Factors Affecting Dissemination of Bacterial Leaf Spot (*Xanthomonas campetris* pv. *betlicola)* Disease and Identification of Resistant Varieties of Betel (*Piper betle* L.)

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ABSTRACT

Betel is an economically important crop grown in Sri Lanka supplying for both foreign and local markets. Bacterial leaf spot caused by *Xanthomonas campestris* pv. *betlicola* is the most serious disease in betel, which can cause severe losses resulting low profits. A study was carried out to examine the factors affecting to the dissemination of this disease and to evaluate the varietal resistance. A survey was conducted with 30 farmers each from Kurunegala and Gampaha districts to find the factors affecting the spread of this disease. Laboratory experiment was done to evaluate resistant varieties using four commonly available betel varieties. Environmental and field conditions of betel growing areas were studied. Results of to this study the rainfall had an effect on the dissemination of this disease. For the use of, the disease severity revealed that the least susceptible variety was Gatathodu.

KEY WORDS: Bacteria leaf spot, Betel, Diseases severity, Dissemination of disease, *Xanthomonas campestris* pv. *betlicola*,

INTRODUCTON

Betel vine (*Piper betle* L.) belongs to the family Piperaceae and is a perennial aromatic creeper grown for its leaves (Singh *et al.*, 2002). It is an economically important crop, which earns nearly 200 million rupees per annum as foreign income (Anon, 2002).

Disease problems, water deficiency and high cost of fertilizers are constrains in betel cultivation. Among the diseases, the major problem is bacterial leaf spot disease caused by *Xanthomonas campestris* pv. *betlicola.* (Khare *et al.*, 1994).

This disease is prominent in certain areas. Less information on factors affecting dissemination of that disease is a reason for highly spread of this disease.

Burning affected plants, use of disease free water source, pest control, sanitation, alternation of cultural practices and selection of disease free planting materials are some of the bacterial leaf spot management practices (Senevirathna and Rathnasoma, 2002).

Rabindran and Marimuthu promote the use of bordeaux mixture to control bacterial leaf spot of betel in India (Rabindran and Marimuthu ,1988). The Betel Research Station, Narammala advice the use of a fungicide mixture including copper fungicide to control this disease (Senevirathna and Rathnasoma, 2002). However deposition of these chemicals on betel leaf surface resulting accumulation of heavy metal ions and toxic compounds in human body when betel leaves are chewed.

Less information is available on factors that may influence the disease spread in Sri Lanka.

The primary objectives of this study are to identify the responsible factors for dissemination of this disease and test this causal organism for their reaction against different betel varieties.

MATERIALS AND METHODS

This study was carried out at the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura premises and several farmer fields at Kurunegala and Gampaha districts in Sri Lanka, from February to August 2006.

Experiment 1

Factors affecting the dissemination of bacterial leaf spot on betel

A survey was done to gather details and data that were related to dissemination of this disease. A questionnaire was given 30 farmers each from Kurunegala and Gampaha districts. Disease availability and rainfall was considered in May of 2005 to July of 2006.

Experiment 2

This study was carried out at the Horticulture Laboratory in wayamba University of Sri Lanka.

a) Isolation of causal organism

Betel leaves with the disease symptoms were cultured on the Potato Dextrose Agar (PDA) media.

b) Confirmation of causal organism

Isolated bacterial cultures were tested for adopting following three methods.

I. *Gram discrimination*

The KOH test was done to confirm the *Xanthomonas* spp.

II. *Culturing leaf spot on Yeast Dextrose Calcium carbonate media (YDC)*

Drop of diluted bacterial solution spread on YDC media.

III. *Koch postulate*

Bacterial cultures isolated from leaf spots were re-inoculated on to healthy leaves of betel. Mixture of carborundum and bacterial suspension was rubbed

on healthy betel leaves to inoculate causal organism. Appearance of the disease symptoms was observed and the resulting leaf spots again cultured to confirm the causal organism.

c) Varietals evaluation

Commonly available four varieties of betel; Ratadalu (V1), Maneru (V2), Gatathodu (V3) and Galdalu (V4) were used to evaluate the resistance. Bacterial suspension was artificially inoculated to betel leaves of each four varieties. Leaves separated from plants and placed on humidity chamber. Mixture of carborundum and bacterial suspension was rubbed on different varieties of betel leaves.

The experiment was laid out in CRD with six replications. Varieties (V) were used as treatments. Following parameters were recorded: Disease severity in 1st, 3rd, 5th and 7th day.

Disease severities, as percentages, were assessed by using scales given in table 1.

Table 1 - scale of disease severity:

Scale	Severity of the infection
1	No any visual symptoms
2	Leaf spot area between 0.5 – 1 (cm)
3	Leaf spot area between 1 – 2 (cm)
4	Leaf spot area between 2 – 3 (cm)
5	Leaf spot area between 3 – 4 (cm)
6	Leaf spot area between 4 – 5 (cm)

Disease severity (%) was computed using the following formula.

$$\text{Disease severity \%} = \frac{nt}{NT} \times 100$$

Where,

n = number of leaves in each scale

t = scale

N = total number of observations

T = maximum scale

The recorded data were statistically analyzed using MINITAB software.

RESULTS AND DISCUSSION

Experiment 1

Factors affecting Dissemination of bacterial leaf spot in betel

Survey data showed that the disease incidence higher in October, November in 2005 & May and June in 2006.In August, September, 2005 and February 2006 show less disease incidence (Figure1.).

Figure 2 shows the rainfall distribution in the survey area May, 2005 to July, 2006. It was observed that highest rainfall during October, November in 2005 and May June in 2006.Lowest rainfall amount was showed August, September, 2005 and February 2006 (Figure 2.)

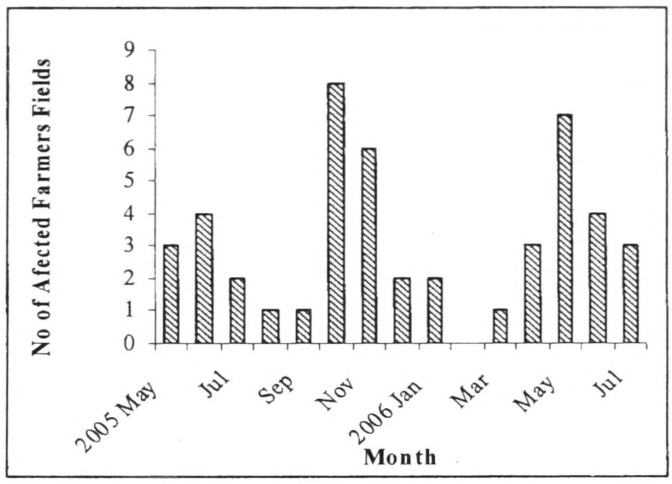

Figure 1 -Number of disease affected farmer fields from May, 2005 to July, and 2006:

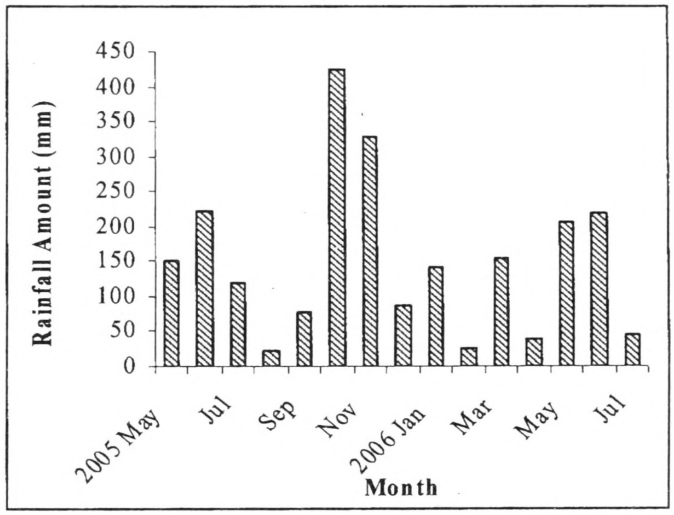

Figure 2 - Monthly rainfall in 2005 to 2006:

It was revealed that there was an association between amount of rainfall and the disease dissemination. When the rainfall was higher, the number of field with the disease was higher. This agrees with Senevirathna and Rathnasoma, (2002).

Experiment 2

a) Isolation of causal organism

Both cultures and sub cultures on PDA media produced yellow colonies that resembled bacteria.

b) Confirmation of causal organism

I. *Gram discrimination*

In KOH test, cultures of bacteria were sticky and slimy upon lifting with the inoculation loop. Isolated bacteria were recognized as gram-negative.

II. *Culturing with Yeast Dextrose Calcium carbonate medium (YDC)*

Yellow coloured colonies were observed on YDC medium. It was obvious that the bacteria were *Xanthomonas* spp (Norman, *et al.*, 1997).

III.. *Koch postulate*

Isolated cultures were shown to induce the disease. Thus it was *Xanthomonas campestris* pv.*betlicola.*

Table 1 - Mean of disease severity (arc sine) with time:

Variety	Disease Severity (Arc Sing √ %) Transformed Mean			
	1st Day	3rd Day	5th Day	7th Day
V1 (Ratadalu)	29.30[a]	39.91[a]	50.12[a]	63.84[a]
V2 (Maneru)	31.18[b]	40.46[a]	50.66[a]	64.07[a]
V3 (Gatathodu)	25.82[c]	37.09[a]	47.66[a]	53.14[b]
V4 (Galdalu)	29.99[a]	39.37[a]	50.11[a]	60.72[a]
LSD	1.26	4.29	5.12	6.48
CV%	9.2	9.09	8.57	9.28

A mean in the same column followed by a common letter are not significantly difference

c) Varietal evaluation

The disease severity of V1 and V4 not shows significantly different and disease severity of the V1, V2, and V3 were significantly different from each other in 1st day. Minimum disease severity can be seen in V3 (Table 1.).In 3rd and 5th day there was no any significant difference between V1, V2, V3, and V4.In 7th day V3 was significantly different from V1, V2 and V4 (Table 1.). These results revealed that the disease severity of V3 was significantly different from others in 1st day and 7th day showing less disease severity denoting less susceptibility to the bacterial leaf spot disease than other varieties.

When considering all these varieties the V1, V2, V4 have large and succulent leaves while V3 has small and less succulent leaves. This may be the reason for the less susceptibility of the V3 for the bacterial leaf spot disease. The causal organism *Xanthomonas campestris betlicola* attacks more to the succulent and fertile leaves (Senevirathna and Rathnasoma, 2002).

CONCLUSIONS

Results of the study reveal that the higher rainfall highly affects to spread of bacterial leaf spot disease. The variety Gatathodu has less susceptibility to the bacterial leaf spot disease than other varieties.

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