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Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India Morphology of *Puccinia horiana* Henn., the causal agent of chrysanthemum white rust occurred in West Bengal

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#### Abstract

Chrysanthemum is one of the most important flower crops in India. The chrysanthemum white rust (CWR) disease caused by *Puccinia horiana* (Henn.) was observed on chrysanthemum (cv. Meri Gold) in West Bengal for the first time. Initial symptoms was appeared light green to yellow, slightly raised spots mostly on the lower surface of leaves, which later, turned into pinkish brown to dark brown necrotic lesion surrounded with light green to yellow hallo. The fungus produced only two spore stages, teliospores and basidiospores. Studies under the microscopes revealed that the telial pustules were found mostly on the lower side of the leaves. The teliospores were thin walled, bicelled, pedicellate, pale yellow, oblong to oblong-clavate. The average size of teliospores was 47.85µm X 13.48µm. The teliospores could germinate frequently into a promycelium *in situ*, from the apical telial cell and were tubular, mostly segmented, club shaped. The basidiospores were oval in shape.

Keywords: Chrysanthemum white rust (CWR), Puccinia horiana, teliospores

### Introduction

Chrysanthemum (*Chrysanthemum sinensis* L.) is one of the oldest flower crop grown in the world. It is known as "Queen of East" in European countries. It is the top dollar earning flower in United States. In India, total area and production of flower are 200170 MT and 20090 ha, respectively (Anonymous, 2015-16)<sup>[1]</sup>. Chrysanthemum flower cultivation is also very popular in West Bengal occupying 6<sup>th</sup> position in production (7880 MT) and 8<sup>th</sup> position in area (520 ha) in India (Anonymous, 2015-16)<sup>[1]</sup>. In West Bengal, the main chrysanthemum growing districts are Purba Medinipur, Paschim Medinipur, Darjeeling, North 24-Paraganas, Nadia, South 24-Paraganas and Howrah.

The major threat in successful and profitable cultivation of chrysanthemum is the fungal diseases and among them, one of the most important diseases is white rust. Puccinia horiana Hennings is a microcyclic autoecious rust fungus that causes chrysanthemum white rust. The teliospores are normally formed on the lower side of the leaves and can germinate in situ into a promycelium without a period of dormancy. Leaf wetness and a high relative humidity are essential for basidiospore formation, survival, and infection ((Firman and Martin 1968<sup>[2]</sup>; Kapooria and Zadoks 1973 [3]). Puccinia horiana was first detected in Japan in 1895 (Hennings 1901<sup>[4]</sup>; Hiratsuka 1957<sup>[5]</sup>). Currently, the pathogen has been reported in most chrysanthemum growing countries like China, South Africa, Brazil and Australia (Whipps 1993 <sup>[6]</sup>; Anonymous, 2004 <sup>[7]</sup>). Incidence of the chrysanthemum white rust is first reported from Bengaluru, Karnataka state (Sriram et al., 2015<sup>[8]</sup>; Thakur et al., 2017<sup>[9]</sup>), Nasik district of Maharastra (Barhate et al., 2015<sup>[10]</sup>), Kothagiri Hills of Nilgiris District and Yercaud Hills of Salem District (Deepa et al., 2015 [11]) in India. However, the disease is recently noticed in severe form at Ranaghat-II block of Nadia district in the year 2018, which is one of the most important flower growing areas of West Bengal. The disease has not been reported previously from West Bengal.

In this paper, we report the outbreak of chrysanthemum white rust (CWR) in the State of West Bengal, India along with the symptomatology of the disease and illustrate morphological details of teliospores of *P. horiana* collected from natural infections in the farmers' field.

## Materials and methods

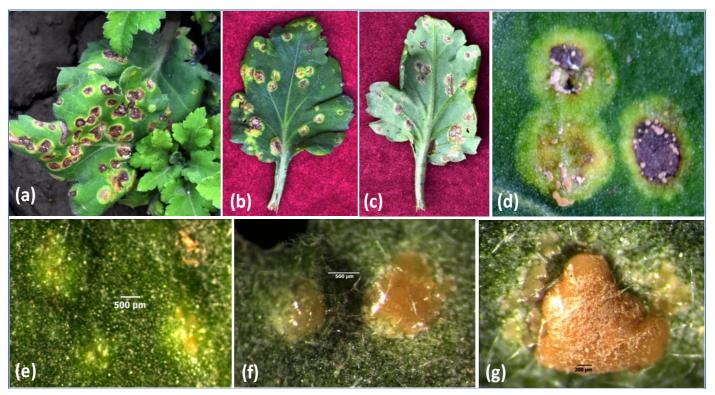
A field survey was conducted at the farmers' field growing chrysanthemum at Nadia district of West Bengal. The chrysanthemum white rust (CWR) disease caused by *Puccinia horiana* was noticed on Meri Gold variety grown in farmers' fields at Ranaghat-II block of Nadia district, West Bengal in 2017-18.

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The fields were exhibiting putative signs and symptoms of natural CWR infections. Incidence of the disease was 80-100%. The infected leaves along with the branches were collected from the field and replanted in earthen pot for further study. The pots were kept inside an isolated and protected area. Some healthy rooted seedlings/cuttings of the cultivar were planted in pot for fresh inoculation and multiplication inside the protected green house. For new propagation, infected leaves were macerated in sterilized distilled water and sprinkled onto healthy chrysanthemum plants with periodic misting. Development of symptoms took place in approximately 15 to 20 days after inoculation. The symptomatology of the disease and teliospores morphology of the pathogen was studied from the fresh infected leaves of P. horiana collected from the pots using stereo and light microscope. Fresh sample of leaves with pustules were free hand sectioned and mounted in 0.05% lactoglycerol cotton blue and the sections were viewed under microscope at different magnifications.

# **Results and Discussion**

Initial symptoms were appeared as numerous, greenish white spots on both the surface of the infected leaves. The spots on the lower surface were slightly raised due to formation of rust pustules. Size of the spots may vary and was up to 5 mm in diameter. Later, the spots turned into pinkish brown to dark brown necrotic lesions which were surrounded by light green to yellow hallo (Fig 1). Two or more necrotic spots were found to be coalesced and gave rise to burning symptoms. Pinkish buff coloured telia pustules were developed mostly on the lower surface of the leaves. In advanced stage, the numerous pustules were also observed on the upper surface. Under stereo microscope, telia pustules were buff coloured covered with slimy layer at early stage and turned into waxy at later stage (Fig 1). All these characteristics, like, symptoms of the disease, pustules development and their uniqueness were at par as described by earlier worker (Firman and Martin, 1968<sup>[2]</sup>; Punithalingam, 1968<sup>[12]</sup>; Kapooria and Zadoks, 1973<sup>[3]</sup>; Sriram et al., 2015<sup>[8]</sup>; O'Keefe and Davis, 2015 [13]).

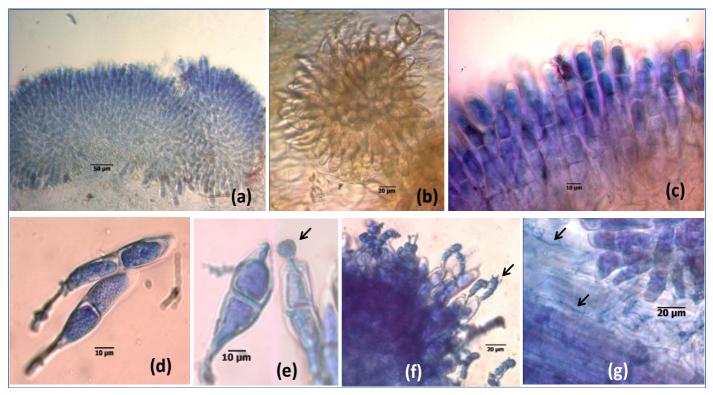


**Fig 1:** Symptoms of chrysanthemum white rust infected by *Puccinia horiana* [(a) Infected plant, (b) upper surface and (b) lower surface of infected leaf] and development of teliospore pustule viewed under stereo microscope [(d) on leaf lesion, (e) initial development of pustules, (f) active developmental stage of pustules and (g) matured pustule]

The fungus produced only two spore stages, dikaryotic or diploid teliospores and haploid basidiospores. The pathogen, autoecious microcyclic rust, was reported to produce only teliospores and basidiospores by earlier worker (Firman and Martin, 1968<sup>[2]</sup>; Punithalingam, 1968<sup>[12]</sup>; Kapooria and Zadoks, 1973<sup>[3]</sup>; O'Keefe and Davis, 2015<sup>[13]</sup>). Pycnia and

Uredial stages were not found as reported previously (Kapooria and Zadoks, 1973<sup>[3]</sup>; O'Keefe and Davis, 2015<sup>[13]</sup>). However, anastomosis within infected chrysanthemum tissues has been reported (O'Keefe and Davis, 2015<sup>[13]</sup>). The intercellular mycelia were found in our study also (Fig 2).

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**Fig 2:** Morphological study of pustules and teliospore of *Puccinia horiana* under light microscope [(a), (b) & (c) Teliospores in pustules, (d) Teliospores, (e) germinating teliospores, (f) basidia and sterigmata and (g) intercellular hyphal growth.

Under light Microscopic examination, an enormous numbers of teliospores were formed in the pustules (Fig 2). The teliospores were formed mostly on the lower side of the leaves. The teliospores were thin walled, bicelled, pedicellate, pale yellow, oblong to oblong-clavate, slightly constricted at the middle. Morphometric study of reproductive structures of *Puccinia horiana* was depicted in the table (Table 1).

Reproductive structures	Mean (µm)	Range (µm)
Length of teliospore	$47.85 \pm 4.97$	36.85 - 58.80
Width of teliospore	$13.48 \pm 1.75$	9.80 - 16.75
Length of teliospore pedicel	$46.92 \pm 4.86$	38.24 - 55.47
Thickness of teliospore cell wall	$1.58\pm0.32$	0.84 - 2.34
Thickness of apical cell wall of teliospore	$5.47 \pm 0.85$	2.58 - 7.14
Length of basidium	$27.85 \pm 3.41$	17.08 - 32.73
Width of basidium	$5.61 \pm 1.21$	3.04 - 8.28
Length of basiospore	$5.89 \pm 1.10$	3.92 - 7.25
Width of basiospore	$3.60\pm0.63$	2.91 - 4.66

Table 1: Morphometric study of reproductive structures of Puccinia horiana on chrysanthemum sinensis.

The average length and width of teliospores, length of pedicel, thickness of spore wall and apex spore wall were 47.85 µm, 13.48 µm, 46.92 µm, 1.58 µm and 5.47µm respectively. The morphometric studies of reproductive structures were also reported by several workers (Göre, 2008 <sup>[14]</sup>; Kapooria and Zadoks, 1973<sup>[3]</sup>; Backer, 2011<sup>[15]</sup>; O'Keefe and Davis, 2015<sup>[13]</sup>) and the shape and size of the teliospores were more or less similar to our study. Sriram et al. (2015)<sup>[8]</sup> reported that the teliospores were typically two celled, oblong with a pedicel of 20-40µm in length and the dimensions of teliospores was 27-41µm x 11-18µm. The size of teliospores as reported by O'Keefe and Davis (2015)<sup>[13]</sup> was 28-43µm x 8-18µm and the shape was oblong or clavate. The thickness of teliospore wall was ranging from 0.84µm to 2.34µm and the apex wall was 2.58µm to 7.14µm. The apex wall of teliospore was thicker than the spore wall. Göre (2008) <sup>[14]</sup> reported the thickness of spore wall and apex wall of teliospore as 1µm to 2µm and 4µm to 9µm respectively. The teliospores could germinate in situ into a promycelium, which were formed from the apical telial cell as reported by O'Keefe and Davis

(2015) <sup>[13]</sup>. The promycelia were found to be tubular, mostly segmented, club shaped at apical portion of cell, and sometimes branched. The size of basidia was measured by 17.08 - 32.73 $\mu$ m x 3.04 - 8.28 $\mu$ m. The basidiospores were oval in shape and 5.89 $\mu$ m X 3.60 $\mu$ m in size. The symptom alogical study of the disease and the morphological characteristics of teliospores confirmed the fungus as *P. horiana* as described by Kapooria and Zadoks (1973) <sup>[3]</sup>, Sriram *et al.* (2015) <sup>[8]</sup> and O'Keefe and Davis (2015) <sup>[13]</sup>. Under favourable conditions, teliospore produced basidiospore.

In conclusion, this is the first record and outbreak of white rust disease caused by *P. horiana* on chrysanthemum cultivar grown in West Bengal. The morphological characteristics confirmed the fungus as *P. horiana*.

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