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First report of *Puccinia horiana* Henn. causing white rust of chrysanthemum in Himachal Pradesh

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Abstract
The chrysanthemum plants especially of Calabria and Bonita group varieties showed rust symptoms for the first time which outbreaks in first week of April, 2018 in Seobagh, Kullu district of Himachal Pradesh during periodical disease surveys. The typical symptoms constituted as small pinhead flecks with blister-like round swellings having white crusty growth on the lower surface of the leaves and pale-green to sunken yellow spots on upper corresponding surface. The incidence ranged between 14.35- 47.36 % while the disease severity index found within 9.62 to 47.36 per cent. The pathogen identified as *Puccinia horiana* Henn. and required about 11-12 days for expression of the symptoms under pathogenicity test.

Keywords: Chrysanthemum, rust, Kullu district, symptoms, pathogenicity test

Introduction
Chrysanthemum (*Dendranthema grandiflora*) belonging to the Asteracea family is an important commercial cut-flower crop in the state of Himachal Pradesh. India is the second largest producer of flowers behind China and Himachal Pradesh has emerged as the most suitable place due to wide variations in prevalence of agroclimatic zones where commercial cultivation has come up in an enormous way. Flower production is covered in an area of 719.05ha and mostly confined to major districts like Solan, Shimla, Sirmaur, Kangra, Mandi, Kullu, Bilaspur, Una, Chamba (Directorate of Horticulture, 2016) [7]. Chrysanthemum alone occupies an area of 190.66 and 86.08 ha in Himachal Pradesh with a production and productivity of 68,864,000 and 7,626,400 and 1,721.60 and 1,525.28 of as loose and cut flowers, respectively (Singh, 2017) [14].

Material and Methods
A regular survey in the month of March to May, 2018 conducted for the occurrence of fungal diseases in chrysanthemum grown in Kullu district. Both the government and private chrysanthemum fields and poly houses were examined for the presence of diseases, if any. The disease incidence and disease severity were recorded in frequently grown commercial varieties which were raised by the growers. The per cent disease incidence of the disease was calculated as per the formula:

$$\text{Disease Incidence (\%)} = \frac{\text{Diseased plant}}{\text{Total number of plants examined}} \text{ X } 100$$

While the disease severity was enumerated as per the scale 0 to 5 followed by Hanudin *et al.* (2017) [8] with slight modification where 0= No spot (symptomless);1= Very low, infection detected only on lower plant leaves and the intensity not exceed than 5 % from total leaf area; 2=5-15% Low, infection detected on lower plant leaves and the intensity ranges 5-15 % from total leaf area; 3=15-25% Medium damage, infection detected on middle and lower plant leaves and the intensity ranges 15-25 % from total leaf area; 4=25-40% Heavy damage, infection detected on upper, middle and lower plant leaves and the intensity was not more 40 % from total leaf area; 5=>40%.Very heavy damage, the infection is prominent on all the leaf parts, extend to stem, flowers and leaves droop and hang on to the plants, dry and dead.

The disease index was determined following the Mc Kinneys scale (1923) [11]:

$$\text{Per cent disease index (I)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of plant examined} \times \text{Maximum grade}} \text{ X } 100$$

The diseased leaves samples after bringing to the laboratory were examined critically under stereo zoom microscope and compound microscope for the presence of the fungal structures for their morphological characters and identity. For ascertaining the pathogenicity of the pathogen, the teliospores scraped from the rust pustules were collected and the healthy leaves of the chrysanthemum plants grown in pots were inoculated with teliospore suspension (12-15x 10^3 ml^{-1}) prepared in sterilized distilled water. The spore suspension was sprayed uniformly with the help of an atomizer on the lower side of the leaves after little swabbing with the wetted cotton. The plants were kept covered with moistened perforated polyethylene bags for 24h to maintain high humidity and leaf wetness for development of typical symptoms upto the time period of symptom expression under polyhouse conditions within a temperature range of 18-20 ºC as the disease normally appears at low temperature regimes. The experiment was replicated five times with three plants per replication. Control leaves were sprayed only with free distilled water without pathogen spores.

Results and Discussion

The disease become apparent for the first time during 1st week of April,2018 in chrysanthemum grown under polyhouse conditions by a private grower at Seobagh, Distt Kullu of Himachal Pradesh. The disease was first observed as small pinhead flecks on the lower surface of the leaves. After 3-4 days pustules developed as blister-like round swellings having white crusty growth with chlorotic spots or pale-green to sunken yellow spots on upper surface corresponding to underside lesions. As such the plants later exhibited raised, buff or pinkish, waxy pustules on the corresponding lower surface which often have the whitish crusty growth turning greyish after 6-8 days and necrotic areas on the upper surface of the leaves appeared after 11-12 days. The disease affected mainly the leaves, but under severe infestation, spread is seen onto the stems, bracts, or even the flowers. Portions of the leaf may fall out, giving a shot hole or tattered appearance. Severe infections may result in premature defoliation. The lower leaves were found severely affected. Similar types of the symptoms were also reported by Dickens, 1970 who showed that the progress of infection may lead to necrotic flecking with occasional pustules on the flowers. Under severe infection, the pustules coalesced and lead to complete drying and wilting of leaves, hang on to the stem and finally cause death of the plant.

Fig 1: Symptoms on upper side as pale yellow of rust pustules infecting chrysanthemum

The Calabria group variety was highly infected followed by Bonita group variety with 36.8 to 47.36 % and 14.35 to 31.16% incidence and disease severity index of 17.35 to 24.76 and 9.62 to 11.29%, respectively throughout the growing season. However, the variety no. 24 growing adjoining to the Calabria and Bonita was totally free from rust disease. The average number of lesions/leaf in observed varieties were Calabria: 12-105, Bonita: 3-37 and No. 24: 0. The flower lost due to the fungus may reach 80 % in India in state like Tamil Nadu, during the outbreak seasons (Dheepa *et al*., 2015) [5]. Alaei *et al*. (2009) [1] while screening found about 12 species including chrysanthemum, Nipponanthemum and Leucanthemella were attacked by the rust pathogen. The pathogen is capable of attacking the plant enzymatically to penetrate the leaf cuticle in order to colonizes both inter- and intracellularly the mesophyll tissue (Bonde *et al*., 2015) [3]. However, De Baker (2011) [4] in Indonesia observed to have little damage from this disease in the following cultivars: Albert Heijn (Royal and White), Finmark, Freedom, Hawaii, Helsinki, Kes, Majesty, Paso Doble, Statesman, Tiger, Tigerrag, and Westland (winter and yellow).

Observation under stereo zoom and compound microscope revealed the presence of raised, dull white, waxy telia covered with pubescence of the leaf. The formation of telial spores were prominent which were usually hypophyllous, rarely epiphyllous, compact, and often bicelled, pedicellate, oblong to clavate, smooth, and yellowish to gray with pale-yellow cell wall measuring 55-58 µm length x 12-17 µm wide at 400× magnification. The basidiospores however were hyaline, slightly curved, broadly ellipsoid to fusiform, 7-14 x 5-9 µm. Thus after comparing the morphological characters of the fungus with those of the already existing records, the rust fungus was identified as *Puccinia horiana* Henn. and the disease was recognized as Chrysanthemum white rust. The disease in fact originated from Japan but until 1963, rust pathogen was confined to China and Japan thereafter spread to Eastern parts, South Africa and from there to Europe (Yamada, 1956, Hennings, 1901 and Lelliot, 1984) [16, 9, 10]. Walker (1983) [15] critically reviewed the spreading pattern of the disease. The similar outbreaks of white rust have also been observed in Ontario, Canada but later it was evident in other parts of the world (Pemberton, 1988; Bonde, 2015) [3]. Walker (1983) [15] recorded the disease from Australia whereas Dheepa *et al.* (2015) [5] found its occurrence in polyhouses at Kothagiri Hills of Nilgiris District and Yercaud Hills of Salem District of Tamil Nadu province in India in 2013 on a variety, Saffin Pink.

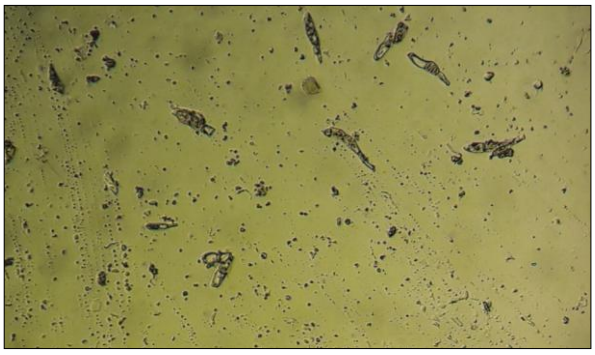

Fig 2: *Bicelled teliospores* of *Puccinia horiana* Henn.

The pathogenicity studies indicated that the disease symptoms developed on inoculated leaves within 14-15days after inoculation. The lesions started initiating after 10-12 days, showing minute dot like light pale green pustules of nearly 1-4mm size generally noticed on underside of the leaves of

affected plants with corresponding depressed yellow spots on the upper surface of the leaf. Later the pustules enlarged in size, appeared pinkish and waxy, and covered the entire lower surface of the leaves. The causal pathogen was re-isolated from lesions to confirm Koch's postulates. No disease symptoms were observed on water sprayed leaves. Microscopic observations of the rust pustules further confirmed the presence of bicelled teliospores of *Puccinia horiana* Henn. Indicating the rust identity. To our knowledge, this is the first report of the disease occurrence in Kullu district in Himachal Pradesh. According to Punithalingam, 1968 the pathogen is of prime importance from quarantine point of view. Therefore the most care should be taken to mitigate the losses as early as possible.

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