0.5 unit increments) at 10°C, 14°C, 20°C, 27°C and 30°C under 0, 6, 12, 18 and 24 hr of light (intensity 1000 lux) following De and Roy[6] and De[7].

For studying nuclear behaviour the uredospores showing development of different stages of infection structure were fixed in equal parts of propionic acid and ethyl alcohol for 24 hr. The slides were then washed in 70% alcohol for 5 min, transferred to alcoholic-HCl-Carmine stain solution for 24 hr at 60°C, rinsed with 70% alcohol and mounted in 45% propionic acid following De[8].

No significant effect of photoperiod on uredospore germination of *P. arachidis* was observed whereas temperature and pH greatly influenced its uredospore germination and infection strucuture development. The uredospores did not germinate at 14°C or 30°C while 3–7% of them germinated within 40–48 hr in pH 5.5 at 27°C but these germlings remained nondifferentiated without undergoing any nuclear division and a septum was laid down between the two nuclei (figure 7).

Maximum germination was noticed at 20°C in water of pH 5.5 and in this optimal condition 84% of the spores germinated within 20–24 hr and formed infection structure. Host stimulus[9], heat shock[10] or nutrient[11] was found to be essential for differentiation of germlings of some rust fungi but these factors did not affect infection structure development of *P. arachidis*.

The uredospores were spherical to oval, yellowish brown, thick-walled, echinulate and contained 2 nuclei (figure 1). Single germ tube was produced by a spore (figure 2) into which both the nuclei of the uredospore migrated. No nuclear fusion was observed within the uredospores. Similar observations have been recorded by Chinnappa and Sreenivasan[12]. The germ tube extended into long, unbranched, aseptate hypha with rounded apical end, the protoplasm occupying a relatively constant volume at the tip of the germ tube, while the rest of it remained almost empty. After attaining a length of 2500 μ the germ tube ceased its forward growth. The apical portion of the germ tube containing cytoplasm and 2 nuclei became cut off from the rest of it by a septum. The process of 2 nuclei dividing mitotically to form 4 nuclei (figure 3) has also been reported in some other rust fungi[12–14]. The apical portion of the germ tube containing 4 nuclei gradually swelled (figure 4) to form quadrinucleate appresorium (figure 5). From the appresorium an infection peg developed, the tip of which swelled to form substomatal vesicle. The 4 nuclei of the appresorium moved one after another into the substomatal vesicle through the infection peg. After migration of all the 4 nuclei into the substomatal vesicle a septum was laid down between the infection peg and the substomatal vesicle (figure 6). In contrast to the observation of Grambow and Muller[14] working with *Puccinia graminis* f sp *tritici*, further divisions of the 4 nuclei inside the substomatal vesicle have never been observed. The 4 nuclei possibly migrated in pairs into the infection hypha, but this point could not be decided and needs further investigation.

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STUDIES ON NON-SPECIFIC ESTERASES IN SEBACEOUS GLANDS OF EXTERNAL PORTION OF VAGINA IN THE BAT, *CYNOPTERUS SPHINX SPHINX*

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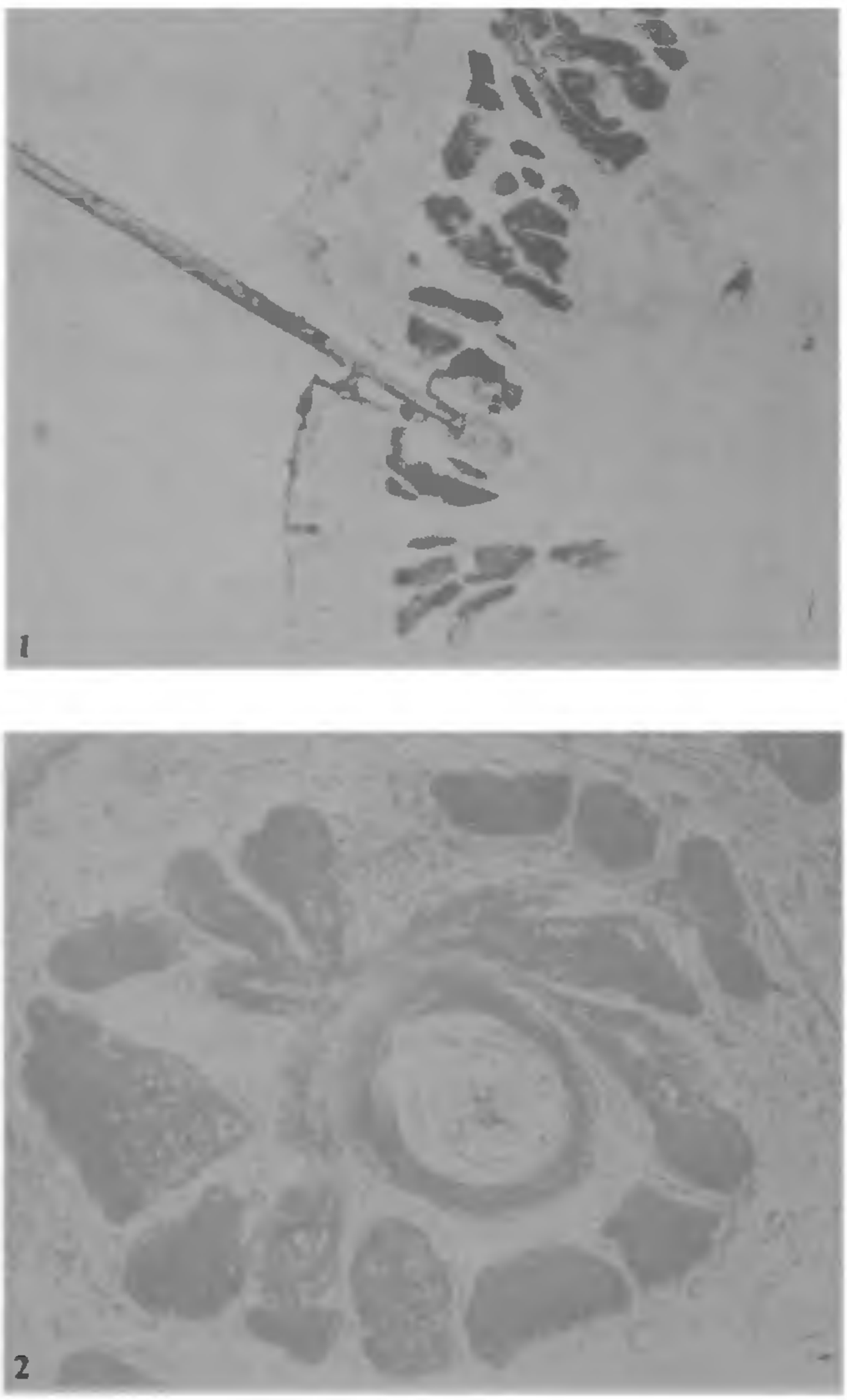
THE phenomenon of lordosis is found in many vertebrates and for this reason secondary sexual characters

are present in many vertebrates. These characters are controlled by sex hormones. Such non-gonadal sensitive sites have been reported in man[1], in uropygial glands of male ducks[2] and in Indian weaver bird *Ploceus philippinus*[3-5]. Non-specific esterase was reported as hormone-sensitive and the glands reported on the external portion of vagina are specific to that region and are many in number. In this paper the non-gonadal steroid site is reported in megachiropteran bat.

The adult female bats *Cynopterus sphnix sphinx* were collected during their active breeding period. The animals were killed by decapitation, the external vaginal portions were dissected out and fixed in cold (4°C) Banker's fixation. Following fixation (24 hr) the tissues were transferred to Holt's gum surcose[6]. The sections were cut at 5–6 μm on a Lipshaw cryostal at −25°C. Before incubation, the sections were washed with chilled distilled water.



Figures 1, 2. **1.** V. S. of vaginal skin showing hair shaft. Note the intense staining of non-specific esterase in sebaceous glands. **2.** T. S. of skin passing through hair follicle. Note the hair root at the centre and glands around it.

The following two histochemical techniques were employed for enzyme localization: (1) α-napthyl acetate (Sigma) as a substrate with Fast Blue B as a coupler[7]. (ii) 5-Bromoindoxyl acetate (Sigma) as substrate with ferri-ferrocyanide as redox buffer[8].

The females of *C. sphinx sphinx* megachiropteran frugivorous bat experience two pregnancies in quick succession. The first lasts from November to March and the second from March or April to July[9].

The enzyme activity in the epithelial cells of sebaceous glands appeared as diffused cytoplasmic in the form of granules. The granular non-specific esterase activity was localized in supranuclear region of holocrine cells (figure 1). In epithelial cells of sebaceous glands, the non-specific esterase activity was very high due to rising titer of estrogen level in blood. The sebaceous glands in other parts of skin of bat are very scanty. But many sebaceous glands are distributed at the site of hair root in the vaginal region (figure 1, 2). Non-specific esterase staining was very scanty during sexually inactive period.

Studies on non-specific esterases in sebaceous glands associated with vaginal or pubis region revealed that the glands show intense staining during active breeding. It has been reported that the non-specific esterase activity in endometrium is sensitive to ovarian steroid hormones[10]. It has also been reported that the neutral lipids are depleted by non-specific esterases in the endometrium of rat[11]. However, only 17β-steroid dehydrogenase cannot be considered for the detecting non-gonadal steroid structures, because neutral lipids oı cholestrol and the non-specific esterases are equally important in steroid synthesis.

The results in the present studies reveal that the vaginal skin glands appear to be non-gonadal steroid structures. Such a type of non-gonadal structure is also found in other bats. Non-gonadal steroid site has been confirmed in *Hipposideros speoris*. In this bat nasofrontal gland is the non-gonadal steroid structure which undergoes cyclic variations according to the male sexual cycle. This gland is present on the nose of male bats[12]. The gland is meant for attracting females during breeding season. No doubt the vaginal skin glands in Indian short-nosed bat are also important for attracting males and play an important role in localized sweating. Besides, energy is made available by

mobilizing lipids by non-specific esterases at the time of labour.

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ISOLATION OF TENUAZONIC ACID, A PHYTOTOXIN FROM *ALTERNARIA CRASSA* (SACC.) RANDS CAUSING LEAF BLIGHT AND FRUIT ROT OF *DATURA STRAMONIUM* MILL

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LEAF blight and fruit rot disease of *Datura stramonium* caused by *Alternaria crassa* was observed in the experimental fields of this Institute. The symptomatology of the disease indicated that the pathogen might elaborate a toxic metabolite during pathogenesis. Tenuazonic acid produced by *A. alternata*[1], *A. longipes*[2], *A. kikuchiana* and *A. mali*[3] and *Pyricularia oryzae*[4] has been shown to play an important role during pathogenesis. The present studies report the isolation of tenuazonic acid from *A. crassa* infecting *D. stramonium*, its phytotoxic effect on the host plant and the role in disease syndrome.

A pathogenic strain of *A. crassa* isolated from the leaves of *D. stramonium* was employed for these studies. The pathogen was grown in several 500 ml Erlenmeyer flasks filled with a nutrient liquid medium (g/l: glucose, 40; $(NH_4)_2HPO_4$, 2; KH_2PO_4, 11; $MgSO_4\ 7H_2O$, 0.5; KCl, 0.5 and yeast extract, 1.0). The inoculated flasks were incubated for 20 days at 25 $\pm$ 2°C. The culture was filtered through Whatman No. 1 filter paper and the filtrate was used to isolate the toxic metabolite following the method of Janardhanan and Husain[5].

Tenuazonic acid isolated from the culture filtrate of *A. crassa* was identified by TC on silica gel G using chloroform : methanol (90 : 10 v/v) solvent system. The compound was identified by UV and IR spectral analyses.

Toxicity of tenuazonic acid was tested on germinating seeds of *D. stramonium* at 10 μg to 100 μg/ml concentration. Toxicity was also tested on the leaves of *D. stramonium*. A drop of 200 μg/ml toxin was placed on the leaves followed by a fine needle prick. In both the experiments sterilized distilled water was used as control.

Results indicated that *A. crassa* produced significant amount of a toxic metabolite, tenuazonic acid, *in vitro*. TLC analysis of the toxin and the reference compound (tenuazonic acid) showed identical R_f value (0.4) and characteristic colour reaction when sprayed with methanolic $FeCl_3$. The compound had a UV absorption maxima at 220, 277 nm in acidic and 240, 280 nm in alkaline phase. IR spectrum revealed characteristic peaks at 3200, 3040, 1620, 1430 cm^{-1}. Thus the TLC combined with UV and IR spectral analysis confirmed the identity of the compound as tenuazonic acid.

The tenuazonic acid isolated from *A. crassa* induced typical necrosis on *D. stramonium* leaves 24 hr after application. The symptom of the toxicity consisted of necrotic spots surrounded by chlorotic halo which enlarged further resulting in death and defoliation. The toxic symptoms elaborated were almost similar to natural infection. Tenuazonic acid also induced complete inhibition of root and shoot elongation of germinating seeds of *D. stramonium* at 100 μg/ml concentration.

The present studies reveal that tenuazonic acid produced by *A. crassa* has a significant role during the leaf blight and fruit rot disease of *D. stramonium*. The