International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 10 No. 4, 2024
https://www.theaspd.com/ijes.php

Studies on Taxonomy and Phytopathology of *Pseudocercospora* species invading Medicinal Plants of Family - Solanaceae in North-Eastern Uttar Pradesh, India

Fakhare Do Alam[1], Dilip Kumar Tripathi[2], Shailendra Yadav[3], Supriya Pandey[4] and Neeraj Srivastasva[5*]

[1-4]Ph.D. Research Scholars, Applied Mycology Lab, Department of Botany, St. Andrew's (Post-Graduate) College, Gorakhpur – 273001, U.P., India
[5]Professor, Department of Botany, St. Andrew's (Post-Graduate) College, Gorakhpur – 273001, U.P., India
***Corresponding Author:** Neeraj Srivastava. Email: mycology1999@gmail.com

Abstract
The North-Eastern region of Uttar Pradesh, India, boasts a diverse array of biological species, including micro- and macro-fungi. *Pseudocercospora* Speg. is a plant pathogenic fungus responsible for various diseases in different plants of economic importance. In the current study, while conducting a survey of villages, agricultural fields and woodlands of Gorakhpur district, two plant-pathogenic species of *Pseudocercospora* have been collected and documented, named - *Pseudocercospora solanicola* and *Pseudocercospora withaniae*, causing infection to two plants of medicinal importance of Family – Solanaceae - *Solanum nigrum* and *Withania somnifera*. Morpho-taxonomic descriptions of these two *Pseudocercospora* species have been done, including Camera Lucida drawings and photomicrography. Phyto-pathological studies have also been conducted, with reference to decrease in photosynthesizing regions of leaves. It is determined that these *Pseudocercospora* species influence the efficacy along with therapeutic qualities of these medicinally important plants.

Keywords: *Pseudocercospora*, Leaf Spot Fungi, Morpho-taxonomy, Phytopathology, Solanaceae, Medicinal Plants.

INTRODUCTION

District Gorakhpur of North-Eastern Uttar Pradesh, India, is situated along river Rapti, at the base of the Himalayas (Geographical Location - 26.7637152° N; 83.4039116° E; Yearly Average Temperature ranging from 26 to 27°C; Altitude – 246 ft.). Gorakhpur boasts a diverse array of plant life, encompassing both - forest vegetation and crops of agricultural, horticultural, and medicinal significance.[1] It has a subtropical, warm, and highly humid climate, especially during the monsoon season from July to November, which fosters the growth of different pathogens, especially fungal, bacterial, and viral pathogens, among others.[2]

The fungus genus *Pseudocercospora* was established by Carlos Luigi Spegazzini in 1910 (Type Species - *Pseudocercospora vitis*)[3], and was redefined by Ellis in 1971.[4] It belongs to Division – Ascomycota (Systematic Position: Kingdom – Fungi, Division – Ascomycota, Class – Dothideomycetes, Order – Capnodiales, Family – Mycosphaerellaceae). Species of *Pseudocercospora* have been reported as plant pathogens, causing a wide range of necrotic and non-necrotic leaf spots and heavy damage to economically important plants[5, 6]. These are predominantly reported from tropical regions of the world.[7] About 1,517 species of this pathogenic fungus genus have been listed by *"Species Fungorum"*[8], while the *"Global Biodiversity Information Facility (GBIF)"* lists about 1540 species.[9] The genus is characterized by pigmented conidia and conidiophores without thickened hila and conidiogenous scars.[10, 11]

District Gorakhpur has been reported since long to harbour numerous novel fungi, including species of *Pseudocercospora*. In India, the biodiversity of this fungus genus has been compiled by Prof. Kamal[12] and his associates.[13, 14, 15, 16, 17, 18, 19]

MATERIALS AND METHODS

Villages and forest regions of district Gorakhpur were explored for collection of diseased plant specimens. The collected diseased leaves of *Solanum nigrum* and *Withania somnifera* were flattened, and dried for preparation of Perpetual Herbarium for submission in Applied Mycology Lab. as Isotype. The Herbarium Cryptogamae Indiae Orientalis (HCIO) at Indian Agricultural Research Institute (IARI), New Delhi was accessed to examine afflicted samples collected. Morpho-taxonomy, photomicrography, and Camera Lucida illustrations of *Pseudocercospora* species invading these two medicinally significant plants were carried out. The phytopathology of these pathogenic fungi was studied in terms of reduction in photosynthetic regions of leaves, which was in contrast to the leaf sizes of healthy leaves, and increase in the sizes of leaf spots at one-week intervals.

HOST PLANTS UNDER STUDY

Two medicinally important plants, named *Solanum nigrum* L. and *Withania somnifera* L. were collected during survey, infected with *Pseudocercospora* species.

I. *Solanum nigrum* L. (Family – Solanaceae)

Solanum nigrum L. (Family – Solanaceae), often referred to as "Black Nightshade", is a standing, spreading, branched, unarmed, suffrutescent, yearly herb found as a weed in various locations of India, including Gorakhpur district of North-Eastern Uttar Pradesh.

Medicinal Properties

The plant serves as an abundant source of riboflavin, nicotinic acid and ascorbic acid, beta carotene and citric acid. In addition to this, fruit also holds aglycone with steroidal properties, glycoalkaloids, specifically Solamargine and α & β Solanigrine.

The plant is utilized in diverse ways in Ayurvedic and ethnomedicine to manage jaundice, dysentery, fever, and different stomach and liver disorders.[20, 21]

II. *Withania somnifera* L. (Family - Solanaceae)

Withania somnifera (Family-Solanaceae) commonly known as "Ashwagandha or Indian Ginseng", is of Indian origin and is one of the most powerful medicinal plants for more than 3,000 years.[22, 23]

Medicinal Properties

It is commercially cultivated for its roots, a natural rich source of Glycowithanolides, Tannins, Potassium Nitrate, *etc.*, which are an anti-inflammatory, anti-tumor, anti-oxidant, anti-ulcer, and regulator of the nervous system and sleep. [24, 25]

OBSERVATIONS AND RESULTS

Two species of *Pseudocercospora* invading leaves of these two medicinally important plants have been described and illustrated as follows:

(I). *Pseudocercospora solanicola* Singh *et al.*

On leaves of *Solanum nigrum* L. (Family – Solanaceae).

(1). Symptoms on Leaves (Fig. 2a and 2b)

Leaf spots are initially hypophyllous, but become amphiphyllous at advanced stage of infection; greyish to dark black, circular to subcircular, black with yellowing margins on the adaxial leaf surface, and black and velvet-like on abaxial leaf surface, with solid texture. Colonies growing at a very slow pace, measuring 11–12 mm in diameter.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 10 No. 4, 2024
https://www.theaspd.com/ijes.php

Fig. 1. *Solanum nigrum* L. (Family – Solanaceae)
(Healthy Leaves)

Fig. 2a **Fig. 2b**

Fig. 2. *Solanum nigrum* L. (Family – Solanaceae)
(Diseased Leaves with considerate reduction in photosynthetic areas)

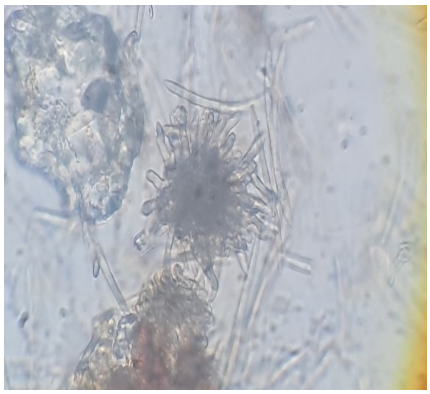
(2). Morpho-taxonomy of Pathogen - *Pseudocercospora solanicola* Singh *et al.* (Fig. 3a, 3b and 3c)

Conidiogenous cells incorporated, apical, polyblastic, light olive-green, sleek, bent, scar not thickened.

Conidiophores are grouped in fascicles of 20–31 in divergent arrangements; smooth, 0–5 septate, infrequently branched, straight to bent, geniculate, subcylindrical 21.5–44 × 3–4 µm.

Conidia solitary, acropleurogenous, holoblastic, light brown, subcylindrical, apex blunt to slightly sharp, base obconically shortened, direct to curved, 4–7 septate, 31.0–80.5 × 3.5–4.5 µm, hilum not thickened.

Holotype: HCIO - 48722; Isotype: SAC/AML/NS - 10044.



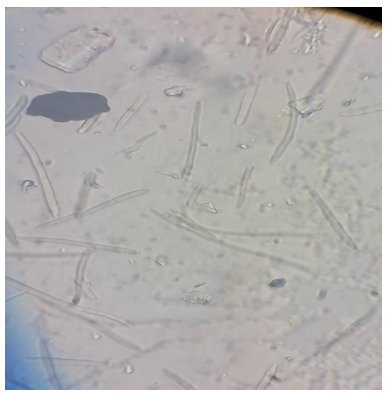


Fig. 3a. Stroma showing Conidiophores **Fig. 3b. Conidia**

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 10 No. 4, 2024
https://www.theaspd.com/ijes.php

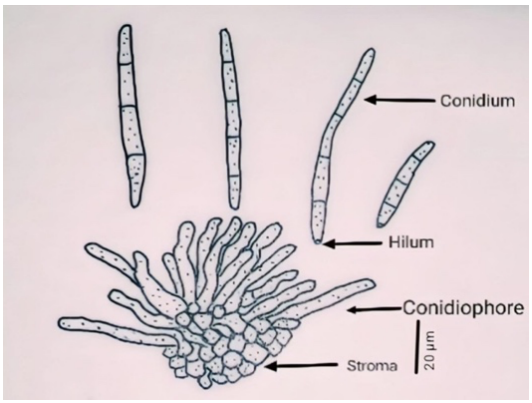


Fig. 3c. Camera Lucida drawings
(Stroma, Conidiophores and Conidia)

Fig. 3. Photomicrograph of V.S. Leaf passing through Leaf Spot

(3). Phytopathology of *Pseudocercospora solanicola*

The leaf spots on diseased leaves were measured and compared with the leaf areas of healthy leaves. It is concluded that this fungus causes about 50 - 55% reduction in the photosynthetic areas of *Solanum nigrum* leaves, resulting into considerable loss of photosynthetic productivity of the host plant. It directly affects its medicinal properties.

These leaf spots were measured thrice, at one-week intervals and gradual increase in size of leaf spots were recorded.

(II). *Pseudocercospora withaniae* (H. Syd. & P. Syd.) Deighton

On leaves of *Withania somnifera* L. (Family – Solanaceae).

(1). Symptoms on Leaves (Fig. 5)

Leaf spots are amphigenous, almost circular, well-defined, spread evenly over the whole leaf surface, reaching up to 4.0 mm in size. Initial symptoms are characterized by chlorotic spots, on adaxial and abaxial, both surfaces of leaves. These spots are gradually converted into grey, black or brown lesions. The small leaf spots gradually merge to form larger, irregular spots, and ultimately, rapid and premature defoliation occurs.

Fig. 4. *Withania somnifera* L. (Healthy Leaves)

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 10 No. 4, 2024
https://www.theaspd.com/ijes.php

Fig. 5. *Withania somnifera* L.
(Diseased Leaves with considerate reduction in photosynthetic areas)

(2). Morpho-taxonomy of Pathogen - *Pseudocercospora withaniae* (H. Syd. & P. Syd.) Deighton (Fig. 6a, 6b and 6c)

Microscopic analyses during initial and advanced phases of infection revealed the formation of conidia on conidiophores.

Mycelium inner, light olive green, branched and partitioned.

Stroma substomatal, well-developed, pseudoparenchymatous, light olive-hued, extending up to 30 µm in width.

Conidiophores found in fascicles of 12-30, linear or slightly curved, macronematous, mononematous, unbranched, with 0-1 transverse septa, olive-hued, measuring 15 - 40 × 3 - 5 µm.

Conidiogenous cells are terminally fused, cylindrical, polyblastic, and cicatrized.

Conidia are dry, holoblastic, acropleurogenous, solitary or occasionally linked, obclavato-cylindrical, straight to curved, smooth-edged, light olivaceous, 1-5 transversely septate, possessing a rounded tip and an obconically clavate base, measuring 25-100×4-5 µm.

Holotype: HCIO - 50131; Isotype: SAC/AML/NS - 10047.

Fig. 6a. Stroma showing Conidiophore

Fig. 6b. Conidia

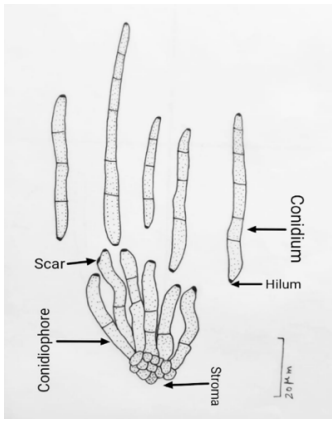


Fig. 6c. Camera Lucida drawings (Stroma, Conidiophores and Conidia)

Fig. 6. Photomicrograph of V.S. Leaf passing through Leaf Spot

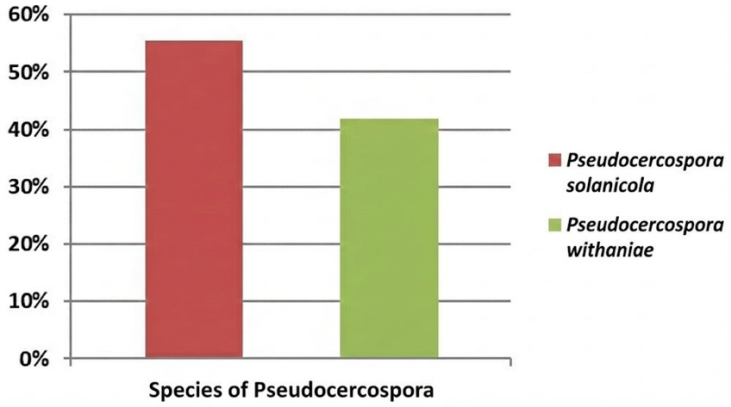
(iii). Phytopathology of *Pseudocercospora withaniae*

The areas of leaf spots of infected leaves are measured and compared with the total leaf areas of disease-free healthy leaves. It is estimated that this fungus causes approximately 40 to 45% reduction in the photosynthetic areas of the leaves, and ultimately affecting its medicinal properties by causing heavy reduction in photosynthetic productivity of the host plant.

Leaf spots were measured thrice, at one week interval. Gradual and continuous increase in the size of leaf spots was recorded. Also, small spots coalesce to form larger spots in due course of time.

DISCUSSION AND SUMMARY

The present investigation clearly shows that species of *Pseudocercospora* produce smaller to larger leaf spots on both, adaxial and abaxial surfaces. In advanced stage of infection, the smaller leaf spots coalesce to form larger and irregular spots. As such, photosynthetic areas of leaves are considerably reduced, resulting into reduction in the productivity of host plants. Also, the disease causes rapid and premature drying and shedding of infected leaves. This results into less production of medicinally and pharmacologically important compounds and secondary metabolites of plants. Therefore, the infected and diseased plants show comparatively less medicinal value/importance as compared to healthy plants.



Percent Reduction in Photosynthetic Areas of Leaves

If a comparison is made between two species of *Pseudocercospora*, it is clear that *Pseudocercospora solanicola* is showing more reduction in photosynthetic areas of leaves of *Solanum nigrum* (50 - 55%), as compared to less reduction caused by *Pseudocercospora withaniae* (40 – 45%).

ACKNOWLEDGEMENTS

Authors are thankful to HCIO (Herbarium Cryptogamae Indiae Orientalis), Indian Agricultural Research Institute (IARI), New Delhi for providing facilities to study diseased specimens deposited there. We are also thankful to the Principal, St. Andrew's College, Gorakhpur for providing infrastructural lab. facilities.

Authors Contributions: All authors contributed significantly to the final manuscript.
Statements and Declarations: Authors declare no conflict of Interest.

REFERENCES

[1.] Srivastava, Neeraj. 2007. Foliicolous hyphomycetous fungi invading plants of Family – Lamiaceae in North-Eastern Uttar Pradesh, India. J. Liv. World. 14(2), pp. 78-82.
[2.] Srivastava, Mamta, Arya, Mithilesh Kumar, and Srivastava, Neeraj. 2011. Cellulolytic fungi causing biodeterioration of Webster's dictionary in Gorakhpur. International Journal of Biological Technology

Vol. 2 (Special Issue), pp. 216-220.
[3.] Spegazzini C.L. 1910. Mycetes Argentinenses (Series V). Anales del Museo nacional de Historia natural, Buenos Aires. 20 (13), pp. 329–467.
[4.] Ellis, M.B. 1971. Dematiaceous Hyphomycetes. – 608 p., Commonwealth Mycological Institute, Kew, U.K.
[5.] Lieberei, Reinhard. 2007. South American Leaf Blight of the Rubber Tree (*Hevea* spp.): New Steps in Plant Domestication using Physiological Features and Molecular Markers. Ann. Bot. 100 (6), pp. 1125 – 1142. doi:10.1093/aob/mcm133. PMC 2759241. PMID 17650512.
[6.] Kumar, Shambhu, and Singh, Raghvendra. 2015. *Pseudocercospora bischofigena*, a new anamorphic fungus from North-Eastern Uttar Pradesh, India. Czech Mycology. 67. pp. 39-44. 10.33585/cmy.67105.
[7.] Kirk P.M., Cannon P.F., Minter D.W., and Stalpers J.A. 2008. *Dictionary of the Fungi* (10th ed.). Wallingford, UK: CABI. p. 567
[8.] "*Species Fungorum* - Search Page - *Pseudocercospora*". 2023 (September). www.speciesfungorum.org.
[9.] "*Pseudocercospora*". 2023 (September). www.gbif.org.
[10.] Crous P.W. and Braun U. 2003. *Mycosphaerella* and its anamorphs: 01. Names published in *Cercospora* and *Passalora*. – p. 571, C.B.S., Utrecht.
[11.] Braun U., Nakashima C., and Crous P.W. 2013. Cercosporoid fungi (Mycosphaerellaceae) 1. Species on other fungi, Pteridophyta and Gymnospermae. – IMA Fungus. 4. pp. 265–345.
[12.] Kamal. 2010. Cercosporoid Fungi of India. – vii + 351 p., Bishen Singh Mahendra Pal Singh Publication, Dehradun, India.
[13.] Kamal, and Singh, R.P. 1980. Fungi of Gorakhpur. XIX. *Pseudocercospora*. Sydowia. 33, pp. 157-161.
[14.] Rao, H.S.G., A.S. Moses, and P.N. Singh. 1996. Some new species of Pseudocercospora from India. Mycological Research, 100 (9). pp.1071-1074. https://doi.org/10.1016/S0953-7562(96)80215-2.
[15.] Rao, H.S.G., S. Narayan, and Archana Singh. 1998. New species of *Pseudocercospora* from India. Mycological Research, 102(2), pp. 157-162. https://doi.org/10.1017/S0953756297004784.
[16.] Kumar, Shambhu. 2011. Two new species of *Passalora* and *Pseudocercospora* from North-Eastern Uttar Pradesh, India. Mycotaxon. https://doi.org/10.5248/117.137.
[17.] Ranjan Rajiv. 2014. *Pseudocercospora eugeniae-heynianae* sp. nov. on *Eugenia heyneana* from Tilkoma forest, Gorakhpur, U.P., India. International Journal of Applied Research, 1(1), pp. 454-457.
[18.] Singh, Archana, Singh, P.N., Nath, Gopal, and Dubey, Nawal Kishor. 2021. Phylogeny and taxonomy of a novel species of *Pseudocercospora* from India. Turkish Journal of Botany, 45 (2). https://doi.org/10.3906/bot-2012-27.
[19.] Singh, Archana; Yadav, Sanjay; Singh, Raghvendra; and Dubey, Nawal. 2022. Taxonomy and Phylogeny of a new species of *Pseudocercospora* on *Solanum nigrum* from India," Turkish Journal of Botany, 46 (5). https://doi.org/10.55730/1300-008X.2726.
[20.] Jain, S.K. 1991. Dictionary of Indian Folk Medicines and Ethnobotany. Deep Publication, Paschim Vihar, New Delhi. pp. 168. https://agris.fao.org/agris-search/search.do?recordID=US201300721288
[21.] Prajapati, N.D., Purohit, S.S., Sharma A.K., and Kumar, T. 2003. A Handbook of Medicinal Plants: A Complete Source Book. Agrobios (India). pp. 480-481.
[22.] Pandit, S., Chang, K.W., Jeon, J.G. 2013. "Effects of *Withania somnifera* on the growth and virulence properties of *Streptococcus mutans* and *Streptococcus sobrinus* at sub-MIC levels". Anaerobe. 19, pp. 1–8.
[23.] Cheah, K.L., Norhayati, M.N., Yaacob, L.H., *et al.* 2021. "Effect of Ashwagandha (*Withania somnifera*) extract on sleep: A systematic review and meta-analysis". PLOS ONE. 16 (9) e0257843. doi: 10.1371/journal.pone.0257843. ISSN 1932-6203.
[24.] Pasricha, T. 2024 (March). "Advice | Should I take ashwagandha for sleep? Here's what the science says". The Washington Post. ISSN 0190-8286.
[25.] Philips, C.A. and Theruvath, A.H. 2024 (April). "A comprehensive review on the hepatotoxicity of herbs used in the Indian (Ayush) systems of alternative medicine". Medicine (Baltimore) (Review). 103 (16) e37903.