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Effect of Sakkara Brewing on the Severity of Powdery Mildew Disease of Luffa (*Luffa acutangula*) and Cucumber (*Cucumis sativus* L.) under Greenhouse Condition

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***Abstract*— *Powdery mildew is one of the major production constraint of cucurbits in almost all parts of Sri Lanka.The disease can be controlled with fungicides. However, bio control agents or organic compounds provide economically sound, practically feasible and environmentally safe approach. "Sakkaraa" brewing (SBr) is a fermented aqueous drink based on cane sugar and yeast (Saccharomyces cerevisiae). Most of the studies assessing the efficacy of yeast as a bio control agent, however, have focused on its effects against some fungi. Two experiments were conducted in parallel to identify the effect of SBr on severity of powdery mildew of Luffa and cucumber varieties under greenhouse condition. Six luffa varieties and twelve cucumber varieties were grown in pots and artificially inoculated with powdery mildew pathogen. Layout of the factorial experiments involving crop varieties and SBr treatments was completely randomized block design with four replications. About 15 days after inoculation of spore suspension of pathogen and when powdery mildew symptoms were well appeared, started the application of diluted SBr on six Luffa varieties and twelve cucumber varieties as an aqueous spray and untreated plants of each Luffa and cucumber variety were kept as control. Disease evaluation and measurements of percentage disease severity index (DSI (%)) of powdery mildew on plants were performed and recorded at flowering stage and fruiting stage. Microscopic observations confirmed that causal agent of powdery mildew of Luffa and cucumber in the country was Podosphaera xanthii. Results of DSI % of powdery mildew in both experiment showed that there was a significant difference between SBr applied treatment and control both at flowering and fruiting stage. Luffa varieties did not show significantly different of DSI (%) of powdery mildew. Popular Luffa variety Naga recorded highest DSI (%) when compared with other tested varieties. Cucumber varieties showed significant difference of DSI (%) of powdery mildew at flowering and fruiting stages. Cucumber var. KWxG17(S) Green and Var. Tunnel Green showed significantly lower DSI (%) of powdery mildew compared to other tested varieties. Results revealed that SBr has remarkable ability of control of powdery mildew and provides an opportunity to produce an effective control tool to protect Luffa and cucumber varieties from powdery mildew disease.*

***Keywords*— Powdery mildew, Sakkara Brewing (SBr), Luffa, Cucumber.**

I. INTRODUCTION

Powdery mildew is one of the most prevalent and aggressive diseases that affect leaves in cucurbits [1]. The infection is evident by the development of white mycelia and conidia, mainly on leaves and stems, but it can also affect fruits and floral structures. Severely infected leaves may become chlorotic, or even necrotic and brittle. Consequently, it decreases the photosynthetic potential, and concomitantly lowers the fruit quality and yield [2]. *Podosphaera xanthii*[syn. *Sphaerotheca fuliginea*

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(Schlecht) Pollacci] and Golovinomyces *orontii* (syn. *Erysiphe cichoracearum* DC. Ex Mérat) are the most important powdery mildew pathogen species of cucurbits [3].

"Sakkaraa" brewing (SBr) is a fermented aqueous drink based on cane sugar and yeast (*Saccharomyces cerevisiae)*. SBr an intricate process encompassing mixing and further elaboration of essential raw materials, including cane sugar, water and yeast. It contains 2.4 x 10^4 yeast cells per $1mm^3$, 2.2% ethanol, 4% methanol and pH of the SBr is 3.36.

Yeasts such as *Debaryomyces hansenii*, *Kluyveromyces* spp., and *Saccharomyces cerevisiae* have been tested for their ability to suppress mycological growth and limit mycotoxin production on foods such as grapes, coffee beans, cereals, peanuts, and dairy products [4], [5], [6]. Several studies have demonstrated an efficient antagonistic activity of yeast against *Botrytis cinerea* [7], [8], [9]. When evaluating the effects of increasing antagonistic yeast concentrations on the inhibition of *B. cinerea* conidial germination, it was observed that there is a dose-dependent response. As yeast concentration increased, inhibition of *B. cinerea* conidial germination increased [10]. Inhibition of *B. cinerea* conidial germination could be due to the parasitism exerted by the yeast [11]. and enzyme action, such as quitinases and β-1,3 glucanases, which degrade the *B. cinerea* cell wall and produce cytological damage [12], [13].

Leaf-colonizing yeasts are widely used as biological control agents to protect against diverse foliar pathogens such as powdery mildew fungi, *Aspergillus flavus*, *Botrytis*, and *Ustila gomaydis* [14], [15]. *Pseudozyma flocculosa* is a basidiomycetous fungal yeast that has been extensively characterized as an effective control agent for powdery mildew fungi, which are ubiquitous phyllosphere pathogens of numerous field and greenhouse crops. *P. flocculosa* was first isolated as an antagonist of cucumber powdery mildew under different environmental conditions [16]. An early study reported that *P. flocculosa* does not penetrate plants but induces rapid plasmolysis of powdery mildew cells, which suggests that *P. flocculosa* secretes an antibiotic or other bioactive agent that affects powdery mildew cells [17]. Subsequent work showed that *P. flocculosa* culture filtrates produced the same effects (rapid cell plasmolysis) on powdery mildew fungi [18]. Molecular and biochemical analyses identified the antibiotic glycolipid flocculosin, and found that flocculosin production was strongly correlated with *cyp1* expression, which encodes a mono oxygenase with a crucial role in fungal growth inhibition [19].

To date, research on powdery mildew disease in Sri Lanka was mainly directed towards the development of control methods using fungicides. The chemical control of powdery mildew may be ineffective due to development of resistance of pathogen to some fungicides [20], [21]. Sakkaraa brewing applied Luffa and cucumber plants showed superior results in contrast to untreated control with enhancing flowering as well as fruit setting performances [22]. Therefore, two experiments were conducted in parallel to study the effect of Sakkara brewing (SBr) on control of powdery mildew on Luffa and cucumber under greenhouse condition.

II. METHODOLOGY

2.1 Identification of pathogen

Powdery mildew affected Luffa and cucumber leaves were collected from farmers' field in different locations in the cucurbits growing regions. Morphological features of mycelia and conidia of sixteen diseased samples were microscopically observed to identify the powdery mildew pathogen of Luffa and cucumber [24].

2.2 Greenhouse experiments

Two separate experiments were conducted in parallel with six Luffa varieties and twelve cucumber varieties were grown in 15 cm-diameter plastic pots and randomly arranged on greenhouse benches. The soil was a composition of 1:1compost and top soil. Layout of the factorial experiments involving Luffa and cucumber varieties and SBr treatments was completely randomized design with four replications. The plant growth was conducted under controlled conditions (relative humidity was above 85% and temperature 25^0C – 28 ^{0}C). Once every two days, plants were watered to saturation and standard crop management practices were done throughout the study. Also, experiments were repeated twice.

2.3 Pathogen Inoculation in green house

For pathogen inoculation, 15-days old plants with 4 fully-expanded true leaves were chosen with a 28 ^{0}C temperature and 85% relative humidity in the greenhouse. Thereafter, spore suspension was prepared from freshly sporulating leaves by immersing a few pieces of the leaves in 200 ml distilled water and adjusted to the number of $5x10^4$conidia/ml with the aid of a haemocytometer. The upper surface of the leaves was inoculated by spraying uniformly with a hand sprayer until tiny water droplets covered the leaf surface but not flawed.

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2.4 Application of Sakkara Brewing (SBr) on plants

About 15 days after inoculation and when powdery mildew symptoms were well appeared, started the application of diluted Sakkara brewing (yeast cells 1.5×10^8conidia/ml) on six Luffa varieties and twelve cucumber varieties of both experiment as an aqueous spray continued 4 times with 10 days intervals and untreated plants were kept of each luffa and cucumber variety as control.

2.5 Data collection and analysis

Disease evaluation and measurements on plants were performed and recorded at flowering stage and fruiting stage. The severity of powdery mildew was recorded by using 0-9 scale [25] as given below. Percent disease severity index (DSI %) was calculated by using formula given by Wheeler [26].

Table1. Rating scale for DSI of powdery mildew on leaves

Rating Scale	Description
0	No symptom of powdery mildew on leaves.
1	Small scattered powdery mildew specks covering 1 % or less leaf area.
3	Small powdery lesions covering 1-10 % of leaf area.
5	Powdery lesions enlarged covering 11-25 % of leaf area.
7	Powdery lesions coalesce to form big patches covering 26-50 % leaf area.
9	Larger powdery patches covering 51 % or more of leaf area and defoliation occur

Formula wherein DSI (%) = [Sum of numerical values/ (number of leaves rated maximum rating)] 100.

III. DATA ANALYSIS

The data obtained were tabulated and analyzed subjected to the Analysis of Variance procedure of Statistical Analysis System (SAS) 9.1 software. Duncan's New Multiple Range Test was performed to compare the differences among treatment means at p=0.05.

IV. RESULTS AND DISCUSSION

4.1 Pathogen identification

Morphological characters of powdery mildew isolates of Luffa and cucumber leaves collected from open field in different locations were as follows: powdery mildew fungi were found on the leaves on the upper side and underside. Microscopic observation revealed that fungi colonies were white to brown, irregular. Hyphae were hyaline and septate. Conidiophores hyaline, straight, unbranched. Conidia formed singly at the apex of the conidiophores. Fibrosin bodies were observed in conidia [24]. Based on the morphological characteristics especially on the presence of fibrosin bodies in conidia, the identity of powdery mildew fungi of Sri Lanka was *Podosphaera xanthii.*

4.2 Effect of foliar application of SBr on DSI (%) of Powdery mildew of Luffa Varieties at Flowering and Fruiting Stages.

Varieties	**DSI (%) at flowering stage**			**DSI (%) at fruiting stage**		
	Spraying	None spraying	Mean	Spraying	None spraying	Mean
N1-1	16.03	46.74	31.39	10.31	45.30	27.80
N3-2	34.34	50.90	42.62	19.58	60.35	39.96
N5-3	26.53	39.90	33.22	18.30	38.96	28.63
N3-4	33.14	46.43	39.79	12.90	46.39	29.64
Gannoruwa Ari NAGA	13.99	35.31	24.66	11.76	47.33	29.55

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Mean	20.45 24.08^{b}	66.47 47.63^{a}	43.46	13.15 14.33^{b}	80.64 53.16^{a}	46.89

Note: Means followed by the same letter/s along the row are not significantly different at p=0.05 level.

Results revealed that it has no significant interaction between DSI (%) of powdery mildew of crop varieties and Sakkara Brewing treatment at flowering as well as fruiting stages of both experiment. All Luffa varieties showed significantly lower DSI (%) of SBr treated pots compared to control pots both at flowering and fruiting stage. However, varietal difference of DSI (%) of powdery mildew was not significant among Luffa varieties. Popular Luffa variety Naga recorded highest DSI (%) when compared with other tested varieties. In general, lower DSI (%) was recorded on leaves of treated plants at fruiting than flowering stage (Table 4.2).

4.3 Effect of foliar spraying of SBr on DSI (%) of Powdery mildew of Cucumber Varieties at Flowering and Fruiting Stages.

DSI at flowering stage				**DSI at fruiting stage**		
Varieties	Spraying	None spraying	Mean	Spraying	None spraying	Mean
KW x G17 (S) Green	6.27	12.99	9.63 ^{p}	1.29	10.78	6.04 ^{p}
KW x M2 (Self)	8.01	40.53	24.27 ^{pq}	3.33	55.67	29.49 ^{qr}
Champion x US Cucumber	18.78	46.33	32.56 ^{q}	11.3	40.24	25.77 ^{qr}
Shani x White Jade	16.85	40.92	28.89 ^{pq}	22.71	68.28	45.49 ^{r}
NS46 (Self)	24.43	43.12	33.78 ^{q}	21.45	62.37	41.91^{qr}
White Jade x Champ	27.20	45.28	36.24 ^{q}	17.17	67.64	42.4 ^{qr}
EFDAL outcross	8.04	42.64	25.34 ^{pq}	4.00	47.58	25.79 ^{qr}
R2 x M2 (self)	17.44	37.49	27.47 ^{pq}	11.73	48.88	30.3 ^{qr}
Tunnel Green	1.39	5.40	3.40 ^{p}	0.26	9.49	4.87 ^{p}
Champion	16.94	36.9	26.92 ^{pq}	11.37	73.53	42.45 ^{qr}
Kalpitiya White	8.64	42.73	25.69 ^{pq}	7.36	70.94	39.15 ^{qr}
Gannoruwa White	10.25	20.55	15.40 ^{pq}	2.12	26.79	14.45 ^{pq}
Mean	13.69 a	34.57 b		9.5 a	48.51 b	

Note: Means followed by the letter/s along the row/column are significantly different at p=0.05level.

All cucumber varieties showed significantly lower DSI (%) of SBr treated pots compared to control pots both at flowering and fruiting stage. Varietal difference of DSI (%) of powdery mildew was also significant among cucumber varieties at flowering as well as fruiting stage. Cucumber var. KWxG17(S) Green and Var. Tunnel Green showed significantly lower DSI (%) of powdery mildew compared to other tested varieties. In general, lower DSI (%) was recorded on leaves of treated plants at fruiting than flowering stage (Table 4.3).

For the bio control yeasts so far studied in detail, multiple mechanisms such as competition for nutrients and space, secretion of enzymes, toxin production, release of volatile organic compounds (VOCs), mycoparasitism and induction of resistance in plants are likely to be involved in the antagonistic function [27], [28], [29]. The basis of the antagonistic properties of yeast against pathogens has been previously described and includes: competition for nutrients, pH changes on the plant surface, production of ethanol and biosynthesis of killer toxins called mycocins [30]. Nevertheless, despite all these beneficial traits, the commercial application of yeast in the field as bio control agents has shown an inconsistent efficacy compared to synthetic fungicides [31]. However, results of the both experiments have shown that SBr clearly suppress the powdery mildew disease of Luffa and cucumber plants.

To date, research on powdery mildew disease in Sri Lanka was mainly directed towards the development of control methods using fungicides. Application of agro-chemicals are the major plant protection method over decades even though they are associated with many disadvantages including their expensive applications, environmental pollution and human health hazards due to excessive usage. This has emerged a worldwide huge trend to explore other environmental friendly alternative methods for plant protection. “Sakkaraa” brewing is a low cost, fermented aqueous drink based on cane sugar and yeast (*Saccharomyces cerevisiae).*

V. CONCLUSION

Sakkara brewing provides an opportunity to produce a safe, environmental friendly, effective bio-control tool to protect Luffa and Cucumber crops from powdery mildew disease caused by *Podosphaera xanthii* under greenhouse condition.

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