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Evaluation of Desiccated Coconut Water and Dairy Whey on Soybean [*Glycine max* (L.) Merr.] Seed Germination

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Abstract

The uncontrolled dumping of agricultural and industrial wastes and waste water has serious environmental and public health consequences. In order to utilise these wastes for agricultural purposes, the response of soybean seed germination to the desiccated coconut water and dairy whey was examined. In this investigation the nutritional content analysis and enumeration of microorganisms found in desiccated coconut water and dairy whey obtained, respectively from coconut mills and dairy were carried out. Different microorganisms were isolated and characterized morphologically and biochemically characterized. The results showed that the desiccated coconut water included a diverse microbial population such as bacteria (286.4 × 104 cfu mL^{-1}), yeast (8.89 × 104 cfu mL^{-1}), actinomycetes (1.00 × 104 cfu mL^{-1}), free living nitrogen fixers (26.7 × 104 cfu mL^{-1}), phosphate solubilizing bacteria (33.3 × 104 cfu mL^{-1}), *Pseudomonas* sp. (73.3 × 104 cfu mL^{-1}) and coliforms (76.7 × 104 cfu mL^{-1}). Whereas, in dairy whey, microorganisms such as bacteria (153.3 × 104 cfu mL^{-1}), yeasts (3.30 × 104 cfu mL^{-1}), actinomycetes (0.12 × 104 cfu mL^{-1}) and coliforms (56.7 × 104 cfu mL^{-1}) were found. The effect of desiccated coconut water and dairy whey on soybean seed germination was studied by pre-treating the seeds and examining different seed quality indicators. Compared to all other treatments and the control, seeds pre-treated with 10 per cent desiccated coconut water and 10 per cent whey had significantly higher germination percentage (98.00 and 97.33), shoot length (13.58 cm and 14.30 cm), root length (21.94 cm and 20.12 cm), seedling dry weight (404.28 mg and 100.19 mg), seedling vigour index-I (3481 and 3351) and seedling vigour index -II (39619 and 39019), respectively.

Keywords : Desiccated coconut water, Dairy whey, Germination percentage, Soybean

Industrialization has aided in the growth of a country's economy, but its negative consequences, such as pollution posing major issue. All industrial sectors, particularly the food industry, have had a significant environmental impact due to excessive water use and high effluent generation per unit of production (Karthikeya *et al*., 2015).

There are around 266 desiccated coconut (DC) units in India, with an average capacity of 9345 nuts per ha. In Karnataka there are about 45-50 DC units, mostly in coconut-growing areas, with 6892 nuts per ha. (Anonymous, 2020). During the processing of desiccated coconut powder, the desiccated industries produce a lot of waste water, including 1500 to 2000 litres of coconut water, 7000 to 8000 litres of washed water and about 800 to 1000 litres of pasteurized water, which is discharged as an effluent from desiccated coconut powder producing industries with a capacity of 1000 kg per day (Anonymous, 1993).

Coconut water was utilized as a growth supplement and the optimum medium for microbial development and plant tissue culture in micro propagation (Sathiyavimal *et al*., 2014). Coconut water is medically sterile and microbe-free while it is in drupe form. Coconut water is reported to contain considerable levels of essential elements that help to set right nutritional imbalances and other health issues. It appears to have the similar impact on plants, fostering robust root systems, faster growth and development according to research findings (Sandoval *et al*., 2014).

Increased demand for milk and milk products around the world has resulted in massive growth in the dairy industry (Chokshi *et al*., 2016). Fast industrial growth on the other hand, increases not only production of

desired product but also releases the harmful compounds into land and water reservoirs. This discharge has negative impact on environment and poses substantial health risks to individuals (Porwal *et al*., 2015). The dairy industry is an example of a global food industry that produces a variety of products such as milk, milk powder, butter and cheese while also producing solid and liquid waste (Jaganmai and Jinka, 2017). Whey is a by-product of the dairy industry that was formerly dismissed as inconsequential and was either used as animal feed or discarded. Approximately 145 million tonnes of whey is produced globally each year, the quest for innovative whey applications is continued. Several researches have been conducted in recent years to determine the importance of whey in terms of nutritional content and the qualities of its constituents (Silviya *et al*., 2016).

The dairy waste poses a serious environmental danger due to its high organic content. Every year, around 4 to 11 million tonnes of dairy waste are dumped into the environment, posing a major threat to biodiversity. The reduction of dissolved oxygen is one of the most important issues produced by the direct release of waste water into the environment. Fat effluents such as oil and grease build a film on the water surface preventing oxygen transport and as a result, aquatic creatures and plants fall in perilous survival situations (Rosa *et al*., 2009).

The germination is a crucial step that assures reproduction and as a result, governs population dynamics making it a crucial test for crop productivity (Radosevich *et al*., 1997). The dairy whey contains a mix of organic and inorganic nutrients and has been shown to improve seed germination (Subramani *et al*., 1999). Plant susceptibility to effluent salinity varies from species to species (Ramana *et al*., 2002). The effect of different concentrations (10-100 per cent) of dairy effluent on seed germination in some vegetables like Lady's Finger (*Abelomoschus esculentus*) and Guar (*Cymopsis tetragonoloba*) was studied in the laboratory and results showed that wastewater irrigation increased growth and nutrient content in both crops (Sharma *et al*., 2011).

Soybean (*Glycine max*) is a good model plant used to study rhizosphere interactions since soybean plants have symbiotic relationships with rhizobia and arbuscular mycorrhizal fungi and which secretes isoflavones and saponins into the soil. Considering all the above aspects, a series of experiments were conducted to determine the biotic, abiotic aspects and effect of desiccated coconut water and dairy whey on soybean seedling germination.

Material and Methods

Isolation and Characterization of Microorganisms in Desiccated Coconut Water and Dairy Whey

The fresh samples of desiccated coconut water and dairy whey were collected in the sterilized plastic containers and brought to the laboratory from desiccated coconut mills and Karnataka Milk Federation (KMF), respectively. Using a standard plate count technique, microorganisms present in desiccated coconut water and dairy whey were enumerated (Bunt and Rovira, 1955). The isolates were purified and the cultures were maintained on slants for further studies.

The morphological characteristics of the colony such as elevation, opacity, cell shape and Gram reaction were studied as described by (Pelczar, 1957) and Schaad's descriptions (1992). The biochemical assays such as catalase, indole, methyl red, voges proskauer test and citrate production of cultures were examined as per by consulting the Pelczar's (1957) methodology and laboratory guide for bacterial identification (Schaad, 1992).

Analysis of Mineral Contents of Desiccated Coconut Water and Dairy Whey

All the elements were determined using the standard and accepted analytical procedure and methods adopted for plant sample analysis and are given below.

Parameters	Method	References
Nitrogen (%)	Kjeldahl digestion and distillation method	Piper (1966)
Phosphorus (%)	Diacid digestion and vanadomolybdate yellow color spectrophotometry	
Potassium and Sodium (%)	Diacid digestion and flame photometry	

Parameters	Method	References
Calcium (%)	Diacid digestion and versenate titrometry	Jackson (1973)
Magnesium (%)	Diacid digestion and versenate titrometry	
Sulphur (%)	Diacid digestion and turbidometry	
Fe, Mn, Zn and Cu (ppm)	Diacid digestion and atomic absorption spectrophotometry	

Effect of Desiccated Coconut Water and Dairy Whey on Germination Percentage, Shoot Length, Root Length & Vigour of Soybean Seeds

An experiment was conducted in the Department of Seed Science and Technology, University of Agricultural Sciences, GKVK, Bengaluru. Certified *soybean* cv. JS 335 seeds were obtained from NSP, GKVK, Bengaluru and soaked in two effluents *viz*. desiccated coconut water and dairy whey at various concentrations of 0, 10, 15, 20, 25 and 100 per cent, and were placed on top of a clean filter paper and air dried to their normal moisture content. The germination test was conducted with four replicates of 50 seeds in each treatment, held at a temperature of seed germinator maintained at 25 ± 1°C and 90 ± 2 per cent RH using the ISTA's 'between paper' method (Anonymous, 2014). On two sheets of 'germitest' paper, four replicates of 50 seeds were placed, covered with another sheet and rolled. The paper was moistened with 2.5 times its mass of distilled water and the rollers were kept in the germinator at a temperature of 25°C and a relative humidity of about 90 ± 2 per cent. The seedlings were counted on every day and the results were expressed in percentages from the 5th to the 8th day after sowing (last count).

i)
$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

After the germination test, the lengths of several randomly selected normal seedlings were measured and they were subsequently dried for 24 hours in an oven at 80°C. The mean seedling length and dry weight for each treatment for each replication were determined by weighing the dried seedlings. According to Abdul-Baki and Anderson (1973), the vigour index was determined by multiplying the germination percentage by the seedling length.

ii) Seedling vigour index I (SVI) = Germination (%) x Mean seedling length (cm).

iii) Seedling vigour index II (SVII) = Germination (%) x seedling dry weight (mg).

Results and Discussion

Isolation of Microorganisms in Desiccated Coconut Water and Dairy Whey

The data presented in Table 1 revealed that the desiccated coconut water and dairy whey samples recorded microbial population *viz*., bacteria (286.4 x 10^4 cfu mL^{-1}), yeasts (8.89 x 10^4 cfu mL^{-1}), actinobacteria (1.00 x 10^4 cfu mL^{-1}), free living N_2 fixers (26.7 x 10^4 cfu mL^{-1}), *Pseudomonas* sp. (73.3 x 10^4 cfu mL^{-1}), PSB (33.3 x 10^4 cfu mL^{-1}), coliforms (76.7 x 10^4 cfu mL^{-1}) in desiccated coconut water. Microbial population in dairy whey was *viz*., bacteria (153.3 x 10^4 cfu mL^{-1}), yeast (3.30 x 10^4 cfu mL^{-1}), actinomycetes (0.12 x 10^4 cfu mL^{-1}) and coliforms (56.7 x 10^4 cfu mL^{-1}) (Plate 1).

These findings were in line with the reports of Umesha and Narayanaswamy (2015). The previous study explained how mature nuts collected on the ground or from a coconut garden as well as those nuts severely broken during harvesting or transportation, which is then allowed for the seepage of coconut water, which is an appropriate transporter for most organisms. (Nandana and Werellagama, 2001). The pollutants must have entered the system during the washing of de-shelled coconut pieces. Microorganisms could be found in the washing water, utensils that came into touch with grated coconut milk, coconut shell, air and handlers. (Priyanthi, 1997). By performing morphological and biochemical tests on the isolates, they were investigated and described (Plate 2). The isolated colonies were mostly round, smooth, convex, whitish, opaque and rod-shaped. The majority of them were positive for catalase, indole test and Gram negative reactions (Mohsin *et al.,* 2014).

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TABLE 1

Microbial population of desiccated coconut water and dairy whey

Samples	Population ($\times 10^4$ cfu mL^{-1})						
	Bacteria	Yeasts	Actinomycetes	Free living nitrogen fixers	Phosphate solubilizing bacteria	*Pseudomonas* sp.	Coliforms
Desiccated coconut water	286.4	8.89	1.00	26.7	33.3	73.3	76.7
Dairy whey	153.3	3.30	0.12	-	-	-	56.7

Note: '-' No population

Bacterial culture

Free living N_2 fixers

Yeast

Phosphate Solubiling Bacteria

Pseudomonas sp.

Plate 1 : Microorganisms isolated from desiccated coconut water and dairy whey

Mineral Content of Desiccated Coconut Water and Dairy Whey

The concentration of mineral elements of desiccated coconut water and dairy whey obtained from desiccated coconut industry are presented in Table 2.

Control (only distilled water)

10 % - Desiccated coconut water

10 % - Dairy whey

Plate 2 : Effect of desiccated coconut water and dairy whey on seed germination of soybean

TABLE 2

Nutrient composition of desiccated coconut water and dairy whey

Samples	N	P	K	S	Na	Ca	Mg	Zn	Cu	Fe	Mn
	(%)							(ppm)			
Desiccated coconut water	0.67	0.38	2.30	1.61	0.86	0.12	0.50	22.60	18.60	95.65	84.00
Dairy whey	0.085	0.169	0.192	0.007	0.039	0.103	0.009	2.348	0.006	1.068	0.002

TABLE 3

Effect of different concentrations of desiccated coconut water on germination percentage, growth parameters and vigour index of soybean

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedlingdry weight (mg)	Seedling vigour index-I	Seedling vigour index-II
T_1:Control water	92.67 ^{bc}	18.43 ^{c}	11.66 ^{b}	369.45 ^{d}	2788 ^{c}	34235 ^{d}
T_2:10%-Desiccated coconut water	98.00 ^{a}	21.93 ^{a}	13.58 ^{a}	404.28 ^{a}	3481 ^{a}	39619 ^{a}
T_3:15%-Desiccated coconut water	96.00 ^{ab}	19.96 ^{b}	12.00 ^{b}	387.57 ^{b}	3068 ^{b}	37206 ^{b}
T_4:20%-Desiccated coconut water	92.00 ^{bc}	18.67 ^{c}	11.64 ^{b}	375.99 ^{c}	2788 ^{c}	34591 ^{c}
T_5:25%-Desiccated coconut water	91.33 ^{c}	15.78 ^{d}	11.26 ^{b}	363.30 ^{e}	2469 ^{d}	33181 ^{e}
T_6:100%-Desiccated coconut water	72.67 ^{d}	12.33 ^{e}	7.36 ^{c}	328.37 ^{f}	1430 ^{e}	23861 ^{f}
S.Em.±	0.62	0.38	0.36	1.51	54.97	136.88
C.D. at 1%	2.69	1.64	1.54	6.53	237.46	591.30

The desiccated coconut water and dairy whey contained mineral elements respectively *viz.,* N (0.67 % and 0.085 %), P (0.38 % and 0.169 %), K (2.30 % and 0.192 %), S (1.61 % and 0.007 %), Na (0.86 % and 0.039 %), Ca (0.12 % and 0.103 %), Mg (0.50 % and 0.009 %), micronutrients like, Zn (22.60 ppm and 2.348 ppm), Cu (18.60 ppm and 0.006 ppm), Fe (95.65 ppm and 1.068 ppm) and Mn (84.00 ppm and 0.002 ppm).

When the results from the two samples were compared, it has shown that natural coconuts and whey both contain some amount of all important elements. (Chuku and Kalagbor, 2014). Similar results were reported by Abdul and Zafar, (2011) and Ibe *et al*. (2013). Whey results are in accordance to Anonymous, (2016).

Effect of different Concentrations of Desiccated Coconut Water and Dairy Whey on Germination Percentage, Growth Parameters and Vigour Index of soybean

The results revealed that seeds treated with 10 per cent desiccated coconut water and dairy whey had higher seed germination than control treatment and was statistically on par with 15 per cent. Desiccated coconut water and dairy whey were found to have a substantial impact on germination (per cent), shoot length (cm), root length (cm), seedling dry weight (mg) and seedling vigour index (Table 3 and 4).

TABLE 4

Effect of different concentrations of dairy whey on germination percentage, growth parameters and vigour index of soybean

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedlingdry weight (mg)	Seedling vigour index-I	Seedling vigour index-II
T_1: Control (Water)	91.33 ^{c}	18.33 ^{b}	11.93 ^{b}	367.70 ^{c}	2764 ^{c}	33583 ^{d}
T_2: 10%-Dairy whey	97.33 ^{a}	20.12 ^{a}	14.31 ^{a}	400.88 ^{a}	3351 ^{a}	39018 ^{a}
T_3: 15%-Dairy whey	95.33 ^{ab}	19.34 ^{ab}	13.92 ^{a}	375.29 ^{b}	3170 ^{a}	35777 ^{b}
T_4: 20%-Dairy whey	93.33 ^{bc}	18.00 ^{b}	13.74 ^{a}	375.15 ^{b}	2962 ^{b}	35014 ^{c}
T_5: 25%-Dairy whey	92.67 ^{bc}	15.72 ^{c}	11.68 ^{b}	365.53 ^{c}	2538 ^{d}	33872 ^{d}
T_6: 100%-Dairy whey	70.67 ^{d}	13.70 ^{d}	6.00 ^{c}	328.87 ^{d}	1392 ^{e}	23240 ^{e}
S.Em.±	0.58	0.45	0.33	1.42	63.88	127.3698
C.D. at 1%	2.49	1.95	1.43	6.14	275.96	550.2084

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Due to the seed treatment with desiccated coconut water and dairy whey, all the seed quality indicators exhibited a substantial change. The seeds treated with 10 per cent desiccated coconut water and dairy whey (T_2) had the highest seed germination (98.00 and 97.33 per cent, respectively) among the different treatments, followed by T_3 (96 per cent and 95.33 per cent, respectively) with desiccated coconut water and dairy whey. T_6 had the lowest germination (72.67 and 70.67 per cent, respectively) in desiccated coconut water and dairy whey (Table 3 and 4) (Plate 2). These findings are consistent with those of previous soybean researchers (Imran Baig, 2005 and Reddy *et al*., 2017).

Similarly, the seedling length differed significantly due to desiccated coconut water and dairy whey. Among the treatments, (T_2) recorded significantly higher shoot length (13.58 and 14.3 cm) and root length (21.94 and 20.12 cm) as compared to control in desiccated coconut water and dairy whey, respectively (Table 3 and 4, Fig. 1 and 2). These findings are in agreement with the reports of Suma and Srimathi (2014).

The seedling dry weight differed significantly due to desiccated coconut water and dairy whey treatment. The seedling dry weight which is directly dependent on the total seedling length (shoot and root length) and significantly higher seedling dry weight was noticed with the seeds treated with 10 per cent desiccated coconut water and dairy whey (T_2) (404.28 and 400.88 mg, respectively) and was superior to control (T_1) (369.45 and 367.70 mg, respectively), the minimum seedling dry weight (323.37 and 328.90 mg) was recorded in T_6 of desiccated coconut water and whey, respectively as indicated in Table 3 and 4.

The seedling vigour index is the most important aspect of seed quality as it decides the vigour level or performance of a seed. Significantly the highest seedling vigour index I (3481 and 3351) and seedling vigour index II (39619 and 39019) were recorded in T_2 of desiccated coconut water and dairy whey, respectively.

While the lowest seedling vigour index I (1430 and 1392) and II (23891 and 23240) were observed in T_6 of desiccated coconut water and dairy whey, respectively. The beneficial effect of desiccated coconut water and dairy whey might be the reason for better seedling growth and vigour. It might be also due to enhanced metabolic activities in early phases of germination. Geetharani *et al.* (2006) also obtained similar results in chilli, Vinodkumar *et al.* (2013) in pigeon pea, Suma and Srimathi (2014) in sesame and Verma & Verma (2014) in soybean.

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