



# **STUDIES ON BACTERIAL WILT DISEASE OF SOYBEAN**

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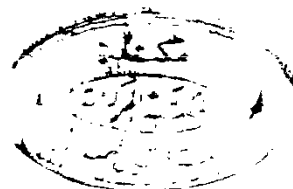
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## INTRODUCTION

Soybean, Glycine max (L.) Merr., is one of the most important leguminous crops in the world, particularly in the developing countries due to its high protein and oil content. Soybean was known in Egypt since 1932. However, it was only cultivated in recent years at a large scale as an important leguminous crop. The area cultivated with soybean reached 144 . 355 feddans in 1981 producing about 1.15 ton to feddan (Ministry of Agriculture 1983).

The cultivation of such crop in Egypt, usually faces many problems, such as the failure of inoculation with nodule bacteria, and the bacterial and fungal diseases that attack the growing plants and cause high loss in the crop yield. One of the most important disease recently observed in some regions of the country, is the wilt disease caused by Corynebacterium flaccumfaciens (Hedges, 1926). Lawson. The infected plants show wilting and shrivelling of their leaves accompanied with dull green or brownish green discoloration and the whole plant may be dead before it has developed more than the first pair of leaves (Hedges, 1926 and Elliott, Charlote (1950).

Therefore, it was found of interest to study this disease focussing on the following aspects:

- 1- Isolation of the causal pathogen from different infected plant parts.
- 2- Identification studies.
- 3- Factors affecting the disease incidence.
  - a) Methods of inoculation.
  - b) Air temperature and relative humidity and disease development.
- 4- Effect of disease on crop yield.
- 5- Pectinolytic and cellulolytic activities of the pathogen.
- 6- Sensitivity to different antibiotics.

## REVIEW OF LITERATURE

### 1- Characteristics of the Causal Organism :-

Elliott, Charlotte (1930) described Corynebacterium flaccumfaciens (Hedges, 1920) as a causal organism of bean wilt. She found that it was a motile organism by a single polar flagellum. The cells ranged between 0.3-0.5 X 0.6-3.0  $\mu$ . No chains, spores or capsules are formed. The organism was aerobic, Gram positive and not acid fast. On beef agar medium it formed cream to yellow, round, Smooth, Wet-Shining, Flat, Semi-Opaque colonies with granular entire margins and viscid consistence. Nitrates were not reduced, and gelatin was slowly liquefied. No gas or acid was formed from dextrose, lactose, sucrose, or glycerin, milk curdled and peptonized, and litmus milk reduced. It formed a moderate to strong sediment viscid in beef broth. Ammonia was produced, no indole, or hydrogen sulphide was formed, little or no growth in Cohn's and term's solutions. She also found that its optimum temperature was about 31°C with a maximum of 36-40°C, and a minimum below 15°C. The thermal death point was found to be about 60°C.

Walker (1952) observed that Corynebacterium flaccumfaciens had a single polar flagellum and produce cream to yellow colonies on nutrient agar.

Dunleavy (1962) noticed several important differences in serological and biochemical characteristics between Corynebacterium species. He found that the cells were small and Gram variable. Indole was produced and gelatin was liquefied by the C. flaccumfaciens. No curd was formed in milk and nitrates were reduced to nitrites.

Kiraly et al. (1974) showed that the bacterium Corynebacterium flaccumfaciens is a short non-sporing rod with a rounded end of 0.6-3 X 0.3-0.5  $\mu$  in size. Electron microscopic investigation indicated that it had a one polar flagellum. It liquefied gelatin, Gram positive. Starch was weakly hydrolyzed. Peptonization in litmus milk commences on the 5<sup>th</sup> day and the milk turned pale pink. Nitrite was not formed from nitrate. Indole was not produced in peptone water culture.

They also found that nitrate was not reduced by the organism. Ammonia was formed but it could not be produced in peptone water culture. Hydrogen sulphide did not develop. Acid was produced from glucose, galactose, arabinose, xylose, raffinose, sucrose, maltose, mannitol, mannose, fructose, salicin and dextrin. A little acid was formed from lactose and dextrin after 15 days. Acid was not produced from dulcitol and asparagine.

Sinclair and Shurtleff (1975) mentioned that colonies on agar were small, opalescent and became brown. The causal organism was a motile, gram-positive rods 0.3 to 0.5 X 0.6 to 3.0 um, with a polar flagellum. Colonies on beef agar were yellow, circular, smooth, flat or slight convex, wet shining semi-opaque, had an entire margin and were slightly viscid.

They found that the optimum growth temperature was 35°C to 37°C, while the maximum one was 42°C and the minimum was 10°C. Nitrate was not reduced and gelatin was slowly liquefied. It fermented some carbohydrates without a gas production.

#### 2- Symptoms of Soybean Wilt :

Hedges(1926) mentioned that wilting and death of bean seedlings, wilting, dwarfing and sometimes breaking over of older bean plants could be caused by C. flaccumfaciens. Stomatal infection did not occur. Artificial infections were produced upon soybean plants, however the disease was not known to occur upon this crop.

Hedges(1926)and Elliott,Charlotte(1930)found that the wilting and shrivelling of the leaves were sometimes accompanied with a dull green or brownish green discoloration, and the

whole plant may be dead before it has developed more than the first pair of leaves. Pod infection may be observed on green or just yellowing pods. On green pods, the diseased area may be a rather sickly green or a yellowish green, or it may be somewhat darker than the rest of the pod and more or less watery looking. The causal organism was found as a thick bright yellow layer underneath the white seed coat.

Walker (1952) showed that a bacterial wilt, being incited by a seed-borne pathogen, was widespread and occurred in conjunction with one or more of the bacterial blights and was often obscured by the latter. Spotting of leaves and pods may prevent the development of leaves and eventually die. Lesions may extend along the pod suture, and the stem begin to canker.

Dunleavy and Kunkel (1962) isolated a nonidentified Corynebacterium sp. which caused a severe stunting of soybean seedlings. He found that the bacteria were transmitted in the vascular elements of dormant seeds obtained from eight soybean producing states. Infected seedlings were extremely spindly and rate of growth was greatly reduced.

He noticed that the two-year old infected seeds did not germinate and the growing points of seedling were killed

or at least seriously damaged. Therefore a spindly branch developed from both cotyledon and buds. Infected plants developed few pods and did not mature normally. The bacteria produced internal necrosis of the cotyledons of seeds near the Central Vascular bundle.

Dunleavy (1962) microscopically examined the infected seeds and found a high bacterial population in pigmented portions of seed coats. He also observed that pigmentation usually spread in streaks of 0.1 to 4.0 mm wide but sometimes formed as an irregular halo around the hilum. Pigmentation was most frequently observed on the portions of the seed coat over the radical of the embryo only on the outer layer of cells of the seed coat.

Rousseaux and Hoffelt (1975) stated that in witches-brooms induced by C. flaccumfaciens after systemic infection or a local infection of pea plants at leaf axiles, no mutual inhibition was observed between shoots stimulated by bacteria. The growth of these abnormal shoots was reduced and finally stopped.

Miller and Pollard (1976) found that this seedborne disease attacks the very young seedlings. Young plants two to three inches tall are usually killed when attacked. If they survive, they may live throughout the season. The leaves may

become flaccid and limp on the stems and branches, especially during the warmest part of the day. If the vascular bundles become clogged, hence cutting off the water supply, the leaves will turn brown and drop-off. Sometimes when these typical symptoms are not apparent, golden-yellow necrotic leaf lesions can be seen. They also observed water-soaked spots on infected pods. They added that wilt was more conspicuous on ripe pods where it produced an olive-green color.