APOTHECIAL DEVELOPMENT IN *PSEUDOPEZIZA RUBIAE* T. S. RAMK & K. RAMK

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Abstract

Apothecial development in *Pseudopeziza rubiae* T. S. Ramk & K. Ramk parasitic on *Rubia cordifolia* Linn. was observed to be initiated by one to several symmetrical hyphal cells, tapering apically into a prolongation. During development of the ascocarp, the paraphyses grew centripetally. The initiating coil retained its integrity in the middle of the primordium and formed ascogenous system. This ascogenous system was observed to lead to the formation of asci and ascospores. As the asci grew, an excipulum was differentiated which later developed into a cup-shaped apothecium.

Introduction

The genus *Pseudopeziza* Fuck. with more than 25 species is world wide in distribution. In India it is represented by 7 species (Kalani, 1965). *Pseudopeziza rubiae* T. S. Ramk & K. Ramk parasitises one of the important medicinal plants, *Rubia cordifolia* Linn. The paper deals with the developmental morphology of the apothecia of *Pseudopeziza rubiae* T. S. Ramk & K. Ramk.

Material and methods

Stems and leaves of *Rubia cordifolia* infected by *Pseudopeziza rubiae* were collected from Mahabaleshwar (Maharashtra State) at periodic intervals from September to February and fixed in FAA. The fixed material after 24 hours were rinsed in 70% alcohol, and then embedded in paraffin. Microtome sections of the embedded material were cut to the thickness of 5-7 μm. Heidenhain's haematoxylin stain was used with traces of sodium carbonate to improve the staining. Orange G was used as a counterstain. Observations and photomicrographs were made using Olympus PM6 Camera.

Observations

Morphology

In *Rubia cordifolia* Linn, the infection occurs on both stem and leaves, the old stem gets severe infection than the young ones. The infection is initiated in the month of September in the form of circular-irregular spots which mature by the end of November-December. As the infection spreads, these spots slowly enlarge attaining a size upto 0.3 cm. The infected spots are irregular, circular, scattered on the upper surfaces of the leaves or even in interveinal regions. These are greyishbrown in colour. A single infected spot consists of 3-6 disc-shaped apothecia, sub-endophytic measuring up to 140-460 μm in diameter. Asci are elongate, clavate with short stalks and measure 60-85 $\times$ 12-20 μm. Ascospores are uninucleate, elliptic, more or less broadly rounded at one end measuring 4-6 $\times$ 15-25 μm in dimensions.

Formation and development of apothecia

The intracellular mycelium of *Pseudo-*

peziza rubiae was observed to grow within epidermal and parenchyma cells of *Rubia cordifolia*, eventually digesting and replacing the cells with the developing ascoma (Pl. 1, figs. 1, 2). When viewed from the leaf surfaces, the ascomata appeared as disc-shaped and were near the centres of brown to purple lesions on the leaves. Ascomata were common in both upper and lower epidermis of the leaf.

Ascoma formation in the upper epidermal region was observed to begin with proliferation of hyphae in epidermal cells and parenchyma cells. It was noted that hyphae of the ascomal initial sometimes extended as far as the lower epidermis. As a result of which infected epidermal cells collapsed leaving persisted host cell walls, whereas palisade cells became swollen and distorted in shape due to growth of internal mycelium. Ascogonial coils were formed in the hyphal complexes in infected palisade parenchyma cells of the host (Pl. 1, fig. 4) while a few hyphae were observed to surround ascogonial coils that were formed in spongy parenchyma cells of the host. The host cell walls were retained around the area where the coils were formed.

The ascogonial coils were seen consisting of several uninucleate cells and taper apically into a prolongation which traced a tortuous path through the outer portion of ascoma body and finally emerged at the upper leaf surfaces. Those deeply staining extensions of the ascogonial coils were trichogynes (Pl. 1, fig. 5).

The brown-black, one-celled spermatia were obstricted from the pointed tips of the sterigmata. They were typical, round or narrowly elliptical structures ranging from 3.5-6.0 μm in length. Constant occurrence of the spermatia as well as these spermatia were found to be firmly attached to the tip of trichogynes (Pl. 1, fig. 6), thereby indicating that the spermatization might be external.

When the ascomal initial was about 60-80 μm in diameter, a locule appeared in the centre by disintegration of cells in the central area (Pl. 2, fig. 8). The cells of inner walls immediately produced paraphyses formed from uninucleate and binucleate cells located in the host parenchyma at the base of the developing ascoma.

The ascogonial coil was observed to gradually loose its coiled appearance and came to lie on the subhymenial region. No cell fusions that might explain the origin of the binucleatc condition of ascogonia were observed. Chains of binucleate ascogenous cells could be observed to grow among the cells of the developing ascoma, presumably derived from ascogonia.

The observations showed that the ascogenous hyphae started growing through the tissue of the young ascoma to give rise to asci. A series of asci formed from the croziers and the ascomata developed to a stage at which many asci, each containing a single large nucleus, were prcsent (Pl. 2, figs. 9, 10). 2-nucleate and 4-nucleate asci were formed by a final division resulting in 8-nucleate asci (Pl. 2, fig. 12). By this time the ascoma consisted of a large central hymenium surrounded by a brown pseudoparenchyma which formend a rind like excipulum (Pl. 2, fig. 10), As the asci continued their growth, the overlying layer of the excipulum was broken and a portion of it was torn away (Pl. 2, fig. 11). In this way the ascoma was transformed into a cup-like apothecium.

Apothecia are 160-340 μm in length with depth of 120 μm. Mature asci are typically clavate with short stalk and measure 35-80 $\times$ 10-12 μm and ascospores are uninucleate, ovo-ellipsoidal and hyaline.

Discussion

It was concluded from the present study that in *Pseadopeziza rubiae* infecting *Rubia cordifolia*, there was a aggregation of hyphae in host cells by the time ascogonia were recognizable. Similar aggregation of hyphal mat has also been observed by Meyer and Luttrell (1986) in forma specialis *Pseudopeziza trifolii*.

The ascomal initials of *P. rubiae* consisted of an ascogonium surrounded by excipular tissues. The excipulum (Korf, 1973) has a covering layer that consisted of hyphae within and immediately beneath the host epider mis. Such excipular tissue was also observed by Meyer and Luttrell (1986) in *P. trifolii* except the covering layer of the excipulum was not highly developed. In *P. rubiae* a distinct well developed layer forming a ring was also evident.

Our observation in *P. rubiae* regarding ascomata opening in the late mesohymenial stage, when some mature asci were present (Brummelan's 1967, terminology) are similar to those of Meyer and Luttrell (1986)

in forma specialis *P. trifolii*, Jones (1930) observed 'trichogynes' in *P. trifolii*, such trichogynes were absent in *P. medicaginis* as reported by Tewari (1973) and forma specialis *P. trifolii* by Meyer and Luttrell (1986). But in *P. rubiae* elongated distinct trichogynes were seen during the present studies. Presence of a trichogyne in heterothallic species of *Ascobolus* have been reported by Dodge (1912, 1920), Gwynne-Vaughn and Williamson (1932), Bistis (1956, 1957), Gamundi and Ranalli (1966) and Ranalli and Gamundi (1957). But a trichogyne has been reported to be absent in homothallic by Gwynne Vaughn and Williamson (1933) and Paden (1973).

In *P. rubiae* dikaryophase has been observed to be initiated by plasmogamy between the ascogonium and spermatium. While Kristinsson (1967) reported the dikaryophase initiation by plasmogamy between the ascogonium and hyphal cells, condition similar to our observations was recorded in *P. trifolii* Meyer & Luttrell (1986).

The concurrent appearance of spermatia and archicarps with projecting trichogynes might be indicative of a spermatial fertilization in *P. rubiae* as recorded in *Diplocarpon maculatum* by Stowell and Backus (1957).

Jones (1930) diagrammed abortive ascogonial coils in *P. trifolii* that occurred in closed proximity to certain vegetative cells in substomatal cavities. The vegetative cells became multinucleate and gave rise to ascogenous hyphae. Ascomatal development therefore appeared to have proceeded apogamously from these multinucleate cells. While Mayer and Luttrell (1986) reported that in forma specialis of *P. trifolii*, the ascogenous hyphae typically arose from multinuclate primordial cells rather than from ascogonia in different strains of this fungus, our observations for *P. rubiae* revealed that ascogenous hyphae were initiated by the dikaryotization of fertile ascogonium with spermatium.

Thus, *Pseudopeziza rubiae* showed cleistohymenial phase of development and the paraphyses appeared to arise in centripetal manner and may be termed as Nectria type of development.

Acknowledgements

The authors are thankful to Head of Botany Department, Shivaji University, Kolhapur for providing the laboratory facilities and to Principal Dr K, R. Yadav, The New College, Kolhapur for constant encouragement.

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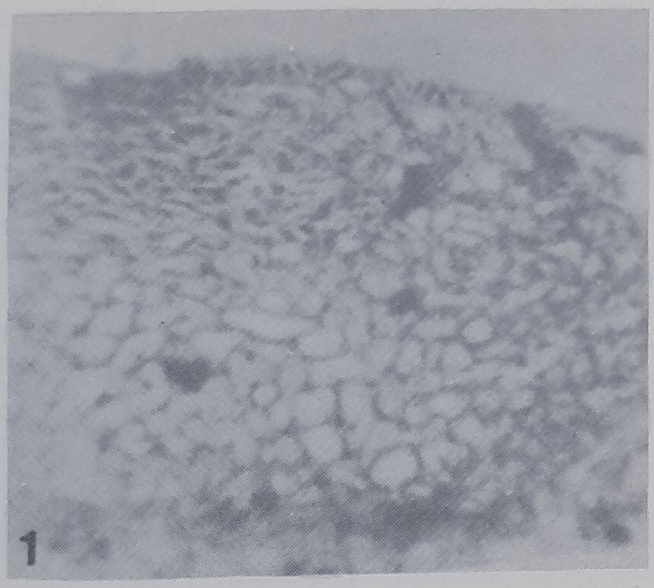
Explanation of Plates

Plate 1

1-2. A section showing mycelium of *Pseudopeziza rubiae* below the epidermis froming a mat, × 450 and × 675.
3. Mycelium showing uninucleate cell, × 450.
4. One to few ascogonial coils in ascocarp initial, × 1000.
5. Section showing trichogyne protruding.
6. A group of spermatia attached to the tip of trichogyne, × 450.

Plate 2

7. Section showing development of apothecium, × 315.
8. Primordium with centripetal paraphyses, the apical paraphyses are beginning to elongate downwards and the initiating hyphae has lost its coiled structure, × 450.
9-10. Section showing developmental aspect of apothecium, × 315.
11. Section showing the upper tissue tornway, × 315.
12. A mature apothecium showing developing asci and ascospores, × 150.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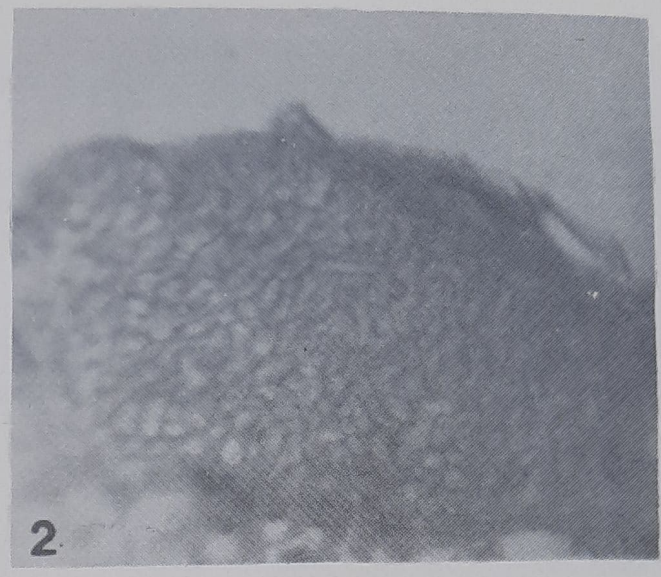
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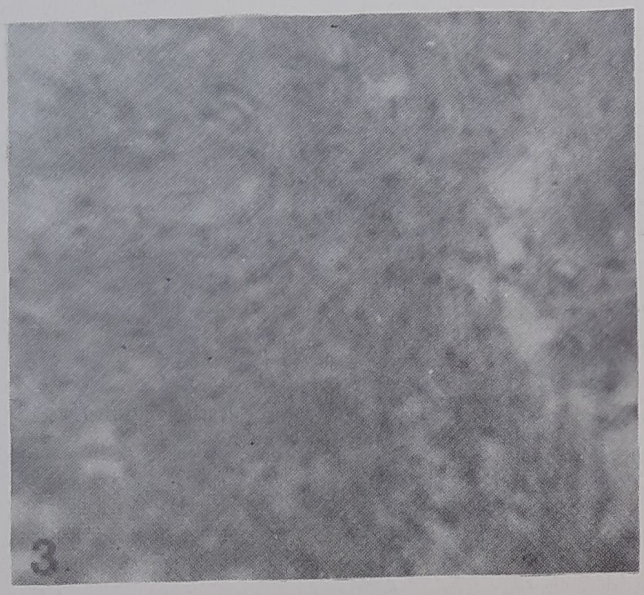
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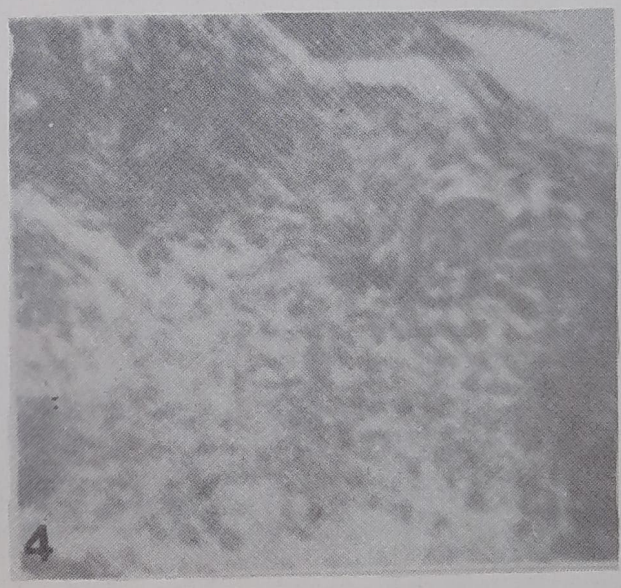
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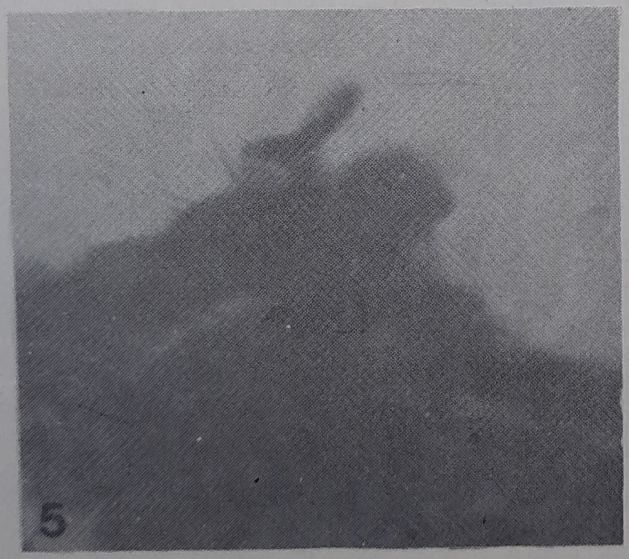
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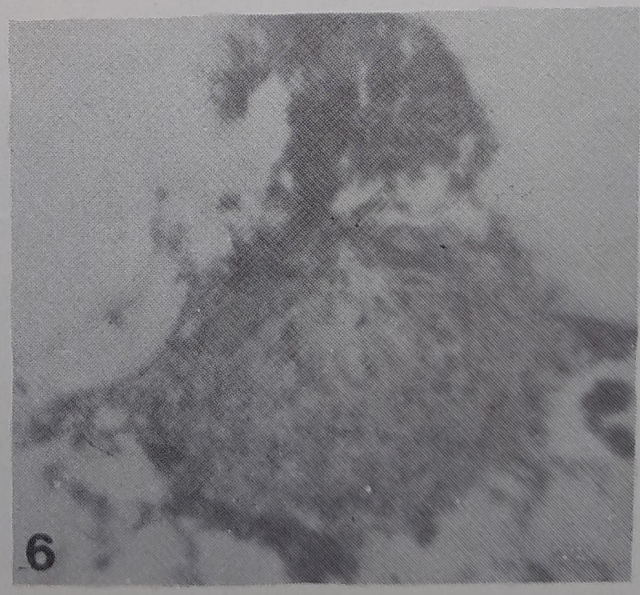
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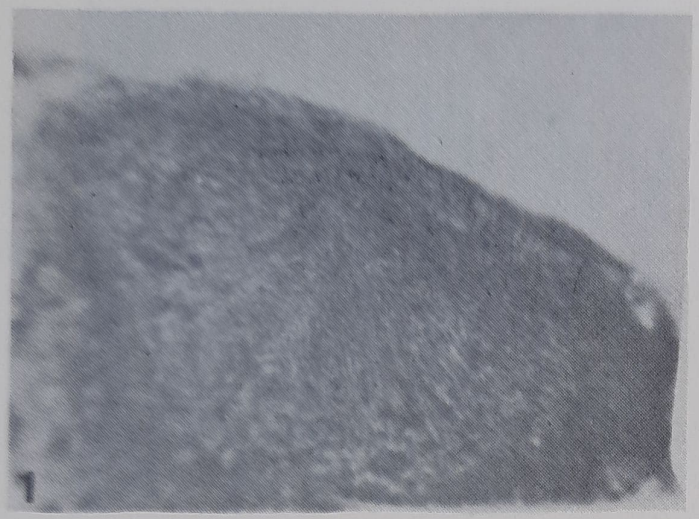

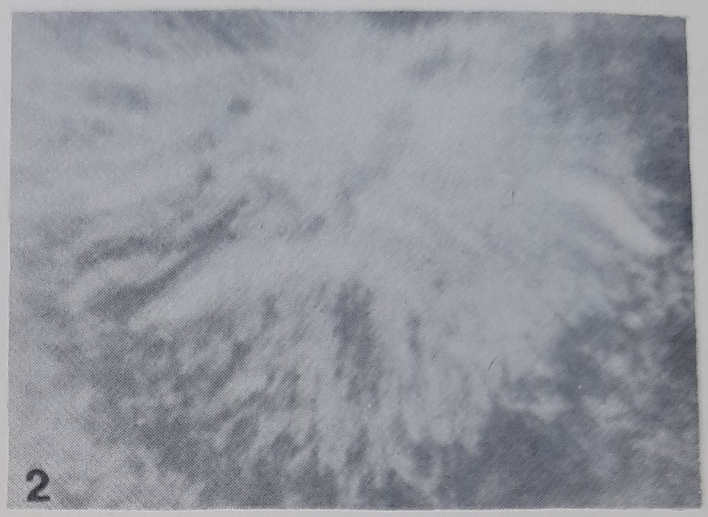

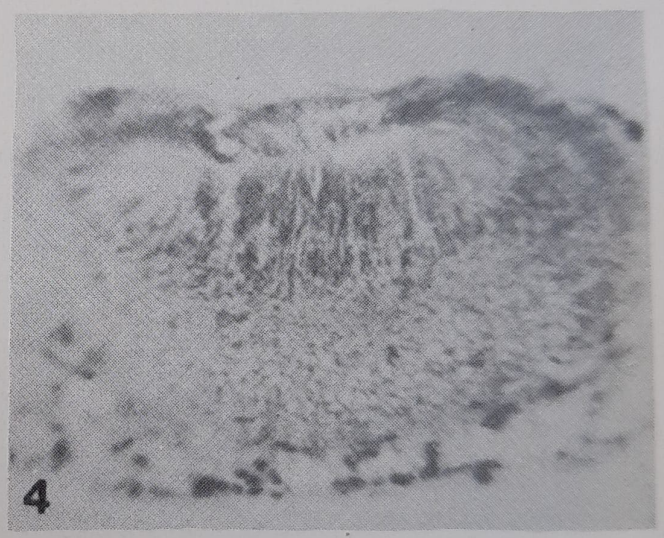

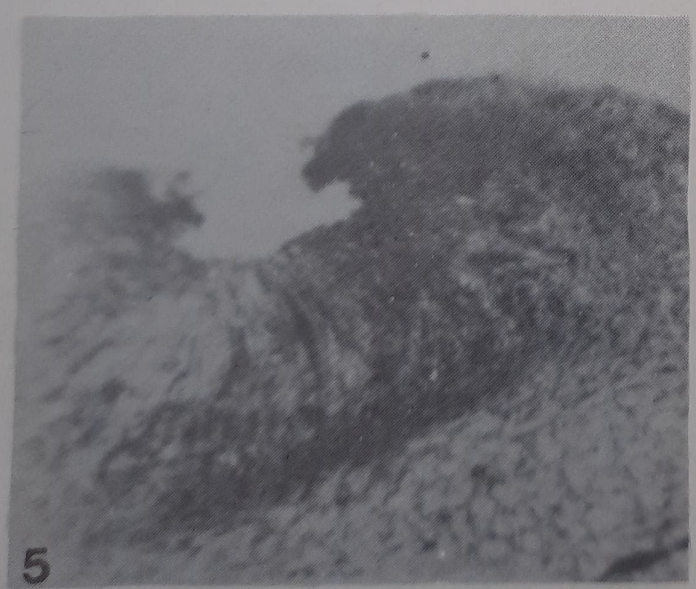

Thite & Nagaraja—**Plate 2**