

## Demonstration of Koch's postulates by using plant fungi

### **History:**

Diseases can be spread by air, water, food, and human and animal vectors. In 1854, English physician John Snow found a relationship between polluted water and disease. Then, in 1884, German microbiologist Robert Koch isolated the bacteria *Vibrio cholera*, the cause of cholera, from water taken from Germany's Elbe River, thus proving the relationship between polluted water and this disease. Koch went on to formulate an established set of procedures to isolate and identify the causative agent of a particular microbial disease.

### **Koch's postulates:**

A set of criteria to be obeyed before it is established that a particular organism causes a particular disease. The organism must be present in every case and must be isolated, cultured and identified; it must produce the disease when a pure culture is given to susceptible animals/plant; and it must be recoverable from the diseased animal/plant.

### **The following four steps, which are still used today, are known as Koch's Postulates:**

Postulate 1: A specific organism must always be observed in association with the disease.

Postulate 2: The organism must be isolated from an infected host and grown in pure culture in the laboratory.

Postulate 3: When the organism from the pure culture is inoculated into a susceptible host organism, it must cause the disease.

Postulate 4: The infectious organism must be re-isolated from the diseased organism and grown in pure culture.

**Name of the disease: Sheath blight of rice and the casual organism is *Rhizoctonia solani***

**Sheath blight of Rice:**

Symptoms are usually observed from tillering to milk stage in a rice crop and include the following:

- Oval or ellipsoidal greenish gray lesions, usually 1-3 cm long, on the leaf sheath, initially just above the soil or water level in the case of conventionally flooded rice.
- Under favorable conditions, these initial lesions multiply and expand to the upper part of the sheaths, the leaves, and then spread to neighboring tillers belonging to different hills (transplanted rice) or plants (direct-seeded rice).
- Lesions on the leaves usually have irregular lesions, often with gray-white centers and brown margins as they grow older.

***Rhizoctonia solani*:**

The form genus *Rhizoctonia* traditionally includes filamentous soil fungi that do not produce asexual spores, possess brown pigmented hyphae, and possess right angled ( $\sim 90^\circ$ ) branching points with constrictions.

**Materials needed:**

- Sterile Water
- Dissecting and compound microscopes
- Dissecting needles and alcohol or sterile (autoclaved) toothpicks
- Bunsen burners or candles
- Microscope slides and cover slips
- Paper towels
- Petri dishes
- Plastic containers with healthy rice plant
- Culture medium etc.

Postulate 1: A specific organism must always be observed in association with the disease.

**Procedure:**

1. Collected disease specimen from the field
2. Study the characteristics symptoms of the disease specimen
3. Prepare the temporary slide from disease specimen
4. Identify the microorganism by using standard catalog

Postulate 2: The organism must be isolated from an infected host and grown in pure culture in the laboratory.

Procedure

1. Isolate the microorganism by using standard method
2. Identify the microorganism by using standard catalog
3. Culture the isolate microorganism on culture medium
4. Repeatedly transfer of the microorganism from one culture media to another unless getting pure culture

Postulate 3: When the organism from the pure culture is inoculated into a susceptible host organism, it must cause the disease.

Procedure

1. Select healthy, similar age and species of the plant for inoculation.
2. Inoculate by using standard method with isolated pure culture microorganism
3. After inoculation then, keep for incubation at least 7 days and allow to grow plant disease at 28°C under 14-h days.

Postulate 4: The infectious organism must be re-isolated from the diseased organism and grown in pure culture.

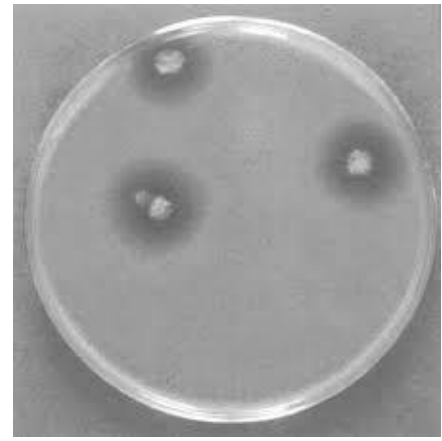
Procedure

1. Observe the infected area of the plant
2. Aseptically transfer of the microorganism from inoculated plant by using standard method in culture medium.
4. Make a data chart for recording the observations
5. Identify the microorganism under microscope and compare with previous one.

**Remark:** This pathogen is absolutely identical with previous one. As a result, it concluded that the disease was Sheath blight of rice and the casual organism was *Rhizoctonia solani*.



A



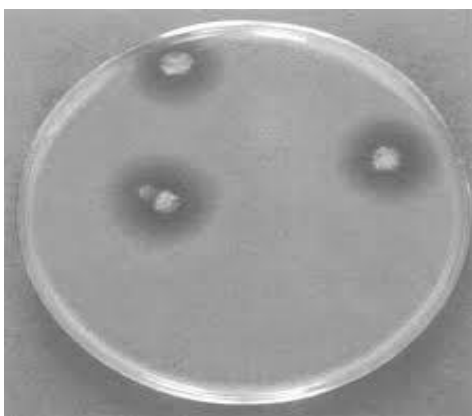
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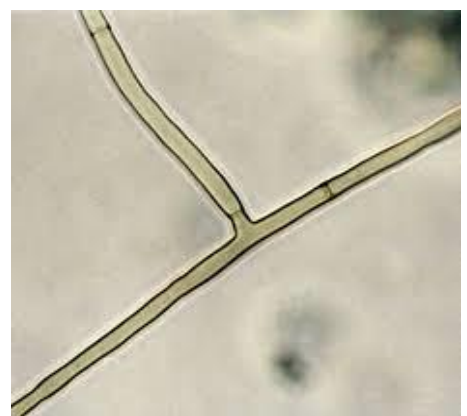
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D



E



F

Figure A: **ASSOCIATION**

Rice plant + *Rhizoctonia solani*

Figure B-C: **ISOLATION**

Culture the *Rhizoctonia solani* on medium

Pure culture of *Rhizoctonia solani*

Figure D: **INOCULATION**

Inoculate with isolated *Rhizoctonia solani*

Figure E-F: **RE-ISOLATION**

Pure culture of *Rhizoctonia solani*

Identify the *Rhizoctonia solani* under microscope