the both sides of leaves, in advanced stage necrotic spots shed off and form holes. This is commonly known leaf spot disease of mulberry (Chattopadhyay *et al*. 2002) Siddaramaiah and Hegde (1990) studies on change in biochemical constituents of *Cercospor*a infected mulberry leaves and found that infection by the pathogen induced change in the chemical constituents like total amino acids, phenol and sugars. Sundares *et al.* (1988) reported that diseases leaves are biochemically poor in nutritive value and indicated the reduction of moisture, protein and sugar contents. Ghose L. (2003) studies that due to changes of moisture, ash, protein, lipid, sugar, reducing sugar, non- reducing sugar, total sugar, vitamin-C, phenol, pectin and mineral contents in infected leaves, the metabolic process of infected plants is altered as compared to healthy ones which leads to cause yield reduction of mulberry leaves. Chikkaswamy and Paramanik (2014) and Dutta *et al*. (2013) reported that the incidence of leaf spot was found maximum during the winter season. Siddaramaiah *et al*. (1978) observed on leaf spot of mulberry in the humid condition. Sukumar and Ramalingam (1989) also reported that the disease spread primarily with rain droplets through conidia.

CONCLUSION

The result of the study that both of the pathogens *Cercospora moricola* and *Pseudocercospora mori* of mulberry plant leaf can be reduced by general sanitation and proper management, which includes the elimination of susceptible weeds and infected debris around the mulberry plantation, can also help reduce the chance of infestation.

ACKNOWLEDGEMENTS

We express our cordial thanks to Professor Aquil Ahmad, Principal Gandhi Faiz-e-Aam College Shahjahanpur and Dr. Atul Singh, Joint Director and Incharge Pathology Section, Uttar Pradesh Council of Sugarcane Research Institute, Shahjahanpur for providing the facilities for isolation of pathogens in their laboratory. Special thank are due to Dr. V. Elangovan, Professor, Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, for his valuable suggestions during present research work.

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