

the excitability is increased, the heart may not relax properly.

5. Acetylcholine in small concentrations potentiates the response to electric current in frog stomach (Singh, 1939). It is possible that chemical transmission potentiates electrical transmission.

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January 19, 1945.

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A NEW BACTERIAL LEAF-SPOT ON PIPER BETLE

A NEW type of bacterial leaf-spot disease was observed on *bangla* variety of pan (*Piper betle*) at Ramtek during December 1943. So far, under field conditions, the disease has not been observed to occur on *kapuri*, *kakher* and *gangari* varieties. The mode of infection and symptoms of this leaf-spot disease are entirely different from those described by Raghunathan (1926, 1928), Park (1934), Nirula (1931) and Asthana and Mahmud (1944). As far as known to the authors this disease has not so far been reported from anywhere else.

The earliest symptom of the disease is the presence of extremely minute pale-yellow spots either on the lower or upper sides of the leaves. Within a couple of days the initial spots turn dark-purple in colour and are slightly raised. There are no corresponding spots or discolouration on the other side of the leaf. Yellow coloured zones with water-soaked areas are not formed round these spots as in the leaf-spot disease described by Raghunathan (1926). The spots may appear inbetween the veins or on or along the veins. In the former cases they are more or less round or roughly angular while in the latter they are irregularly elongated or branched like fern leaves. They vary considerably in size, measuring from 1 mm. to 1 cm. across, and are generally apparent only on one side of the leaves. In cases where rotting has advanced considerably these spots are visible on both the sides. The infected leaves gradually turn yellow and fall off. The disease has not been observed to cause any damage to roots, stems or petioles.

Healthy leaves of *kapuri* and *bangla* varieties were artificially inoculated by pure cultures of the pathogen. Inoculations were either carried out by spraying the leaves with a suspension of the bacterium in sterile distilled water or simply by smearing the leaves with the pure cultures of the organism. Under both the methods positive results were obtained on either of the varieties. *Kapuri* variety proved as susceptible to the disease as *bangla*. Under moist conditions the symptoms of the disease appeared within 12 to 18 hours. Infection appeared with equal readiness on both the sides of the leaves of all ages. On re-isolation the same pathogen was isolated from all the infected leaves. Inoculations of roots, stems and petioles gave negative results.

Parenchymatous cells are chiefly infected, the pathogen being intra-cellular. In the earlier stages of infection the organism is found only in the epidermal cells but later on it invades spongy and palisade cells. In highly advanced stages of rotting the pathogen is occasionally seen in the phloem and xylem vessels as well, though mostly in the former. The invaded cells slightly enlarge in size, turn dark-lemon and disintegrate. The leaves turn yellow and gradually drop off. Two to three spots are enough to kill a leaf. In some cases the parasite enters the host through the stomata but in others the entry appears to be directly through the epidermal wall. There are no stomata on the upper side of pan leaves of *bangla* and *kapuri* varieties yet the entry of the organism is easily effected by spraying or keeping a small bit of inoculum there. In some cases the organism has been found to enter through the stomata on the underside of the leaves while in others the epidermal cells are clogged but the neighbouring stomata and the cells beneath them are absolutely free of it. All these show that the presence of stomata or wound are not at all essential for the entry of this bacterium into the host cells.

On bouillon-agar plates the organism produces sky-white colonies within 12 to 18 hours which later on turn maize-yellow. The colonies are round, thin and flat, glistening and marked with ridges. In texture they are dry and brittle. The colonies have a distinct central area surrounded by an outer ring and a lobed margin; the lobes occasionally branching out fern-like and appear fan-shaped. On bouillon-agar streak the growth is echinulate in formation but in stab cultures it is filiform.

The pathogens are rod-shaped bacterium, measuring $1.2 \times 2.5 \mu$ and occur in pairs or in chains of 3 to 12 or even more cells at a time. They are fairly motile both in solid and liquid media and in young and old cultures. Twenty-four to 48 hours-old cultures show very brisk motility of the sinuous swimming type, rarely straight and frequently with spells of quick and sudden tumbling on the short axis. At 30° to 38° C., the sporulation, even in young cultures, is in abundance. The spores remained viable even when they were heated to 90° C. for ten minutes.

The characteristics of the pathogen as described above are quite different from those of *Bacterium betle* Reg. (1926, 1928). It is presumed to be a new species and is provisionally named *Bacillus betle*.

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