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Pivotal Role of Residual Coconut Water and Spent Wash on Phyllosphere and Rhizosphere Microflora of Gherkin (*Cucumis sativus* L) Under Glass House Condition

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A B S T R A C T

Application of residual coconut water and spent wash obtained from desiccated coconut mills has relatively enhanced the phyllosphere and rhizosphere microflora of gherkin under glass house condition. Among the different concentrations of residual coconut water and spent wash, the 10 per cent of spent wash showed higher phyllosphere populations at 45 days after sowing, such as bacteria, yeast and actinobacteria (0.316, 0.295 and 0.133 x 10^4 cfu /cm^2) and rhizosphere microflora *viz*., bacteria, fungi and actinobacteria (58.33 x 10^5, 10.80 x 10^4, 14.00 x 10^3cfu g^{-1} of soil) and beneficial microflora like *Azotobacter* sp, Phosphate solubilizing bacteria and *Pseudomonas* sp. (14.67, 9.00 and 18.30 x 10^5cfu g^{-1} of soil) as compared to control.

Introduction

The epiphytic phyllosphere and rhizosphere harbours a diverse group of microorganisms including bacteria, fungi, yeasts, algae and in some situations protozoans and nematodes (Morris and Kinkel, 2002). These heterogeneous microbial populations have a vital effect on the crop improvement, as they are powerful forces for specific enzyme mediated fundamental metabolic processes (Ahmed and Kibret, 2014).

These microorganisms were exposed to rapidly fluctuating conditions such as temperature, water content, drought and exogenous application of nutrients (Hirano and Upper, 2000). The plant associated microbes were stimulated by application of exogenous nutrients like coconut water. The presence of carbohydrates, amino acids and organic acids in coconut water acts as nutrient source for microorganisms. The growth promoting substances like auxin content of coconut water stimulates the release of saccharides from the plant cell wall and microbes utilized these compounds (Goldberg, 1980, Van derwal and Leveau, 2011) and formation of root architecture and photosynthetic activity of plants. Due to this effect, the plant produces sugars, amino acids and other organic acids in the form of root exudates in the rhizosphere. These exudates favoured colonization of microorganisms in the rhizosphere (Farhatullah *et al.*, 2007).

The use of locally available inputs or other growth enhancing product like the coconut water needs to be given importance. It is rich with different nutrients, phytohormones, enzymes and minerals.

Residual coconut water and spent wash are available from desiccated coconut industries. It is organically rich and therefore may contribute pollution to our environment if allowed to stagnate in open or into water bodies. Coconut water can be used directly on plants as it serves as a nutrient and organic matter (Genaro, 2013). Therefore, present study was undertaken to determine the effect of residual coconut water and spent wash on epiphytic microflora of gherkin under greenhouse condition.

Materials and Methods

Source of residual coconut water and spent wash

The matured coconuts are used for production of desiccated coconut powder in coconut industry. The desiccated coconut industries discharge residual coconut water and which were collected at two different stages from Maruthi desiccated coconut mills at Kaidal gate, Tiptur taluk, Tumkur district.

Stages of sampling

Stage-1

Residual coconut water- this water was collected after nut breaking. It constitutes only coconut water.

Stage-2

Spent wash – this was collected after de-shelled coconut pieces were washed (out let). This stage contains- residual coconut water, coconut milk and water used for washing the de-shelled coconut pieces.

Details of pot experiments

Pot culture experiment was conducted in the glass house at Department of Agricultural Microbiology, UAS, GKVK, Bengaluru. Ajax variety of Gherkin (procured from the Nunhem's Pro Agro seeds Pvt. Ltd. Bengaluru) cultivar was used in the study. The crop was sown on 25th January, 2015 and crop was maintained up to 45 days. Freshly collected residual coconut water and spent wash were sprayed at different intervals at 15 and 30 days after sowing. The initial chemical and biological properties and the average values along with methods followed are provided in table 1.

Experimental design and treatment details for pot experiment study

The experiment had seven treatments with three replications, laid out in completely randomized block design and the treatments are as follows,

T_1: Control (water spray only)
T_2: 10 % - Residual coconut water
T_3: 15 % - Residual coconut water
T_4: 20 % - Residual coconut water
T_5: 10 % - Spent wash
T_6: 15 % - Spent wash
T_7: 20 % - Spent wash

Isolation of phyllosphere microorganisms

The phyllosphere microorganisms were isolated from gherkin leaves at different intervals. The leaf samples were collected from each treatment at regular interval and kept in the sterile polythene cover and then brought to the laboratory in the icebox and samples were analyzed within 24 hrs. The leaf of all ages was cut randomly into a 10 mm disc and 30 leaf discs were placed in 100 ml sterile water blank and shaken for 20 minutes in a rotatory shaker at 100 rpm. After shaking, the 10 ml of suspension was taken and transferred to 90 ml sterile water blank and shaken for few seconds. Sample (1 ml) from 90 ml was transferred to 9 ml sterile water blank (10^2) and serially diluted up to 10^4dilution. The microorganisms *viz.*, bacteria, yeast and actinomycetes were isolated using a spread plate technique by plating on specific nutrient media and plates were incubated at 30 ± 1 ^{0}C for a week the colonies which emerged were counted (Aneja, 2003).

Enumeration of rhizosphere microorganisms

The population of rhizosphere microorganisms in soil was determined by serial dilution plate count method. Rhizosphere soil samples were collected treatment wise at different intervals. Ten grams of soil (treatment wise) weighed and mixed in 90 ml sterilized water blank to give 10^1 dilutions. Subsequent dilutions up to 10^5 were made by transferring serially 1 ml of each dilution to 9 ml sterilized water blanks. The population of bacteria, fungi, actinomycetes, *Azotobacter* sp., *Pseudomonas* sp. and phosphate solubilizing bacteria were determined in respective medium. Plates were incubated at 30 ± 1^0 C for a week and the colonies which emerged were counted.

Results and Discussion

The phyllosphere microorganisms of gherkin such as bacteria, yeast and actinobacteria were significantly influenced by the foliar application of residual coconut water and spent wash at different intervals and the results are presented in table 2. Before spraying (15 days after sowing), the bacteria, yeast and actinobacteria recorded maximum (0.106, 0.106, 0.045 x 10^4 cfu cm^2) and minimum (0.064, 0.064, 0.020 x 10^4 cfu cm^2). However, at 30 days after sowing there was significant difference between the populations among the treatments. Higher bacterial, yeast and actinobacterial populations were recorded at foliar application of 10 per cent spent wash (0.209, 0.290 and 0.063 x 10^4 cfu cm^2). A similar trend was observed at 45 days after sowing. Higher bacterial, yeast and actinobacterial populations were found in the treatment at 10 per cent spent wash (0.316, 0.295 and 0.113 x 10^4 cfu cm^2) compared to control.

The proliferation of phyllosphere microorganisms at all the stages of crop growth was maximum in treatment at 10 per cent residual coconut milk. This may be because coconut water consists of considerable amounts of sugars, amino acids, mineral salts, vitamin B complex, vitamin C and cytokines *etc*. Microorganisms require a source of carbon and nitrogen for maintenance and growth, all these are present in coconut water (Vigliar *et al.,* (2006), Mishra and Srivastava (1974), Chikere and Azubuike (2014) and Shannon *et al.*, (2008).

The rhizosphere microflora of gherkin was significantly influenced by the foliar application of residual coconut water and spent wash at different intervals and the results are presented in tables 3 and 4. Bacterial population at15 DAS (before spraying) the bacterial population in rhizosphere was 18.33 to 20.33 x 10^5 cfu g^{-1} of soil. However, higher bacterial population in rhizosphere at 30 and 45 DAS was recorded in the treatment 10 per cent spent wash (42.00 and 58.33 x 10^5 cfu $g^{-1}$of soil). Whereas, the fungal populationat 15 DAS, the fungal population in rhizosphere ranged from 2.33 to 3.70 x 10^4cfu g^{-1} of soil and at 30 and 45 DAS the higher fungal population was recorded in 10 per cent spent wash (11.30 and 10.80 x 10^4 cfu g^{-1} of soil respectively) and rest of the treatments differed significantly. Similarly actinobacteria population at 15 DAS in rhizosphere of gherkin ranged from 1.67 to 3.60 x 10^3 cfu g^{-1} of soil. At 30 and 45 DAS, application of 10 per cent spent wash recorded significantly higher (12.63 and 14.00 x 10^3 cfu g^{-1} of soil respectively) actinobacteria population and lower actinobacteria population was recorded with the application of 10 per cent residual coconut water (6.33 and 8.60 x 10^3 cfu g^{-1} of soil, respectively). The *Azotobacter* population in rhizosphere of gherkin before spraying (15 DAS) ranged from 3.00 to 3.67 x 10^5 cfu g^{-1} of soil. Whereas at 30 and 45 DAS higher *Azotobacter* population in rhizosphere was recorded in the treatment 10 per cent spent wash (10.00 and 14.67 x 10^5 cfu g^{-1} of soil). The *Pseudomonas* population in rhizosphere of gherkin before spraying (15 DAS) ranged from 2.00 to 3.03 x 10^5 cfu g^{-1} of soil and at 30 and 45 DAS was *Pseudomonas* population was significantly higher in treatment 10 per cent spent wash (16.70 and 17.23 x 10^5 cfu g^{-1} of soil) Phosphate solubilizing bacterial (PSB) population before spraying (15 DAS) the phosphate solubilizing bacterial population ranged from 1.00 to 2.13 x 10^5 cfu g^{-1} of soil. Whereas, at 30 and 45 DAS, the maximum phosphate solubilizing bacteria (8.33 and 9.00 x 10^5 cfu g^{-1} of soil) was observed in treatment 10 per cent spent wash.

Table.1 Initial chemical and biological properties of soil used for pot experiment

Sl. No.	Particulars	Value	Method employed
Chemical properties			
1	pH	6.73	Potentiometry (Piper, 1996)
2	EC (dSm^{-1})	0.12	Conductometry (Jackson, 1973)
3	Organic carbon (%)	0.41	Wet oxidation titrimetry (Walkey and Black, 1934)
4	Available N (kg ha^{-1})	336.17	Alkaline permanganate digestion and distillation (Subbaiah and Asija, 1956)
5	Available P_2O_5 (kg ha^{-1})	31.20	Spectrophotometry (Jackson, 1973)
6	Available K_2O (kg ha^{-1})	195.30	Flame photometry (Jackson, 1973)
Biological properties			
7	Bacteria	16 x 10^5 cfu g^{-1} soil	Serial dilution plate count technique (Bunt and Rovira, 1955)
8	Fungi	5 x 10^4 cfu g^{-1} soil	
9	Actinomycetes	7 x 10^3 cfu g^{-1} soil	
10	*Azotobacter* sp.	6 x 10^5 cfu g^{-1} soil	
11	*Pseudomonas* sp.	8 x 10^5 cfu g^{-1} soil	
12	P solubilizing bacteria	5 x 10^5 cfu g^{-1} soil	

Table.2 Effect of residual coconut water and spent wash on phyllosphere microflora of gherkin under glass house condition

Treatments	**Bacteria**			**Yeast**			**Actinobacteria**		
	10^4 cfu /cm^2								
	BS	AS		BS	AS		BS	AS	
	15 DAS	**30 DAS**	**45 DAS**	**15 DAS**	**30 DAS**	**45 DAS**	**15 DAS**	**30 DAS**	**45 DAS**
T_1: Control (water spray)	0.106	0.144	0.153	0.064	0.116	0.140	0.020	0.025	0.031
T_2: 10 % - Residual coconut water	0.103	0.188	0.266	0.078	0.278	0.287	0.034	0.049	0.106
T_3: 15 % - Residual coconut water	0.099	0.161	0.205	0.085	0.251	0.263	0.033	0.040	0.080
T_4: 20 % - Residual coconut water	0.092	0.115	0.177	0.092	0.203	0.241	0.026	0.032	0.071
T_5: 10 % - Spent wash	0.085	0.209	0.316	0.106	0.290	0.295	0.045	0.063	0.113
T_6: 15 % - Spent wash	0.064	0.173	0.238	0.113	0.262	0.271	0.032	0.044	0.094
T_7: 20 % - Spent wash	0.067	0.154	0.191	0.099	0.214	0.250	0.030	0.036	0.076
S. Em. ± **C. D. at 1 %**	**NS**	**0.43** **1.32**	**0.42** **1.30**	**NS**	**0.33** **1.01**	**0.41** **1.25**	**NS**	**0.49** **1.49**	**0.36** **1.10**

Table.3 Effect of residual coconut water and spent wash on rhizosphere microorganisms of gherkin under glass house condition

Treatments	**Bacteria (10^5) cfu g^{-1} of soil**			**Fungi (10^4) cfu g^{-1} of soil**			**Actinobacteria (10^3) cfu g^{-1} of soil**		
	BS	AS		BS	AS		BS	AS	
	15 DAS	**30 DAS**	**45 DAS**	**15 DAS**	**30 DAS**	**45 DAS**	**15 DAS**	**30 DAS**	**45 DAS**
T_1: Control (water spray)	19.00	32.32	43.67	2.33	8.33	10.60	1.67	9.00	10.60
T_2: 10 % - Residual coconut water	18.33	24.73	31.80	3.00	5.03	4.00	3.00	6.33	8.60
T_3: 15 % - Residual coconut water	18.67	28.67	36.70	3.70	6.00	5.62	3.60	7.40	9.33
T_4: 20 % - Residual coconut water	19.63	38.60	55.30	3.33	9.70	8.90	3.33	10.90	12.67
T_5: 10 % - Spent wash	18.60	42.00	58.33	3.30	11.30	10.80	3.30	12.63	14.00
T_6: 15 % - Spent wash	18.67	32.03	40.50	3.00	7.67	6.10	3.00	8.03	10.00
T_7: 20 % - Spent wash	20.33	26.50	33.10	2.33	5.60	4.30	2.33	7.17	9.03
S. Em. ± **C. D. at 1 %**	**NS**	**0.95** **2.88**	**1.02** **3.10**	**NS**	**0.43** **1.32**	**0.33** **1.01**	**NS**	**0.37** **1.14**	**0.30** **0.93**

Table.4 Effect of residual coconut water and spent wash on beneficial microorganisms in rhizosphere of gherkin under glass house condition

Treatments	***Azotobacter* sp. (10^5) cfu g^{-1} of soil**			**Phosphate solubilizing bacteria (10^5) cfu g^{-1} of soil**			***Pseudomonas* sp. (10^5) cfu g^{-1} of soil**		
	BS	AS		BS	AS		BS	AS	
	15 DAS	**30 DAS**	**45 DAS**	**15 DAS**	**30 DAS**	**45 DAS**	**15 DAS**	**30 DAS**	**45 DAS**
$\mathbf{T_1}$: Control (water spray)	3.03	6.67	9.00	1.30	6.00	8.03	2.67	10.60	14.50
$\mathbf{T_2}$: 10 % - Residual coconut water	3.00	4.33	5.60	1.67	2.33	4.30	2.00	8.10	10.40
$\mathbf{T_3}$: 15 % - Residual coconut water	3.60	5.30	6.33	1.00	3.50	7.20	2.50	9.00	11.00
$\mathbf{T_4}$: 20 % - Residual coconut water	3.33	8.90	14.03	2.00	6.60	8.40	3.03	14.33	17.23
$\mathbf{T_5}$: 10 % - Spent wash	3.67	10.00	14.67	1.33	8.33	9.00	2.33	16.70	18.30
$\mathbf{T_6}$: 15 % - Spent wash	3.50	6.33	8.10	1.50	4.67	7.90	2.00	9.63	12.13
$\mathbf{T_7}$: 20 % - Spent wash	3.33	5.07	7.00	2.13	3.00	6.33	2.30	8.60	10.90
S. Em. ± **C. D. at 1 %**	**NS**	**0.30** **0.93**	**0.25** **0.76**	**NS**	**0.24** **0.79**	**0.21** **0.66**	**NS**	**0.37** **1.14**	**0.41** **1.26**

The rhizosphere microorganisms recorded maximum in treatment 10 per cent spent wash and was on par with 20 per cent residual coconut water. These results are in line with the findings of Kiran *et al.,* (2015), who pointed out that organic manures not only help to supply nutrients but also act as a food for microorganisms and encourage the multiplication of their population. The foliar spray of coconut milk to the crop will enhance the photosynthetic activity of plant. It will alter the plant root architecture and enhance the root exudates in the rhizosphere environment reported by Caers and Vending (S1986).

The fungal population decreased at 45 DAS in coconut water treated pots, it is possible that fungi did not react as fast as bacteria to the addition of C substrates with the organic fertilizers. It has also been observed that bacterial proliferation after the addition of labile organic substrates had antagonistic effects on fungal growth (Meidute *et al.,* 2008).

In conclusion the study shown that epiphytic (phyllosphere and rhizosphere) microflora of gherkin has been enhanced by the application of residual coconut water as well as spent wash.

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