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RESEARCH ARTICLE

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ABSTRACT

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Keywords:

Mechanical transmission- *Watermelon bud necrosis virus*- Efficacy of different buffers Watermelon, *Citrullus lanatus* (Thumb.) is an annual trailing creeper belonging to the family cucurbitaceae. It occupies a pivotal position among fruit vegetables. Among the diseases affecting watermelon, bud necrosis disease has emerged as a serious threat in India. Recently, bud necrosis a *Tospovirus* disease, has emerged as a serious problem of watermelon cultivation in Kerala. For the biological characterization of the virus mechanical transmission was standardized with different buffers at four pH level (7.0, 7.2, 7.4 and 7.6). Under artificial inoculation, symptom initiated as curling, crinkling and mottling of leaves. Citrate phosphate buffer 0.1 M (pH 7.2) showed the maximum transmission (72.22 %) with minimum incubation period (8 days). This is the first report on the efficacy of Citrate phosphate buffer.

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INTRODUCTION

Watermelon is an important summer vegetable crop of India. The cultivation of watermelon is often hindered many biotic constraints such as diseases. Viral disease is a serious problem in cultivation of cucurbits in India. A total of 14 viruses have been reported to infect watermelon naturally. Among them, bud necrosis is an emerging disease and causing significant yield reduction in watermelon cultivation. Recently, in Kerala this disease is posing a serious threat to watermelon growers, but it was not received any attention so far. Hence the investigation was carried out with the objective of biological characterization of watermelon bud necrosis virus through mechanical transmission.

MATERIALS AND METHODS

The mechanical transmission of *Watermelon bud necrosis virus* was standardized with different buffers at four pH level (7.0, 7.2, 7.4 and 7.6). For the sap transmission, young leaves of watermelon plants with bud necrosis symptoms were selected, washed with tap water, dried with blotting sheet and weighed separately for preparation of standard extract. Buffer volume equal to the weight of the leaves was added to chilled mortar and ground with the pestle. After thorough grinding, the homogenized leaf pulp was filtered through double layered muslin cloth to get filtered standard extracts of the leaves. A

pinch of carborandum powder (600 mesh) was added to the extracted sap. Cotton pad soaked in standard extract was rubbed on the primary leaves of test plants, in one direction only that was from the petiole to the apex of the leaf by supporting the leaf from the centre with a cardboard. After five minutes of inoculation, test plants were washed with distilled water to remove excessive inoculum and extraneous particles. The numbers of treatments were twenty and each treatment was replicated thrice. Plants inoculated with buffer without the infected sap served as control. The inoculated plants were kept in insect proof net house and observed daily for the development of symptoms.

RESULTS

The standardization of mechanical transmission was carried out with variety Durgapura Lal using five different buffers at pH range, *viz.*, 7.0, 7.2, 7.4 and 7.6. The result showed significant difference in the disease transmission with variation in buffer and pH level. Of the different buffers tested, citrate phosphate buffer at pH 7.2 showed maximum disease incidence (72.2%) compared to others at all pH levels and was on par with the potassium phosphate buffer. Acetate buffer (pH 7.6) showed minimum disease incidence (5.55 per cent). The absolute control plants without inoculation of infected sap remained healthy (Table 1).

Buffer	Mea	Mean			
рН	7.00	7.2	7.4	7.6	
Citrate phosphate	66.67	72.22	44.44	38.89	54.17
Potassium phosphate	61.11	66.67	38.89	38.89	51.38
Sodium phosphate	27.78	38.89	22.22	11.11	25.00
Acetate	11.11	22.22	5.55	5.55	11.11
Boric acid borax	38.89	44.44	27.78	22.22	33.33
Mean	40.00	48.89	27.78	23.33	

 Table 1 Standardization of different buffers at different pH

 level based on the per cent disease incidence

CD (0.05) buffer = 8.76, CD (0.05) pH = 7.83, CD (0.05) interaction (NS)

Incubation period was the other factor involved in selection of suitable buffer. Taking into consideration of the incubation period for the symptom appearance, significant difference was observed between the selected buffers and the four pH level used for each buffer.



Curling and leaf distortion

(9 days). Acetate buffer (pH 7.6) showed the maximum incubation period (20 days).

 Table 2 Standardization of different buffers at different pH level based on the incubation period

Buffer	Incu	Mean			
pН	7.00	7.2	7.4	7.6	
Citrate phosphate	9.0	8.0	11.0	14.0	10.50
Potassium phosphate	10.0	9.0	11.0	14.0	11.00
Sodium phosphate	13.0	12.0	14.0	16.0	13.75
Acetate	15.0	15.0	18.0	20.0	17.00
Boric acid borax	13.0	11.0	14.0	17.0	13.75
Mean	12.00	11.00	13.60	16.20	

CD (0.05) buffer = 0.70, CD (0.05) pH = 0.63, CD (0.05) interaction = NS

According to the results, citrate phosphate buffer (0.1 M, pH 7.2) was selected as the best buffer for mechanical transmission.



Crinkling and mottling on leaf



Bud necrosis



Yellowing and bud necrosis

Plate 1 Symptoms developed under artificial condition

Among the selected buffers, citrate phosphate buffer (pH 7.2) showed minimum incubation period (8 days) for the symptom appearance followed by potassium phosphate buffer (pH 7.2)

In mechanically inoculated plants, symptoms initiated as curling of younger leaves in about 8 days after inoculation which later spread to other leaves. Later the leaves showed crinkling and mottling, leaf size was reduced, became malformed and was rough and brittle. Flowering started 40 days after sowing and flowers were less in number. Unopening of flower buds and bud necrosis was also observed in the flowering stage (Plate 1).

DISCUSSSION

Transmission is an important experimental tool to establish the etiology of viral diseases and plays vital role in disease spread. With this view, an attempt was made to understand the mode of transmission of the watermelon bud necrosis disease through sap. In the present study, the mechanical transmission was standardized using five different buffers each at four pH range, viz., 7.0,7.2, 7.4 and 7.6. The result indicates that the different buffers at varied pH level used for each buffer shows significant difference in per cent transmission of the disease. Of the buffers used 0.1 M (pH 7.2) showed maximum disease incidence of 72.22 per cent with minimum incubation period of 8 days and was selected as the best buffer for mechanical transmission. The early report on the successful transmission was by Singh and Krishnareddy (1996).

According to this report, the leaves of watermelon showing bud necrosis symptom was extracted using 0.1 M phosphate buffer pH 7.0 and on mechanical inoculation it produced localized followed by systemic infection on watermelon and localized chlorotic lesions on cowpea. Hence, this is the first report on the efficacy of citrate phosphate buffer (pH 7.2), on the transmission of *Watermelon bud necrosis virus*.

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