The Pharma Innovation Journal 2022; 11(7): 3005-3008

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(7): 3005-3008

www.thepharmajournal.com
Received: 19-05-2022
Accepted: 28-06-2022

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Effect of vegetarian *Kunapajala* on pigments and soluble protein content in rice

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DOI: https://doi.org/10.22271/tpi.2022.v11.i7al.14463

Abstract
Ancient Indian study of plants, known as 'Vrikshayurveda', was meticulously organized into 325 Sanskrit slokas and published in a book of the same name by Surapala about 1000 years ago with number of recommendations for promoting plant development and warding off illness. "*Kunapajala*" is referred here as a plant nutrient solution. *Kunapajala* is a liquid ferment from animal wastes that contained animal flesh, dung, urine, bone marrow and skin. The fermented product contains essential components such as amino acids, sugars, fatty acids, keratins, macro and nearly all micronutrients in available form. Y.L. Nene formulated special *Kunapajala* of vegetable origin instead of raw meat as people showed reluctance towards using raw meat as 40% of the people do not eat meat. As organic farming and natural farming are the need of the hour with reduced usage of synthetic fertilizers, a study was formulated with vegetarian *Kunapajala* in rice to study its effect on pigments, soluble protein and plant height. Field experiment was conducted during summer 2021 at Wetland farm of TNAU Coimbatore in RBD with three replications and rice variety CO-51. The experiment had 10 treatments *viz.*, T_1 - Foliar spray of water, T_2 - Foliar spray of water + cow urine (9:1), T_3 - Foliar spray of *Kunapajala* 3%, T_4 - Foliar spray of *Kunapajala* 3% in water + cow urine (9:1), T_5 - Soil application of *Kunapajala* 10%, T_6 - Foliar spray of water + Soil application of *Kunapajala* 10%, T_7 - Foliar spray of *Kunapajala* 3% + Soil application of *Kunapajala* 10%, T_8 - Foliar spray of water + cow urine (9:1) + Soil application of *Kunapajala* 10%, T_9 - Foliar spray of *Kunapajala* 3% in water + cow urine (9:1) + *Kunapajala* 10% soil application, T_{10} - Control (no foliar spray and soil application). Observations were recorded for the parameters like chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, soluble protein and plant height at 25, 40 and 55 DAT (10 days after each spraying). Growth parameters of rice like plant height, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and soluble protein were significantly influenced by foliar spray and soil application of *Kunapajala* along with foliar spray of cow urine. Among all the treatments, plots provided with foliar spray of *Kunapajala* 3 % in water + cow urine (9:1) + *Kunapajala* 10 % soil application (T_9) produced plants with greater height (35.07, 55.07 and 81.25 cm), more content of chlorophyll a (2.25, 3.09 and 3.11 mg g^{-1}), chlorophyll b (2.04, 2.81 and 2.88 mg g^{-1}), total chlorophyll (4.28, 5.90 and 5.99 mg g^{-1}), carotenoids (3.61, 4.82 and 4.81 mg g^{-1}) and soluble protein (11.34, 15.09 and 18.75 mg g^{-1}) at 25,40 and 55 DAT i.e., ten days after every spray respectively and followed by Foliar spray (3 %) and soil application of *Kunapajala* (10 %) (T_7) and Foliar spray of cow urine and soil application of *Kunapajala* (T_8). Least amount of chlorophyll a (1.04, 1.16 and 1.76 mg g^{-1}), chlorophyll b (0.91, 1.18 and 1.27 mg g^{-1}), total chlorophyll (1.18, 2.82 and 3.03 mg g^{-1}), carotenoids (1.86, 2.76 and 3.11 mg g^{-1}) and soluble protein (10.6, 14.16 and 17.11 mg g^{-1}) with the smaller plant height (30.40, 50.17 and 74.53 cm) were seen in control plot (T_{10}) no foliar spray and soil application.

Keywords: Vrikshyurveda, special *Kunapajala* (vegetarian based), soluble protein, pigments, plant height and rice

Introduction
Pollution has an impact on our agriculture system. The use of synthetic fertilizers, pesticides, and other agricultural methods to increase crop yields adds to environmental degradation. In the long run, these methods degrade soil fertility by upsetting the nutrient balance. By utilizing environmentally friendly, animal and plant based organic resources that are extremely enriched in nutrients needed by crop plants, organic farming offers a natural method of crop growing to address the issue at hand. Organic manures improve microbial activity and soil condition that helps in offering stable yields, greater soil health, organic food, and a decrease in the use of synthetic fertilizers which reduce environmental issues. Various agricultural strategies are being used to lessen environmental issues, but organic farming without a doubt is the best scientifically proved environmentally friendly strategy for maintaining the ecological balance of our agricultural and ecological systems.

A thousand years ago, organic farming began to develop. By utilizing natural resources, ancient farmers begin crop production along the river belts. The use of organic agricultural inputs by farmers at the period is briefly mentioned in Indian texts like the Ramayana, Rigveda, and Mahabharata. The cycle of dead creatures and foul waste particles that returned to earth in the form of nutrients is mentioned in the Ramayana. The Mahabharata (5500 BC) contains a description of the kamadhenu cow that relates to its function in agricultural operations. Similar to this, in the Kautilya Arthashastra of 300 BC, Kautilya makes mention of oil cake and animal excrement. In the Brihad-Sanhita, Varahmihir discusses many types of manures and manuring techniques for various crops. In the Atharva Veda-II and Rigveda, respectively, organic and green manure are mentioned. Similarly copy of Surapala's manuscript 'Vrikshyurved' from the Bodlein collection in Oxford, acquired by Dr. Y.L. Nene got published in 1996. Vrikshyurveda recommends a number of techniques for agriculture, one such suggestion is to utilise liquid manure known as *Kunapajala* to improve plant growth and development.

Building blocks of organic farming: The method of organic farming depends on a number of different building blocks, some of which include organic manure, crop rotation, vermicomposting, nitrogen-fixing microorganisms, organic residue, crop residue, bio fertilisers, and bio pesticides. Other important building blocks include kitchen waste, sludge, and biogas. These have been shown to be highly helpful in preserving the health and texture of the soil. Their use promotes sustainable agriculture and is environmentally friendly. In addition to these, the organic farming strategy adheres to several fundamental ethical standards for health, the environment, justice, and care. These results are in accordance with Edmeades (2003), who showed managing crop nutrients with organic sources is environmentally friendly and improves the quality of the soil and water, by enhancing soil's physical, chemical, and biological properties, these procedures restore the soil's natural ability to supply nutrients (Reganold 1995; Yadav *et al*. 2000). Application of organic amendments enhances soil biological activity, soil organic carbon (SOC), and infiltration rate (Kundu *et al*. 2002).

Due to lengthy decomposition process of solid manures, liquid organic manures like Panchagavya and *Kunapajala* are provided to crops so, that plants can take nutrients quickly due to their fast decomposition process. As per Vrikshayurveda, *Kunapajala* is recommended for better propagation of various plants. Hence, present study is carried out to know the effect of *Kunapajala* on rice through the soil and foliar application.

The most significant quantitative character in crop, yielding ability, is reliant on the growth of other crop characters. The rice plant at any given time is made up of leaves that are physiologically at various ages (Ramasamy 2000). The photosynthetic activity rate is conditioned by the amount of photosynthetically active pigments (Koch, 1976; Richardson *et al*., 2002). Nitrogen is the essential of all the amino acids in plant structures which are the building blocks of plant proteins, important in growth and development of chlorophyll. According to Alluwar and Deotale (1991) leaf area and yield are correlated, which shows that chlorophyll and leaf area are crucial for influencing output (Raj and Tripathi, 1999). Hence, observations on physiological parameters like pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) and soluble proteins has been carried out to know the effect of rice growth with liquid organic manure.

Materials and Method

***Kunapajala* preparation:** *Kunapajala* was prepared following the procedure outlined by Nene (2012). It is prepared by cooking items soybean meal 500 g + paneer 500 g, tofu 500 g, rice husk 500 g, sesame oil cake 500 g and black gram 250 g together in 5 l or more water. After the liquid has cooled, transfer to a 100 l non corrosive container and add ingredients cattle dung 5 kg, cattle urine 7.5 l, honey 125 g, ghee 125 g and milk 500 ml and water to make up the volume to 50 l. Stir the mixture twice every day in clockwise and anticlockwise. Twelve hours before straining the fermented *Kunapajala*, stir the mixture thoroughly allow to settle, so that the supernatant can be easily removed. For spraying, fine filtering will be necessary. Make the final volume to 100 l.

Field experiment was conducted during Summer 2021 at Wetland farm of TNAU Coimbatore with rice variety CO-51. The organic carbon content of the soil is 0.42%, pH - 7.93, EC (dSm^{-1}) - 0.55, Available N - 291 kg/ha, Available P - 14 kg/ha, Available K - 461 kg/ha was recorded in O_3 field of organic block, wetland farm of TNAU, Coimbatore where the experiment was laid out during 2021-22. Experiment was laid out with 3 replications and 10 treatments *viz.*, T_1 - Foliar spray of water, T_2 - Foliar spray of water + cow urine (9:1), T_3 - Foliar spray of *Kunapajala* 3%, T_4 - Foliar spray of *Kunapajala* 3% in water + cow urine (9:1), T_5 - Soil application of *Kunapajala* 10%, T_6 - Foliar spray of water + Soil application of *Kunapajala* 10%, T_7 - Foliar spray of *Kunapajala* 3% + Soil application of *Kunapajala* 10%, T_8 - Foliar spray of water + cow urine (9:1) + Soil application of *Kunapajala* 10%, T_9 - Foliar spray of *Kunapajala* 3% in water + cow urine (9:1) + *Kunapajala* 10% soil application, T_{10} - Control (no foliar spray and soil application). The experiment was carried out with the common package of practices developed for organic rice farming by Department of Sustainable Organic Agriculture, Tamil Nadu Agricultural University, Coimbatore.

Kunapajala spraying was done at 15, 30 and 45 DAT and observations were recorded at 25, 40 and 55 DAT i.e., 10 days after every spray. Chlorophyll content in leaves was estimated using 80% acetone method (Arnon, 1949) expressed in mg/g fresh weight of leaf and protein content is estimated by using Lowry's *et al.* (1951) method expressed in mg/g fresh weight.

Results and Discussion

Liquid organic manure has an intense effect on the quantities of photosynthetic pigments and protein content of plant. Growth parameters of rice like plant height, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and soluble protein were significantly influenced by foliar spray and soil application of *Kunapajala* along with foliar spray of cow urine. Foliar spray of *Kunapajala* 3 % in water + cow urine (9:1) + *Kunapajala* 10% soil application (T_9) recorded highest plant height (35.07, 55.07 and 81.25 cm), higher content of chlorophyll a (2.25, 3.09 and 3.11 mg g^{-1}), chlorophyll b

(2.04, 2.81 and 2.88 mg g^{-1}), total chlorophyll (4.28, 5.90 and 5.99 mg g^{-1}), carotenoids (3.61, 4.82 and 4.81 mg g^{-1}) and soluble protein (11.34, 15.09 and 18.75 mg g^{-1}) at 25,40 and 55 DAT i.e., ten days after every spray respectively. This might be due to quick absorption and assimilation of macro and micro nutrients available in *Kunapajala* and cow urine improved the metabolic activities and cell division resulting in increased plant height and pigment content. These findings are in agreement with that of Vasmathi (2001) and Sanjuthi *et al*. (2008). At the same time growth parameters of control plot (T_{10}) with no foliar spray and soil applications, showed lowest amount of chlorophyll a (1.04, 1.16 and 1.76 mg g^{-1}), chlorophyll b (0.91, 1.18 and 1.27 mg g^{-1}), total chlorophyll (1.18, 2.82 and 3.03 mg g^{-1}), carotenoids (1.86, 2.76 and 3.11 mg g^{-1}) and soluble protein (10.6, 14.16 and 17.11 mg g^{-1}) content with least plant height (30.40, 50.17 and 74.53 cm), and was on par with T_1 (foliar spray of water alone). Foliar spray of water alone (T_1) did not have much influence on growth parameters of rice and recorded lower values in comparison with other treatment plots.

Following (T_9) Foliar spray of *Kunapajala* (3%) in water + cow urine (9:1) + *Kunapajala* (10%) soil application, foliar spray (3%) and soil application of *Kunapajala* (10%) (T_7) and Foliar spray of cow urine (9:1) and soil application of *Kunapajala* (10%) (T_8) showed better results on growth of rice plant. Growth parameters like chlorophyll a (2.23, 2.91 and mg g^{-1}), chlorophyll b (2.05, 2.70 and 2.82 mg g^{-1}), total chlorophyll (4.27, 5.61 and 5.83 mg g^{-1}), carotenoids (3.58, 4.78 and 4.79 mg g^{-1}), soluble protein (11.03, 15.03 and 18.52 mg g^{-1}) and plant height (34.87, 55.00 and 80.05 cm) of T_7 were observed greater than those of growth parameters of T_8 *viz.*, chlorophyll a (2.19, 2.71 and 2.83 mg g^{-1}), chlorophyll b (2.00, 2.61 and 2.74 mg g^{-1}), total chlorophyll (4.18, 5.32 and 5.57 mg g^{-1}), carotenoids (3.62, 4.62 and 4.59 mg g^{-1}), soluble protein (11.01, 14.81 and 18.47 mg g^{-1}) and plant height (34.77, 54.77 and 76.57 cm) with minute difference. From this is it seen that foliar and soil application of *Kunapajala* showed better results than foliar spray of cow urine with soil application of *Kunapajala*. This is due to the role of organic fertilization in improving soil physical and chemical properties and making it more fertile which helps in increasing the nutrient content of the plants, especially nitrogen and magnesium that enter in chlorophyll (Abdlrahman and Ramathan, 2015, AL-Hamdany and Hadie, 2017) [1, 2]. Liquid organic manure contains high amount of nitrogen, which can influence the chlorophyll content and plant growth as mentioned by Hokmalipour and Darbandi (2011) [6]. Application of *Kunapajala* along with cow urine might have increased cell division and metabolic activity resulted in higher pigment content, soluble protein and plant height which is due to nitrogen present in *Kunapajala*.

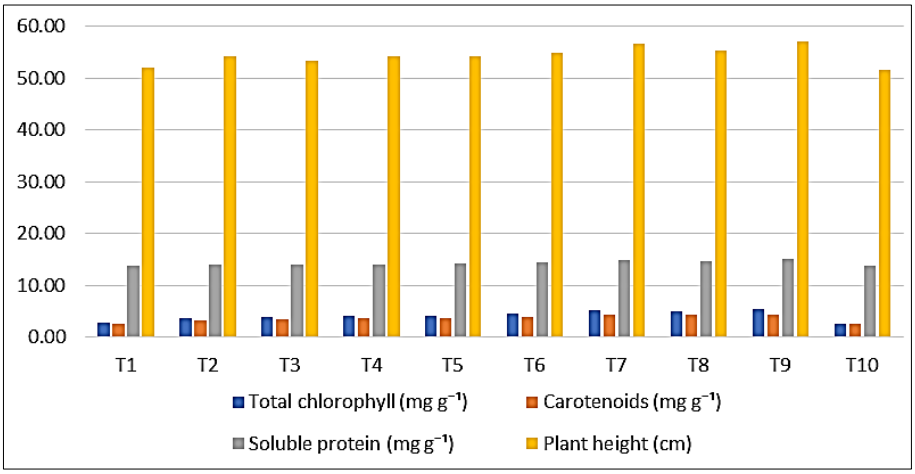

Fig 1: Effect of physiological parameters like pigments and soluble protein on plant height.

Physiological parameters like pigments and soluble protein shows significant effect on plant height (Fig 1.) which may be due to nutrients, primary and secondary elements like nitrogen, phosphorus, potassium, magnesium, calcium etc. provided by *Kunapajala* and cow urine. These elements are useful in development of chlorophyll and protein, which are useful in photosynthesis and other metabolic process which are helpful for plant growth and development. These results are in accordance with Zhilin *et al.* (1997) [14] stated that plant height is increased significantly due to nitrogen application. Nitrogen application directly increased the amount of chlorophyll and leaf surface area, which accelerated the photosynthesis process and produced more sugar (Dikshit and Paliwal, 1989) [5] which is used for plant development like height of plant.

Table 1: Effect of *Kunapajala* and cow urine on chlorophyll 'a', chlorophyll 'b' and total chlorophyll.

Treatment		Chlorophyll a (mg g^{-1})			Chlorophyll b (mg g^{-1})			Total chlorophyll (mg g^{-1})		
		25 DAT	40 DAT	55 DAT	25 DAT	40 DAT	55 DAT	25 DAT	40 DAT	55 DAT
T_1	Foliar spray of water	1.12	1.65	1.76	0.93	1.44	1.56	1.98	3.09	3.32
T_2	Foliar spray of water + cow urine (9:1)	1.76	1.96	2.08	1.49	1.75	1.84	3.23	3.72	3.92
T_3	Foliar spray of *Kunapajala* 3%	1.83	2.06	2.18	1.62	1.86	1.99	3.44	3.92	4.17
T_4	Foliar spray of *Kunapajala* 3% in water + cow urine (9:1)	1.87	2.31	2 .42	1.65	2.08	2.19	3.53	4.39	4.61
T_5	Soil application of *Kunapajala* 10%	1.88	2.28	2.31	1.66	2.06	2.18	3.53	4.34	4.49
T_6	Foliar spray of water + Soil application of *Kunapajala* 10%.	2.05	2.54	2.66	1.80	2.32	2.44	3.85	4.86	5.10
T_7	Foliar spray of *Kunapajala* 3% + Soil application of *Kunapajala* 10%.	2.23	2.91	3.01	2.05	2.70	2.82	4.27	5.61	5.83

T_8	Foliar spray of water + cow urine (9:1) + Soil application of *Kunapajala* 10%.	2.19	2.71	2.83	2.00	2.61	2.74	4.18	5.32	5.57
T_9	Foliar spray of *Kunapajala* 3% in water + cow urine (9:1) + *Kunapajala* 10% soil application	2.25	3.09	3.11	2.04	2.81	2.88	4.28	5.90	5.99
T_{10}	Control (no foliar spray and soil application).	1.04	1.64	1.76	0.91	1.18	1.27	1.89	2.82	3.03
	SEd	0.052	0.030	0.046	0.058	0.129	0.053	0.061	0.123	0.075
	CD (P=0.05)	0.110	0.063	0.096	0.122	0.271	0.112	0.129	0.258	0.157

Table 2: Effect of *Kunapajala* and cow urine on carotenoids, soluble protein, and plant height.

Treatment		Carotenoids (mg g^{-1})			Soluble protein (mg g^{-1})			Plant height (cm)		
		25 DAT	40 DAT	55 DAT	25 DAT	40 DAT	55 DAT	25 DAT	40 DAT	55 DAT
T_1	Foliar spray of water	2.01	2.95	3.08	10.04	14.15	17.03	30.37	50.83	74.80
T_2	Foliar spray of water + cow urine (9:1)	2.83	3.56	3.59	10.33	14.39	17.14	32.23	52.33	77.90
T_3	Foliar spray of *Kunapajala* 3%	2.92	3.88	3.47	10.30	14.40	17.09	32.13	52.07	76.03
T_4	Foliar spray of *Kunapajala* 3% in water + cow urine (9:1)	3.08	4.08	3.63	10.49	14.49	17.28	33.37	52.80	76.47
T_5	Soil application of *Kunapajala* 10%	3.18	4.19	3.89	10.39	14.43	18.02	33.33	53.20	76.20
T_6	Foliar spray of water + Soil application of *Kunapajala* 10%.	3.29	4.28	4.06	10.76	14.62	17.89	33.50	54.00	77.31
T_7	Foliar spray of *Kunapajala* 3% + Soil application of *Kunapajala* 10%.	3.58	4.78	4.79	11.03	15.03	18.52	34.87	55.00	80.05
T_8	Foliar spray of water + cow urine (9:1) + Soil application of *Kunapajala* 10%.	3.62	4.62	4.59	11.01	14.81	18.47	34.77	54.77	76.57
T_9	Foliar spray of *Kunapajala* 3% in water + cow urine (9:1) + *Kunapajala* 10% soil application	3.61	4.82	4.81	11.34	15.09	18.75	35.07	55.07	81.25
T_{10}	Control (no foliar spray and soil application).	1.86	2.76	3.11	10.06	14.16	17.11	30.40	50.17	74.53
	SEd	0.082	0.071	0.090	0.076	0.078	0.343	0.376	0.338	1.476
	CD (P=0.05)	0.172	0.150	0.190	0.160	0.165	0.720	0.791	0.711	3.102

Conclusion

Results of this research revealed that application of foliar spray of *Kunapajala* (3%) in water + cow urine (9:1) + *Kunapajala* (10%) soil application as significantly increased parameters like chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, soluble protein and plant height followed by foliar spray (3%) and soil application of *Kunapajala* (10%) which is on par with foliar spray of cow urine (9:1) and soil application of *Kunapajala* (10%) and least values of recorded growth parameters are seen in plot provided with foliar spray of water alone which is on par with control (no foliar spray and soil application).

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