

Contributions of rust fungi and host plants from Thandiani region, Abbottabad, Pakistan

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ABSTRACT: During 2018, survey of rust fungi and their associated host plants was attempted in thandiani regions of Pakistan. In this paper descriptions and illustrations of six rust species viz. *Melampsora euphorbiae*, *Phragmidium barclayi*, *Puccinia ambegua*, *P. calcitraipae*, *P. mentha* and *P. ustalis* have been provided on the hosts such as *Euphrbia helioscopia*, *Rubus lasiocarpus*, *Gallium aprine*, *Cirsium vulgare*, *Origanum vulgare* and *Ranunculus muricatus*. The *Melampsora euphorbiae*, *Puccinia ambegua* and *Puccinia ustalis* collected as a new host record from Abbottabad. This research work is an addition to available data related to the Uredinales of Pakistan with special reference to the Thandhiani area, Abbottabad District, Khyber Pakhtunkhwa.

Key words: Thandiani regions, Pucciniales, Taxonomy, Uredinales.

INTRODUCTION

Rust fungi (Pucciniales) constitute one of the largest and diverse groups of plant pathogens that can affect almost all the plants in the biosphere [1]. The biggest genus in the family Pucciniales, *Puccinia* has over 4,000 species that are distinguished by generating pedicellate and two-celled teliospores [2]. There are more than 66 species in the genus *Phragmidium*, and 17 of those have been found in Pakistan [3]. The rust fungi, which affect many angiosperm and gymnosperm trees as well as cereal and legume crops, are among the most pervasive plant infections [4]. Due to the way rust fungi obtain nutrition from living host cells, they are both biotrophs and obligatory parasites that must have a living host to complete their life cycles [5]. The majority of rust fungi are highly specialized diseases unique to certain host species, in part because of these parasitic traits. By means of wind-borne basidiospores, aeciospores, and Urediniospores, rust fungi can spread widely over large geographic areas. They are also frequently highly genetically diverse for races or pathotypes that can be identified by their virulence or pathogenicity to distinct host genotypes. The Abbottabad district of Khyber Pakhtunkhwa (KP), which is located in northern Pakistan, is one of the regions where rust fungus are expanding. To date, over 66 species have been described there, along with new hosts and local dispersion data. For the purpose of researching rust fungus, the northwest Pakistani region

of Murree, which has a diversified floristic region and is one of the most favorable places for rust fungi, is being researched [6]. The rust infections, which are brought on by fungi, are unique in that they are biotrophic, meaning that they need a living plant host to obtain their nutrients. Both the hosts they target (known as *formae speciales*, or *f. sp.*, for example, wheat leaf rust cannot attack oat, and vice versa) and the cultivars they attack are extremely specialized. In the latter, rust strains differ in their pathogenicity to particular resistance genes and can attack some kinds but not others. These races are referred to as physiological races. Rust diseases are very harmful because they multiply quickly on plants (from infection to new spores in 8–10 days), create enormous quantities of spores (1 trillion stem rust spores per acre at moderate infection levels), and travel great distances by wind. Wheat stem rust has moved from South Africa to Australia via the Indian Ocean on westerly winds, whereas soybean rust travelled from Africa to North America via hurricane transport across the Atlantic Ocean. The rust diseases that affect crops in Canada include flax rust, sunflower rust, soybean rust, stem, leaf, and stripe rust of wheat, stem rust of barley, crown and stem rust of oat, and rust illnesses [7].

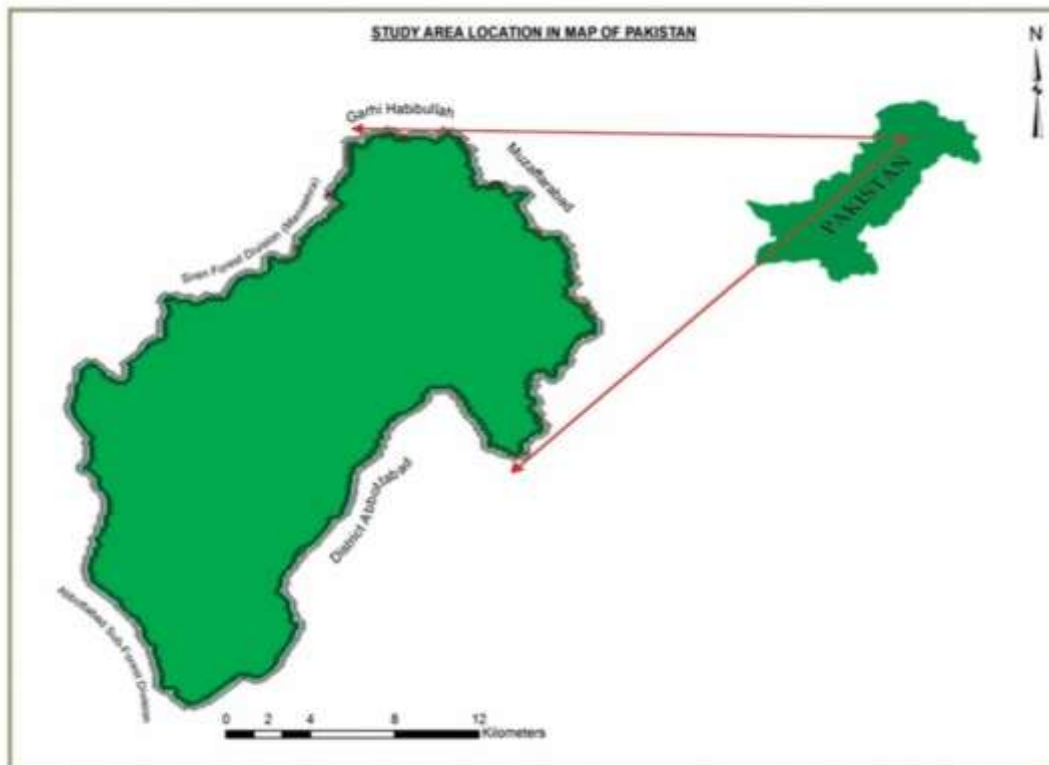


Fig 1: Map of Study Area

MATERIALS AND METHODS

Collections of rust affected plants were carried out from the study area.

a. SURVEY AND COLLECTION

The surveys were conducted for the collection of rusted plants from Thandhiani, District Abbottabad in different seasons in 2018. The plants were collected which were attacked by rust fungi. The plants which were healthy and green with flower or inflorescence and fruits were given special attention during identification for exact host identification. During collection three pictures of every infected plant were taken. Samples were carefully and artistically pressed and then these collected samples were kept among the blotted papers and newspaper. With the passage of time, every day the specimen were exposed to air under strict surveillance and the newspapers and blotted papers were changed to make it dry. The above-mentioned process was repeated again and again to make these plants completely dry. For taking clear and high mega pixel pictures, modern digital camera was used and pictures were taken. Alcoholic solution of Mercuric Chloride (Hg Cl_2) was used to poison these specimens and to prevent them from decomposing fungal attack and insect damage [8].

b. LAB WORK

The infected host plants were photographed by the help of digital camera. Sori was studied under the stereomicroscope with magnification power of 25 to 50. Photographs were taken with the help of camera attached to microscope. After that rust spores were mounted in lectophenole and semi-permanent slides were prepared by cementing the cover slips with the help of nail lacquer. The slides were slightly heated by using the spirit lamp. Due to heating the spore were dispersed. The spores were then measured by an ocular micrometer (Zeiss Eye Piece Screw Micrometer) and photographed under a Leitz HM-LU compound light microscope. At least 30 spores were measured for each spore stage, including the smallest and the largest spores found. Lucida camera (Ernst Leitz Wetzlar Germany) was used for making the line drawings of spore and Paraphyses. After taking all the necessary data specimens were technically described. Host plants were identified by comparing with flora of Pakistan and already identified specimens of herbarium and by consulting the experts [8]. The descriptions were compared with available data for accurate identification of the rust species. The infected plants were preserved and submitted to the herbarium of Department of Botany Hazara University (HUP).

RESULTS

1. *Melampsora euphorbiae* (Ficinius and C. Schuib.) (Plat 1, Figs. A–H)

Aeciospores as well as Spermogonia absent or unknown. Uredia, amphigenous, spread or circinate, yellowish, Uredinospore globose, sub globose or ellipsoid, 17.5 - 20.5 x 13 - 18 μm . Paraphyses capitate, thick walled 14.3 - 30 μm . Telia sub-epidermal, amphigenous, brownish. Teliospores are cylindrical oblong, yellowish brown, 36 - 66 x 9 - 14 μm .

Material examined: On *Euphorbia helioscopia*, with stage II and III, pakistan, khyber pakhtunkhwa province, district Abbottabad, Thandiani region, at 2790 m. a. s. l., 15 september, 2018. R # 01.

Comments: This rust fungus has previously been reported on *Euphorbia hypericifolia* L. and *E. helioscopia* L., from Gilgit, Karachi and Muzaffarabad [9]. It is a new record for Thandiani region.

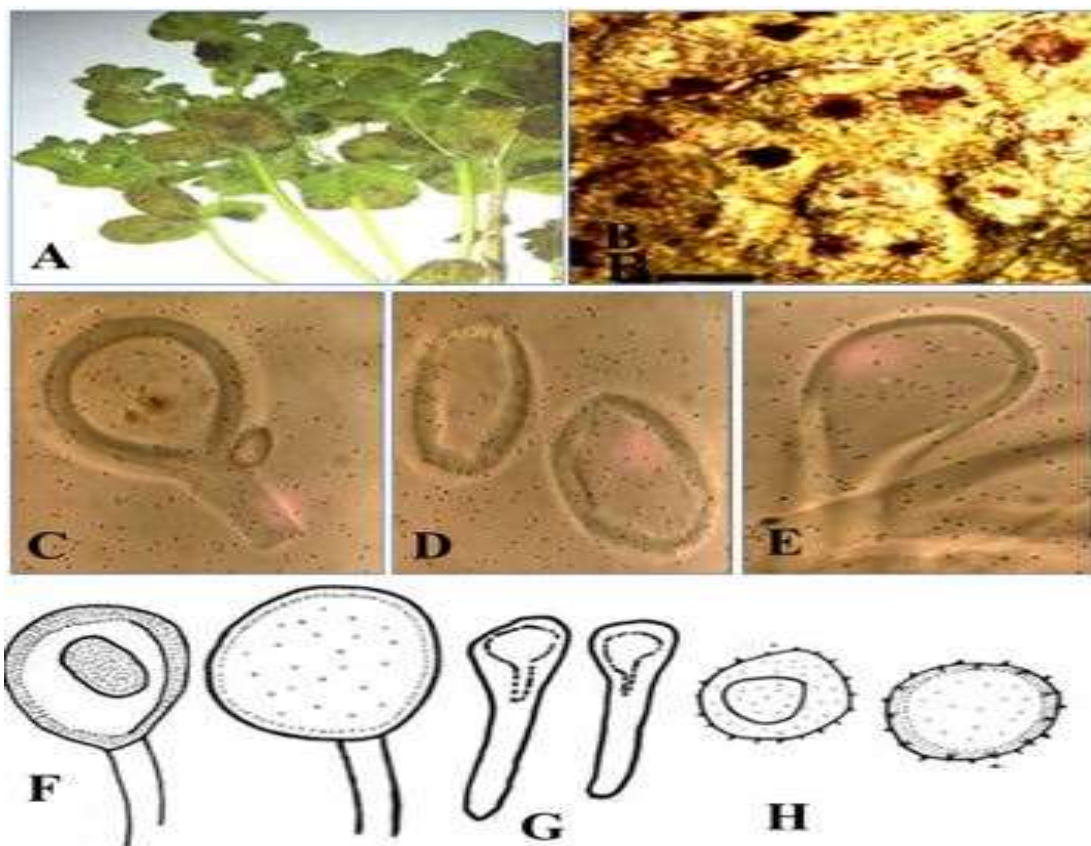


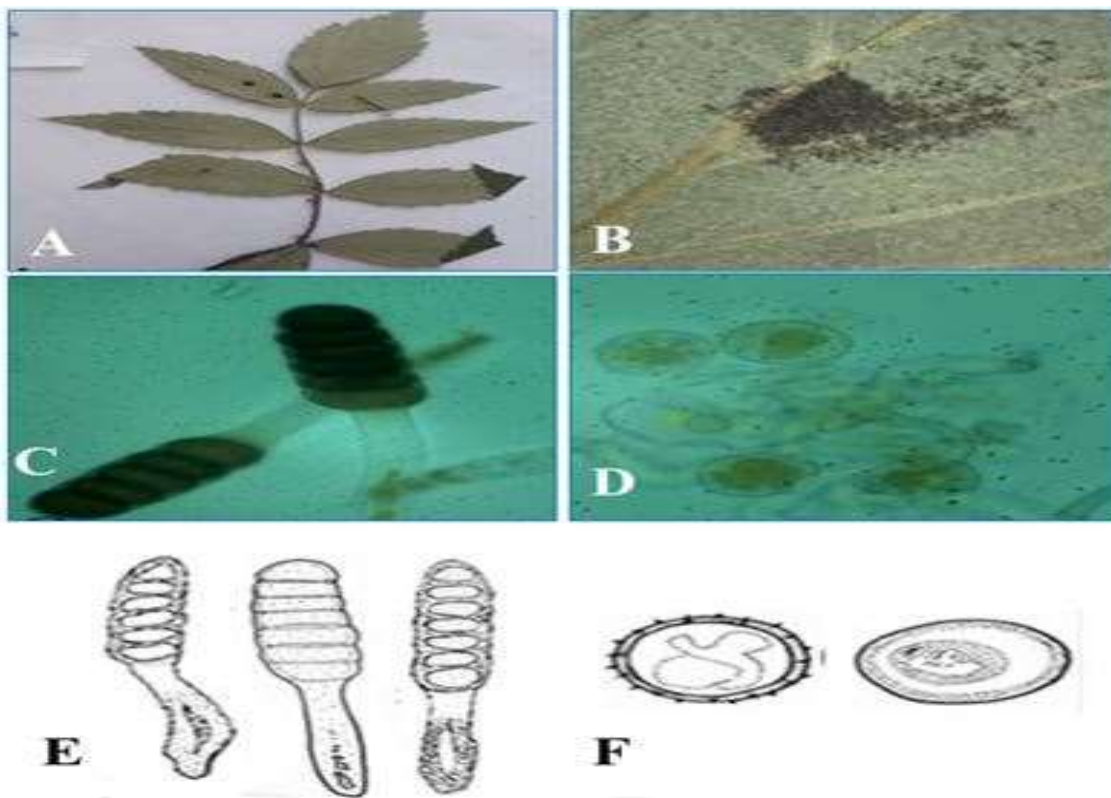
Plate 1, Figs, A–H: (A) *Melampsora euphorbiae* Infected host plant (B). Cross section of infected leaf showing uredinium (D) Uredinospore (E) Paraphyses (F-G) Line drawing of paraphysis (H) Urediniospores. Scale bar for A = 2.6 cm, B = 29 mm, C = 14 μm , D = 13.5 μm E = 7.6 μm , F=5.6 μm and G&H= 6.5 μm .

2. *Phragmidium barclayi* Dietel. (Plat 2, Figs. A–F)

Uredinial and telial stages present. Aceia and Spermogonia absent. Uredinia, light brown, spread, biaxial, 2 – 3.9 mm. and uredinal spore mostly ellipsoid and their color is yellow, $9.3 - 14.12 \times 13 - 21 \mu\text{m}$. germs pores obscured, wall thickness $1.24 - 1.44 \mu\text{m}$. Telia brown dark, hypophyllous and scattered about 4mm in diameter, and spore are slightly cylindrical at base and composed of 3 to 7 cell, apex $4.4 - 5.7 \mu\text{m}$. Teliospores about $25 - 34 \times 73 - 117 \mu\text{m}$, and wall length $2.3 - 3.2 \mu\text{m}$. Pedicel are hyaline, $16.6 - 19.3 \mu\text{m}$, broad, up to $137 \mu\text{m}$ long.

Material examined: Pakistan, Khyber Pakhtunkhwa, Abbottabad District, Thandiani region, at 2770 a.s.l., On *Rubus niveus* II and III stages, 30 September, 2018. R # 2.

Comment: *Phragmidium barclayi* has been reported on *Rubus lasiocarpus* in KP and ChanglaGali by Ahmad [10], *Phragmidium barclayi* on *Rubus spediunculosus* is again described from swat. It is a new record for Abbottabad district.



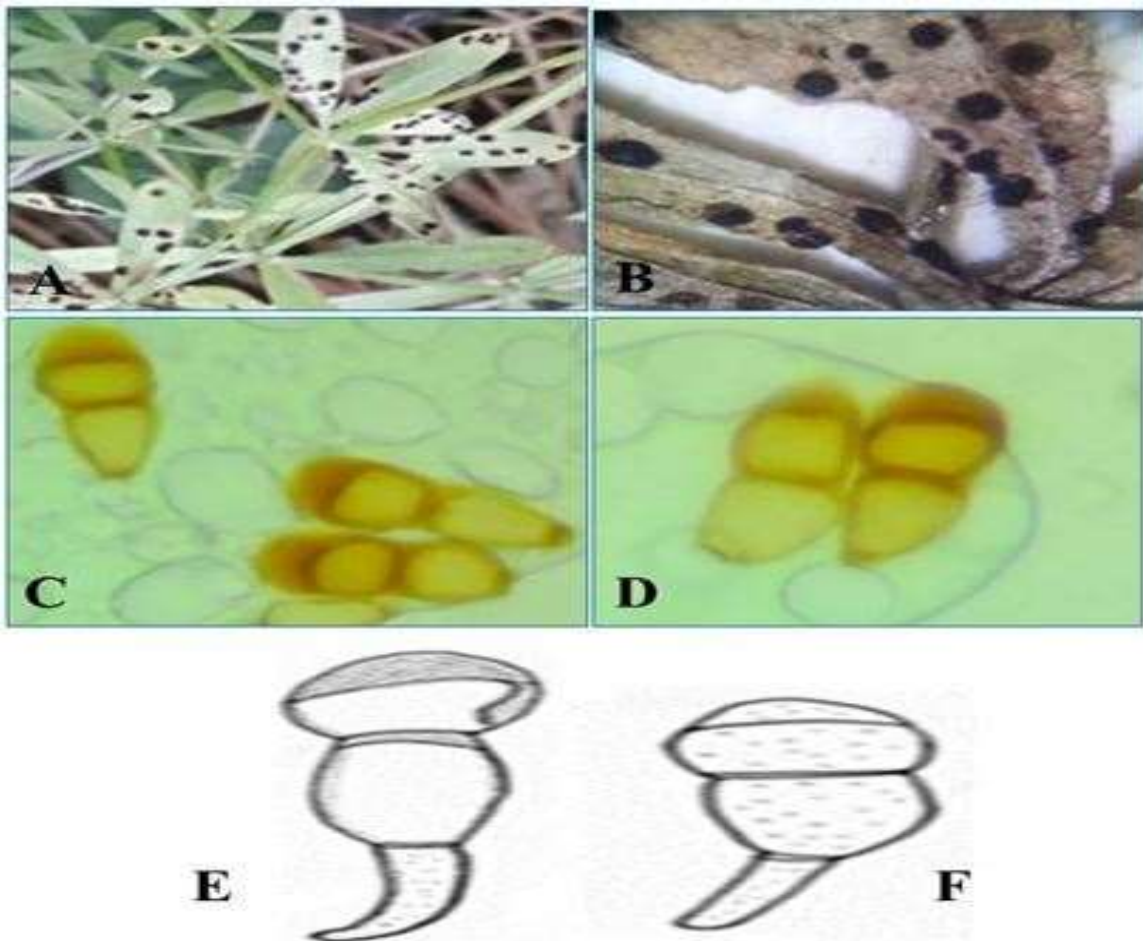
Plat 2, Fig. A-F: *Phragmidium barclayi* **A.** *Rubus niveus*, **B.** Telialori **C.** Teliospores **D.** Uredinospore, **E.** Line drawing of Teliospores **F.** Urediniospores, **A** = 1.6 cm, **B** = 1.3 mm, **C** = $32.5 \mu\text{m}$ **D** = $9 \mu\text{m}$, **E**= $30.5 \mu\text{m}$ and **F** = $8.8 \mu\text{m}$.

3. *Puccinia ambigua* (Alb.) (Plat 3, Fig. A – F)

Spermogonia and aecia, not found. Uredinia hypophyllous, large may be dense, dark brown, 1.3 to 1.5mm. Telia clavate, ellipsoidal, abaxial, conical, thickened, 1.3 to 1.5 mm, teliospores dark brown, hyaline to pale yellowish, 38. 24-57.77 μm \times 13. 35-20.66 μm . Pedicel 2.35-6.35 \times 7.88-27.97 μm , and hyaline.

Material examined: Pakistan, Khyber Pakhtunkhwa, Pakistan, Khyber Pakhtunkhwa, Abbottabad Thandiani region, at 2740 m a.s.l., On *Gallium aprine* L., II and III stages, October, 2018. R # 03.

Comment: The uredinial stage has been reported on *Gallium aprine* from Murree Ghoregalii by [11]. It is new recorded for Abbottabad.



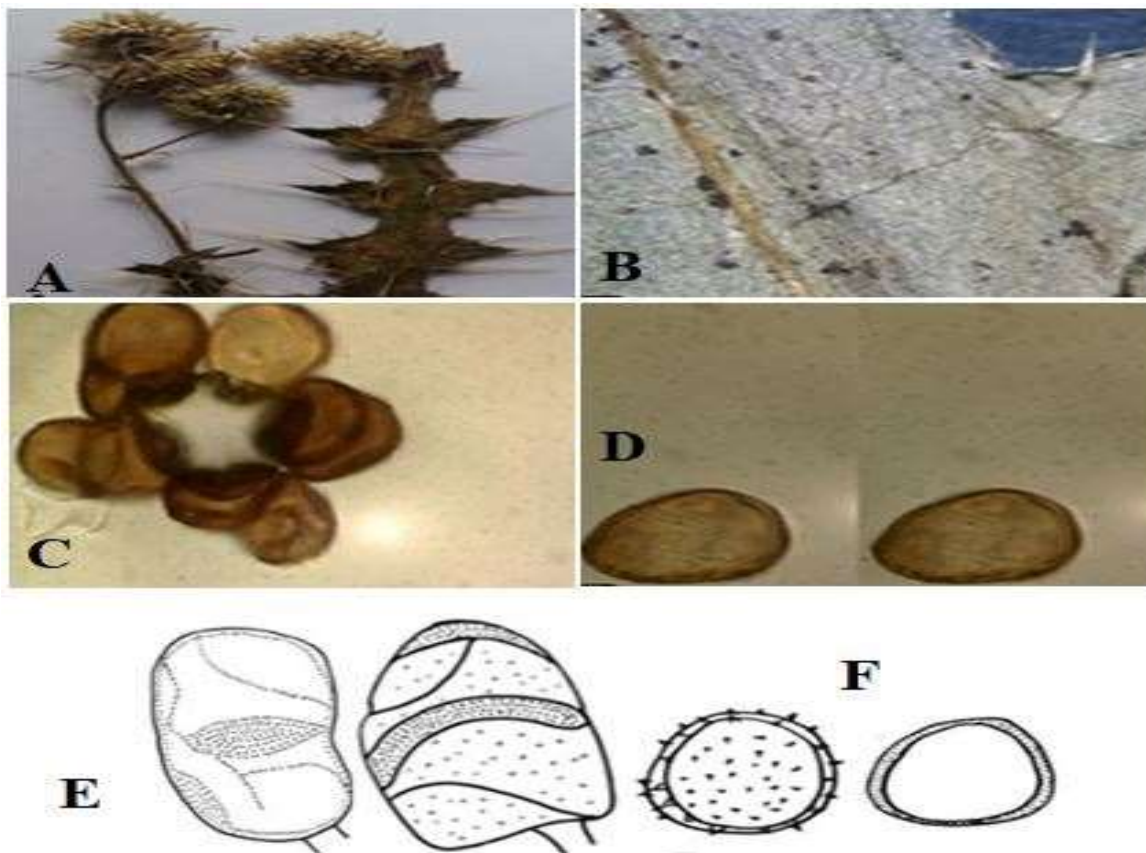
Plat 3, Fig: A-D: *Puccinia ambigua* **A.** *Gallium aprine* **B.** Telia **C.** and **D.** Teliospores, **E&F.** Line drawings of teliospores. Scale bar: **A** = 1.3 cm, **B** = 1.6 mm, **C** = 22.1 μm , **D** = 25.11 μm **E&F** 20 μm

4. *Puccinia calcitraipae* DC. (Plat 4, Fig. A-F)

Aecia and Spermogonia are totally absent. Uredinia hypophyllous, spread curved, brawny to pale redish. Urediniospores globose to ellipsoids. 19-24 x 23.22–27 μ m. Telia hypophyllous infrequently, spotted; into groups various size dark brown color. Teliospores generally ellipsoidal or fusiform 15–27 x 18-40 μ m. Yellow to brown, germ pore apical or sub-apical. Pedicle hyline.

Material examined: *Puccinia calcitraipae* on *Cirsium vulgare* with II and III, stages, Pakistan, Khybar Pakhtankhwa Provinc, Districtt, Abbottabad, Thandiani region, 2600 m.a.s.l., 10 septembar, 2018. R # 04.

Comment: *P. calcitraipae* has been reported on *Centaurea bruguieriana* (DC.) from Peshawar; on leaves of *Cardus edelbergii* Rech. f., and from Batakundi, Shogran (Kaghan valley) and Kalam (Swat), on leaves of *Cirsium arggracanthum* Dc,c.*Walichii* Dc and *Cnidus* from Maindam, Kalam, Changla Gali, and Shogran [12].



Plat 4. Fig: A - D: *Cirsium vulgare*. **A.** Host Plant. **B.** Infected leaf showing telium. **C.** Urediniospores **D.** Telio-spores scale bar A =3 cm, B = 1 mm, C = 7 μ m, D=4 μ m, Line drawing of **E.** Teliospores **F.** scale bars **E**= 4 μ m, **F**= 2 μ m.

5. *Puccinia mentha* Pers. (Plat: 5, Fig: A – E)

Spermogonia and aecia unknown. Uredinia brown, abaxial, naked, uneven. Urediniospores hyaline, sub-globose, $18 - 20 \times 19 - 28 \mu\text{m}$; wall echinulate, $1.4 - 2.3 \mu\text{m}$ broad, Telia, brown to black, wall 2-3 μm thick, verruculose, pedicle little, typically broken.

Material examined: Pakistan, Khyber Pakhtunkhwa, Abbottabad District, Thandiani region, 2730 m a. s. l., On *Mentha longifolia*, with II, III stage, 8 Sep. 2018. R# 05.

Comments: *P. mentha* has been reported on *M. Sylvester's* L., from Peshawar, Mingora, Kaghan Valley, Challianwala, Quetta, Poonch and Muree Hills; on *Origanum vulgare* L. from ChanglaGali and Kaghan Valley [12, 13]. Recently it has been reported from Battagram. It is first time reported for study area.

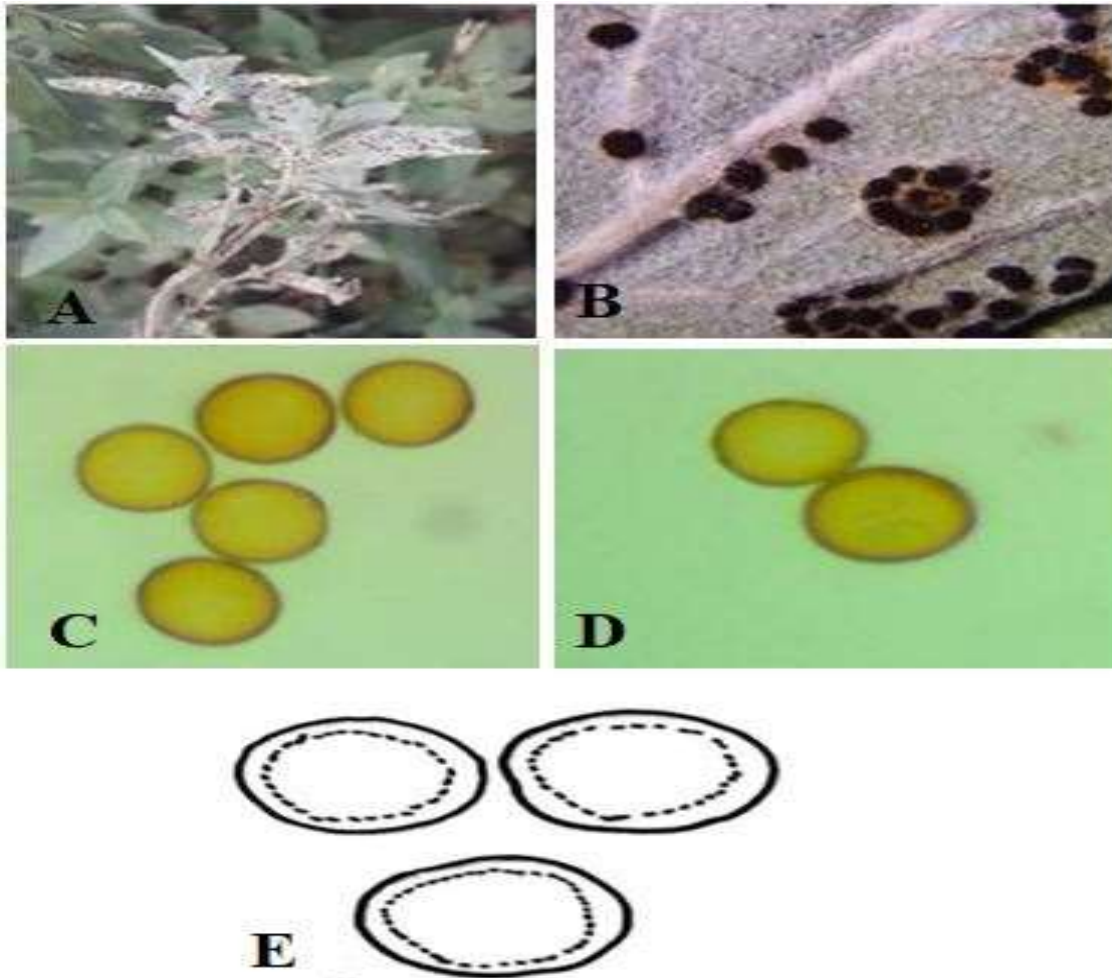


Plate 5, Figs, A–E: *Puccinia menthae* A. *Mentha longifolia* L., B. Telial. C D. Urediniospores. Scale bar: A = 1 cm B = 1mm C = 10 μm D = 7 μm and E= Lining drawing of Urediniospores scale bar A = 4.2 μm .

6. *Puccinia ustalis* Bark. (Plate 6, Figs. A–F)

Spermogonia, aecia unknown. Uredinia hypophyllous, dark brown to blackish spots, scattered or aggregated in large groups, compact. Urediniospores ovoid, $9.8\text{--}23 \times 11\text{--}22 \mu\text{m}$, wall $0.6\text{--}1.7 \mu\text{m}$ in thickness. Telia usually hypophyllous, dark brown to blackish spots, scattered or aggregated in large groups, compact. Teliospores mostly two celled, occasionally 1 or 3 celled, teliospores co-exist. ellipsoid, oblong to cylindrical or irregular, conical or rounded above and narrowed below, constricted at the septum, yellowish brown, pale brown basally, 2 celled spores $10\text{--}17.2 \times 31.6\text{--}62.2 \mu\text{m}$, smooth, $1\text{--}1.4 \mu\text{m}$ thick at side, epically $2\text{--}8 \mu\text{m}$ thick. Germ pore one per cell, apical or sub apical in distal cell, in basal cell near the septum or obscure. Pedicel pale yellow to hyaline, deciduous, $6\text{--}9 \mu\text{m}$ long, deciduous.

Material examined: *P. ustalis* on *Ranunculus muricatus* with stage II & III, Pakistan, Khyber Pakhtunkhwa Province, District Abbottabad, Thandiani region, at 2600 m.a.s.l., 15 september, 2018. R # 06.

Comments: *P. ustalis* has been reported on *Anemone obtusiloba* D. Don. from Changla Gali and Nathia Gali by Ahmad (1956a, b); on *R. diffusus* DC. from Dunga Gali KP (NWFP) [14] and from Kaghan Valley [15]. *Ranunculus muricatus* is a new addition to the rust flora of Pakistan on the base of uredinal stage and new host.

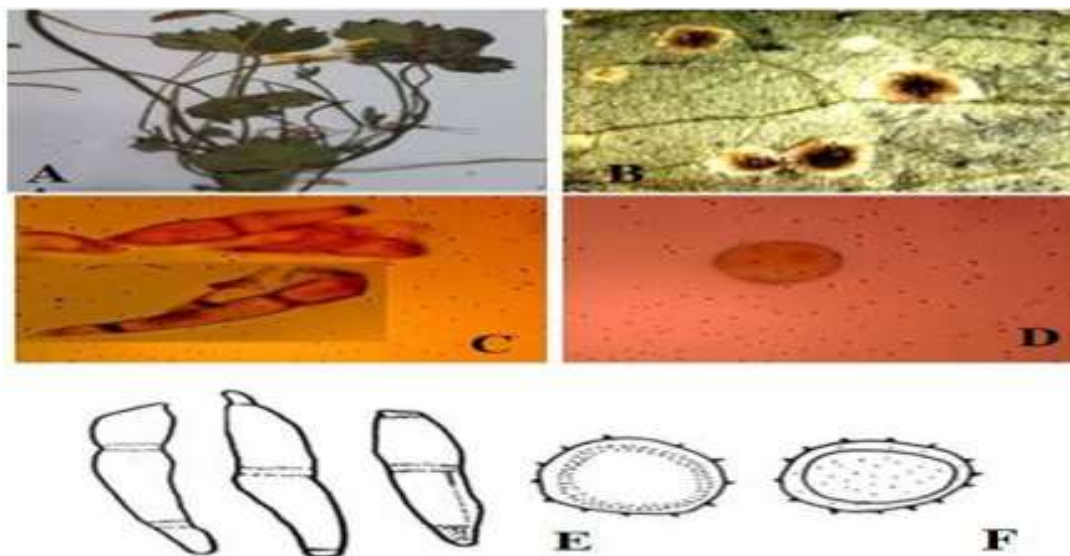


Plate 6, Figs. A–F: *Puccinia ustalis* (A). Infected Leaves of *Ranunculus muricatus* (B). Section of the sorus showing teliospores (C) Urediniospore (D). Teliospores. Scale bar for A = 1.9 cm, B = 5 μm , C = 15 μm D = 6.5 μm , E, Lining drawings of Teliospores, Urediniospore. scale bar E = 14 μm , F = 7 μm .

DISCUSSION

The current research project was undertaken to investigate rust fungi of Thandhiani region, District Abbottabad, Khyber Pakhtunkhwa. To collect the data for the research, various selected sites were visited and specimens were collected in September to October 2018. A figure of specimens was composed and after careful morpho-anatomic search six genera, consisting of six rust species have been identified on six different host plant species [16]. It is one of the largest group of fungi which is round about 7000 – 8000 species. It attacks nearly all types of plants. It may affect angiosperm as well gymnosperm, Pteridophytes, bryophytes. The unique character of Puccinia is that it may be two or three celled and having smooth walls and having well develop pedicel. The spore of Puccinia wall having various pigmentation as well as two or more germ pores in each cell [17]. The current research from Abbottabad Thandhiani, one Taxon M. Euphorbiae has been received and it was recognized on the basis of spore morphology and the pattern of disease. From Muzaffarabad, Karachi and Gilgit, the rust fungi Euphorbiae hypericifolia was reported previously [18]. From Charsadda on leaves, Nathiagali, Quetta, Peshawar, Muree, Lahore, Kaghan Valley, Swat, Wazirabad, Challianwala [19]. It was concluded that for this study area, it was a new record. *Phragmidium barclayi* has been reported from *Rubus lasiocarpus* in KP and Changla Gali by Ahmad [20]. *Phragmidium barclayi* on *Rubus pedunculatus* is again described from swat with latest illustrations. It is a new record for Abbottabad district. *P. ambegua* has been reported on *gallium aprine* L., but it was represented by uredinal stage only in Muree hills, Ghoregalii by [21], Telial stage of this fungus is an addition to the rust flora of Pakistan. It is also a new record for Abbottabad District, Thandhiani region. *Calcitraipae* previously reported on *Centaurea bruguieriana* (DC.) Bornm. *Ranunculus muricatus* is a new addition to the rust flora of Pakistan on the base of uredinal stage and new host.

CONCLUSION

Our finding result show that District Abbottabad is very rich and show a great diversity of fungi especially rust. Among the reported genera, *Puccinia* was recorded as the largest genus with 4 species followed by *Uromyces* with 04 species. From the study it can be concluded that District Abbottabad, which is located in moist temperate region and covered by 70% of coniferous forest, has enormous potential of fungal diversity. The area needs to be explored further in future for very valuable species of other groups.

REFERENCES

1. Cummins GB, and Hiratsuka Y. 2003. Illustrated genera of rust fungi, 3rd ed., American Phytopathological Society, St. Paul, MN.
2. Mahadevakumar S, Szabo LJ, Eilam T, Anikster Y, Janardhana GR. 2016. A New Rust Disease on Wild Coffee (*Psychotria nervosa*) Caused by *Puccinia mysuruensis* sp. nov., *Plant Disease* 100(7): 1371–1378.
3. Ali B, Sohail Y, Mumtaz AS, Berndt R. 2017. *Phragmidium punjabense*, a new species of rust fungus on *Rosa brunonii* in the outer Himalayan ranges of Murree, Pakistan. *Nova Hedwigia* (accepted).
4. Kolmer, J.A.; Ordonez, M.E.; Groth, J.V. The rust fungi. *Encyclopedia of Life Sciences*; John Wiley & Sons, Ltd.: Chichester, UK, 2009; pp. 1–8. Available online: www.els.net (accessed 17 October 2012).
5. Mendgen, K.; Hahn, M. Plant Infection and the establishment of biotrophy. *Trends Plant Sci.* 2002, 7, 352–356.
6. Ali B, Sohail Y, Tasmia B, Mumtaz AS. 2016. Biogeography of rust fungi and their hosts in Pakistan. *Science International* 28(5) 4777–4781.
7. Fetch, T., McCallum, B., Menzies, J., Rashid, K., & Tenuta, A. (2011). Rust diseases in Canada. *Prairie Soils and Crops*, 4, 87-96.
8. Haq, F.U. H. Ahmad and M. Alam. 2010. Species diversity of vascular plants of Nandiar valley western Himalaya, Pakistan
9. Ahmad, S., S.H. Iqbal & A.N. Khalid. 1997. *Fungi of Pakistan*. Sultan Ahmad Mycological Society of Pakistan. Department of Botany, University of Punjab, Lahore, Pakistan, 1-248.
10. Ahmed, S., S. H. Iqbal and A. N. Khalid. 1997. *Fungi of Pakistan*. Sultan Ahmed Mycol. Soc. of Pakistan Dep. Of Botany, Uni. of the Punjab Lahore Pakistan, Quaid-e-Azam Campus, Lahore, Pakistan. pp. 120–121.
11. Ali, S.I. and M. Qaiser. 1986. A phytogeographical analysis of the Phanerogams of Pakistan and Kashmir. *Proc. Royal Soc. Edinburg.* 89: 89-101.
12. Ahmed, S., S. H. Iqbal and A. N. Khalid. 1997. *Fungi of Pakistan*. Sultan Ahmed Mycol. Soc. of Pakistan Dep. Of Botany, Uni. of the Punjab Lahore Pakistan, Quaid-e-Azam Campus, Lahore, Pakistan. pp. 120–121.

13. Fiaz, M., Jabeen, S., Imran, A., Ahmad, H., & Khalid, A. N. (2014). Macrolepiota distribution extends to the montana temperate forests of Pakistan. *Mycotaxon*, 129(1), 197-208.
14. Afshan NS, Khalid AN, Iqbal SH, Niazi AR, Sultan A. 2009. Puccinia subepidermalis sp. nov and new records of rust fungi from Fairy Meadows, Northern Pakistan. *Mycotaxon* 110: 173–182.
15. Ahmad S and Lodhi SA. 1953. Some new or unreported fungi from West Pakistan. *Sydowia* 7(1–4): 266–269.
16. Ono, Y. 1992. Uredinales collected in Kaghan Valley, Pakistan. *Cryptogamic Flora of Pakistan*, 1(1): 217-240.
17. Okane, I., M.Kakishima&Y.Ono. 1992. Uredinales collected in Muree hills, Pakistan. *Cryptogamic Flora of Pakistan*, 1(1): 185-196.
18. Cummins, G.B. and Y. Hiratsuka. 2003. Illustrated genera of rust fungi. Third ed. American psychological society. PS Press. Paul, MN, 1-230.
19. Ahmad, S., S.H. Iqbal &A.N. Khalid. 1997. *Fungi of Pakistan*. Sultan Ahmad Mycological Society of Pakistan. Department of Botany, University of Punjab, Lahore, Pakistan, 1-248.
20. Ahmed, S., S. H. Iqbal and A. N. Khalid. 1997. *Fungi of Pakistan*. Sultan Ahmed Mycol. Soc. of Pakistan Dep. Of Botany, Uni. of the Punjab Lahore Pakistan, Quaid–e–Azam Campus, Lahore, Pakistan. pp. 120–121.
21. Ono, Y &Kakishima, M. 1992.Uredinales collected in Swat Valley, Pakistan. *Cryptogamic Flora of Pakistan*, 1(1): 197-216.

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