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## *Pseudocercospora zizyphae-onopleae* sp. Nov. on *Zizyphus oenoplia* from Ramgarh Forest, Gorakhpur U.P., India

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### **Abstract**

During survey for the foliicolous fungi from diversified habitats of Ramgarh Forest, Gorakhpur U.P. India we came across an important plant of the locality, *Zizyphus oenoplia*, (Rhamnaceae) commonly known as the jackal jujube, small-fruited jujube or wild jujube, known as Makora in Hindi is a flowering plant with a broad distribution through tropical and subtropical Asia and Australasia. It is a spreading, sometimes climbing, thorny shrub growing to 1.5 m in height. The leaves are simple, alternate, ovate-lanceolate, acute and oblique. The flowers are green, in sub sessile axillary cymes. The fruit is a globose drupe, black and shiny when ripe, containing a single seed. On critical study the living leaves were found to be infected with *Pseudocercospora*. Since, it has also been customary for plant pathologists and mycologists to describe as new any *Cercospora* or *Pseudocercospora* found a host for the first time (Ellis, 1971), this undescribed taxa has been described and illustrated as *Pseudocercospora zizyphae-onopleae* sp. nov. Rajiv Ranjan. The review of available literatures reveals that there has been no record of this fungus from India on this host so far. Therefore, this host of the new species is a new record to Indian mycoflora from Ramgarh Forest, Gorakhpur U.P. India

**Keywords:** Foliicolous fungi, pseudocercospora, Gorakhpur, morphotaxonomic treatment, camera lucida.

### **1. Introduction**

The leaves provide a very suitable habitat for the growth & development of fungal pathogen by providing ample surface area and nutrient supply. Such leaf inhabiting fungi are known as foliicolous and the invaded area of the leaf appears as leaf spot or leaf lesion. Taxonomic studies of such fungal forms have been generally considered as only of academic interest but the taxonomic treatment of a fungal organism in the first requirement for any studies concerning its biology. Correct identification of a fungus absolutely free from ambiguities is vital for its employment in applied disciplines. In fact without being equipped for ascertaining the correct identity of a fungal pathogen all studies concerning its phytopathological aspects would be misleading. The weed and forest plants serve as reservoirs of leaf spot pathogens which on getting opportunity may spread to agriculture and horticulture plants.

India is the one of the twelve mega biodiversity countries of the world, has two of the world's eighteen biodiversity hot spot located in the Western Ghats and in the Eastern Himalayas. In North of North Tarai Forests, the Himalayas rise as a virtual wall beyond the snow line. Above the alluvial plain lies the Tarai strip, a seasonally marshy zone of sand and clay soils. The Tarai has higher rainfall than the plains, and the downward-rushing rivers of the Himalayas slow down and spread out in the flatter Tarai zone depositing fertile silt and reproductive means during the monsoon season and receding in the dry season. The Tarai, as a result has high water level and is characterized by moist sub-tropical conditions and a luxuriant turnover of green vegetation all the year around. The climatological and topographical conditions favor the luxuriant growth and development of foliicolous fungi. This North Eastern Tarai region of U.P. is next only to Eastern and Western Ghats, as one of the hottest spots for biodiversity in general and the diversity of fungal organism inhabiting plant leaves in particular offers an ideal opportunity for the morphotaxonomic exploration of fungal organism in general and foliicolous fungi in particular. Keeping this in view we surveyed the locality of Forest (Jungle) Ramgarh Urf Chawri is a locality in Gorakhpur, Gorakhpur District, Uttar Pradesh, India. Sahara Estate (5.44 Km), Mohaddipur (8.62 Km), Taramandal (11.29 Km),

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Transport Nagar (12.94 Km) are the nearby areas to Jungle Ramgarh Urf Chawri. Domane, Rampur, Upadhavlee, Chhoti Semari, Jungle Ramgarh Urf Sarka, Jangl Ramgarh Urf Chawri, Taldohr, Jangalsikari Urf Khoraba, Vahrampur, Raneedeh, Konee, Belwar, Maheemath, Jangal Ayodhya Parsad, Semra Ayodhya Parsad, Siktaur, Gaighat Buzurg, Gorakhpur, Chapha, Bishunpur Khurd, Raiganj, Korar, Chhinuteejam, Tal Kandala, Ramnagar Karjaha, Khorabar Urf Soobabazar, Gorakhpur, Jangl Ram Lakhan, Motiram Adda are the nearby cities to Jungle Ramgarh Urf Chawri in Gorakhpur district during February 1999 to August 2001.

## 2. Scope of study

The Foliicolous Fungi causes huge loss every year in different parts of the world. The fungal pathogens producing leaf spots infect a large variety of hosts including most of the crops, forests and other plants. The destruction caused by these enemies of leaves is a serious problem before us. The focus of this research is identification & documentation of foliicolous fungi which will assist in the discovery of new fungicides and ideas to overcome from the severity of these enemies of nature as well as in the protection of floral diversity from the infection of these pathogens and also in the conservation of valuable flora of the area.

## 3. Materials and Methods

The climatic condition favors the growth of various types of phanerogamic vegetation along with seasonal and annual crops and other plants. With a view to study the foliicolous fungi in their natural habitat, frequent collection trips will be arranged. The following articles would be required for collecting foliicolous fungi-collection containers, hand lens, pruning scissor or secateurs, light plant pressures, blotting paper, paper envelop, field note book etc.

### Laboratory processing and preliminary examination Preparations

- Photograph of both host and pathogen will be taken.
- Scrap mount: If the organisms are superficially attached with the host tissue scrap mounts are made by a sharp razor or scalpel.
- Collodion Preparation: -A drop of collodion solution is applied to a colony on the leaf. The fungus gets embedded entirely and the dried film is peeled off readily from the host surface. Removal of colliding by acetone on a glass slide gives undisturbed preparation.
- Squash preparation: The fruiting body is mounted, cleared and examined. Then the preparation is tapped vigorously and reheated. In this way the fruiting body is broken and content is released.
- Hand cut Section preparation: A hand cut section of infected tissue is made with sharp razor to study immersed or semi-immersed fungi. Section cutting for host parasite interaction / relation.

### Staining and Mounting

For routine microscopic study in the lab temporary slides are made in different type of stains and mountings according to nature of fungal forms involved.

- **Lacto-phenol cotton blue:** The lacto-phenol mounting fluid is used for mounting-colored fungi. For locating cytoplasm, septa, guttules other structures and hyaline forms 0.05-0.01 % cotton blue is added.
- **Poly-vinyl Alcohol:** Benson 1969 is used in routine staining and mounting.

- **Lacto-fuchsin:** By this cell walls are stained more clearly, rapidly and with more suitable color specially for photography [Carmichael 1955] <sup>[15]</sup>. Slides prepared in mountants are sealed with wax or commercial good quality nail polish and are stored for further study.

**Camera Lucida:** Drawings will be made of the distinctly different taxa of generic or species rank so as to show the morpho taxonomic features of vital importance.

**Morpho taxonomic treatment:** Hitherto undescribed forms of foliar fungi will be executed with the help of present literature and expertise available at hand.

- New taxon will be described in English or Latin or both as and when required.
- Material (holotypes) will be deposited in recognized Herbaria for accession no.

During collection, infected leaf samples were taken in separate polythene bags. Suitable mounts of surface scrapping and hand cut sections were prepared from infected portions of the leaf samples. Slides were prepared in cotton-blue lacto phenol mixture & were examined. Camera Lucida drawing were made and the morpho-taxonomic determination of taxa was done using available literature and with the help of resident's expertise available. The fungal taxa were identified using microscopic preparation.

## 4. Result and Discussion

The author surveyed during February, 1999 to August, 2001 in diversified habitats of Ramgarh Forest, Gorakhpur U.P. India for the collection, study and documentation of the leaf spot micro fungi infecting variety of the angiosperms has resulted in abundant gathering of the fungal specimens. During survey the author came across an important plant of the locality, *Ziziphus oenoplia*, (Rhamnaceae) commonly known as the jackal jujube, small-fruited jujube or wild jujube, known as Makora in Hindi is a flowering plant with a broad distribution through tropical and subtropical Asia and Australasia. On critical study the living leaves were found to be infected with *Pseudocercospora*. Since it has also been customary for plant pathologists and mycologists to describe as new any *Cercospora* or *Pseudocercospora* found on a host for the first time (Ellis, 1971) <sup>[22]</sup>, this undescribed taxa has been described and illustrated as *Pseudocercospora zizyphi-onopleae* sp. Nov. Rajiv Ranjan. The holotype specimen has been submitted in HClO, IARI New Delhi for allotment of accession number. The Accession no. is 43861.

*Pseudocercospora zizyphi-onopleae* Rajiv Ranjan sp. Nov.

**Maculae:** Amphigenae, irregulars, griseae vel in obscure brunneum desinentes et coronis flavidis circumdatae, discretiae vel coalescentes et mortuae, denique marginibus obscure orchraceis circumdatae.

**Coloniae:** Epiphyllae, in areolis obscure brunneis vel mortuis restrictae, irregulars, fusco ivaceae vel atrae, paulum punctiformes

**Mycelium:** Exhyphis immerses, ramosis, septatis, levibus, hyalines compositum.

**Stromata:** Bene evoluta, substomatica vel superepidermalia, pseudoparenchymatosa, olivaceo-brunnea, 20.0-47.0 µm diam

**Conidiphora:** Dense fasciculate, valdeconferta, omnifasciculo innumerabiles amplexentia, per stomata vel epidermidem ruptam emergentia, macronematosa, mononematosa, septate, ramose, sinuosa vel flexuosa, pallid vel mediocriter olivaceo-grisea, cylindrica, plus minusve geniculate, denticulis distinctis vel nullis ornate, levia,  $19.0-74.0 \times 4.0-5.0 \mu\text{m}$ .

**Cellulae conidiogena:** integratae, terminalis, polyblasticae, sympodiales, saepe distinct denticulatae.

**Conidia:** Sigularia, sicca, acropleurogena, pleurumque obclavata, vel raro obclavato cylindrica, recta vel subarcuata apicibus plerumque subacuta, raro obtusa, basibus obconico-truncata, pallid egrisea, laevia vel raro an septa constricta, septis 3-pluribus transversis divisa, hilo non incrassato  $33.0-84.0 \times 2.5-4.0 \mu\text{m}$ .

In foliis vivis *zizyphi oenopliae* Mill. (Rhamnacearum), Feb. 1998, Ramgarh Forest, Gorakhpur, leg. R.R. Srivastava, M.L.K./R.R.- 721 holotype, HCIO-43861 isotypus.

**Leaf spots:** Amphigenous, more or less irregular, grayish to dark brown with yellowish halos, remaining discrete or coalescing and becoming necrotic with distinct dark on margin in due course.

**Colonies:** Epiphyllous, localized to dark brown or necrotic areas, irregular, dark olivaceous to blackish, somewhat punctiform.

**Mycelium:** Hyphae immersed, branched, septate, smooth,

hyaline.

**Stromata:** well developed, substomatal or subepidermal, pseudoparenchymatous, olivaceous brown,  $20.0-47.0 \mu\text{m}$  in diam.

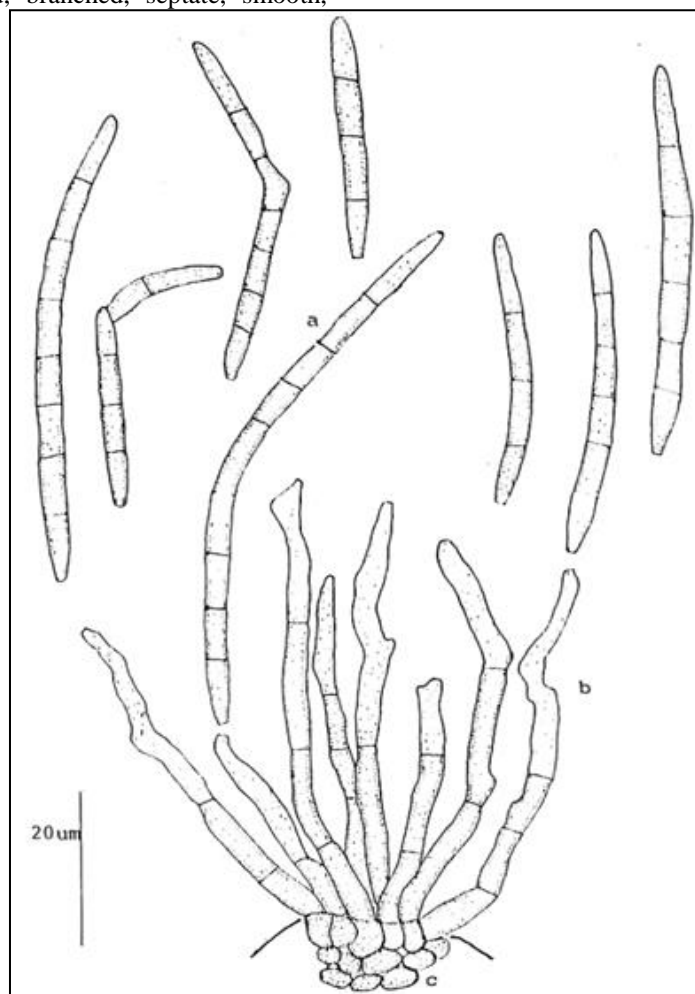
**Conidiophores:** Densely fasciculate, much crowded, many per fascicle, emerging through the stomata or rupture epidermis, macronematous, mononematous, septate, unbranched, mostly tapering towards apices, sinuous and/ or flexuous, light to mid olivaceous grey, cylindrical, more or less geniculate, with or without distinct denticles, smooth-walled,  $19.0-74.0 \times 4.0-5.0 \mu\text{m}$ .

**Conidiogenous cells:** Integrated, terminal, polyblastic, sympodial, often distinctly denticulate.

**Conidia:** Solitary, dry, acropleurogenous, mostly obclavata to seldom obclavato-cylindric, straight to slightly curved or sometimes showing one or more abrupt curvatures, with mostly subacute to rarely obtuse apices and obconico truncate bases, light grey, smooth to rarely somewhat constricted along septa, transversely 3-pluri septate, hila un thickened,  $33.0-84.0 \times 2.5-4.0 \mu\text{m}$ .

On living leaves of *Zizyphus oenoplia* Mill. (Rhamnaceae), Feb. 1998, Ramgarh Forest Gorakhpur, leg. R.R. Srivastava, M.L.K./R.R.- 721 holotype, HCIO-43861 isotype.

The present fungus can be compared with *P. alphonitiae* (Petra) Deighton, the only species of *Pseudocercospora* recorded on Rhamnaceae so far (Deighton, 1976a). The following table shows this comparison:



A. Conidia, B. Conidiophore, C. Stroma

**Fig 1:** *Pseudocercospora zizyphi-onopleae* Sp. Nov.

**Table 1:** Shows the species *Stroma Conidiophores* and *Conidia*

Species	Stroma	Conidiophores	Conidia
<i>P. Asiatica</i> (Proposed sp.)	Pseudoparenchyma oliv. Br.20.0-46.0 µm diam	Erect, tapering towards apices, light to mid olive grey 18.5-7.3 × 3.0-4.0 µm.	Obclavate ocylendiric bases obconico truncate light grey, 3-9 or more septate 32.0- 83.0 × 2.3-4.0 µm
<i>P.alphitoniae</i> (Petrak) Deighton	Absent	Erect, tapering towards spices, 250-500 × 5.0-8.0 µm.	Obclavate to fusoid, bases almost truncate, 1-6 trans. Septate 25.0-66.0 × 8.0-13.0 µm.

It is apparent from the above table that the present collection resembling *P. Alphitoniae* in structure of only conidiophores, shows marks divergence from the latter in having well developed stomata and light to mid olivaceous grey, much shorter and narrower conidiophores. It further differs from the said species in having fairly longer but much narrower and light grey conidia with obconico truncate bases and larger number of septa. It is also noteworthy that two species of *Pseudocercospora* has ever been reported on the host species in question. Obviously, the author's collection cannot be accommodated with *P. Alphitoniae* and its description as a new species is reasonable. Survey of Literature Kamal *et al.*, 1986 [25]; Ellis, 1971, 1976 [22, 23]; Deighton, 1967, 1973, 1974, 1976, 1979 [17-21]; Braun, 1987, 1988 a, b, 1991 [10-14]; Braun *et al.*, 1992 [14]; Bagyanarayan and Braun, 1991, 1992, 1999 [1-6]; Bagyanarayan *et al.*, 1991, 1992, 1994, 1995 [1-6, 14]; Vasudeva, 1963 [26]; Chupp, 1954 [16]; Bilgrami *et al.*, 1979, 1981, 1991 [7-9] reveals that there is no record of *Pseudocercospora* species of this type on the host family. Therefore, it is described and illustrated as a new species to accommodate it.

## 5. Conclusion

The Region of Ramgarh Forest, Gorakhpur U.P. India is rich in phytodiversity in general as well as the diversity of fungal organisms inhabiting plant leaves in particular and it provides great scope for the study of foliicolous fungi. The correct identity of a fungus absolutely free from ambiguities is vital for its employment in applied disciplines in general and it is more so for plant pathology where precision of details about the biology of the pathogen is primarily conditioned by its identity. In fact, without being equipped for ascertaining the correct identity of a fungal pathogen all studies concerning its psychopathological aspects would simply be misleading. However, the end is still not insight and further investigation is warranted.

## 6. Acknowledgement

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