

SHORT COMMUNICATION**FIELD SANITATION AND THE OCCURRENCE OF BROWN SPOT DISEASE OF RAMBUTAN (*NEPHELIUM LAPPACEUM*) FRUITS**D. SIVAKUMAR^{*1}, R.S.WILSON WIJERATNAM¹ and R.L.C. WIJESUNDERA²¹*Industrial Technology Institute, 363 Baudhaloka Mawatha, Colombo 7*²*Department of Botany, University of Colombo, Colombo 3**(Received: 06 October 1997 ; accepted: 09 April 1999)*

Abstract: Rambutan fruits harvested from fields where leaf litter was allowed to collect showed a higher incidence and severity of brown spot caused by *Gliocephalotrichum microchlamydosporum*. *G.microchlamydosporum* was also the predominant pathogen present in leaf litter. Apart from *G.microchlamydosporum*, two other postharvest fungal pathogens, *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* were also isolated from leaf litter.

Key words: Brown spot disease, *Gliocephalotrichum microchlamydosporum*, rambutan, leaf litter

INTRODUCTION

Rambutan (*Nephelium lappaceum*) fruits have a high export potential due to their exotic flavour and colour. The discolouration of the skin, moisture loss and postharvest diseases are the three major problems that restrict their export. The postharvest losses in rambutan due to diseases, is reported to be about 30-40% in the Philippines¹. Brown spot (*Gliocephalotrichum microchlamydosporum*), anthracnose (*Colletotrichum gloeosporioides*) and stem end rot (*Botryodiplodia theobromae*) have been identified as the major postharvest diseases in rambutans grown in Sri Lanka.² These three diseases also have been reported from Philippines¹ and Thailand.³ The fungal pathogens infect fruits in the field and *G.microchlamydosporum* causes symptoms immediately² while *C.gloeosporioides* and *B.theobromae* remain quiescent⁴ until fruit ripening take place.

Postharvest pathogens have the ability to survive in plant or leaf debris.^{5,6} Thus, inoculum is present in the field throughout the year, when sanitation methods are not practiced. During wet weather, the survival structures of pathogens in leaf debris germinate and invade to infect the inflorescence or immature fruits.⁵ The objective of this study was to determine whether field sanitation has any effect on the occurrence of brown spot diseases of rambutan.

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METHODS AND MATERIALS

Determination of disease incidence and severity

Fruits were obtained from six fields in Pasyala in the Gampaha district (Western Province of Sri Lanka). The fields were similar in size and tree density. All the selected fields were cultivated with Malwana rambutan special selection 1. The fields were categorized into two groups as follows:

Group 1 (3 fields) - leaf litter allowed to collect under trees.

Group 2 (3 fields) - leaf litter routinely removed.

The removal of the litter was started in the previous season, after harvest. The leaf litter was removed weekly and after removal, it was burnt completely.

Sampling:

Twenty fruits of colour stage 1 (pericarp and spintern 100% green) were harvested randomly from the lower branches. Fruits were harvested from each field during 1993 & 1996 early growing season (5 weeks after fruit set) by standing under the tree using a pair of scissors. Harvested fruits were packed in ventilated plastic crates, covered with 0.7 nm polythene and transported to the laboratory within 3 h at 20°C. In the laboratory, samples were arranged on plastic trays lined with moist tissue paper. The trays were covered with tightly fitting glass sheets and incubated for 5-6 days at 28°C and at 80% RH. Observations were made on the formation of localized dark brown spots or areas on colour stage 1 immature fruits.

Disease incidence (DI) was determined as a percentage of number of fruits with brown spots per total number of fruits incubated.

Disease severity (DS) was determined on a 0-5 scale as follows:

- 0 = fruits with no symptoms
- 1 = fruits with light brown spots, water soaked lesions on the pericarp and pulp
- 2 = fruits with grayish brown mycelia on infected surface
- 3 = dark brown fruits with oozing pulp
- 4 = heavily infected fruits having yellowish mycelia with spores
- 5 = dark brown mummified, dried fruits

Experimental design:

A randomized complete block design was adopted with twenty replicates. Mean values were analyzed at 5% level of significance (DMRT- Duncan's Multiple Range Test). The experiment was repeated twice.

Isolation of *G.microchlamydosporum* from fruits with brown spot symptoms

The fruits showing brown spot symptoms in the above experiment were removed and from each fruit, approximately 1 mm thick piece of pericarp was cut and surface sterilized by immediately dipping in 1% NaOCl solution for 3 min. These cut pieces were transferred onto a sterile filter paper to remove excess NaOCl. All surface sterilized pericarp tissues were plated on PDA medium (containing 200ppm streptomycin). All culture plates made in this experiment were incubated at room temperature (28°C) for 3-4 days. The emergence of the *G.microchlamydosporum* colonies on the PDA plate was observed and the organism was purified by repeated subculturing. The isolated organism was identified as *G.microchlamydosporum* by observing its vegetative structures under the microscope. The pathogenicity of the fungus was proved by adopting Koch's postulates.

The % isolation from the treated and control fields was calculated as follows:

$$\% \text{ Isolation} = \frac{\text{No. of brown spots from which fungus was isolated}}{\text{Total no. of brown spots}} \times 100$$

This experiment was repeated twice. A complete randomized design was adopted and the mean values were analyzed by DMRT at 5 % level of significance.

Isolation of postharvest pathogenic fungi from leaf litter

Leaf litter (mixture of dried leaves 50g) was collected into a clean plastic bag randomly from underneath trees from fields of group 1. Five replicate samples were collected at a time. The collected leaves were cut into small pieces, 0.2g of the litter was placed in the PDA incorporated with 200ppm streptomycin, and the plates were incubated at 28°C for 5-7 days. Thirty PDA plates were made for one replicate leaf litter sample. Observations were made on emergence of the three postharvest pathogens, *G.microchlamydosporum*, *C.gloeosporioides* and *B.theobromae*. Their presence on the artificial medium was confirmed by observing the cultural characteristics and vegetative and sporulating structures. The percentage isolations of these organisms per total weight of leaf litter were computed. This study was conducted during 1994-96 early growing season (5 weeks after fruit set). A complete randomized design with five replicates was adopted in this study. The mean observations were analyzed by DMRT at 5 % level of significance.

RESULTS

Disease incidence and severity:

Incidence of brown spot disease and its severity were significantly higher in fruits obtained from fields where leaf was allowed to collect under trees, compared with fields where leaf litter was routinely removed (Table.1).

Table 1 : Incidence & severity of brown spot disease and percentage isolation of brown spot pathogen from infected fruits obtained from fields where leaf litter is removed routinely and leaf litter is accumulated under the trees.

| Leaf litter | Disease incidence | Disease severity | % Isolation of brown spot pathogen |
|--------------------------------|--------------------|------------------|------------------------------------|
| Allowed to collect under trees | 40.4 ^a | 3 | 46.40 ^c |
| Removed routinely | 18.43 ^b | 1 | 17.45 ^d |

Similar letter in same column indicates no significant difference at DMRT 5% level of significance.

The percentage isolation of *G.microchlamydosporum* from diseased fruits of colour stage 1 was significantly higher in fields where leaf litter was removed routinely (Table 1).

All three pathogens, *G.microchlamydosporum*, *C.gloeosporioides* and *B.theobromae* were isolated from leaf litter. *G.microchlamydosporum* was isolated in a significantly higher percentage than *C.gloeosporioides* and *B.theobromae* (Table 2).

Table 2 : Percentage isolation of the three major post harvest fungal pathogens of rambutan from leaf litter.

| Name of pathogen | % Isolation |
|------------------------------|--------------------|
| <i>B. theobromae</i> | 15.8 ^a |
| <i>C. gloeosporioids</i> | 26.17 ^b |
| <i>G.microchlamydosporum</i> | 35.2 ^c |

Similar letters in same column indicate no significant difference at DMRT 5% level of significance.

DISCUSSION

The general cultural practice among rambutan growers in Sri Lanka is to allow the leaf litter to collect under the tree as a source of manure. This study clearly shows that accumulation of leaf litter leads to significantly high levels of the brown spot disease. The causative fungus of the brown spot disease has the ability to survive in leaf litter as chlamydospores and as a saprophyte². Hence, the presence of leaf litter is a constant source of fungal inocula, especially during wet weather where rain splash can carry the inocula on to the surface leaves and fruits. Once the pathogen lands on the fruit surface it gains entry via cuts produced by insect bites or injuries caused during careless harvesting². It is evident that the occurrence of brown spot could be reduced by routine removal of litter. However, since leaf litter is used as a source of manure, it is suggested that the growers be advised to remove leaf litter from their fields at least during periods of wet weather.

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