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Research Article

# Soft rot of Jackfruit (*Artocarpus heterophyllus*) by *Rhizopus artocarpi* (Berk. & Broome) Boedijn, in Andhra Pradesh, India.

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**Abstract**: Jack fruit (*Artocarpus heterophyllus*) is most widely cultivated in Andhra Pradesh, india. It has high nutritional values, medicinal values, rich phytochemical compositions, minerals etc. Such crop plants are infected by soft rot causing fungi by *Rhizopus artocarpi* (Berk. & Broome) Boedijn, in Andhra pradesh, India. The flowers and fruits are severally damaged by soft rot fungi. So this soft rot fungus was isolated from fruits and identified as *R. artocarpi* (Berk. & Broome) Boedijn. The soft rot fungus is grown on PDA medium and cultural characters are studied. The antifungal test is done by using fungal extracts from *Phelinus noxius* and *Ganoderma lucidum* and leaf extract *Prosopis juliflora* (Sw.) DC. In early stage of infection, *Rhizopus* spores deposit on moist fruit surface, get germinates and mycelia grow into the tissues of fruit. The infection produces a layer of black spores on the fruit surface. The fruit becomes soft, watery and brown spots develop on the fruit. In culture on PDA medium it is heavily growing and spreading. It produces sporangia with spores, and then it becomes brownish black with maturity of fungal colony. For biocontrol of soft rot fungi, 20% methanolic extract is more effective than 5, 10, and 15% concentrations. The methanolic extract showed 100% inhibition of both soft rot fungi when compared to water extract. For the first time fungal extracts were used to control the soft rot fungus causing disease in Jackfruits.

**Keywords**: Soft rot, *Rhizopus artocarpi* rot, Jack Fruit, Biocontrol, India

## Introduction

The genus Artocarpus belongs to the family Moraceae and is distributed across India. There are 18 artocarpus species found in India (Ahmedullah and Nayar, 1986). The name Artocarpus is derived from the Greek words artos (bread) and carpos (fruit) (Bailey, 1942). The common name of "jackfruit" used by the physician and naturalist Garcia de Orta in his 1563 book "Colóquios dos simples e drogas da India" (IPGRI, 2000). It is native to South and Southeast Asia and Originated in the south-western rain forests of India. Artocarpus is one of the major keystone species in Western Ghats (Isaac and Nair, 2006). The genus is receiving importance for agroforestry, plantation in forestry and afforestration programmes due to wide range of utilities like fruits and timbers, ayurvedic, culinary uses, etc. However, very limited studies are available in jackfruit production, marketing and value addition (Chowdhury *et al.,* 2012; Sharma *et al.,* 2013). The Artocarpus species have been used by traditional folk medicine in India. It is considered as rich source of carbohydrates, minerals, carboxylic acids, dietary fibers and vitamins such as ascorbic acid and thiamine (Lin *et al.,* 2000), Manganese and magnesium (Barua and Boruah, 2004), potassium, calcium and iron (Goldenberg, 2014) elements are found in seed. Seeds contain two lectins namely jacalin and artocarpin (Theivasanthi and Alagar, 2011). Jacalin has used for the evaluation of the immune status of patients infected with human immunodeficiency virus (Haq, 2006). It also has antioxidant activity (Biworo, 2015). It act against inflammation, malarial fever and skin disease (Khan *et al.,* 2003), anti-bacterial and anti-helmintics (Soeksmanto *et al.,* 2007). Jack leaves are commonly used to heal ulcers. Its leaves have the potential of curing diabetics due to the presence of hypoglycemic and hypolipidemic substances (Prakash *et al.,* 2009). The leaves and stem also have sapogenins, cycloartenone, cycloartenol, β-sitosterol and tannins (Sathyavathi *et al.,* 1987). Root extract is remedy for skin disorder and asthma (Ferrao, 1999). Fruits and roots used for tapeworm infection (Khan *et al.,* 2003). The fruit contains free sugar (sucrose), fatty acids, ellagic acid and amino acids like arginine, cystine, histidine, leucine, lysine, metheonine, threonine, tryptophan etc. (Swami *et al.,* 2012). Flakes of ripe fruits are rich with nutritive value; every 100 g of ripe flakes contains 287-323 mg potassium, 30.0-73.2mg calcium and 11-19g carbohydrates (Elevitch and Manner, 2006). Lycopene also found in jackfruit pulp (Setiawan *et al.,* 2001). There are 18 carotenoids were successfully separated, identified and quantified and 14 were detected in jackfruit (De Faria *et al.,* 2009). In the present paper, most widely used fruit was destroyed by soft rot causing fungus like *R. artocarpi* in Andhra Pradesh, India. So this soft rot disease is

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controlled by Biocontrol method like leaf extract from *P. juliflora* (Sw.) DC. fungal extracts of *P. noxius*, and *G. lucidum.*

## Materials and Methods

### Isolation and identification of fungi

The fungi associated with the jackfruit samples are isolated by using Potato dextrose agar medium. Fruit samples measuring 5 mm × 5 mm × 1 mm is aseptically removed from the infected jackfruit. Three pieces of jackfruit samples are transferred to Petri plates containing PDA medium amended with 250 µg streptomycin sulphate per ml. The plates were incubated at 25°C for 7days. Once fungal colonies formed in the PDA plates, each colony was transferred to a new agar slant to grow as a pure culture. The culture isolated from naturally infected fruit of jackfruit was identified by mounting culture with lacto phenol-cotton blue stain and observed the morphological characters by using compound microscope. The cultural characters of fungal samples are studied by using Zheng *et al.,* (2007). Pigment production and the colony characteristics were noted. The conidia, hyphae, conidial head, conidiophores, spores, etc; were observed microscopically for morphological identification. The identified fungi confirmed with microbial expert.

### Antifungal test

The extracts were mixed with appropriate volume of medium (PDA) to obtain concentrations ranging from 5 to 25 % in the final volume of 100 ml of medium. This 100 ml medium was dispensed into 100 mm Petri plates in triplicates (Nene & Thapliyal 1979). Fungal isolates was placed in the centre of each plate. Control sets were also prepared without plant extract. The plates were incubated at 25 ±2°C temperature and growth of colony was measured after 7 days of inoculation. The radial growth of mycelium was measured at two points along the diameter of the plate and the mean of these two readings was taken as the diameter of the colony. The growth of the colony in control sets was compared with that of various treatments and the difference was converted into percent inhibition by following formula

$$\text{Percent inhibition} = \frac{\text{Diameter of control set - Diameter of treated set}}{\text{Diameter of control set}} \times 100$$

## Result and Discussion

### Identification of Disease

Soft rot caused by *R. artocarpi* (Berk. & Broome) Boedijn, is observed in jackfruit flowers and fruit grown in Andhra Pradesh, India (Figure A). This soft rot disease is observed in high-rainfall areas and during or after stormy periods. When warm, humid, wet weather coincides with the flowering and fruiting season, soft rot disease is causing total loss of fruit in Jackfruit trees. Spore of *R. artocarpi* deposits on moist young or mature fruit surfaces, the spores germinate and mycelium enters into the tissues of fruit walls. The infected area produces profusely growing mycelium with reproductive structures i.e. sporangia with black spores on the fruit surface (Figure B, C); the fruit becomes soft, and brown to blackish spots develop on the mature jackfruit (Figure D, E). Subsequently, a powdery, fuzzy-looking mass of black spores, white fungal mycelia covers the entire young and mature jackfruit surface and infection becomes sever. The pathogen engulfs the young fruit, resulting in the characteristic black, rotten, shrunken, and sometimes mummified fruit remains. Fruit symptoms can appear on the tree as well as on fruit that are in storage or transit (Figure F). *R. artocarpi* is surviving on decaying plant litter or in the soil to initiate new infections.

Disease symptoms were as those described by Crisanto (1924) and Chauduri (1948, 1949). Early stages of the infected male inflorescence and young fruits showed first as a water soaked area, and then as the fungus developed a greyish mass appeared which gradually became denser forming a black growth with erect black sporangia. The young sporangiophores formed a whitish fringe on the edge of the black growth. The mycelium eventually covered the entire male inflorescence and fruit. The infected fruit rotted slowly, mummified and eventually fell from the tree. Based on the above symptoms and disease cycle the soft rot disease in Jack fruit is caused by *R. artocarpi* (Berk. & Broome) Boedijn.

**Table 1**: Anti-fungal activity of leaf extract and fungal extracts aginest *Rhizopus artocarpi* fruit rot

| Soft rot fungi/ plant extract | Methanolic | | | | Water | | | |
|---|---|---|---|---|---|---|---|---|
| | 5% | 10% | 15% | 20% | 5% | 10% | 15% | 20% |
| | *Prosopis juliflora* (Sw.) DC. | | | | | | | |
| *R. artocarpi* | 73.77 | 100 | 100 | 100 | 85.87 | 93.22 | 95.88 | 97.74 |
| *R. stolenifer* | 65.23 | 70.45 | 100 | 100 | 50.67 | 79.90 | 85.75 | 95.34 |
| | *Phelinus noxius* | | | | | | | |
| *R.artocarpi* | 63.24 | 95.45 | 100 | 100 | 75.34 | 83.45 | 90.23 | 96.65 |
| *R. stolenifer* | 75.29 | 80.48 | 100 | 100 | 60.11 | 75.21 | 82.75 | 93.55 |
| | *Ganoderma lucidum* | | | | | | | |
| *R. artocarpi* | 68.23 | 98.78 | 100 | 100 | 56.34 | 75.45 | 88.27 | 99.63 |
| *R. stolenifer* | 65.29 | 81.35 | 100 | 100 | 58.18 | 64.28 | 79.10 | 98.46 |

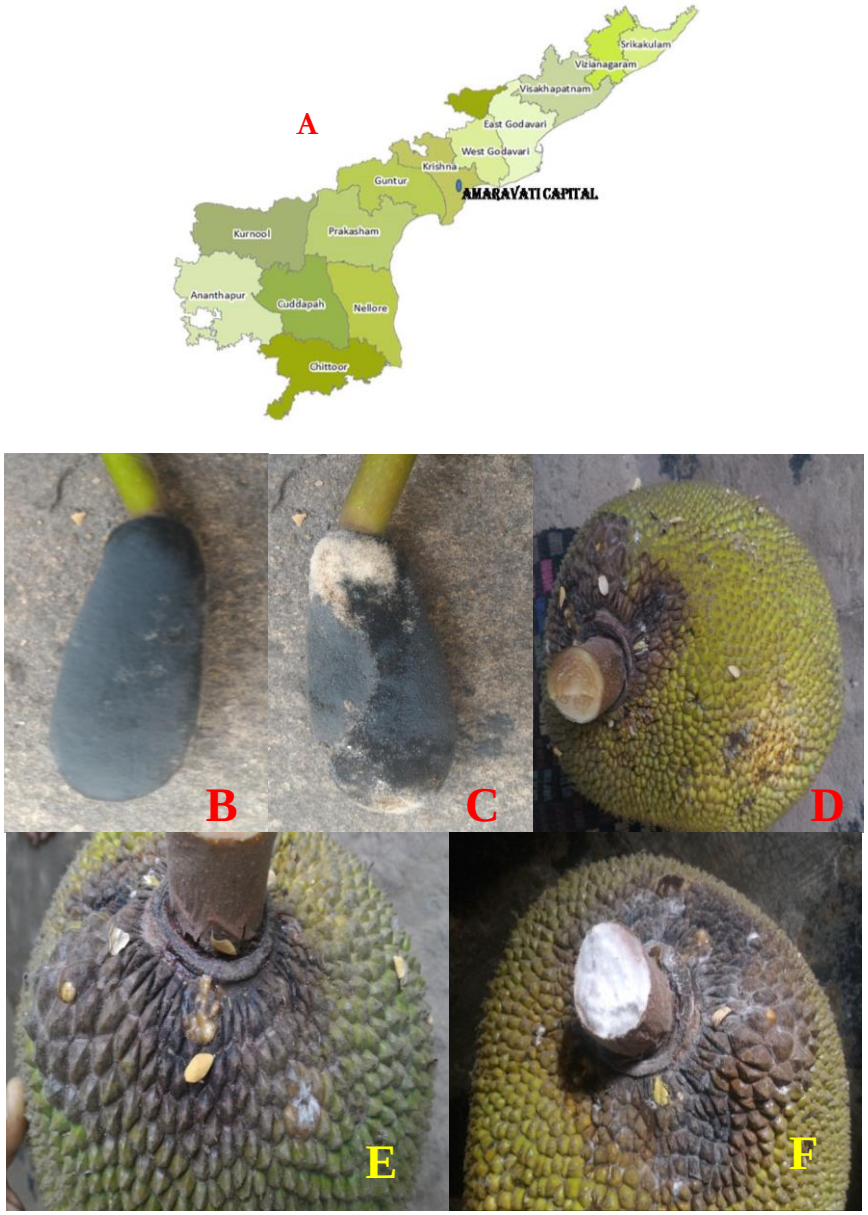


**Figure A.** Map of Andhra Pradesh showing soft rot diseased area, **B.** Blackish sporangiophores and sporangia of *Rhizopus artocarpi* on whole young jackfruit (*Artocarpus heterophyllus*) **C.** white mycelium with sporangiophores and Blackish sporangia on young jackfruit **D.** The brownish black coloured spots developing on mature Jackfruit near stalk. **E.** Enlarged view of infected area on mature jackfruit showing black coloured spots with soft tissue. **F.** On mature Jackfruit, the mycelium patch on stack and reddish brown to black patches with soft, rotten tissue near the stalk.

**Isolation**

The fungal association with the jackfruit trees growing in kitchen garden was surveyed in 2015 to 2018. The fungal isolates are purified and grown on Potato dextrose agar medium slants to study the cultural characters. The fungal pathogen isolated from the jackfruit is identified as *R. artocarpi* (Berk. & Broome) Boedijn. The symptom produced by fungal pathogen was observed on flower buds especially male flowers and young as well as mature fruits

**Phenotypical identification of Pathogen**

***Rhizopus artocarpi*** (Berk. & Broome) Boedijn, Sydowia 12(1-6): 328 (1959)

The pathogen first appears as white cottony colonies on potato-dextrose-agar at 27°C. It is heavily spread in the petriplate by the presence of sporangia with spores, and then it becomes brownish black with maturity of fungal colony, it able to spread by fixing stolons at various points to the substrate by rhizoids in the petriplate. The sporangiophore measures up to 42 x 1100-3200 µm in size. These are smooth walled, non- septate, light brown, simple. They arise in groups of 3-5 from

stolons opposite with rhizoids. The sporangia are 120-380 µm in diameter, globose with flattened base, white at first, then becomes black with maturity of sporangia. Each sporangium has thousands of spores. The columella measure 68-308 x 60-150 µm, are subglobose or dorsiventrally flattened, light brownish grey, and are umbrella-shaped when dehisced. The collar is absent. The apophysis is present and visible below young columellae. The rhizoids and stolons are transparent in young and becomes dark brown with age. The sporangiospores are brownish-black, 7.5- 9 x 18 - 24 µm in size, irregularly and round to oval in shape. They are heterothallic and strongly striate with homogeneous content. The zygospores are produced when compatible isolates are grown together. They measure 113-190 (220) µm, are globose, and compressed between suspensors, brownish-black, thickwalled and verrucose. The suspensors measure 52-100 µm wide, swollen, and usually unequal and somewhat granular.

Cultural characters of *R. artocarpi* (Berk. & Broome) Boedijn, isolated from jack fruit is compared with *R. nigrican* by Crisanto (1924). *R. nigricans* Ehrenberg is peculiar because the maximum growth temperature is somewhat low and because it practically lacks saccharifying and fermenting activities. So *R. artocarpi* show maximum growth rate at 27°C than *R. nigrican*. It has saccharifying and fermenting activities when compared to *R. nigrican* fungi.

**Antifungal test**

The soft rot fungi like *R. artocarpi* and *R. stolenifer* are isolated from jackfruit and pure cultures were maintained on Potato Dextose Agar medium. It is evident from Table 1., that leaf extracts of *Prosopis juliflora* (Sw.) DC., fungal extracts from *Phelinus noxius* and *Ganoderma lucidum* was tested against two soft rot fungi in vitro. In most of the casese soft rot fungal growth was inhibited by 20% methanolic extract than 5, 10, and 15% concentrations. The methanolic extract showed 100% inhibition of both soft rot fungi when compared to water extract. The highest inhibition of *R artocarpi* is observed in case of 15 and 20% methanolic extracts of *P. juliflora, P. noxius,* and *G. lucidum*. The highest inhibition of soft rot disease by *R artocarpi* and *R. stolenifer* in Jackfruit was observed in case of 20% water extract of *G. lucidum*.

There was variation in Minimum Inhibitory Concentration (MIC) of ridomil among the 8 isolates on agar plates so MIC on the agar plate ranged from 550 to 2200µg/ml (Dalavi *et al.,* 2016a). In the present paper, the soft rot fungal growth is inhibited by leaf extracts of *P. juliflora* and fungal extracts of *P. noxius* and *G. lucidum* which shown 100% inhibition. Mixture of different fungicides proved to be a useful approach for the management of fruit rot of jackfruit caused by *R. artocarpi*. Mixed and alternate use of fungicide must have a different mode of action which is significant for induction of resistance (Dalavi *et al.,* 2016b). In the present paper, biocontrol method is used to control soft rot causing fungi like *R.artocarpi* in jackfruit.

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